

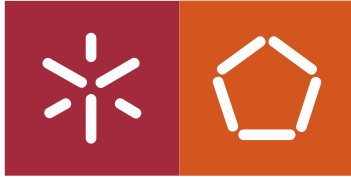
Universidade do Minho
Escola de Engenharia

Telma Sofia Alves Dantas

Natural-inspired tooth replacement:
design and strategies

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Natural-inspired tooth replacement: design and strategies

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Programa Doutoral em Líderes para Indústrias Tecnológicas

Trabalho realizado sob a orientação de

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abril de 2022

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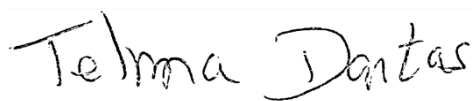
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Statement of Integrity

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Telma Sofia Alves Dantas

RESUMO

Substituição dentária de inspiração natural: design e estratégias

A perda de dentes é uma condição que afeta pessoas de várias faixas etárias. Pode ser provocada por diferentes condições como a higiene oral deficitária, factores associados a condições ambientais e/ou hábitos de vida, mesmo doenças como a doença periodontal, bruxismo ou outras sistémicas, de que constitui exemplo a diabetes mellitus. Hoje em dia substituir um dente por um implante é um processo relativamente comum. Contudo, as atuais estratégias aplicadas apresentam inúmeras limitações, nomeadamente a falha do implante devido à sua fraca estabilidade, e consequente falta de adesão óssea funcional, definida como osseointegração.

Os implantes análogos de raiz personalizados surgiram, assim, como uma alternativa aos convencionais, com o objetivo de proporcionar ao paciente uma solução que preserve mais tecidos duros e moles, com um tempo de cicatrização reduzido.

Neste sentido, o objetivo deste projeto de doutoramento é o desenvolvimento de um implante dentário que se adeque às características de cada pessoa e assim melhorar o desempenho do implante melhorando o processo de osseointegração e minimizando os riscos inerentes a tal cirurgia, bem como os custos relacionados com os implantes dentários.

A estratégia desenvolvida inclui a aquisição da geometria real do dente ou alvéolo através de Tomografia Computorizada de Feixe Cónico (CBCT), modelação 3D do implante e fabrico por Controlo Numérico Computadorizado (CNC). Para assegurar a estabilidade mecânica e biológica do implante, técnicas como a incorporação de microcanais na superfície e outras características de superfície foram exploradas e avaliadas. Para analisar e avaliar a estratégia desenvolvida foram feitos testes como a validação *in silico*, hidrofiliabilidade da superfície e adesão bacteriana. Além disso, para validar o processo global de aquisição de imagem e manufatura do implante, foi realizado um estudo numa mandíbula de um cão cadáver.

Espera-se que o desenvolvimento deste produto conduza ao início de uma nova era de implantes dentários, uma vez que permitirá a produção de implantes dentários semelhantes ao dente natural com um melhor desempenho biológico e mecânico, com o objetivo principal de melhorar a qualidade de vida do paciente.

Palavras-Chave: Implantes dentários análogos de raiz, Customização, CAD/CAM, CNC, Zircónia.

ABSTRACT

Natural-inspired tooth replacement: design and strategies

Tooth loss is a problem that affects both old and young people. It may be caused by several conditions, such as poor oral hygiene, lifestyle choices or even diseases like periodontal disease, tooth grinding, or diabetes. Nowadays, replacing a missing tooth by an implant is a very common process. However, many limitations regarding the actual strategies can be enumerated, namely implant failure due to weak mechanical and biological stabilities, and consequent lack of functional bone adherence, defined as osseointegration. Customized root-analogue implants have, therefore, emerged as an alternative to conventional ones, aiming to provide the patient with a solution that preserves more hard and soft tissues, with a reduced healing time.

In this sense, the aim of this PhD project is the development of a dental implant that fits each person's characteristics and thus improve the implant performance by improving the osseointegration process and minimizing the risks inherent to such surgery as well as the costs related to dental implants. The developed strategy encompasses the CBCT scan of the real tooth or socket geometry, implant 3D modelling and CNC manufacturing techniques. To ensure both mechanical and biological stabilities, techniques such as the incorporation of surface micro-channels and other surface features were explored and evaluated. *In silico* validation, as well as bacterial adhesion to different surfaces, were also assessed. To validate the overall image acquisition process and implant manufacturing, a study was performed in a cadaver dog mandible.

The development of this product is expected to lead to the beginning of a new era of dental implants since it will allow producing natural tooth-like dental implants with enhanced biological and mechanical performance, with the main goal of improving the patient's quality of life.

Keywords: Root-analogue dental implants; Customization, CAD/CAM, CNC, Zirconia.

CONTENTS

Acknowledgments.....	iii
Resumo.....	v
Abstract.....	vi
Contents.....	vii
List of Symbols, annotations and abbreviations	xii
Figures	xv
Tables	xix
Chapter 1 Introduction	1
1.1. Motivation	2
1.1.1. Leaders for Technical Industries Doctoral Program	3
1.2. Objectives	3
1.3. Structure of the Thesis	4
Chapter 2 State of the Art	7
2.1. Jaw Characterization	8
2.1.1. Jaw Anatomy.....	8
2.1.2. Teeth Anatomical Structure.....	10
2.1.3. Bone Classification	12
2.1.4. Bone Geometry Evaluation After Tooth Extraction	14
2.2. Dental Implants.....	16
2.2.1. Materials Used in Dental Implants.....	17
2.2.2. Dental Implants Types and Geometries	21
2.2.2.1. Root-analogue Dental Implants.....	23
2.2.3. Dental Implants Market.....	24
2.2.4. Dental Implants Failures	26
2.3. Manufacturing Techniques.....	28
2.3.1. CAD/CAM Technology	29
2.3.1.1. Digital Radiography	30
2.3.1.2. Clinical Applications of CAD/CAM	32
2.4. Dental Implants Surface Modifications	35
2.4.1. Surfaces for Improved Mechanical and Biological Stabilities.....	36
2.4.1.1. Machining.....	36
2.4.1.2. Sandblasting.....	36

2.4.1.3.	Acid-etching	37
2.4.1.4.	Anodization	38
2.4.1.5.	Laser Treatment.....	39
2.4.1.6.	Plasma Spraying	39
2.4.1.7.	Other Coatings	40
2.4.2.	Surfaces for Antibacterial Purposes	41
	References	42
Chapter 3 Subperiosteal dental implants: Past or future?.....		54
A critical review on clinical trials/case reports and future directions.....		54
	Abstract	55
3.1.	Introduction.....	55
3.2.	Methods.....	56
3.2.1.	Search Strategy	56
3.2.2.	Study Selection.....	56
3.2.3.	Data Collection and Extraction.....	57
3.3.	Results.....	57
3.3.1.	Study Selection.....	57
3.3.2.	Materials and Surface Coatings	61
3.3.3.	Design and Manufacturing Techniques.....	62
3.3.4.	Methods of Implantation	64
3.3.5.	Complementary Strategies	65
3.3.6.	Main Clinical Outcomes	66
3.4.	Discussion	67
3.4.1.	Number of Surgeries and Clinical Procedure	68
3.4.2.	Materials, Aesthetics and Bacterial Affinity.....	69
3.4.3.	Cost and Customization	69
3.4.4.	Optimal/Improved Solution.....	69
3.4.4.1.	Implant Customization	70
3.4.4.2.	Surgical Procedure	70
3.4.4.3.	Osseointegration and Anti-bacterial Strategies.....	71
3.5.	Conclusions	74
	Acknowledgments.....	75
	References	75

Chapter 4 Customized root-analogue implants: a meta-analysis review on outcomes from clinical trials and case reports	81
Abstract	82
4.1. Introduction.....	82
4.2. Methods.....	84
4.2.1. Search Strategy	84
4.2.2. Study Selection.....	84
4.2.3. Data Collection and Extraction.....	84
4.3. Results.....	85
4.3.1. Study Selection.....	85
4.3.2. Design and Manufacturing Techniques, Implant Material and Surface Treatments	87
4.3.3. Clinical Trials/Case Reports Summary	90
4.4. Discussion	96
4.5. Conclusions	99
Acknowledgments.....	100
References	101
Chapter 5 In silico evaluation of the stress fields on the cortical bone surrounding dental implants: comparing root-analogue and screwed implants	107
Abstract	108
5.1. Introduction.....	108
5.2. Materials and Methods	111
5.2.1. 3D Modelling	111
5.2.2. Finite Element Modelling.....	111
5.2.3. Results Analysis	113
5.3. Results and Discussion.....	114
5.3.1. Stress Distribution and Hydrostatic Pressure in the Peri-implant Cortical Bone	114
5.3.2. Octahedral Shear Strain in the Peri-implant Cortical Bone	118
5.3.3. Implant Displacement.....	120
5.4. Conclusions	121
Acknowledgments.....	122
References	122
Chapter 6 Design and optimization of zirconia functional surfaces for dental implants applications	127
Abstract	128

6.1.	Introduction.....	128
6.2.	Materials and Methods	131
6.2.1.	Specimen Design and Manufacturing	131
6.2.2.	Surface Analysis	133
6.2.2.1.	Scanning Electron Microscopy Analysis and Profilometry.....	133
6.2.2.2.	Surface Wettability	134
6.2.2.3.	Surface Capillarity.....	134
6.3.	Results and Discussion.....	135
6.3.1.	Specimens Characterization.....	135
6.3.2.	Wettability Analysis	139
6.3.3.	Capillarity Analysis.....	141
6.4.	Conclusions	144
	Acknowledgments.....	145
	References	145
	Chapter 7 Bacteria co-culture adhesion on different texturized zirconia surfaces	151
	Abstract	152
7.1.	Introduction.....	152
7.2.	Materials and Methods	156
7.2.1.	Production of Titanium Specimens.....	156
7.2.2.	Production of Zirconia Green Compacts.....	156
7.2.3.	Surface Texturing.....	157
7.2.4.	Zirconia Green Compacts Sintering	158
7.2.5.	Specimens Cleaning Procedure.....	158
7.2.6.	Surface Analysis	159
7.2.7.	Bacterial Adhesion Protocol.....	160
7.2.8.	Statistical Analysis	161
7.3.	Results and Discussion.....	161
7.3.1.	Specimens Characterization.....	161
7.3.2.	Wettability and Capillarity Analysis.....	163
7.3.3.	Bacterial Adhesion.....	165
7.3.3.1.	Mono-cultures.....	165
7.3.3.2.	Co-culture of <i>S. aureus</i> and <i>P. aeruginosa</i>	167
7.3.3.3.	Scanning Electron Microscopy Analysis	169
7.4.	Conclusions	171

Acknowledgments.....	173
References	174
Chapter 8 Customized root-analogue dental implants - Procedure and errors associated with image acquisition and treatment, and manufacturing technology in an experimental study on a cadaver dog mandible.....	182
Abstract	183
8.1. Introduction.....	183
8.2. Materials and Methods	186
8.2.1. Study Design	186
8.2.2. Image acquisition	187
8.2.3. Soft Tissues Removal	188
8.2.4. Image Treatment	188
8.2.5. Implant Manufacturing.....	188
8.2.6. Image Analysis	189
8.3. Results and Discussion.....	189
8.3.1. Soft Tissues Influence on Image Quality	189
8.3.2. Implant Development.....	190
8.3.3. Geometric Differences and Errors	193
8.3.4. Teeth Placement and Final Validation.....	198
8.4. Conclusions	200
Acknowledgments.....	201
References	201
Chapter 9 Conclusions	207
9.1. General Discussion and Conclusions.....	208
9.1.1. Implant prototype	210
9.2. Suggestions for Future Works	211
9.3. Further Contributions	211

LIST OF SYMBOLS, ANNOTATIONS AND ABBREVIATIONS

AACD	American Academy of Cosmetic Dentistry
Al_2O_3	Alumina
AM	Additive Manufacturing
ANOVA	One-way Analysis of Variance
AS	As Sintered
BMD	Bone Mineral Density
BMPs	Bone Morphogenic Proteins
BQI	Bone Quality Index
$\text{C}_{19}\text{H}_{14}\text{O}_5$	Phenol red
CA	Contact Angle
$\text{Ca}_5(\text{PO}_4)_3\text{OH}$	Hydroxyapatite
CAD	Computer-Aided Design
CAM	Computer-Aided Manufacturing
CBCT	Cone-Beam Computed Tomography
CFU	Colony Forming Units
CNC	Computer Numerical Control
Co-Cr	Chromium-cobalt
cpTi	Commercially Pure Titanium
cRAI	Customized Root-Analogue Implants
CT	Computed Tomography
DICOM	Digital Imaging and Communications in Medicine
DLMF	Direct Laser Metal Forming
DMLS	Direct Metal Laser Sintering
E. coli	Escherichia coli
EDAM	Engineering Design and Advanced Manufacturing
FBS	Fetal Bovine Serum
FEA	Finite Element Analysis
H_2SO_4	Sulfuric Acid
HAp	Hydroxyapatite
HCl	Hydrochloric Acid

HF	Hydrofluoric Acid
HNO ₃	Nitric Acid
In1	Incisive 1
In2	Incisive 2
ISO	International Organization for Standardization
IT	Information Technology
LTD	Low-Temperature Degradation
LTI	Leaders for Technical Industries
M1	Molar 1
M2	Molar 2
MC	Micro-channels
MCE	Micro-channels and acid etching
MCI	Mandibular Cortical Index
MIT	Massachusetts Institute of Technology
NT	Natural Tooth
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PBS	Phosphate Buffered Saline
PDL	Periodontal Ligament
PEEK	Poly-ether-ether-ketone
PRF	Platelet-Rich Fibrin
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT	Polished Titanium
PZ	Polished Zirconia
R _a	Roughness Average
RAI	Root-Analogue Implants
RAIF	Root-Analogue Implant with Flaps
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SbE	Sandblasted and Acid Etched
SCG	Subcritical Crack Growth
SEM	Scanning Electron Microscopy
SLA	Sandblasting Acid Etching
STI	Semi-Threaded Implant

STL	Stereolithography
TBZ	Titanium Blasted with Zirconia
TBZA	Titanium Blasted with Zirconia then Acid etched
TCP	Tricalcium Phosphate
TI	Threaded Implant
Ti	Titanium
Ti6Al4V	Titanium-6Aluminum-4Vanadium
TiO ₂	Titanium dioxide
TZP	Tetragonal Zirconia Polycrystal
US	United States
WCA	Water Contact Angles
WHO	World Health Organization
Y ₂ O ₃	Yttria
YSZ	Yttria Stabilized Zirconia
ZrO ₂	Zirconia
β-TCP	Beta-Tricalcium Phosphate

FIGURES

Chapter 2:

Figure 2.1 The facial skeleton: (a) external anatomy of the right side of the skull; (b) internal anatomy of the left side of the skull. Adapted from [1].	8
Figure 2.2 Mandible, right lateral view. Adapted from [1].	9
Figure 2.3 Maxilla, right lateral view. Adapted from [1].	9
Figure 2.4 Median section of a canine tooth and its alveolus. Adapted from [5].	11
Figure 2.5 Different tooth types and respective roots. Adapted from [6].	12
Figure 2.6 Jawbone Quality Index (BQI) representation. Adapted from [9].	12
Figure 2.7 Mandibular Cortical Index (MCI) representation [10].	13
Figure 2.8 Influence of bone density on implant success rate [9].	14
Figure 2.9 Schematic drawing representing the location where the histometric measurements were performed [14].	15
Figure 2.10 Overview of the extraction site after: (a) 1 week; (b), 2 weeks; (c) 4 weeks, and, (d) 8 weeks. B, Buccal; L, Lingual; PM, Provisional Matrix; C, Blood Clot; WB, Woven Bone; BM, Bone Marrow [14].	16
Figure 2.11 The early dental implants made of stone, shells, bones and other materials [16].	16
Figure 2.12 Implant components. Adapted from [18].	17
Figure 2.13 Different implant designs: (a) subperiosteal; (b) endosseous; and (c) transosteal. Adapted from [55].	22
Figure 2.14 Global dental implants market size. Adapted from [68].	25
Figure 2.15 Global dental implants market share. Adapted from [68].	26
Figure 2.16 CAD/CAM process workflow. Adapted from [94].	30
Figure 2.17 CBCT apparatus: (a) equipment; (b) CBCT data acquisition software; (c) CBCT images and 3D reconstruction.	32
Figure 2.18 CAD/CAM applied in dentistry: (a) ceramic crown; (b) software layout [105].	33
Figure 2.19 Surgical guide: (a) surgical guide design and surgery simulation; (b) clinical procedure with the manufactured surgical guide [106].	33
Figure 2.20 Dental implants manufactured by CAD/CAM: (a) titanium root-analogue implant [109]; (b) zirconia root-analogue implant [112].	34
Figure 2.21 Schematic representation of the implant stability [23].	35
Figure 2.22 Schematic representation of the sandblasting process [123].	37
Figure 2.23 Schematic representation of the anodization process [134].	38
Figure 2.24 Schematic representation of the plasma-spraying process [119].	40

Chapter 3:

Figure 3.1 Search strategy flowchart, adapted from [8].	58
Figure 3.2 Schematic representation of an endosseous implant, a subperiosteal implant and an improved solution.	71

Chapter 4:

Figure 4.1 Search strategy flowchart, adapted from [16].	86
--	----

Figure 4. 2 Schematic representation of the materials, design and manufacturing techniques, and surface treatments of the selected and analysed studies.....	90
--	----

Chapter 5:

Figure 5. 1 Schematic representation of the studied models: (a) STI - Semi-Threaded Implant; (b) TI - Threaded Implant; (c) RAI - Root-Analogue Implant; (d) RAIF - Root-Analogue Implant with Flaps; and (e) NT - Natural Tooth.....	111
Figure 5. 2 Boundary conditions: (a) Applied loads; (b) Fixed constraint.	113
Figure 5. 3 Analysed cortical bone volume (partition) for the different models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.	113
Figure 5. 4 Von Mises stress distribution in the cortical bone for the studied models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.	115
Figure 5. 5 Von Mises stress distribution in the circular partition, as a function of the volume, for the different models.	116
Figure 5. 6 Hydrostatic pressure in the circular partition, as a function of the volume, for the studied models.....	118
Figure 5. 7 Percentage of the circular partition volume in the four μ -strain zones: Disuse atrophy (< 50 μ -strain); Steady state (100 - 2000 μ -strain); Physiologic overload (2000 - 4000 μ -strain); and Pathologic overload (> 4000 μ -strain).	119
Figure 5. 8 Implant/Tooth total displacement for the studied models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.....	120

Chapter 6:

Figure 6. 1 Specimens design and final dimensions: (a) P1; (b) P2; (c) P3.....	133
Figure 6. 2 SEM micrographs of the surface topography of the three specimens with the respective micro-channels: (a), (b) P1 specimen; (c), (d) P2 specimen; (e), (f) P3 specimen.	136
Figure 6. 3 Representative figures of the profilometry analysis software outputs: (a) C2 micro-channel depth and width; (b) C2 micro-channel topography.	137
Figure 6. 4 Roughness (mean $\pm$ SD) of the specimens' top surface (P1 TS, P2 TS and P3 TS) and in the bottom of the micro-channels.....	138
Figure 6. 5 Representative images of a water droplet and measured contact angle: (a) immediately after reaching the surface; (b) 10 s later.....	139
Figure 6. 6 Water and FBS contact angles (mean $\pm$ SD) of P1 (a), P2 (b) and P3 (c) specimens, for 0 s and 10 s.	140
Figure 6. 7 Fluid height variation with time, in the P1 micro-channels, during the capillarity test: (a) pigment; (b) FBS.....	141
Figure 6. 8 Fluid height variation with time (mean $\pm$ SD), in the P2 micro-channels, during the capillarity test: (a) pigment; (b) FBS.	142
Figure 6. 9 Fluid height variation with time (mean $\pm$ SD), in the P3 micro-channels, during the capillarity test, for both the pigment and FBS fluids.	143
Figure 6. 10 Representative images of the fluid rise in the capillarity tests: (a) P1; (b) P2; and (c) P3.	143

Chapter 7:

Figure 7. 1 Schematic representation of the produced specimens: (a) Ti- titanium with SLA; (b) AS- zirconia as-sintered; (c) SbE- zirconia with sandblasting and acid etching; (d) MC- zirconia with micro-channels on the surface; (e) MCE- zirconia with micro-channels and acid etching.....	158
Figure 7. 2 Schematic representation of the developed surfaces and tested bacteria.....	160
Figure 7. 3 SEM micrographs of the surface topography of the produced specimens: (a) Ti; (b) AS; (c) SbE; (d) MC; (e) MCE.	162
Figure 7. 4 Contact angles (mean $\pm$ SD) of tested materials, for 0 s and 10 s.	164
Figure 7. 5 Representative images of the fluid rise in the capillarity tests: (a) Ti; (b) AS; (c) SbE; (d) MC; (e) MCE.	165
Figure 7. 6 Bacterial adhesion (CFU/mL) on the tested surfaces: (a) <i>Escherichia coli</i> ; (b) <i>Staphylococcus aureus</i> ; (c) <i>Pseudomonas aeruginosa</i> . The error bars represent the standard deviation. Different letters between distinct columns denote significant differences using one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).	166
Figure 7. 7 Bacterial adhesion (CFU/mL) in co-culture: (a) <i>Staphylococcus aureus</i> ; (b) <i>Pseudomonas aeruginosa</i> . The error bars represent the standard deviation and (*) indicates statistically significant differences ($p < 0.05$).	168
Figure 7. 8 Representative micrographs of the adhered bacteria: (a) and (b) <i>E. coli</i> on Ti and MC, respectively; (c) and (d) <i>S. aureus</i> on Ti and MC, respectively; (e) and (f) <i>P. aeruginosa</i> on Ti and MC, respectively.	170
Figure 7. 9 Representative micrographs of the adhered bacteria in co-culture: (a) Ti; (b) MC. .	171

Chapter 8:

Figure 8.1 Flowchart representing the performed procedure.	187
Figure 8.2 3D reconstructions of the Cone-Beam Computed Tomographies: (a) dog skull with soft tissues- CBCT1; (b) dog maxilla after soft tissues removal- CBCT2; and (c) dog mandible after soft tissues removal- CBCT2.	190
Figure 8.3 Selected teeth: (a) selected teeth from the mandible; and (b) selected tooth from the maxilla.	191
Figure 8.4 M1 tooth segmentation in <i>RadiAnt</i> : (a) application of a threshold based on the image grey levels; (b) selection of the region of interest; and (c) M1 model resulting from the software.	191
Figure 8.5 M1 treatment in <i>Materialise Magics</i> : (a) tooth before artefacts removal; and (b) tooth after artefacts removal and surface smoothening.	192
Figure 8.6 Designing In1 based on the socket geometry: (a) region of interest segmentation; (b) 3D mesh creation in <i>AutoDesk Meshmixer</i> ; and (c) final model.	192
Figure 8.7 Superimposition of the SLT files resulting from the 3D scan (green) and CBCT scan (orange): (a) M1; (b) M2; and (c) In2.	193
Figure 8.8 Deviations of the superimposed surfaces (measurement in millimetres): (a) M1; (b) M2; and (c) In2.	194
Figure 8.9 Original vs. manufactured teeth: (a) M1; (b) M2; (c) In1; and (d) In2.	197
Figure 8.10 X-ray scans after teeth placement: (a) In1; (b) In2 and M1; and (c) M2.	198

Figure 8.11 Distances between the manufactured tooth and the bone, measured in <i>RadiAnt</i> : (a) In1; (b) In2; (c) M1; and (d) M2.....	199
Figure 8.12 Distances between the original tooth and bone, measured in <i>RadiAnt</i>	200

Chapter 9:

Figure 9.1 Implant prototype.....	210
-----------------------------------	-----

TABLES

Chapter 2:

Table 2. 1 Width of the bone tissue at the buccal and lingual walls of the extraction sites. Adapted from [14].	15
---	----

Chapter 3:

Table 3. 1 Summary of the selected articles.	58
Table 3. 2 Comparing subperiosteal and endosseous implants.	67

Chapter 4:

Table 4. 1 Articles included in the review.....	86
Table 4. 2 Summary of the selected articles: implant material, design and manufacturing techniques and implant surface treatments.....	88
Table 4. 3 Summary of the clinical trials/case reports parameters and results.....	91

Chapter 5:

Table 5. 1 Material properties in FEA.	112
Table 5. 2 Average element quality, and number of nodes and tetrahedrons of the mesh of the different models.	112
Table 5. 3 Average μ -strain values for the five models.	118

Chapter 6:

Table 6. 1 Guide for cutting Zirconia with DWX – 50 [37].	131
Table 6. 2 P1 micro-channels dimensions and respective designation.....	132
Table 6. 3 Micro-channels depths and widths acquired by profilometry: CAD model vs. real dimensions.	137

Chapter 7:

Table 7. 1 Mean (SD) of surface roughness of the produced specimens. Roughness of MC and MCE specimens was measured on the top surface.	162
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Chapter 8:

Table 8.1 Milling parameters.	189
Table 8.2 Deviations between the two compared surfaces - 3D scan of the original tooth and developed tooth.....	195
Table 8.3 Comparison of surface area change between the 3D scan of the original.....	195
Table 8.4 Comparison of surface area change between the 3D digital surface models and the fabricated teeth.	196

CHAPTER 1

INTRODUCTION

The first chapter of this PhD thesis aims to present the purpose of this project and includes the motivation and the objectives that led to the development of such work as well as the structure of the thesis.

1.1. Motivation

When I finished my master's degree in Biomedical Engineering, I have started working on an innovative project entitled Hybrid Additive Manufacturing for Bio Inspired Components, which aims to develop new strategies to create and produce advanced materials to implement in the medical field. This project has increased my motivation to develop new products to keep up with the constant advances in medicine and apply all the acquired knowledge in a more industrial and commercial approach.

Nowadays, tooth loss is a very common problem and, as consequence, dental implants are each time more and more usual. In fact, it is estimated that 10% of people will need a dental implant in their lifetime. Despite all the advances in the comprehension of dental implants designs, materials and techniques, there is still a lot of work to do regarding the better use of biomaterials, implant design, surface modification and functionalization of surfaces to improve the osseointegration process and the overall implant performance. Traditional implants have many limitations and, once each patient has a different oral condition, implants may not fit adequately in everyone's characteristics.

Titanium and its alloys have been extensively used in dental implants due to their high corrosion resistance, low weight, biocompatibility, and mechanical properties. However, some drawbacks are associated with these metallic materials. Their dark colour may become an aesthetical issue if gingival recession occurs. Additionally, they tend to release ions when in contact with fluids and their electrical conductivity may compromise the implant performance. In this context, ceramic materials such as Zirconia have also been investigated for such applications. Zirconia is a tooth-like colour ceramic with high wear and thermal resistance, biocompatibility, and low bacterial affinity.

Despite the advantages of Zirconia, the implant osseointegration process also needs to be optimized. The constant developments regarding dental implant materials and geometries are also notorious. However, few investigations have been conducted regarding customizing a dental implant according to each patient's oral condition. Developing a strategy that customizes the implant according to each patient's geometrical and biological characteristics will not only preserve more hard and soft tissue but also improve the healing time and reduce implant failure rates.

Having this in mind, the main outcome of this work is the development of a customized dental implant that pretends to mimic the original tooth geometry and function, using different

techniques to ensure that it fits perfectly in the patient's oral cavity. To achieve this, different technologies and approaches were used: image acquisition techniques, 3D modelling, Computer Numerical Control (CNC) machining, *in silico* and *in vitro* validation, and validation in dog cadaver mandibles. The main motivation of this work is the belief that this new approach will considerably improve the patient's quality of life and well-being, through a less invasive and more comfortable procedure.

1.1.1. Leaders for Technical Industries Doctoral Program

This PhD work aims to focus not only on the development of a new product but also on its integration in the market world, considering other competitor solutions.

The Leaders for Technical Industries (LTI) doctoral program, within the Engineering Design and Advanced Manufacturing (EDAM) area, - MIT Portugal Program- is designed to promote a close relationship between the industrial environment and academia. This PhD program aims to generate new scientific knowledge and engineering solutions that will compete in different industrial sectors.

This is the main reason why this PhD program was chosen for the development of the present research work. This relationship between academia and industry empowered by the LTI doctoral program contributed for a continuous awareness of the market evolution and needs, and, consequently, contributed to the development of an optimized product to fulfil the market needs. Additionally, this MIT graduate program provided me tools to work in a team, improved my management skills, as well as promoted my leadership spirit.

1.2. Objectives

As previously mentioned, the main goal of this PhD work is to develop a strategy to create a customized dental implant that fits each person's characteristics, through a less invasive surgical procedure, improving the patient's quality of life and satisfaction. This approach avoids bone drilling aiming to reduce the healing time, minimize the risks inherent to such surgery, as well as reduce the costs related to dental implants. To achieve such purpose, it is necessary to fulfil the following main objectives:

- To develop a minimally invasive customized dental implant, targeting the improvement of the patient's overall quality of life.

- To validate the implant development strategy.

Some specific objectives were therefore established to ensure that these two general objectives are accomplished:

- To validate the implant geometry *in silico*.
- To implement strategies to promote an effective primary stability, inducing a decrease in the surgery healing time.
- To develop a functionalized implant surface in order to promote a fast osseointegration and avoid bacterial adhesion.
- To get accurate CBCT images to properly obtain the tooth socket geometry.
- To produce the zirconia implant by CNC.
- To test the developed strategy in dog cadaver mandibles.

1.3. Structure of the Thesis

This PhD thesis is organized as a compilation of research papers that are already published, accepted or submitted in international ISI journals. Apart from the research papers, this thesis is also composed by three other chapters: chapter 1, chapter 2 and chapter 10.

Chapter 1 corresponds to the Introduction. In this chapter it is possible to find the motivation and objectives of this research work, as well as the structure of the thesis.

Chapter 2, entitled “State of the Art” presents a literature review on the most relevant concepts related to the developed work. The main purpose of such chapter is to contextualize readers from different backgrounds to the topics that will be discussed in the subsequent chapters. In this sense, the second chapter addresses topics such as the human jaw anatomy, dental implants materials, types, market, failures and manufacturing techniques, as well as the most common dental implants surface modifications.

As previously mentioned, from chapter 3 to 9 it is possible to find research papers that correspond to the work developed in the scope of this PhD project.

Chapter 3 – “Subperiosteal Dental Implants: Past or Future? A Critical Review on Clinical

Trials/Case Reports and Future Directions”- corresponds to a review article on subperiosteal dental implants. This paper addresses topics such as materials, surface coatings, design and manufacturing techniques, methods of implantation, complementary strategies and the main clinical outcomes of subperiosteal dental implants. This paper is published in *Journal of Oral and Dental Health Research*, 2022, 4:1.

Chapter 4 – “Customized Root-Analogue Implants: A Review on Outcomes from Clinical Trials and Case Reports”- also corresponds to a review article. The aim of this review was to collect and analyse the clinical trials and case reports on customized root-analogue implants. The design and manufacturing techniques, implant material and surface treatments and the main clinical outcomes were assessed and discussed. This review article is published in *Materials*, 2021, 14(9), 2296 (a MDPI journal with an impact factor of 3.623, Quartile 2).

Chapter 5 – “In silico evaluation of the stress fields on the cortical bone surrounding dental implants: Comparing root-analogue and screwed implants”- analyses and compares, by means of FEA, the stress fields of peri-implant bone around root-analogue implants and conventional screwed implants. This research paper is published in *Journal of the Mechanical Behavior of Biomedical Materials*, 2020, 104, 103667 (an Elsevier journal with an impact factor of 3.902, Quartile 1).

Chapter 6 – “Design and optimization of zirconia functional surfaces for dental implants applications”- evaluates the hydrophilicity and capillarity of different CAD/CAM zirconia surfaces with micro-channels, targeting the promotion of implants vascularization and consequent improved osseointegration. This article is published in *Ceramics International*, 2020, 46: 16328-16336 (an Elsevier journal with an impact factor of 4.527, Quartile 1).

Chapter 7 - “Bacteria co-culture adhesion on different texturized zirconia surfaces”- investigates the adhesion of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* to zirconia surfaces with different finishings, in comparison with a titanium surface. This research paper is published in *Journal of the Mechanical Behavior of Biomedical Materials*, 2021, 123, 104786 (an Elsevier journal with an impact factor of 3.902, Quartile 1).

Chapter 8 – “Customized root-analogue dental implants - Procedure and errors associated with image acquisition and treatment, and manufacturing technology in an experimental study on a cadaver dog mandible”- aimed to assess the errors associated with the image acquisition, treatment and manufacturing of customized root-analogue implants. The experimental procedure, carried out in a dog cadaveric mandible, encompassed the image acquisition and treatment using different software, and the subsequent CNC manufacturing. This article is submitted in *Journal of*

Dentistry (an Elsevier journal with an impact factor of 4.379, Quartile 1).

The last chapter of the thesis – Chapter 9 - presents a general discussion and conclusions of the developed work, an implant prototype, and some suggestions for future works.

CHAPTER 2

STATE OF THE ART

This chapter presents a literature review of the most important topics related to this PhD work. In this sense, Chapter 2 begins with a brief characterization of the human jaw, including its anatomy, the anatomical structure of teeth, bone classification, and bone geometry evolution after tooth extraction. Then, dental implants are introduced. This subchapter gathers information regarding different concepts: materials, types and geometries, dental implants market, and failures. A special emphasis is given to dental implants manufacturing techniques, in the third subchapter, namely CAD/CAM technology. The last section of this chapter encompasses the most commonly used dental implants surfaces modifications, including both surface modifications for improved implant stability and for antibacterial purposes.

2.1. Jaw Characterization

2.1.1. Jaw Anatomy

The main functions of the facial skeleton are to protect the brain and the sense organs of smell, taste, and sight, to provide a frame that allows soft tissues of the face to facilitate the masticatory process, facial expression, breathing, and speech [1]. The facial skeleton is made up of 14 bones (Figure 2.1) of which only the mandible and the vomer are unpaired [1].

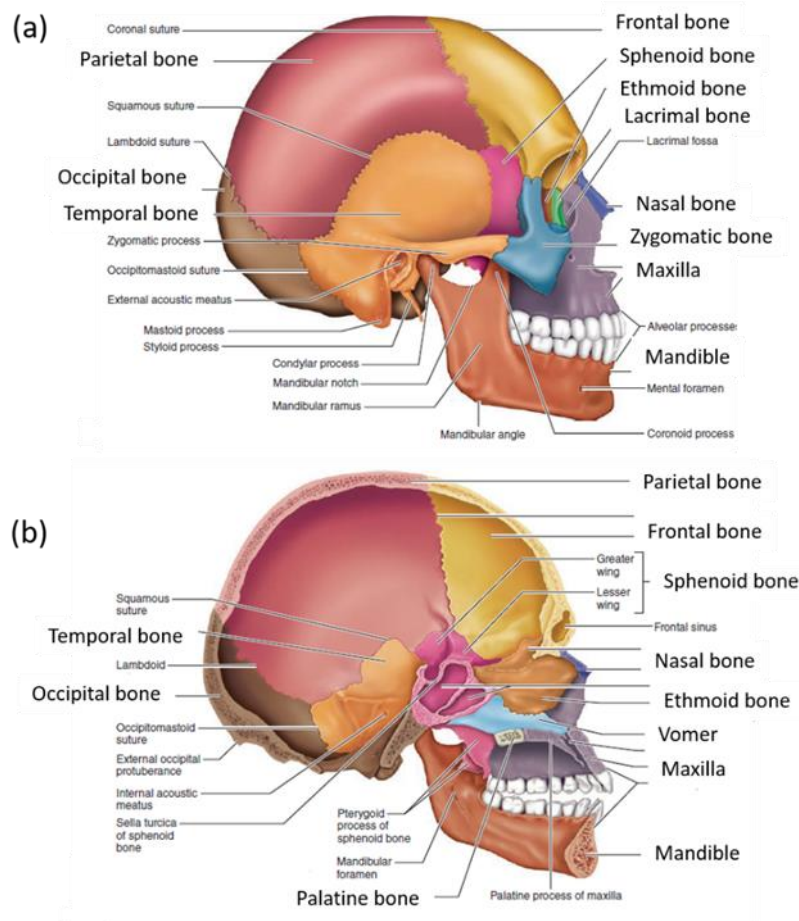


Figure 2.1 The facial skeleton: (a) external anatomy of the right side of the skull; (b) internal anatomy of the left side of the skull. Adapted from [1].

The maxillae, zygomatic, nasals, lachrymal, palatines, and inferior nasal conchae are paired bones. The primary bones of this structure are the mandible, maxilla, frontal and nasal bones, and zygoma[1]. By the age of two, the hemimandibles fuse and form a single bone, composed by a horizontal body with a posterior vertical extension denominated ramus (Figure 2. 2) [1,2]. The mandible has a medullary core with a cortical border with 2-4 mm of thickness [1,3]. The inferior alveolar canal starts at the mandibular foramen and extends inferiorly, anteriorly, and toward the lingual surface in the ramus. In adults, the alveolar canal generally comes in close

proximity to the roots of the third molar tooth. In the mandibular body, the canal extends along the inferior border, adjacent to the lingual surface. The mandible supports the lower dentition, which is typically characterized by two central and two lateral incisors, two canines, two first and two second premolars, and also three sets of molars [1,2].

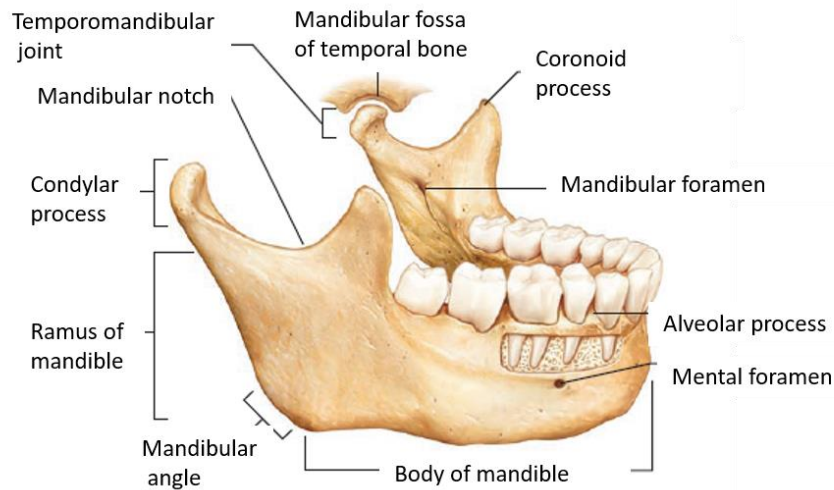


Figure 2. 2 Mandible, right lateral view. Adapted from [1].

In similarity to the mandible, the maxilla also plays several roles: it supports the teeth, forms the roof of the oral cavity, contributes to the lateral wall and roof of the nasal cavity, and supports the maxillary sinus (Figure 2. 3). This facial bone is characterized by its four processes: zygomatic process, frontal process, alveolar process, and palatine process.

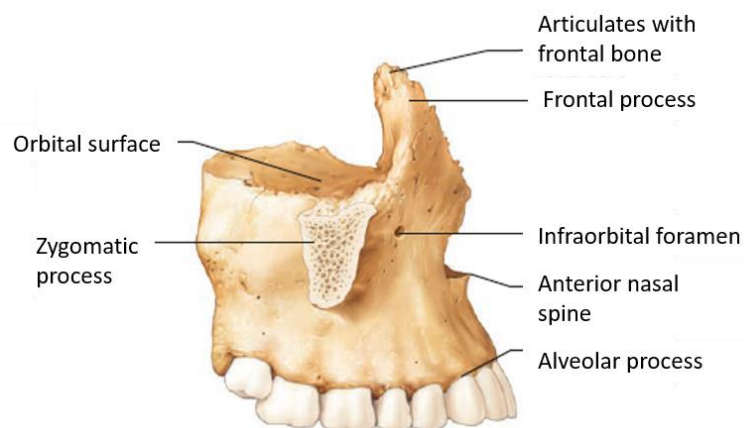


Figure 2. 3 Maxilla, right lateral view. Adapted from [1].

Two maxillary bones are fused in the midline (at the intermaxillary suture) to form the middle third of the face. In the midline of the anterior surface of the maxilla, it is possible to find a

prominence, denominated nasal spine, with a lateral concave border denominated the nasal notch that constitutes the floor of the piriform aperture. The alveolar process of the maxilla supports the teeth – central incisors, lateral incisors, canines, two premolars, and three molars. The teeth roots lead to vertical, wavelike eminences in the anterior face of the maxilla and the canine root is the most prominent. This root forms a vertical ridge denominated canine eminence. The shallow fossae medial and lateral to the canine eminence are denominated incisive and canine fossa, respectively [1,2].

2.1.2. Teeth Anatomical Structure

Teeth are mineral-rich structures that are used mainly to masticate food. They present a very complex structure that varies according to the place where they set (Figure 2. 4) [4]. Each tooth is embedded in a socket called an alveolus, forming a joint called a gomphosis between the tooth and bone. The alveolus is lined by a periodontal ligament, a modified periosteum whose collagen fibers penetrate into the bone on one side and into the tooth on the other. This anchors the tooth firmly in the alveolus but allows for a slight amount of movement under the pressure of chewing. The gum, or gingiva, covers the alveolar bone. Regions of a tooth are defined by their relationship to the gingiva: the crown is the portion above the gum, the root is the portion inserted into the alveolus below the gum, and the neck is the line where the crown, root, and gum meet. The space between the tooth and gum is the gingival sulcus [5]. The teeth enamel is characterized by a matrix of hydroxyapatite (HAp). Dentin, a softer material, acts as a line of defense in case the enamel breaks or dissolves. Cementum is a bone-like tissue that is characterized by high levels of hydroxyapatite and connective proteins. It allows the tooth attachment to the periodontal ligament, which, in turn, ensures that it is firmly held to the jawbone. The pulp contains blood vessels and nerves that act as an indicator of tooth injury or infection [4,5].

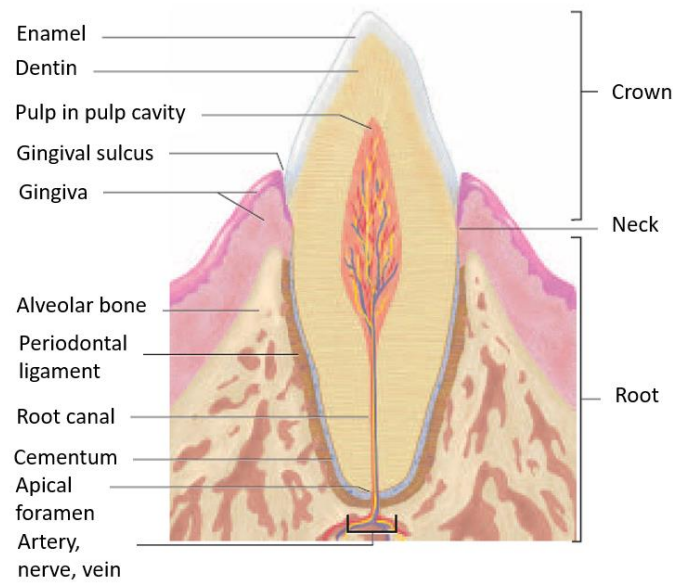


Figure 2. 4 Median section of a canine tooth and its alveolus. Adapted from [5].

Adults normally have 16 teeth in the mandible and 16 in the maxilla. From the midline to the rear of each jaw, there are two incisors, a canine, two premolars, and up to three molars. The incisors are chisel-like cutting teeth used to bite off a piece of food. The canines are more pointed and act to puncture and shred it. They serve as weapons in many mammals but became reduced in the course of human evolution until they now project barely above the other teeth. The premolars and molars have relatively broad surfaces adapted for crushing and grinding [5]. The root of a tooth is characterized by its vulnerability to injury and infection due to the absence of protective enamel. The number of roots and root canals that a tooth has is an important issue when it comes to performing certain treatments. It usually depends on the type of the tooth (Figure 2. 5): the upper incisors, upper canines, and the lower incisors, canines, and premolars are characterized by one root; the upper premolars and the lower molars are characterized by two roots; the upper molars are generally characterized by the existence of three roots [5].

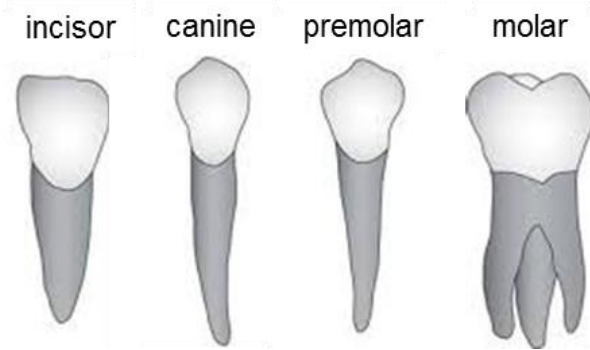


Figure 2. 5 Different tooth types and respective roots. Adapted from [6].

Understanding the anatomy of human teeth is one of the basic functions of dental education, once it is one of the prerequisites for achieving success in any clinical intervention [7]. In this sense, several imaging techniques that allow the three-dimensional analysis of the human teeth structure have already been developed and will later be further explored.

2.1.3. Bone Classification

Quantitative and qualitative measurements of mandibular bone from radiographs have been assessed in order to evaluate 'bone quality'. Some of these techniques require specialized facilities and need high standards radiographies. In general practice, two simple assessments have been used: Bone Quality Index (BQI), and Mandibular Cortical Index (MCI) [8]. From those, BQI describes 'bone quality' according to four different types, regarding the cortical thickness and trabecular pattern (Figure 2. 6):

Type I: homogeneous cortical bone.

Type II: thick cortical bone with marrow cavity.

Type III: thin cortical bone with a dense trabecular bone of good strength.

Type IV: very thin cortical bone with a low-density trabecular bone of poor strength.

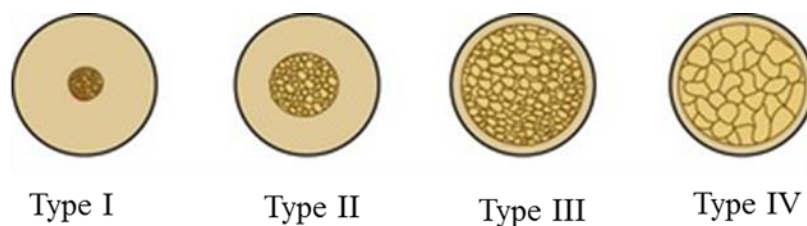


Figure 2. 6 Jawbone Quality Index (BQI) representation. Adapted from [9].

MCI is classified by the appearance of the lower border of the mandibular cortex distally from the mental foramen, according to panoramic radiographs. This index is divided into three types (Figure 2. 7) [8,10]:

C1: endosteal margin of the cortex was even and sharp on both sides.

C2: the endosteal margin showed semilunar defects (lacunar resorption) or seemed to form endosteal cortical residues on one or both sides.

C3: the cortical layer formed heavy endosteal cortical residues and was clearly porous.

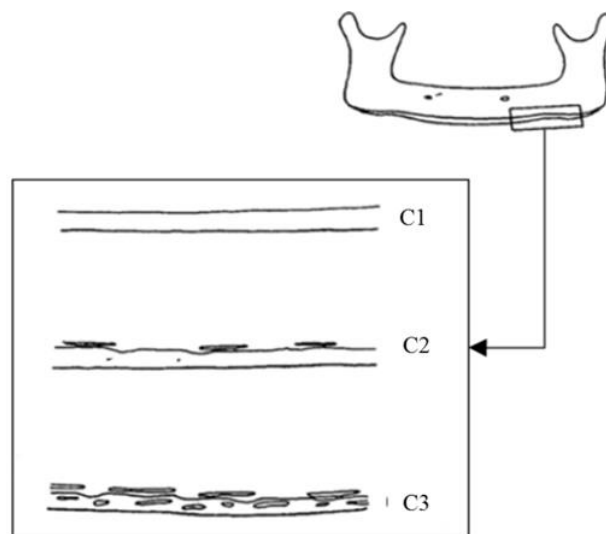


Figure 2. 7 Mandibular Cortical Index (MCI) representation [10].

Bone Mineral Density (BMD) is extremely related to BQI and refers to the density of minerals, such as calcium, in the bones, and is extremely related to the strength of the bones [9]. It is used for clinical issues as an indirect indicator of osteoporosis and fracture risk.

Over the last few years, many investigators have documented the influence of bone density on dental implants' success rate. As seen in Figure 2. 8, higher success rates are found in better bone quality (pink) when compared to poor bone quality (blue) [9].

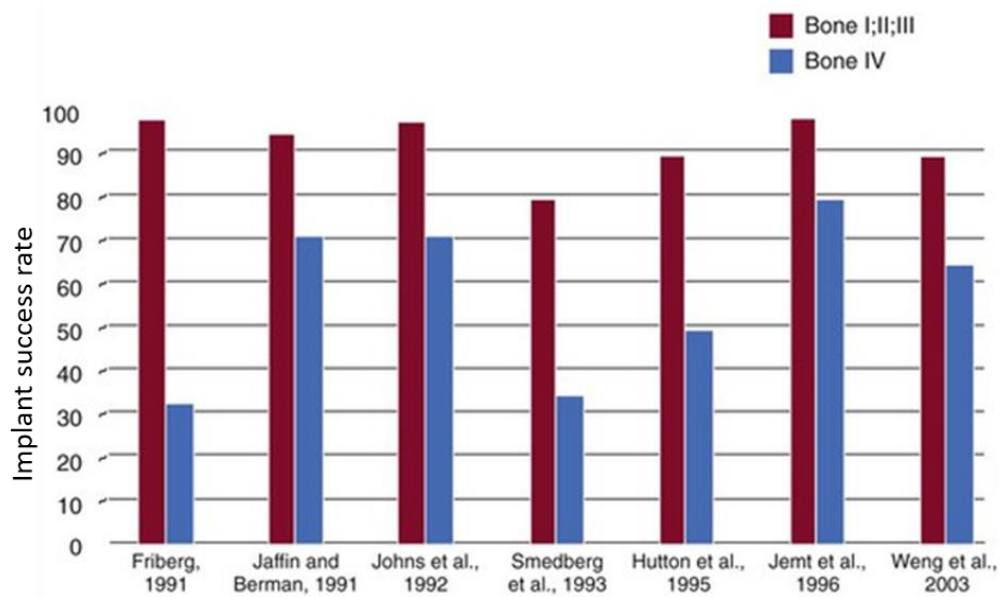


Figure 2. 8 Influence of bone density on implant success rate [9].

2.1.4. Bone Geometry Evaluation After Tooth Extraction

Following a tooth extraction, the geometry of the bone is expected to change since the recovery of the extraction socket starts immediately. From the first week after removal, osteoclasts start to accumulate in the alveolar bone part and bone remodeling lasts for several more weeks [11]. However, if not stimulated, the bone will start to reabsorb. The rate of bone resorption is greatest in the first year, especially in the first 3 months. The bone loss depends on the patient's age as well as noticeable differences between the maxilla and the mandible. Bone loss post-extraction is also influenced by systemic factors such as smoking [12]. One study performed by Elsubeihi, E., et al [13] found that after 14 days of the extraction the BMD is expected to increase about 25%, and between 56 and 112 days after extraction differences were not so significant. They also concluded that healing was associated with a reduction in cross-sectional mandible area of 32.89%, 21% in height, and 12.84% reduction in the mandible width [13].

Lindhe, et al., aimed to evaluate the dimensional changes in the alveolar ridge after premolars tooth extraction, as well as the respective bone remodeling, through an experimental study in dogs [14]. In Figure 2. 9 it is shown a schematic representation of the locations where the measurements were performed.

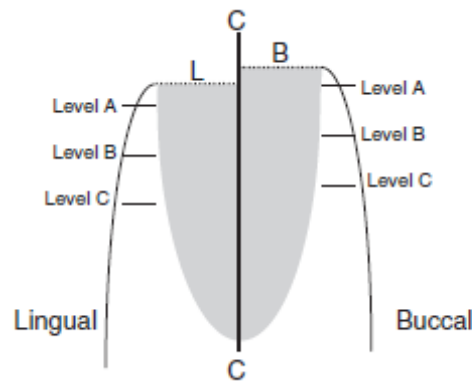


Figure 2. 9 Schematic drawing representing the location where the histometric measurements were performed [14].

Results revealed that the most important alterations occurred during the first 8 weeks after tooth extraction. In Table 2. 1 it is possible to observe the results regarding the width of the bone tissue at the buccal and lingual walls of the extraction sites, during the first 8 weeks.

Table 2. 1 Width of the bone tissue at the buccal and lingual walls of the extraction sites. Adapted from [14].

	1 week		2 weeks		4 weeks		8 weeks	
	buccal	lingual	buccal	lingual	buccal	lingual	buccal	lingual
Level A	0.6 (0.1)	1.4 (0.2)	0.6 (0.1)	1.3 (0.3)	0.7 (0.2)	1.3 (0.1)	0.5 (0.1)	1.2 (0.1)
Level B	1.3 (0.1)	2.0 (0.3)	1.1 (0.3)	1.9 (0.4)	1.1 (0.2)	1.6 (0.1)	1.1 (0.1)	1.7 (0.3)
Level C	2.0 (0.0)	2.8 (0.8)	1.9 (0.4)	2.7 (0.7)	2.0 (0.4)	2.9 (0.3)	1.6 (0.1)	2.7 (0.5)

In the first week of healing, osteoclasts were observed in all areas of the socket, as well as a periodontal ligament characterized by fibroblasts, collagen fibers, and inflammatory cells (Figure 2. 10.a). It is possible to denote large amounts of provisional matrix and a blood clot in the center of the socket. After 2 weeks (Figure 2. 10.b) it is possible to observe large amounts of woven bone (immature bone) in the lateral and apical portions of the socket.

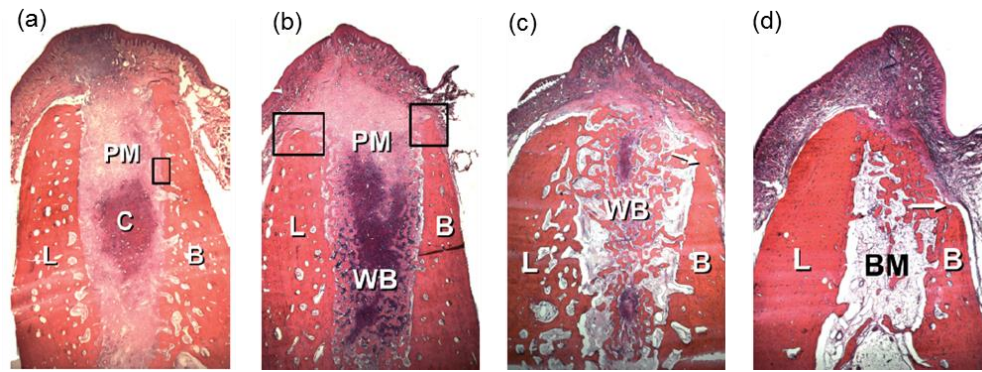


Figure 2. 10 Overview of the extraction site after: (a) 1 week; (b) 2 weeks; (c) 4 weeks, and, (d) 8 weeks. B, Buccal; L, Lingual; PM, Provisional Matrix; C, Blood Clot; WB, Woven Bone; BM, Bone Marrow [14].

Four weeks after the extraction (Figure 2. 10.c), it is possible to observe a structure characterized by newly formed woven bone. The bundle bone of the crestal region of the buccal wall was resorbed and partially replaced by woven bone. Additionally, the marginal portion of the old buccal wall (arrow) is “apical” to its lingual counterpart. After 8 weeks (Figure 2. 10.d), the inner side of the socket was full of bone marrow, and trabecular mineralized tissue. The overall results showed that the biggest dimensional differences were observed in the first two weeks [14].

2.2. Dental Implants

Replacing a missing tooth is a very old process. Stone, shells, bones, gold, or even animal teeth were used by our ancestors to replace a missing tooth (Figure 2. 11). Once this practice was proved to be the cause of infectious diseases or eventually death, evolution had been carried out in order to overcome such issues [15].

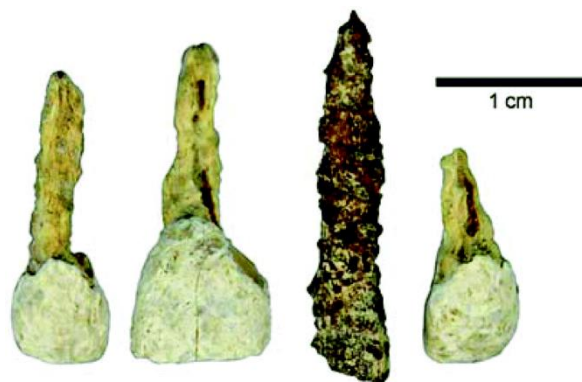


Figure 2. 11 The early dental implants made of stone, shells, bones and other materials [16].

The biggest evolution in the history of dental implants goes back to the 60's when Branemark studied the osseointegration of titanium (Ti) implants [17]. Nowadays, despite all the efforts to implement other materials (such as zirconia – ZrO_2) in the implants industry, Ti and its alloys are still the most used materials in dental implants, with success rates over 90% [17]. The commercially available implants most used in our days are typically composed of three components, as seen in Figure 2. 12: the implant itself, the abutment, and a crown.

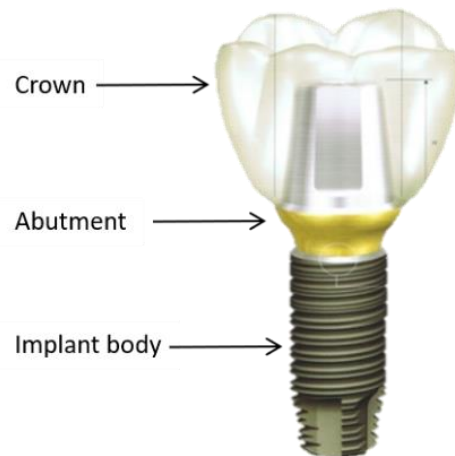


Figure 2. 12 Implant components. Adapted from [18].

The implant is usually a threaded screw that is surgically inserted in the jawbone and aims to replace the natural roots. The abutment is a structure responsible for holding the crown and is screwed into the implant. The crown is the structure that is visible and pretends to mimic the natural tooth.

2.2.1. Materials Used in Dental Implants

The variety of materials used nowadays in dental applications is huge. Metallic materials, such as stainless steel, chromium-cobalt (Co-Cr), or titanium (Ti) and its alloys, ceramics like zirconia (ZrO_2), or even polymers such as poly-ether-ether-ketone (PEEK) have been of great interest for biomedical applications, namely for dental implants [19,20]. When selecting a material for a dental implant, it is crucial to take into consideration its mechanical properties (fatigue and fracture strengths), biocompatibility, and more recently, its aesthetic properties.

Titanium

Titanium and its alloys have been applied in a wide range of biomedical applications, such as hard tissue replacements (artificial bone, joints, bone plates, screws, and dental implants) and

cardiovascular applications (vascular stents and artificial heart valves) [21]. Their excellent mechanical properties, high biocompatibility, and high corrosion resistance make them the metals of choice for biomedical applications, namely for dental applications [20,22]. Ti6Al4V is one of the most required Ti alloys due to its unique properties – high strength, good fracture toughness, low density, low thermal expansion, and good corrosion resistance [23]. This corrosion resistance of Ti6Al4V is related to the formation of a stable and dense oxide layer of Ti (TiO_2) on its surface, responsible for protecting the material and promoting chemical stability in the organism [24,25]. This oxide layer (typically with 2-5 nm of thickness) is spontaneously formed when the surface is exposed to oxygen or environments containing oxygen [26].

Despite all the advantages of Ti6Al4V, its young modulus Ti6Al4V (85-115 GPa) is considerably higher than the one of the cortical bone (8-18 GPa) and consequently loading occlusion can cause stress shielding in the peri-implant area, leading to bone loss and, eventually, implant failure [19,27,28]. On the other hand, Ti6Al4V is characterized by a poor wear resistance that is intensified when in contact with body fluids, resulting in the release of metallic ions to the surrounding tissues [27]. In the specific case of the dental implants, the contact between the abutment and the implant body is characterized by sliding wear and abrasive wear due to the hard particles that come from food intake or tooth brushing [24,25]. The metallic ions that are released from the Ti6Al4V alloy have already been proved to be toxic for the human body and are associated with inflammatory tissue reactions, such as allergic reactions and hypersensitivity [29]. Additionally, the growing concern with aesthetical issues has led to a decrease in the use of titanium as a dental material. Its grey color gets visible and more pronounced when the soft tissues are not healthy, and the dark color appears through the thin mucosa [30].

In this sense, the urge for solutions that overcome these negative aspects associated with titanium implants has led to an increased interest in the development of non-metallic implants.

PEEK

By overcoming some limitations of the titanium, PEEK has been applied in dental implantology since the 90's [21]. PEEK, a high-performance polymer, is characterized by a semi-crystalline, linear, polycyclic, aromatic, and thermoplastic structure [31]. Its young modulus (3-4 GPa), very similar to the cortical bone, is what makes this material so popular in the dentistry field [32]. Moreover, the addition of carbon fibres to the PEEK has been used to increase its elastic modulus to high levels, as demand for specific applications. Due to its good mechanical properties and high biocompatibility this polymer has been applied in different dentistry components: the

dental implant itself, abutments, prosthesis, and also for the manufacturing of screws to bond the implant and abutment [33]. Despite the advantages of this polymer, it has been proved to be a bioinert material and, therefore, does not have osteoconductive properties. Many researchers have compared PEEK implants with titanium and results revealed that PEEK promotes less osteoblast differentiation than Ti [21]. In this sense, efforts have been carried out aiming to increase PEEK's bioactivity, namely by increasing the surface roughness and/or hydrophilicity [34,35].

Zirconia

The drive towards non-metallic implants to satisfy the increasing aesthetic demands and the metal-free request has also empowered zirconia as a promising dental material. Due to its high mechanical strength, excellent wear resistance, and biocompatibility, zirconia is one of the most used ceramics in the biomedical field [36]. ZrO_2 is a white crystalline zirconium oxide and a polycrystalline ceramic with no glassy phase [37]. This ceramic is usually found in three different crystalline forms: monoclinic, tetragonal, and cubic. At room temperature, pure zirconia assumes a monoclinic configuration, and this structure is stable up to 1170 °C. In turn, the tetragonal zirconia phase is stable from 1170 °C to 2370 °C and cubic zirconia is formed at temperatures above 2370 °C up to the melting point - 2680 °C [38]. The transition between the different crystalline forms of zirconia is due to a force on its surface and results in a volumetric change in the crystal where the stress was applied. The cracking energy resultant from the stress creates a tetragonal-monoclinic transition [39].

The previously mentioned characteristics of ZrO_2 (high mechanical toughness and strength) are a consequence of the stabilization of the tetragonal phase of zirconia through the addition of metal oxides such as yttria, ceria, calcia, or magnesia [37]. Although its sintering is much more difficult, yttria (Y_2O_3) stabilized zirconia, also known as tetragonal zirconia polycrystal (Y-TZP) is the kind of zirconia that is most considered for medical use due to its good mechanical properties, excellent chemical stability, and adequate level of oxygen-ion conductivity in both oxidizing and reducing environments [39]. Zirconia's first proposal for medical, namely orthopedic, applications goes back to the 60's. By then, a zirconia hip head was placed in a monkey femur, as an alternative to titanium and alumina components and results revealed no adverse reactions [39].

As far as the dentistry field is concerned, zirconia has been considered for dental applications since the early 90's. In fact, the first research work on zirconia for such purposes was published in 1975, by Cranin et al. [40]. Authors aimed to assess and evaluate the addition of a ceramic coating over a Vitallium implant to increase the implant's biologic acceptability in the oral

environment, in beagles. Zirconia implants have been addressing many limitations of conventional implants. The advantages of zirconia for dental applications are numerous. Its white color, very similar to the natural teeth, improves the aesthetical outcomes of such dental restorations; its excellent flexural strength and fracture toughness allow the implant to withstand the physiological loads from the masticatory muscles [21]; additionally, zirconia excellent osseointegration capabilities, low plaque affinity and good adhesion to bonding substrates are expected to decrease the risk for peri-implant diseases [30,41]. In one study, performed by Wenz et. al [42], a systematic review of the literature was conducted aiming to assess the clinical success of yttria-stabilized zirconia implants and whether its osseointegration is comparable to that of titanium. Osseointegration was evaluated at 4 weeks to 24 months after implant placement and results revealed that zirconia implants osseointegration was comparable (or even better) to that of titanium implants, and the mean bone-implant contact percentage was above 60% in almost all experimental groups. Roehling et. al [43], also evaluated whether zirconia implants demonstrate differences in hard and soft tissue integration compared to titanium implants in preclinical studies. Again, results showed similar qualitative soft tissue integration for zirconia and titanium implants. In another work, Sivaraman et. al [44], aimed to understand if zirconia is a viable alternative to titanium for an oral implant. The main outcomes of such work are that zirconia implants are a promising substitute to titanium standard implants, with a superior soft-tissue response, biocompatibility, and aesthetics, and with comparable osseointegration capability.

Despite these advantages of zirconia, its physical and mechanical properties tend to degrade as a result of a low-temperature ageing process (LTD) [45]. This process is characterized by a slow surface transformation of the tetragonal phase into the monoclinic structure of zirconia, associated with an increase in the volume of the ceramic that stresses the particles and results in subcritical crack growth (SCG), offering a way for water to penetrate inside the material. The stage of the crack growth depends on various microstructure patterns, such as porosity, residual stresses, and particle size [46]. If, in one hand, a certain degree of tetragonal-monoclinic transformation can improve the mechanical properties of Y-TZP, on the other hand, a narrow range exists between improvement and deterioration of mechanical properties [45]. In this sense, some authors have been investigating the effect of LTD on the mechanical properties of zirconia, in the context of dental applications. Att et. al [47], simulated, *in vitro*, the effect of ageing on zirconia used for oral rehabilitation and found that despite reducing the mechanical properties of the ceramic, that decrease occurs within clinically acceptable values. In addition, Alghazzawi et. al [48]

demonstrated that dental procedures do not significantly affect the bulk properties of zirconia (flexural strength and yttria composition), indicating a longer-term *in vivo* function without biomechanical fracture.

2.2.2. Dental Implants Types and Geometries

A dental implant corresponds to the tooth root and is used for the replacement of single or many teeth [21]. To achieve successful implantation, dental implants are expected to stabilize and osseointegrate when set in the body environment. The implant geometry, dimensions, and surface finishing may influence the implant anchorage and osseointegration. Additionally, biological factors such as bone density may also compromise the implant's overall function. Depending on their geometry and their placement within the tissues, dental implants can be classified accordingly to three different categories: subperiosteal implants, endosseous implants, and transosteal implants [49].

Subperiosteal implants have appeared as promising solutions for dental rehabilitation in the late 1940s and, since then, different types of this design evolved up to the 1980s [50]. This type of implant is placed under the periosteum but on, or above, the mandibular bone and has been used in patients who have huge bone resorptions, and, consequently, not enough bone to support endosseous implants [51]. Subperiosteal implants' design is characterized by a metallic framework that usually covers a large surface area, such as completely edentulous mandibles. Additionally, these implants also comprise posts or bars for the subsequent crown attachment (Figure 2. 13.a). Despite having been a common practice for many years, subperiosteal implants are not so common nowadays due to the inherent high complication rates [52]. The positioning of the implant used to be a very demanding process and design flaws used to induce infection and posterior bone loss. Literature reports implant survival rates of only 50-60% for follow up periods of 15 years [53]. Given these outcomes, subperiosteal implants were replaced by endosseous implants, which are by far the most common type of implant placed nowadays [54].

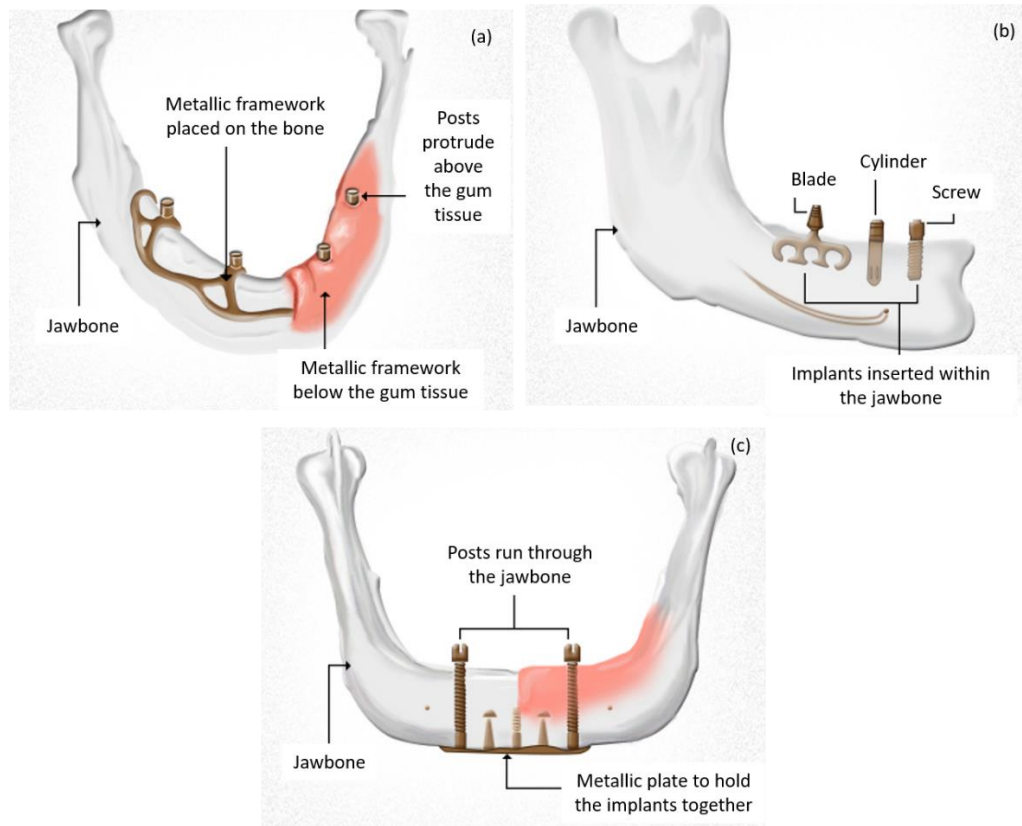


Figure 2. 13 Different implant designs: (a) subperiosteal; (b) endosseous; and (c) transosteal. Adapted from [55].

Endosseous implants (Figure 2. 13.b) are placed within the jawbone after a pilot hole is drilled into the trabecular bone, penetrating the cortical wall once. If the trabecular bone has been partially or completely resorbed, the endosseous implant is anchored in the basal bone beneath [54]. The top of the implant is positioned so that it either protrudes slightly through the cortical plate or is flush with the surface of the bone, for posterior crown attachment. Typically made of titanium, and more recently ceramic materials, endosseous implants are usually shaped like small screws, cylinders, or blades. Screw-type endosseous implants are the most common due to their proven success and great initial retention strength [15,56]. The success of these implants depends on several parameters including the implant diameter, length, and thread pitch, height, and configuration (v-shaped, square-shaped, buttress, and reverse buttress) [15]. Despite overcoming some limitations of the subperiosteal implants and being a widespread treatment, conventional endosseous implants only provide limited options for implant diameter, length, and thread parameters. Moreover, the lack of congruency between the implant and socket may induce plaque formation and accumulation, bone loss (resulting in the implant exposure and consequent aesthetical issues), poor implant stability, and, ultimately, its failure [57]. In this sense, new strategies to overcome the conventional screw-type implants limitations are being developed, namely the design of customized root-analogue endosseous implants (further explored at the end

of this section).

Transosteal implants are the third and last category of dental implants. They are used to support a mandibular denture only when the patient has severe resorption and does not have enough bone support for subperiosteal or endosseous implants [54]. This type of dental implant, only used in the mandible, is composed of a metallic plate and transosteal posts and is characterized for penetrating the cortical bone twice [58]. The metallic plate is held with retentive screws fixed in the mandible inferior border. The posts, in turn, are supported by the plate and extend through the full thickness of the bone to provide implant stability (Figure 2. 13.c). The surgical procedure for the placement of a transosteal implant is quite complicated and invasive since it encompasses the attachment of the plate at the bottom of the jawbone, through an incision made below the chin. The screws and posts are also placed to extend through the bone for posterior crown attachment. Transosteal implants are usually customized to the characteristics of each mandible which make it a very expensive treatment, reason why they are not frequently used [54].

2.2.2.1. Root-analogue Dental Implants

As previously mentioned, endosseous screw-type dental implants have been the gold standard solution for tooth rehabilitation. Despite its clinical success, some aspects still have to be improved. One of the major concerns regarding screw-type implants is the incongruence of the implant and the extraction socket, which results in posterior loss of supporting bone and consequent loss of implant stability [59]. Ensuring that the implant is surrounded by bone during its lifetime is crucial for long-term clinical success. This maintenance of bone around the implant is, therefore, extremely related to the implant's geometry and the stress distribution. Literature indicates that the ideal features encompass a wide implant neck for better stress distribution and a large surface area, with a minimal thread geometry for an improved long-term crestal bone stability [60]. In this sense, efforts are being carried out, aiming to find the optimal implant geometry/dimensions. Recent studies have suggested the development of root-analogue implants, tailored to each patient's condition, with the main focus on reducing the incongruence between the implant and bone [61]. By adapting the implant to the extraction socket instead of adapting the bone to a standardized implant, bone, and soft-tissue trauma is expected to be reduced. This new approach comprises several advantages when comparing to screw-type implants, as, for instance, the less invasive traumatic procedure, since there is no need to drill the bone before placing the implant [62]. Another major difference between these two approaches is the way primary stability is achieved. In conventional screwed implants, primary stability is achieved by the mechanical

interaction between the bone and the implant threads, together with surface modification techniques, whereas root-analogue implants achieve primary stability through a press-fit approach [63]. Customized root-analogue implants were firstly placed by Hodosh et al. [64]. The implant was placed immediately after tooth extraction, in a single-step surgery, reducing the treatment time, and consequently improving the patient's quality of life. The overall costs were therefore reduced and a decrease in the bone resorption and soft-tissue regression due to early functional load was observed [65]. Since then, many studies are being carried out, focusing on the materials and in the design and manufacturing techniques of such dental restorations.

2.2.3. Dental Implants Market

The dental implants market is constantly expanding. This market growth is driven mainly by the growth of the geriatric population and inherent diseases, the increasing prevalence of tooth decay and periodontal diseases and the increasing number of implant-placing dentists [66]. On the other hand, dental injuries resulting from road accidents and sports injuries are each time more common, thus contributing to the demand for dental implants. The World Health Organization (WHO) data indicates that nearly 10 million people are injured or disabled due to road accidents every year. Additionally, oral health and aesthetic reasons are changing the individuals' perception of oral care. According to a survey performed by the American Academy of Cosmetic Dentistry (AACD) in 2015, 86% of the respondents had already been subjected to dental treatments to improve their attractiveness and self-esteem. It is also estimated that, annually, Americans spend about USD 2.75 billion on cosmetic dentistry [66]. In this sense, a boost in the dental implants market growth is expected to happen due to the increasing application of dental implants in numerous therapeutic areas [67]. According to the American Academy for Implant Dentistry, over 15 million people in the U.S. undergo bridge and crown replacements for missing teeth every year, thus increasing the need for dental implants [67]. The National Health and Nutrition Examination Survey estimated that approximately 42% of the children aged 2 to 11 years have tooth decay and almost 23% of them remain untreated. On the other hand, the National Institute of Dental and Craniofacial Research has concluded that tooth loss in American adults begins between the 35 and 45 years, adults above 45 years have already lost a few teeth, and more than 24% of people aged over 74 years have no remaining teeth. Given these numbers, the demand for dental implants is unquestionable [67,68]. Nowadays there are more than 150 dental implants companies spread

all over the world. The most recognized and successful are *Straumann*, *Nobel Biocare (Danaher)*, *Dentsply Sirona*, *Zimmer Biomet* and *BioHorizons* [69]. Other widely known companies are *BEGO Implant Systems*, *Biodenta Swiss*, *Glidewell Laboratories*, *MIS Implants Technologies*, *Neobiotech Co.*, *Neoss*, *Osstem Implant Co.*, *Thommen Medical*, among many others [69]. Each manufacturer produces implants with different characteristics that are more suitable for different clinical conditions. Some of them are prepared to be immediately loaded whereas others need time to recover and to osseointegrate. Among the previously mentioned companies, *Straumann* is nowadays the leading provider of dental implants across the globe. This company has a broad product portfolio in the premium segment of the industry and is followed by *Nobel Biocare* and *Dentsply Sirona*, companies that also have a global presence in the market [68]. According to an analysis performed by the *Fortune Business Insights*, the global dental implants market was valued at USD 3.87 billion in 2018 and is expected to reach USD 5.725 billion by 2026 (Figure 2. 14) [68].

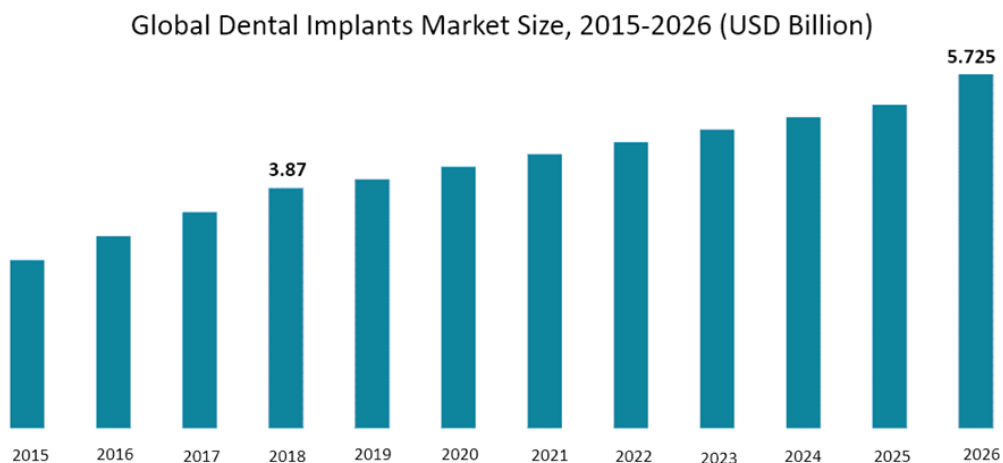


Figure 2. 14 Global dental implants market size. Adapted from [68].

The fastest growing in the dental implants market is happening in the Asia Pacific region, due to an increased incidence of edentulism in developing countries such as India and China, as a result of an increased geriatric population [68,70]. North America, namely the U.S, is the second largest region mainly because of an increased concern with oral care and aesthetics. Europe, in turn, is expected to account for the 43.4% of the global dental implants market million due to the prevalence of aging population, increased population with oral diseases, government investments, and recent innovations. Germany, France, and U.K were among the leading markets for dental implants in Europe in 2018 [70].

In terms of revenue share, Europe dominated the dental implants market in 2019 (Figure 2. 15) [67,68]. According to the European Commission statistics, from 2020 on, a quarter of Europeans will be over 60 years. This aged population is prone to suffer from tooth loss and tooth decay as a result of prescribed medication. The demand for oral care services associated with the geriatric population is, therefore, expected to increase the European dental implants market to a large extent [67].

Global Dental Implants Market Share, by region, 2019

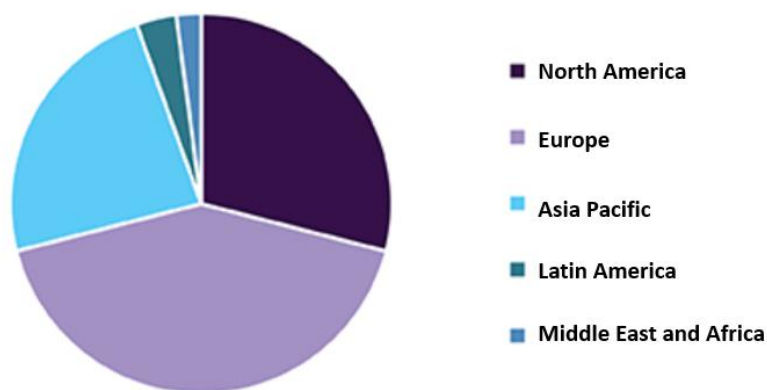


Figure 2. 15 Global dental implants market share. Adapted from [68].

Despite the increased interest in zirconia as a dental material, reports on the dental implants market indicate that titanium implants segment still dominated this market in 2018 and the same trend is expected to be followed until 2026 [66,68,70].

As far as the type of implant is concerned, endosteal implants dominated the market share in 2018. The American Academy of Periodontology stated that implants placed in the bone are the most common type of implant used in dentistry, mainly because of the complex and much more invasive nature of transosteal implants [68].

2.2.4. Dental Implants Failures

Despite being a common and widespread practice for the rehabilitation of missing teeth, dental implants have some limitations, with previous reports of failure rates ranging from 1% to 19% [71]. These failure rates result from a multifactorial process and can be classified into early failures and late failures: early failures are related to failures that happened before the application

of functional load whereas late failures occur after applying occlusal load [71]. Early implant failure corresponds to a failure in establishing the osseointegration of the implant, which means it is solely a biologic complication. On the other hand, a late implant failure corresponds to the failure of either the implant osseointegration or function, resulting from both biological or mechanical complications [71,72]. The biologic complications that lead to early implant failure may be a consequence of factors such as overheating, contamination, and trauma during the surgical intervention, poor bone quality or quantity, and poor primary stability. These biologic mechanisms usually induce the resorption of soft and hard tissues around the implant [73]. Late implant failure, in turn, may be induced as a result of inadequate implant loading design and consequent implant fracture, occlusal trauma, overloading, stress shielding, allergic reactions, bacterial infections, or even poor oral hygiene [71,74].

There are some studies in the literature reporting the factors that influence the failure rates of dental implants. Moy P. et al. [75] conducted a study, between January 1982 and January 2003 to analyse the failure rates of dental implants and determine associated risk factors. Data regarding age, gender, implant location, medical history, and bone quality and volume were recorded and correlations between these factors were used to establish relative risk ratios. Results revealed that increasing age is strongly related to the risk of implant failure. Patients in the 60-79 age group had a significantly higher risk of implant failure when compared to patients less than 40 years. Smoking, diabetes, and postmenopausal estrogenic therapy were proved to have a strong influence on the implant failure rate. On the other hand, gender, hypertension, coronary artery disease, pulmonary disease, steroid therapy, and chemotherapy were not associated with a significant increase in implant failure. The overall results of this investigation reported implant failure rates of 8.16% in the maxilla and 4.93% in the mandible. Raikar S. et al. [76] conducted a study to assess several factors affecting the implants' survival rates. Results revealed that age, length, and diameter of the implant, bone quality and location of the implant are detrimental to the survival of the implants. Furthermore, the authors concluded that implants above 11.5 mm length, with a diameter <3.75 mm, placed in the mandibular posterior region, in type III bone showed the highest failure rates. Sun H. et al. [77] assessed the factors that influence the failure rates of short dental implants and concluded that most failures can be attributed to poor bone quality and machined surfaces. Parihar A. et al. [78] conducted a study to assess the failure rate of dental implants in medically compromised patients (diabetes, osteoporosis, hypothyroidism, organ transplant, and cardiovascular diseases). Results showed that among medically compromised patients, higher

failure rates were found in patients with diabetes. Do T. et al. [71] also summarized the influence of different risk factors on late implant failure. Results indicate that the factors that induce late implant failure may be classified into three different groups: the patient history (radiation therapy, bruxism, periodontitis); clinical parameters such as the bone type and location of the implant; and factors resulting from the doctor's choices, such as low initial stability.

The aforementioned factors, individually or a combination of them, will, eventually, lead to peri-implantitis, an inflammatory process that affects the soft and hard tissues surrounding dental implants [79]. Peri-implantitis is characterized by the creation of peri-implant pocket formation, with subsequent loss of bone around the implant and ultimately its failure [80]. Factors such as diabetes, occlusal overload, genetic factors, smoking habits, periodontal disease, and poor plaque control are reported in the literature for having a huge contribution to the development of peri-implantitis [81,82]. A special concern is given to the formation of plaque in the peri-implant area. This accumulation and progression of inflammatory cells in subepithelial tissue has an adverse effect on peri-implant tissue health leading to considerable supporting bone loss [80,83].

In general, those conditions that were found to be correlated with an increased risk of failure should be considered during implantation planning in order to minimize dental implants failure rates.

2.3. Manufacturing Techniques

As previously mentioned, titanium dental implants are still the gold standard solution for replacing a missing tooth and the variety of techniques to produce this type of implant is huge. Titanium dental implants are often produced by rods of commercially pure titanium (cpTi) or its alloy Ti6Al4V, by a manufacturing process that involves a first milling stage and the posterior application of surface treatments [15,84]. Injection molding has also been applied for the production of such medical devices. This technique is composed of five main steps: selection of the powder and binder, production of feedstock, injection molding, debinding, and sintering [85]. Additive manufacturing (AM) technologies have gained an increased interest in the production of dental implants via 3D printing, mainly due to their simplicity and effectiveness [84,86]. Among the most promising AM technologies for metallic dental implant fabrication is direct metal laser sintering (DMLS). This laser-based technology allows the production of complex structures, layer-by-layer, using powdered metals, radiant heaters, and a computer-controlled laser [87]. Briefly,

objects are fabricated on a moveable platform by applying incremental layers of the desired material. For each layer, the machine lays down a film of powder, which will subsequently be subjected to a high-power laser beam, to fuse the metal powders according to a computer-aided design (CAD) file, resulting in a thin metal layer. This process is repeated, layer by layer, until the desired 3D object is fabricated [87]. With DMLS it is possible to control the porosity of each layer, the pore size, shape and distribution, by changing processing parameters such as the thickness of the layer, the laser power and peak power, the laser spot diameter, the scan speed, and scanning strategy, among others [87]. This AM technique allows the reduction of the manufacturing time and waste, by avoiding post-processing steps [86]. Additionally, recent studies have been reporting DMLS as a promising technique for the production of custom-made root-analogue implants (RAI), by making use of cone-beam computed tomography (CBCT) to fabricate perfect copies of the radicular units to be replaced [88]. M. Figliuzzi et al., fabricated and inserted into a fresh extraction socket a custom-made DMLS titanium root-analogue implant. After 1 year of follow-up, the implant was functional and aesthetically satisfactory, with no bone resorption or soft tissue recessions [88]. The constant developments in the technological industry have led to the introduction of new technologies in the healthcare sector, including the frameworks of modern dentistry [89]. Making use of technology to produce dental applications plays an important role not only in terms of practice in the dental office but also in dental technical laboratories. In this sense, over the last years, dentistry is evolving towards digitalization, in order to simplify the clinical procedures and reduce the treatment and healing times, by adopting new technologies such as Computer-Aided Design/ Computer-Aided Manufacturing (CAD/CAM) [90].

2.3.1. CAD/CAM Technology

The CAD/CAM technology encompasses three main steps/components: an image acquisition equipment (usually a digitalisation scanner or a Computed Tomography (CT) equipment) that transforms geometry into digital data to be processed by the computer; the software that processes the data and produces a digital 3D model for the fabrication of the product; and a production technology that transforms the data into the final product [91]. After image acquisition and 3D modelling, the CAD model is either (1) produced by a 3D printer or (2) manufactured by a milling machine. In this second approach, the CAD model is converted into milling strips for the CAM-processing, and loaded into the milling device, that can have different number of axis (3, 4 or 5 axis) [91]. A computer numerical control (CNC) machining system, uses

power-driven tools to mechanically cut a block of the desired material into the final geometry, according to the computer instructions [92]. In this subtractive manufacturing technique, the milling machine will perform the commands for removing unwanted material from the block, resulting in a product with the final desired geometry.

The application of CAD/CAM in the dentistry field goes back to the 1970's when Dr. Duret fabricated crowns with the functional shape of the occlusal surface, through a system that encompassed an optical impression of the abutment tooth, the designing of the crown and its milling using a numerically controlled milling machine [93]. Some years later, Dr. Moermann (CEREC® system), introduced the concept of this new technology in a dental office at the chairside of patients, by measuring the cavity with an intra-oral camera with the posterior design and carving of an inlay made from a ceramic block [93]. At the beginning of the 1980s, when titanium has emerged as a promising material for dental applications, and since the precision casting of this metallic material was still difficult, Dr. Andersson attempted to fabricate titanium copings by spark erosion. He introduced the CAD/CAM technology as a specialized procedure as part of a total processing system that later evolved to a processing center networked with satellite digitizers targeting the fabrication of frameworks composed only of ceramic materials. These digitalized production systems are nowadays being employed by dental companies worldwide [93].

Producing implants or other dentistry components by this technique has been proved to reduce the production time of the implants and to produce very complex geometries that would be difficult to create with conventional techniques [91,92]. In Figure 2. 16 it is possible to observe a representative image of the CAD/CAM process workflow.

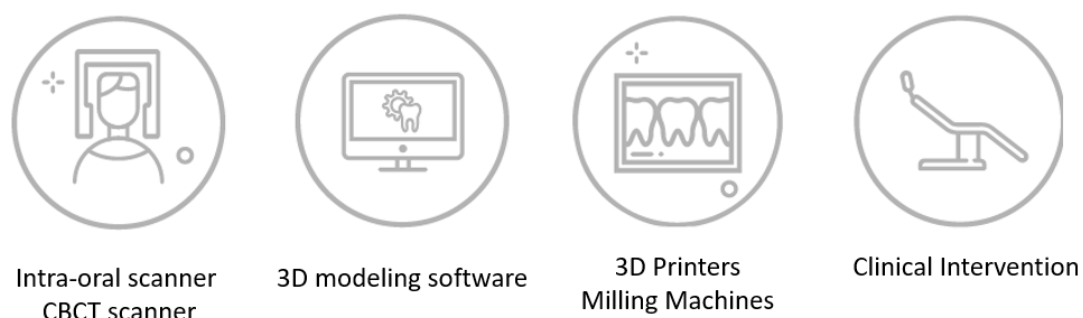


Figure 2. 16 CAD/CAM process workflow. Adapted from [94].

2.3.1.1. Digital Radiography

The current developments in the dentistry field lead to the need for more precise and accurate diagnostic tools, especially imaging methods. These imaging techniques have been used

to evaluate the bone quality and quantity in a diagnostic stage, to evaluate the bone and teeth geometry, and also to create 3D replicas/models of the patient bone/teeth anatomy [95].

Digital imaging has, therefore, been used to diagnose, treat, place, and restore dental implants. It enables the evaluation of the implant sites and provides diagnostic information that other techniques cannot provide [96]. With this technology, analogue photos are converted into digital images to be displayed in a computer that assigns a number to every single shade between white and black, based on the voltage present in the analogue form. A binary image is then created to be used by the technicians or dentists where 0 corresponds to black and 255 refers to white [97]. These digital images can be characterized as intraoral or extraoral depending on whether an X-ray sensor is placed internally or externally in the mouth. The CT technique, which allows to differentiate and quantify hard and soft tissues, has been developed over time also for dental applications, moving from the medical CT to the CBCT [98]. The conventional medical CT is characterized by high levels of radiation exposure and high costs which makes CBCT the most suitable tool for dental practice. CBCT creates images through an X-ray source and detector placed in a moving platform that rotates about a pivot that, in turn, circles the area to be imaged, usually, the patient's head (Figure 2. 17) [99]. This technique generates cone-shaped beams, and the images are obtained in one single rotation, resulting in considerably low levels of radiation. It creates a 3D picture of both hard and soft tissues in a small examination time, reduces the image unsharpness and distortion (related to patient translation and internal movements), and increases the X-ray tube efficiency [96].

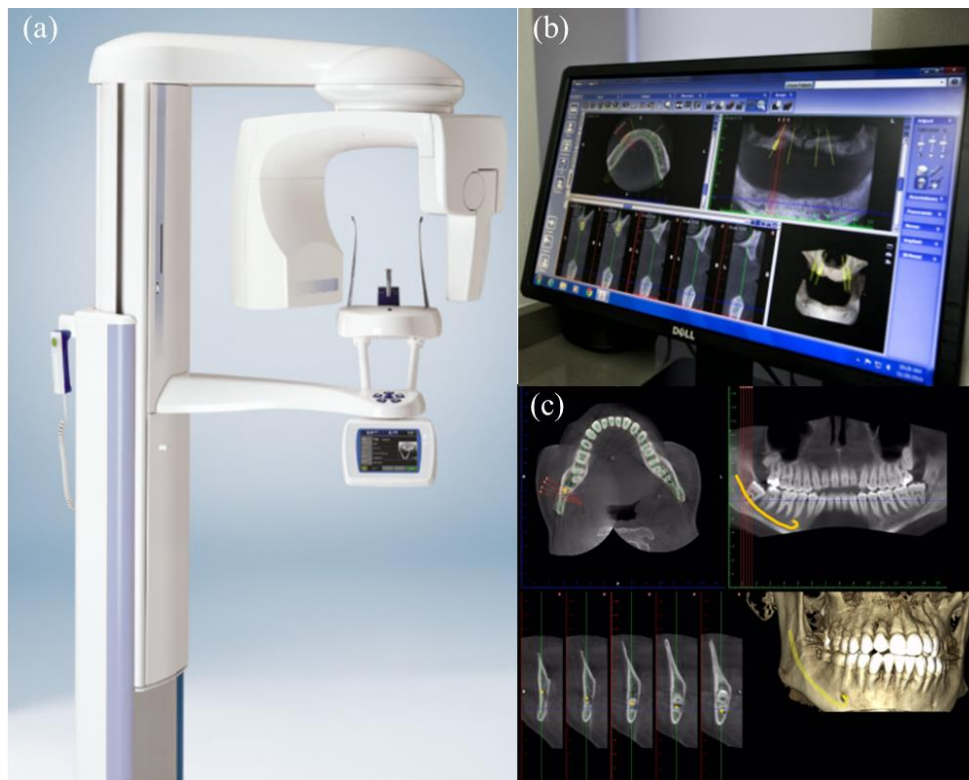


Figure 2. 17 CBCT apparatus: (a) equipment; (b) CBCT data acquisition software; (c) CBCT images and 3D reconstruction.

The accuracy of such technique has been evaluated by Al-Ekrish et al., that found mean absolute errors of 0.49 mm for the overall data [100]. In another study, conducted by Suomalainen, A. et al. results revealed errors in the range of 2.3%-4.7% for CBCT scans [101]. The images acquired by CBCT are of three types: axial images with a computer-generated curve of the alveolar process; alveolar cross-sectional images; and panoramic-like images. The continuity of the cortical bone plates, residual bone in the mandible and maxilla, the relative location of vital structures, and the contour of soft tissues covering the osseous structures are possible to identify with CBCT scans. Also, it allows the measurement of the distance between the alveolar crest and the mandibular canal avoiding impingement of the inferior alveolar nerve [102]. The main advantage of making use of this technology is that it helps to relate the radiographic images with information of a precise anatomic location or potential implant site.

2.3.1.2. Clinical Applications of CAD/CAM

As previously mentioned, CAD/CAM has been applied in the dentistry field aiming to facilitate and reduce the rehabilitation treatment time, but also to overcome the limitations of conventional techniques, namely the difficulty in achieving the total fit of the implants and frameworks [103].

This digital design and manufacturing technique is, nowadays, applied in a large number of clinical applications. It has been used to produce fixed prosthetic inlays, onlays, crowns, and veneers, to manufacture structures for implant bridges, to custom abutments and bars, among many others [89,104]. CAD/CAM allows the production of biocompatible structures with outstanding precision and aesthetic characteristics in a short period of time. In Figure 2. 18 it is possible to observe a ceramic crown manufactured by this technique and an example of a designing software layout (Exocad GmbH) [105].

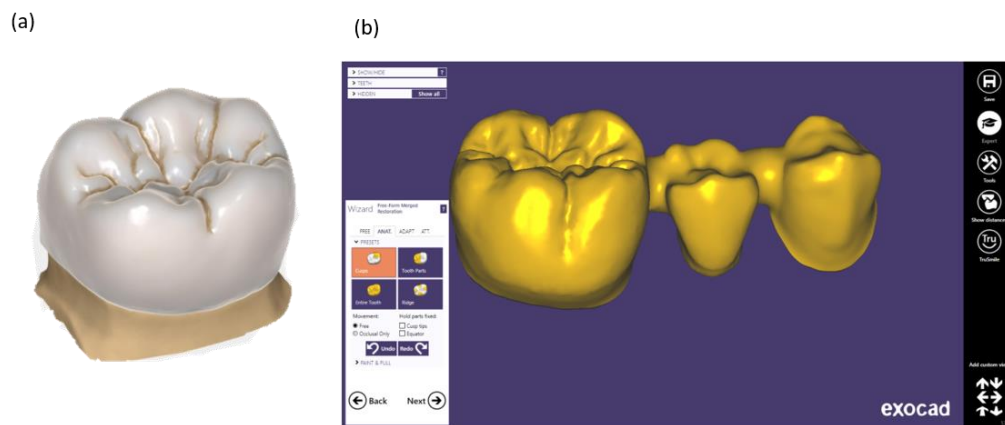


Figure 2. 18 CAD/CAM applied in dentistry: (a) ceramic crown; (b) software layout [105].

Another application of CAD/CAM in this medical field, is the design and production of computerized prototyped surgical guides (Figure 2. 19) to implant placement based on real images of the bone anatomy [104]. In this approach, a virtual surgery simulating the implant placement is performed, by manipulating the CT scan images on a specific software, thus enabling to find the best implant position and bone anchorage [106,107]. Guided surgeries have been reported in the literature as a successful therapy for the rehabilitation of edentulous, partial or single tooth restorations, characterized by optimal aesthetic and functional results [108].

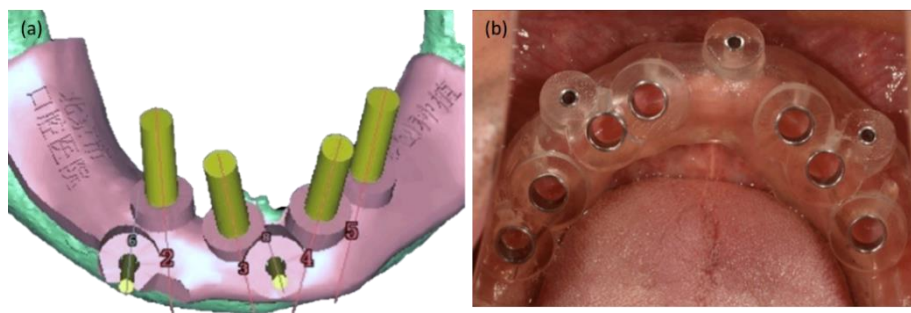


Figure 2. 19 Surgical guide: (a) surgical guide design and surgery simulation; (b) clinical procedure with the manufactured surgical guide [106].

Apart from the previous dental applications of CAD/CAM, this technology has also been applied for the production of totally customized implants.

Chen, J., et al. created a CAD model of a simplified maxillary bone block with 1 mm layer of cortical bone, based on CT scans of the patient. Two types of titanium customized dental implants were designed: a root-analogue implant and a root-analogue threaded implant and a selective laser melting technique was used to print titanium implants [57]. Some other authors have also been developing titanium dental implants from CT scans data, 3D reconstruction of the root, and posterior processing by DMLS [88,109,110]. Pirker, W. et al [65,111,112] has been manufacturing zirconia dental implants based on the laser scanning of the original tooth and posterior implant milling by CNC. In Figure 2. 20 it is possible to observe a titanium and a zirconia dental implant, both manufactured by the CAD/CAM technology.

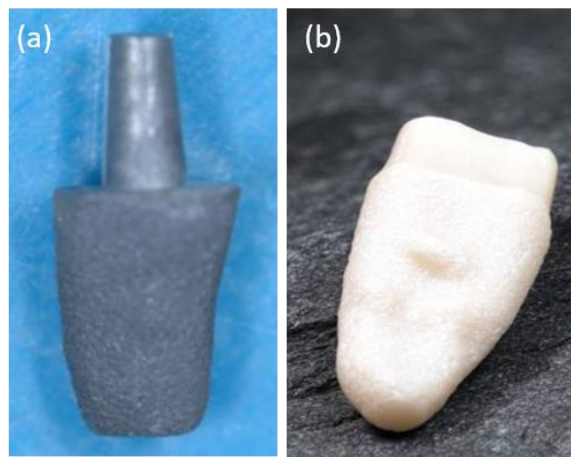


Figure 2. 20 Dental implants manufactured by CAD/CAM: (a) titanium root-analogue implant [109]; (b) zirconia root-analogue implant [112].

It is unquestionable that CAD/CAM systems have revolutionized dentistry and its market. There are numerous studies reporting the successful clinical outcomes of such technique, mainly due to the improved and more accurate implant (or implant parts) fit, when compared to conventional methods [113–116]. It allows the production of both implant parts, such as crowns or abutments, or entire customized implants, making use of different materials, according to each specific clinical situation. In addition, it has become a useful tool for the simulation and planning of implant placement, making surgeries less invasive and more efficient [104]. Despite all the advantages of CAD/CAM technology, it involves several steps that should be carried out with

caution, targeting an optimal clinical performance and balance between all the biological and mechanical factors [104].

2.4. Dental Implants Surface Modifications

The term “osseointegration” was first introduced and developed by Dr. Par-Ingvar Branemark in 1985, to define the direct structural and functional connection between a living bone and the surface of a load-carrying artificial implant, typically made of titanium [117]. This process requires an initial interlocking between the alveolar bone and the implant - primary stability- and later, biological fixation through continuous bone remodelling toward the implant - secondary stability). Osseointegration is a very complex process and there are many factors that influence the formation and maintenance of bone at the implant surface [117]. The implant stability is, therefore, composed of two components: the primary stability, also denominated mechanical stability, and the secondary or biological stability [118]. Primary stability is characterized by the mechanical anchorage of the implant immediately after the implant insertion. There are key factors that influence an implant primary stability: surface roughness, the precision of implant dimensions, thread pitch and respective drills, bacterial adhesion, and many others [23]. On the other hand, secondary stability is related to bone ingrowth and subsequent biologic healing. This biological stability will ensure the prosthesis fixation over time, as well as the osseointegration process, improving the implant longevity [23]. The combination of these two stabilities results in the implant total stability (Figure 2. 21).

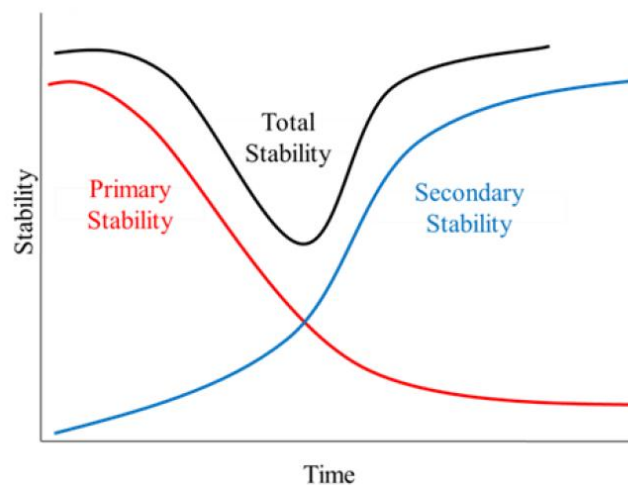


Figure 2. 21 Schematic representation of the implant stability [23].

Over the last years, aiming to enhance dental implants success, different surface treatments have been developed to improve both primary and secondary stabilities.

2.4.1. Surfaces for Improved Mechanical and Biological Stabilities

Surface composition, roughness, and hydrophilicity have been proved to have a huge impact on the implant surface interactions with the surrounding tissues and consequent stability [21]. To induce modifications in these parameters, some techniques have been developed and applied, at the macro, micro, and even nano scales [119].

2.4.1.1. Machining

Machined surfaces are surfaces that after being manufactured are not subjected to any treatment. After being manufactured, implants with machined surfaces are simply cleaned, decontaminated, and sterilized [15]. At the microscopic level, these surfaces are characterized by the presence of marks and grooves resultant from the machining process [120]. The main disadvantage of these surfaces is that the osteogenic cells tend to attach and deposit bone along the surface grooves delaying the surgery healing-time [16,120]. Computer-Aided Manufacturing systems have been used to produce machined ceramic implants and implant parts. On the other hand, the machining of titanium has been proved to be inefficient and a time-consuming process [16].

2.4.1.2. Sandblasting

Sandblasting is one of the most common surface modifications applied in dental applications. This technique is characterized by an increased implant surface roughness resulting from the projection, through a nozzle, of hard ceramic particles, usually alumina (Al_2O_3), hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) or titanium dioxide (TiO_2), at high velocity towards the implant surface (Figure 2. 22) [121,122].

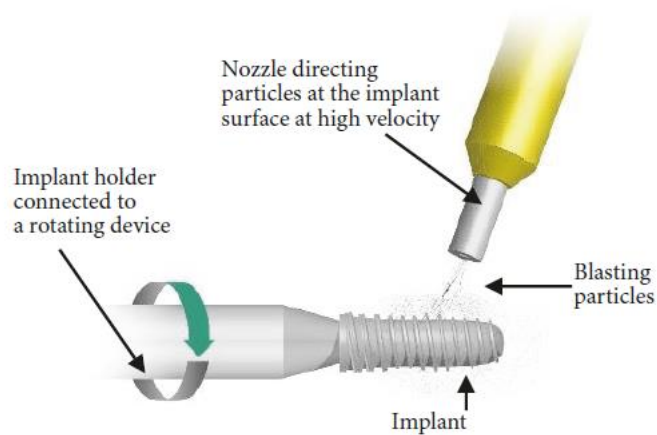


Figure 2. 22 Schematic representation of the sandblasting process [123].

Parameters such as particle size and shape, time of blasting, pressure, and distance from the blasting gun to the implant surface have a huge influence on the final surface roughness. Depending on the selected parameters, surfaces modified by sandblasting may present mean roughness values (R_a) ranging from 300 nm to 3 μm [124,125]. These roughness levels have been proved to promote an improved adhesion, proliferation, and differentiation of osteoblasts when compared with machined surfaces [15,126]. However, some studies suggest that rougher surfaces tend to facilitate the accumulation of bacteria, which is one of the biggest drawbacks of such surface treatment [16,127].

2.4.1.3. Acid-etching

Acid-etching has also been used to modify the surface properties of dental applications. In this technique, the surface to be treated is immersed into an acidic solution, usually of hydrofluoric acid (HF), hydrochloric acid (HCl), nitric acid (HNO_3), or sulfuric acid (H_2SO_4) [119]. These acids can be used separately or in combination (dual-etching). Depending on the characteristics of the bulk material, acid etching can be used whether to increase the surface roughness, by the creation of micro-pits, or decrease its roughness, by the removal of grains and grain boundaries from the surface [16]. Parameters such as the type and concentration of acid, temperature, and soaking time (typically from 1 to 60 min) may be used to control the final surface roughness, which can range from 0.3 to 2 μm [15,16,127]. Apart from surface roughness, acid etching is also capable of changing the wettability of the treated surfaces [128]. Implants with acid-etched surfaces have shown greater resistance in reverse torque removal when compared to machined and titanium plasma spraying ones, which indicates better stability and more efficient osseointegration

[127,129]. Additionally, one study performed by Han A., et al showed that the creation of crater-like topographies on zirconia surfaces by hydrofluoric acid seems to be a promising method for the inhibition of bacterial adhesion and biofilm formation on zirconia [130]. Treating an implant surface with sandblasting followed by an acid-etching treatment has also been used to obtain a dual surface roughness as well as the removal of embedded sandblasting particles. The etching process reduces the highest peaks while smaller pits are created and the average surface roughness will be reduced [131]. Nowadays, modifying implants surfaces with sandblasting followed by an acid etching procedure (SLA) is considered the gold standard surface treatment for titanium dental implants [132].

2.4.1.4. Anodization

Anodization has being applied to titanium (or other metals) surfaces to create dense and stable protective oxide layers, with thickness up to 1 μm [21]. Briefly, this electrochemical process consists of the oxidation of the metallic surface, immersed in an electrolyte, through the induction of an electrical field at the metal/electrolyte interface – Figure 2. 23 [133]. Together with an increase in the oxide layer thickness, this surface treatment allows the creation of nano features with different morphologies and sizes, namely the creation of nano or micro pores along the implant surface [133,134]. The structural and chemical properties of the final surface can be controlled by varying processing parameters such as the electrolyte composition, current, anode potential, and temperature [16].

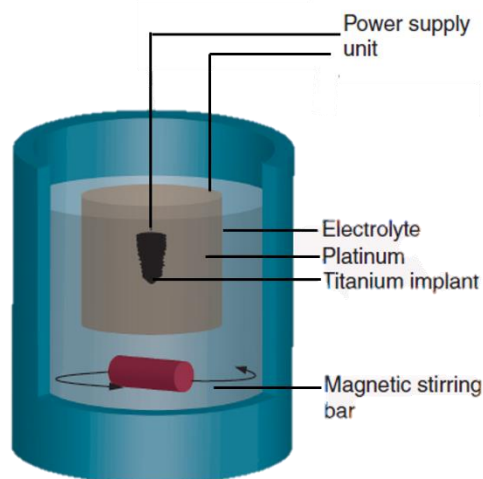


Figure 2. 23 Schematic representation of the anodization process [134].

The main advantages of anodization include improved biocompatibility and increased cell attachment and proliferation [15,135,136]. In fact, such surfaces have already shown satisfactory outcomes as far as the implant osseointegration is concerned, due to an improved mechanical interlocking and biochemical bond between the implant and bone [16,136].

2.4.1.5. Laser Treatment

Laser technology has been applied to create 3D structures at the micro or nano scales in both zirconia and titanium dental implants [16]. This physical technique generates short pulses of light of a single wavelength, providing energy focused on one spot, allowing the rapid production of complex and precise topographies with high resolution [131].

Treating a surface by laser eliminates the risk of surface contamination and was proved to improve the surface wettability which, in turn, is expected to enhance cellular adhesion [137]. This surface treatment has been widely used to fabricate micro-grooved surfaces on zirconia implants. The obtained micro-structure seems to change the collagen fiber orientation, peri-implant bone architecture, and human cell metabolism [138]. Literature reports higher removal torques for implants with laser-treated surfaces, when compared to machined ones [131,139]. Additionally, combining a laser treatment with acid etching has been proven to improve implant osseointegration, when comparing to a surface that was only treated by laser [140].

2.4.1.6. Plasma Spraying

This technique has been applied to titanium surfaces to increase their roughness [21]. It encompasses the projection of different substances (usually Ti or calcium phosphates) into a plasma torch under vacuum, reduced or atmospheric pressure, at high temperature. Argon and oxygen are the most commonly used gases in such applications [16]. The particles are then projected on the implant surface and melt to form a uniform thin film, with a thickness in the range of 30-50 μm and average roughness of 7 μm (Figure 2. 24) [15].

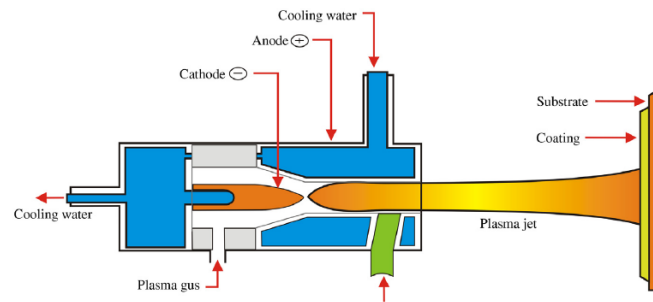


Figure 2. 24 Schematic representation of the plasma-spraying process [119].

The plasma spraying technique increases the surface area and tensile strength at the implant-bone interface [119,141]. One of the major drawbacks of such a technique is the release of metallic particles to the body environment, not only to the implant adjacent tissues but also to organs such as the liver and spleen [119]. Additionally, delamination or resorption of the coating may cause the loss of its mechanical integrity and induce adverse biological reactions such as periimplantitis [142]. Uneven coating thickness and dissimilarities in the composition of the coating have also been reported as a common limitation of this surface treatment [120].

2.4.1.7. Other Coatings

Apart from the previously mentioned surface treatments, dental implants are also commonly coated with a variety of materials and/or molecules depending on the specific requirements [15].

Coating implants with calcium phosphates such as hydroxyapatite or tricalcium phosphate (TCP) is being applied in the dentistry field since the 1980's [21]. These bioceramics, namely HAp, have been used to coat zirconia surfaces with the main goal of modifying them from highly inert to bioactive [21]. HAp has the ability to form a strong bond between the implant and bone due to its similarities in composition to the mineral phase of bone [138]. The bioactivity of HAp is related to the precipitation of a calcium phosphate-rich layer on the implant surface through a solid solution ion exchange at the implant-bone interface [131]. These coatings can be produced by a variety of methods that go from plasma spraying, vacuum deposition technique, electrolyte process, sol-gel and, dip-coating [21,120]. Literature reports an improved bone-implant bond and enhanced cellular proliferation of zirconia implants coated with HAp when compared to uncoated ones [138,143,144]. Other materials such as silica, magnesium, carbon and, graphene are also described in the literature for their favorable biologic properties [138].

Due to the ability of osteoblasts of recognizing specific molecules, it is possible to coat implant surfaces with immobilized molecules to enhance cell attachment, protein deposition and,

mineralization [15]. The most commonly used molecules for such application are amino acid sequences, fibronectin, vitronectin, collagen and also pharmacological substances such as biophosphonates [145].

Despite all the efforts that have been carried out, coating implant surfaces still present some limitations: risk of delamination or even total coating loss are the most reported ones [23,146]. Weak bond between the implant and the coating material and difficulty in achieving a uniform coat thickness have also been reported as possible drawbacks for such surface modification [21].

2.4.2. Surfaces for Antibacterial Purposes

Improving the antibacterial properties of dental implants is one of the biggest challenges in implantology nowadays. The plaque formation is related to the adhesion of microorganisms to the implant surface and has a huge impact on the implant primary stability [147]. It may be a result of poor oral hygiene but is also extremely associated with the implant characteristics [148]. In this sense, morphologically designed surfaces have been modified with the ultimate goal of improving the implant's antibacterial properties [149].

Surface morphology and roughness play an important role in bacterial adhesion [150]. Literature reports that the accumulation of plaque around dental implants is extremely dependent on its surface roughness and rougher surfaces are expected to promote a higher bacterial adherence due to the presence of grooves and pits [147,151,152]. Additionally, recent studies have suggested that the creation of micro or nano topography patterns may be a promising approach for the inhibition of bacterial adhesion [153,154]. As far as zirconia is concerned, implants with surface roughness ranging from 130 to 360 nm are reported as ideal to avoid bacterial adhesion [155].

Another strategy that has been developed is the incorporation of therapeutic concentrations of antibiotics in the implant coatings, namely calcium-phosphate coatings [21,120]. Volker Alt et. Al, combined HAp with gentamicin and antibiotics of a systemic nature and coated them on the implant surface to act as an antibacterial agent [156]. This antibiotic is also expected to remove virulent endotoxins from the implant surface. Results revealed that the developed coating showed significant improvement of infection when comparing to conventional HAp coatings [156].

Al Mugeiren et. Al showed that coating an implant surface with a combination of calcium-phosphate, zinc, and fluoride ions exhibited bioactivity and antibacterial properties [157]. On the

other hand, mimicking human antimicrobial peptides also seem to reduce the bacterial adhesion to implant surfaces [21]. Zeqian Xu et. Al, applied totarol coatings - a natural biocompatible antibacterial agent- on experimental dental implant and abutment surfaces and investigated the antibacterial capability of totarol coatings in a simulated oral environment [158]. Results revealed that totarol exhibited a promising antibacterial effect and was able to inhibit biofilm growth.

Due to the fast degradation of the coatings in the oral environment, these antibacterial effects get sometimes compromised. In this sense, in contrast to antibiotics, silver particles are emerging as promising approaches to avoid bacterial adhesion to implants surfaces [149]. Favorable outcomes regarding the incorporation of silver particles on the implants surface are reported in the literature [153,159].

Despite all the advances in the development of surfaces that effectively avoid bacterial adhesion, this is still one of the biggest challenges in implantology and studies should proceed aiming to find better solutions for such purpose.

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CHAPTER 3

Subperiosteal dental implants: Past or future?

A critical review on clinical trials/case reports and future directions

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Abstract

Subperiosteal implants were first introduced in 1942 in Sweden and were subsequently used worldwide for the treatment of mandibular and maxillary arches with advanced bone atrophy. However, due to the high complication rates and unsuccessful outcomes, this therapy fell in disuse. Advances in digital technology have led to a new interest in subperiosteal implants therapy and investigators are looking for innovative and effective techniques for dental rehabilitation. In this review, a critical analysis on the performance and characteristics of subperiosteal implants was carried out. Parameters such as materials and surface coatings, the design and manufacturing techniques, methods of implantation, complementary strategies and the main clinical outcomes were carefully assessed. Also, a comparison with current dental implants and a proposal for an optimized solution are presented.

Keywords: Dental Rehabilitation; Subperiosteal Implants; Endosseous Implants; Clinical Trials; Customized Solutions; Minimally Invasive.

3.1. Introduction

Dental implants are a very common practice for tooth rehabilitation. Throughout the years many efforts have been carried out aiming to improve their clinical performance and success. Dental implants may be classified accordingly to their materials, their reaction with bone, the treatment options and, their placement within the tissues [1]. As far as the placement within the tissues is concerned, dental implants are classified as endosseous (most commonly used nowadays), subperiosteal and transosteal implants. Subperiosteal implants were first described in the late 1940s and different types of this design evolved up to the 1980s [2]. Subperiosteal implants are custom-fabricated structures designed to rest on top of mandibular bone, under the periosteum [3]. However, due to inappropriate or non-rigid fixation, difficulty in positioning them, and the high complication rates, this type of dental restoration fell into disuse [4]. In fact, in 15-year follow up periods, survival rates of only 50-60% are reported [5]. In this sense, endosseous implants became a better solution for the replacement of a missing tooth, solving several issues associated with subperiosteal implants [4]. Despite being a worldwide practice, endosseous implants are still not well accepted by patients because of their high cost, time of treatment, postoperative complications, and because of the invasive surgeries that are associated with this

type of rehabilitation. Digital technology has led to a new era in the dentistry field. The development of acquisition methods with considerably reduced energy given to the patient, such as the cone-beam computed tomography (CBCT), as well as the developments in the manufacturing techniques and materials, has simplified and improved the procedures related to the placement of dental implants [4]. With the technology that is available nowadays, it is possible to design and fabricate custom-made implants, perfectly adapted to the patient's specific anatomy [6]. These constant technological developments allow the opportunity to revisit some ancient concepts such as the subperiosteal implants, by adapting and improving them, based on a new technological context [7]. In this sense, the aim of this critical review was to collect and analyse clinical trials performed in subperiosteal implants in order to better understand their weaknesses. Additionally, a comparison with conventional endosseous implants was carried out, aiming to propose an alternative solution, that encompasses features of both subperiosteal and endosseous implants, for the design of an optimal solution for dental rehabilitation. The authors believe that minimally invasive procedures together with customized solutions are the future of implantology.

3.2. Methods

This review was carried out according to the PRISMA Statement- Preferred Reporting Items for Systematic Reviews and Meta-Analyses. In this section of the work, the authors describe the search strategy as well as the criteria for the study selection.

3.2.1. Search Strategy

An electronic database search was performed using five databases: PubMed, Google Scholar, Medline, Science Direct, and Scopus. To conduct the search, Boolean operators such as "AND" and "OR" were used to correlate the keywords. The following keywords were explored and inserted with the field tag [Title/Abstract/Keywords]: "subperiosteal" and "dental implants". In addition, the references of review articles were manually searched in order to include all the relevant articles available in the literature.

3.2.2. Study Selection

A screening process was conducted over the titles and abstracts retrieved by the databases search, in order to select the articles for full-text reading. From this screening process and after

duplicates removal, 95 articles were selected for full-text reading. In order to assess their eligibility to be included in this systematic review, the following inclusion criteria were applied: (1) articles written in English, (2) articles reporting clinical trials or case reports, (3) articles regarding subperiosteal implants. Articles not meeting the inclusion criteria were excluded from the review. It is important to mention that the full text of some articles found on the databases were not available online (for being too old) and, therefore, were not included in this analysis. Moreover, review articles and studies performed in animals were also excluded. Articles that met the inclusion criteria were then entirely read and analysed considering the aim of this systematic review.

3.2.3. Data Collection and Extraction

The information extracted from each article was divided into five main groups: the materials and surface coatings; the implant design and manufacturing techniques; the methods of implantation; complementary strategies; and the main clinical outcomes. In this sense, for each of these topics, the authors summarized the information available in the selected literature and the extracted information can be seen in the next section of this work.

3.3. Results

3.3.1. Study Selection

Automatic and manual database searches resulted in a total of 949 records. After duplicate removal, 462 titles and abstracts were evaluated and a total of 95 full texts were selected to assess their eligibility to be included in this systematic review. Of these 95 publications, 62 could not be included in the final analysis. These articles were excluded for ≥ 1 of the following reasons: 1) they did not present any clinical trial nor case report; 2) studies were performed in animals; 3) they were review articles; 4) the articles were not written in English. The application of the exclusion criteria resulted, therefore, in 33 articles to be evaluated and analysed in the scope of this work. In Figure 3. 1 it is possible to observe the PRISMA flowchart used in this selection process.

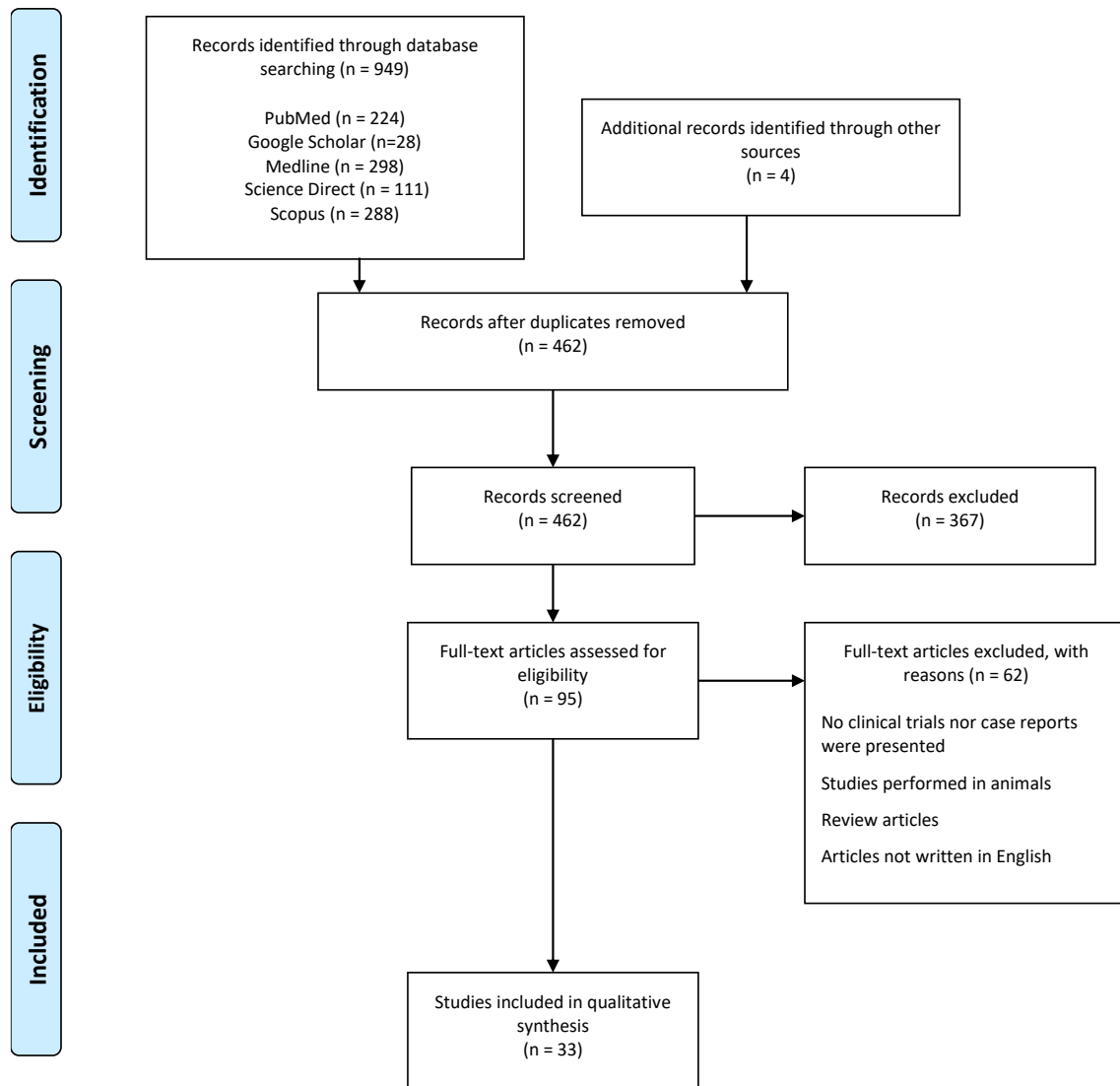


Figure 3. 1 Search strategy flowchart, adapted from [8].

This study analyses relevant articles published in the literature on the clinical performance of subperiosteal dental implants. The selected articles include case reports or clinical trials, the implants' design and manufacturing techniques, the implantation process and report the main clinical outcomes. In Table 3. 1 it is possible to observe the selected articles' author, year of publication and title. Additionally, the number of tested implants and the implant type are also presented. As can easily be seen, articles found in literature and included in this review are dated from 1959 to 2020.

Table 3. 1 Summary of the selected articles.

Authors/ Year of publication	Title	N° of implants	Implant type
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Obwegeser, 1959 [9]	Experiences with subperiosteal implants	35	23 complete and 2 partial mandibular subperiosteal implants 8 complete and 2 partial subperiosteal maxillary implants
Weber, 1968 [10]	Complete bilateral subperiosteal implants for partially edentulous mandibles	1	Complete bilateral subperiosteal implant.
Kratochvil and Boyne, 1972 [11]	Combined use of subperiosteal implant and bone-marrow graft in deficient edentulous mandibles: A preliminary report	1	Combination of subperiosteal implant and bone-marrow graft
Boyne, 1974 [12]	Restoration of deficient edentulous ridges by bone grafting and the use of subperiosteal metal implants	12	Combination of subperiosteal implants and bone-marrow graft
Bodine, 1974 [13]	Evaluation of 27 mandibular subperiosteal implant dentures after 15 to 22 years	27	Complete mandibular subperiosteal implants
Bloomquist, 1982 [14]	Long-term Results of Subperiosteal Implants Combined with Cancellous Bone Grafts	19	Combination of subperiosteal implants and cancellous bone graft
Hess et al., 1982 [15]	Two cases of incompatibility to carbon-coated subperiosteal implants	2	Complete mandibular subperiosteal implants
Kreutz and Carr, 1986 [16]	Bilateral oronasal fistulas secondary to an infected maxillary subperiosteal implant	1	Complete maxillary subperiosteal implant
Kay et al., 1987 [17]	Hydroxyapatite-coated subperiosteal dental implants: Design rationale and clinical experience	339	66 complete maxillary; 165 complete mandibular; 11 unilateral mandibular; 97 unilateral maxillary
Truitt et al., 1988a [18]	Use of computer tomography in subperiosteal implant therapy	41	3 submerged full subperiosteal implants; 33 exposed full subperiosteal implants; 3 circumferential subperiosteal implants; 2 unilateral subperiosteal implants.
Truitt et al., 1988b [19]	Morphologic replication of the mandible using computerized tomography for the fabrication of a subperiosteal implant	10	Mandibular subperiosteal implant
Cranin et al., 1988 [20]	Reconstruction of the Edentulous Mandible with a Lower Border Graft and Subperiosteal implant	15	Combination of subperiosteal implant and lower border bone graft
Falomo, 1988 [21]	A retrospective survey of patients treated with subperiosteal and endosseous implants	16	2 maxillary subperiosteal implants; 14 mandibular subperiosteal implants
Bailey et al., 1988 [22]	The mandibular subperiosteal implant denture: A fourteen-year study	74	Mandibular subperiosteal implants
Fischer, 1993 [23]	CAD/CAM subperiosteal implants in Australia. Case report	1	Mandibular subperiosteal implant
Yanase et al., 1994 [24]	The mandibular subperiosteal implant denture: A prospective survival study	81	Mandibular subperiosteal implants
Bodine et al., 1996 [25]	Forty years of experience with subperiosteal implant dentures in 41 edentulous patients	41	Mandibular subperiosteal implants

Perry, 1998 [26]	Reconstruction of advanced mandibular resorption with both subperiosteal and root-form implants	3	Circumferential mandibular subperiosteal implants
Mansueto, 1999 [27]	Replacement of a mandibular subperiosteal implant	1	Mandibular subperiosteal implant
Fish and Misch, 2000 [28]	Mandibular bone growth induced by a Hydroxyapatite-coated subperiosteal implant: a case report	1	Mandibular subperiosteal implant
Sirbu, 2003 [29]	Subperiosteal implant technology: Report from Rumania	2	Mandibular and maxillary subperiosteal implants
Minichetti, 2003 [30]	Analysis of ha-coated subperiosteal implants	22	6 full maxillary 1 full mandibular 6 unilateral maxillary 9 unilateral mandibular
Moore and Hansen, 2004 [31]	A descriptive 18-year retrospective review of subperiosteal implants for patients with severely atrophied edentulous mandibles	40	Mandibular subperiosteal implants
Lozada et al., 2004 [32]	Immediate functional load of mandibular implant overdentures: A surgical and prosthodontic rationale of 2 implant modalities	5	Mandibular subperiosteal implants
Kusek, 2009 [3]	The use of laser technology (er;cr:ysgg) and stereolithography to aid in the placement of a subperiosteal implant: Case study	1	Mandibular subperiosteal implant
Loperfido et al., 2014 [33]	Severe mandibular atrophy treated with a subperiosteal implant and simultaneous graft with rhBMP-2 and mineralized allograft: a case report.	1	Mandibular subperiosteal implant
Nazarian, 2014 [34]	Placement of a modified subperiosteal implant: A clinical solution to help those with no bone	1	Maxillary subperiosteal implant
Mapkar and Syed, 2015 [35]	Revisiting the maxillary subperiosteal implant prosthesis: A case study	1	Maxillary subperiosteal implant
Peev and Sabeva, 2016 [36]	Subperiosteal Implants in Treatment of Total and Partial Edentulism - A Long Term Follow Up	93	Partial and total subperiosteal implants
Gellrich et al., 2017 [5]	A customised digitally engineered solution for fixed dental rehabilitation in severe bone deficiency: A new innovative line extension in implant dentistry	3	2 mandibular subperiosteal implants 1 maxillary subperiosteal implant
Nguyen et al., 2018 [37]	A subperiosteal maxillary implant causing severe osteolysis	1	Maxillary subperiosteal implant
Cerea and Dolcini, 2018 [38]	Custom-Made Direct Metal Laser Sintering Titanium Subperiosteal Implants: A Retrospective Clinical Study on 70 Patients	70	Maxillary and mandibular subperiosteal implants
Mangano et al., 2020 [4]	Custom-made 3D printed subperiosteal titanium implants for the prosthetic restoration of the atrophic posterior mandible of elderly patients: a case series	10	Mandibular subperiosteal implants

After a careful analysis of the thirty-three articles, authors were able to extract a lot of information to be analysed and compared. In this sense, and as previously mentioned, results of this study were divided into five main groups: the materials and surface coatings; the implant

design and manufacturing techniques; the methods of implantation; complementary strategies; and the main clinical outcomes.

3.3.2. Materials and Surface Coatings

The materials applied for the fabrication of subperiosteal implants have changed throughout the years. The subperiosteal implants first described in literature were made of different biomaterials. Chromium-cobalt-molybdenum alloys (particularly Vitallium) and tantalum are the most reported ones [5,9]. In fact, the first subperiosteal implant, placed in 1948 by Gershkoff and Goldberg [39], was made of Vitallium. Their electric inertness, mechanical strength, hardness, insolubility in body fluids, resistance to corrosion and also their biocompatible nature made these alloys appropriate materials for such applications [17]. Later, titanium and its alloys have also been indicated as good candidates for subperiosteal implants [33].

A controversy regarding the side effects of the metallic materials in the body environment, namely the ions release to the surrounding tissues has led to the development of alternative solutions. In a first attempt, it was thought that the subperiosteal implants would be better tolerated if coated with carbon due to its supposed outstanding biocompatibility [40]. This thin coat over the metallic substrates was thought to minimize the formation of a connective tissue capsule around the implant frame. However, this approach was not much developed since the purported biocompatibility of the carbon-tissue interface was not effectively confirmed. A study performed by Hess [15] reported two cases of incompatibility to carbon-coated subperiosteal implants. Authors believe that the implants failure may be related to the fragmentation of the carbon coating on the implant surface and demonstrated the histopathologic effects that this type of implant material can induce in human tissues that would eventually lead to the implant failure.

Later, in the 1980s, another strategy was implemented and evaluated in this regard - the creation of hydroxyapatite (HAP) coatings over the metallic structures [17,34]. By then it was already known that this ceramic material has a high similarity in composition to the bone mineral, is bioactive and osteoconductive and has a high ability to form a strong interface with bone. The incorporation of HAP over metallic substrates had already been found to combine the excellent mechanical properties of the metallic materials with the biocompatibility and bone bonding characteristics of the ceramic. When comparing coated to uncoated implants, during some experiments in dogs, results revealed that the coated metallic implant induced a stronger implant-bone adherence [17]. Since then, several studies have reported clinical results on the use of HAP-

coated subperiosteal implants. Golec [41] performed a study on the performance of 241 HAP-coated mandibular subperiosteal implants and, in a follow-up period of 7 years, a survival rate of 98% was achieved [31,41]. In another study [24], survival rates of 79% were achieved in a follow-up period of 10 years, and 60% after 15 years. A more recent study [30] evaluated the performance of HAP-coated subperiosteal implants over 10 years and a success rate of 91% was achieved. However, 36% of those implants needed a corrective intervention. Despite these rates not being so high as desirable, the use of HAP coatings over metallic substrates is still recommended since it has been proved to improve the interactions between the implant and bone, to decrease strut dehiscence and to improve the soft tissue environment [34].

3.3.3. Design and Manufacturing Techniques

The design of the subperiosteal implants must allow the transfer of load from the denture to the post, and from the post to the strut structure, with neglectable stress concentrations and static or cyclic fatigue mechanisms [17]. The technique of designing this type of implants was first described by Boyne and Kratochvi [11]. This technique used to start with a direct impression of the bone where the implant would lay, leading to the need of a two-stage surgery, as will later be explained, in the Methods of implantation section. In this method, a previously prepared plastic tray used to be fitted over the exposed bone and over the remaining anterior teeth, followed by some corrections to ensure a good fit between the implant and bone [10]. Then, the tray was filled with elastic materials, namely rubber adhesive. After approximately 10 minutes, the impressions were removed, and the occlusal registration was made [10,25]. It is important to mention that some more recent studies performed this step without the need of using the prefabricated trays, by simply making bone impressions with materials such as polysulfides, silicones, and polyethers [42]. During this procedure, the patient was occluded onto a prepared baseplate and occlusion rim, which was seated on the exposed mandible. The base of the occlusion rim was lined with soft wax to ensure that it would seat directly onto the bone. This record was later used to determine the interocclusal distance and to serve as a guide to the height of the implant posts [10]. Following the impression step, a bone model was made in dental stone and mounted at the proper vertical dimension for the implant fabrication [30]. This method was characterized for inducing a significant postoperative discomfort to the patients due to the excessive bone exposure [14].

In this sense, aiming to reduce the patient discomfort and avoid a two-stage surgery, in 1985 Truitt [43] developed a non-invasive technique for the design of mandibular subperiosteal

implants, based on the computerized tomography (CT) scanning technique. The main advantage of this method is that the clinician is able to obtain the bone model by making use of a CT and a computer-generated model. The CT is performed prior to any surgical intervention, and only one surgery is needed to insert the implant, making the overall process much less invasive [30]. Since the introduction of this new technique, some studies can be found in literature with quite satisfactory results [4,5,18,19,23,34,38]. Apart from its non-invasive nature, this technique also avoids the potential toxic effects of foreign bodies of the impression material and does not require the use of anesthesia [19]. After CT acquisition, stereolithography, has been used to fabricate very precise anatomical models of the patients' jaws anatomy. This highly accurate technology uses the data from the 3D computer model to, layer by layer, fabricate a 3D model of the patient anatomy. The model is then delivered to a dental laboratory to ultimately fabricate the cast framework [3,33]. Some surface treatments such as polishing, sandblasting, acid etching, HAP coating and sterilization are also reported. Direct metal laser sintering (DMLS) is an additive manufacturing technique characterized by its ability to produce custom-made grids and implants, perfectly adaptable to specific anatomical requirements. In this sense, in the past few years, DMLS has emerged as a potential manufacturing technique for the production of subperiosteal implants. One study performed by Cerea [38] evaluated the clinical performance of 70 custom-made DMLS titanium subperiosteal implants and a satisfactory survival rate of 95.8% was reported in a two-year follow up. Briefly, in this technique, the implant was fabricated on a moveable platform by applying layers of grade 5 titanium micro-powders. For each layer, the machine lays down a thin film of the metallic powder with a specific thickness. The laser melts selected areas and the platform then moves down by the pre-established layer thickness, a fresh film of metal powder is poured and the next layer is melted via exposure to the laser source [38]. This process is repeated, layer by layer, until the implant is complete. In this specific case, the implant was then polished by electroerosion and finally sterilized. Even more recently, Mangano [4] evaluated the clinical outcomes of ten subperiosteal implants fabricated by DMLS. Despite the fit of two of the implants not being satisfactory, at the one-year follow-up no implants were lost, leading to a 100% survival rate.

This approach of fabricating subperiosteal implants by DMLS is a novel technique that still requires further clinical evidence to corroborate these positive preliminary clinical outcomes. Clinical studies on a larger number of patients and longer follow-up periods are needed.

3.3.4. Methods of Implantation

As previously mentioned, the first generation of subperiosteal implants used to encompass two surgeries for complete implant insertion. The first surgery was related to the bone impression for the implant design. This surgery was characterized by excessive bone exposure. According to Sirbu [29] this procedure used to start with the sterilization of the oral cavity followed by the administration of anesthetic blocks. A crestal incision was made in the periosteum, around the entire arch. To facilitate reflection of the mucoperiosteal flaps, a vertical anterior relieving incision was also required. The vital bearing areas were thus exposed after flaps reflection with a sharp periosteal elevator. If bone irregularities or protrusions were observed at this time, corrections were made with bone files or rongeur forceps. Cross tongue dorsum ligatures were used to promote an adequate flaps retraction and the impression tray (mentioned in the designing techniques) was then placed or made over the bone [29,44]. At this time, the bone was exposed and prepared for the impression method described in section 3.3.3. An impression was considered adequate if it included the external oblique ridge area, the mental nerve region and the mental symphysis area of the buccal side; and the mylohyoid ridges, the genial tubercles of the lingual side [45].

As far as the second surgery is concerned (that corresponds to the unique surgery in the cases where the bone geometry is acquired by CT), it corresponds to the implant placement procedure. As previously mentioned, with the introduction of the CT for the fabrication of subperiosteal implants, in 1985, this became the only needed surgery for the placement of subperiosteal implants. There is no consensus in the literature regarding the time to wait between the two surgeries. Obwegeser [9] mentioned that, while some previous authors waited three to six weeks between the two surgeries, one to three weeks is the most acceptable time to wait between bone impression and implant placement, in order to ensure a better implant fit. This author refers that waiting three to five weeks for the implant insertion would result in variable degrees of misfit between the implant base and bone and waiting more than five weeks may lead to such a poor fit that implant placement would no longer be recommended [9]. However, a wide range of periods of time can be found in literature as regards the most appropriate time for the second surgical intervention: Kratochvil [11] performed the second surgery ten weeks after the first; Cranin [20] placed the implant 12h after the bone impression procedure; Mansueto [27] performed the second surgery four weeks after the first one; Sirbu [29] waited twenty-eight to forty-five days to place the implant; Mapkar [35] performed the implant insertion eight weeks after bone impression acquisition, claiming it was enough time for the tissues to re-establish blood supply and avoid the

risk of incision line opening; Leake [40] scheduled the second surgery for two or three weeks after the first one; and Truitt [43] inserted the implant twenty days after the first surgical intervention.

This second surgery is more rapid and causes less swelling and discomfort to the patient when compared to the first surgery [42]. In this surgical intervention, the patient was again sedated under local anesthesia, after oral cavity sterilization, and the wounds were then reopened, using the same incision lines [29]. After exposure of the bone, the sterilized implant was inserted under the mucoperiosteal flaps and seated on the bone. The position and fit of the implant were adjusted and screws were used to fix the implant [4,10,11,34,35,38]. There are, however, some authors that reported that there was no need to wire or screw the implant to the bone [9,20]. It was believed that with adequate bone preparation and enough implant framework extension, the implant would be stable enough during the initial postoperative period and after ten days it would already be fixed by connective tissue. The procedure was finally completed by a careful suturing to obtain a tension-free, first-intention closure [4,29,38].

3.3.5. Complementary Strategies

Subperiosteal implants used to be placed in mandibles with extreme bone resorption. In this sense, some strategies used to be applied in order to rebuild the bone and, at the same time, give structural support for the implant base. These strategies were also used to reduce the fit mismatch between the implant and bone when placing the implant [11]. The main advantage of such procedure was the ability to manage and reduce the inaccuracies related to the implant design technique, namely the bone impressions or the CT.

Autologous marrow and cancellous bone grafts used to be very common in the first subperiosteal implants [11,12,14,18,19]. The bone grafts were commonly harvested from the iliac crest, and patients had to be under general anesthesia [14].

In more recent studies, HAP particles were applied to induce bone augmentation and fill the void between the bone and the implant [17,27,30,34]. Additionally, a combination of autogenous bone and hydroxyapatite for augmenting the bone inferior border has also been reported [20]. In another clinical report, performed by Kusek [3], the implant struts were grafted with the patient's blood and beta-tricalcium phosphate (β -TCP) and then covered with a membrane. The development of grafts of bone morphogenic proteins (BMPs) has also been reported by Loperfido [33]. BMPs are multifunctional proteins with a wide range of biologic activities. BMP-2, specifically, had already been reported of being capable of driving multipotent cells into an

osteoblastic phenotype culture. In this sense, these authors treated a severely atrophic mandible with a HAP-coated titanium subperiosteal implant together with a BMP-2/mineralized allograft. Results revealed that no clinical complications were observed and grafting the mandible with mineralized allograft and BMP-2 may lead to considerable bone formation. However, not many studies are available on the literature regarding this matter and therefore further studies are required to confirm the benefits of bone morphogenic proteins when placing a subperiosteal implant [33].

As mentioned in section 3.3.2, strategies to improve the biocompatibility of subperiosteal implants have also been reported. In a first attempt, coatings of carbon were used [15,40] and their clinical outcomes assessed. This strategy did not lead to promising results and therefore did not prevail. These coatings were later replaced by HAP coatings and better outcomes are reported [17,28,32,34].

The constant developments in the field of biomaterials enabled doctors and clinicians to enhance the implant's biocompatibility and rebuild atrophic bones before implant insertion. However, quite invasive surgeries are associated with these procedures, as, for instance, the harvesting of autologous bone from the patient's hip. Further research should be performed in order to create alternative and effective solutions for dental rehabilitation.

3.3.6. Main Clinical Outcomes

One of the main reasons why dental implants fail is the lack of functional bone adherence. Osseointegration is a very complex process and there are many factors that influence the formation and maintenance of bone at the implant surface [46]. The implant material and surface properties, the congruence between the implant and bone, the surgical procedures, among others, have a huge impact on this biologic response. In fact, the lack of direct contact between the implant and bone is expected to lead to fibrous integration, rather than osseointegration [47].

In this sense, the clinical reports evaluated in the scope of this review present varying clinical outcomes. Complications such as pain, swelling, and inflammation have been reported. Removing a subperiosteal implant is characterized by an extremely complicated prosthodontic treatment and, as can be seen in Table 3. 1, at least one-third of the analysed articles reported implant removal during the follow-up period [13,14,38,15,17,21,24,25,27,36,37]. Despite most of the articles not reporting the need for implant removal, complications were commonly observed. Post-operative infection, strut dehiscence, bone resorption, and fibrous encapsulation with

subsequent implant movement were some of the reported complications.

Only a few articles reported satisfactory results [11,12,35,18–20,26,28,31–33]. These satisfactory results mean that, during the follow-up periods, no major complications were observed, and the implants were still in place, which does not necessarily indicate that the implants had been successful.

3.4. Discussion

Subperiosteal implants were used for several years, however, some drawbacks are associated with this kind of dental rehabilitation. The positioning of the implant was a very complex process, and high complication rates have been reported, as described in the previous sections of this work [4]. In this sense, subperiosteal implants have been replaced by endosseous dental implants, first introduced by Branemark. Endosseous implants overcome some limitations of the subperiosteal implants and have been proved to be a reliable and successful solution for dental restoration [48]. Despite being a worldwide practice, standard endosseous dental implants only provide limited options for implant diameter, length, and thread parameters [49]. Additionally, the lack of congruency between the implant and socket is expected to lead to plaque formation and accumulation, bone loss (and consequent implant exposure leading to aesthetical issues), poor implant stability and, ultimately, its failure [50]. In this sense, in this section of the present critical review, the authors aimed to compare and discuss some aspects (positive and negative) related to both subperiosteal and endosseous implants. Moreover, a new optimal/improved solution for dental rehabilitation is proposed based on the limitations and advantages of the current solutions.

In

Table 3. 2 it is possible to observe a summary of the main differences/similarities between subperiosteal and endosseous implants, as well as the parameters that an optimal solution should encompass.

Table 3. 2 Comparing subperiosteal and endosseous implants.

Aspects to compare/discuss	Subperiosteal implants	Endosseous implants	Optimal solution
Number of surgeries	one or two	one	one
Bone drilling	No	Yes	No
Type of procedure	Invasive	Extremely invasive	Minimally invasive
Bone loss	Low	High	Low
metallic/ceramic materials	Metallic	ceramic materials	ceramic
Aesthetics	Unsatisfactory (material +size)	Satisfactory	Good
Lifetime bacterial related diseases expectancy	High	Low/High	Low
Cost	Very high	High	Low
Customization	Yes	No	Yes

3.4.1. Number of Surgeries and Clinical Procedure

When placing a dental implant, the number of surgeries that are necessary to complete the process of implantation plays an important role. The patient will always prefer a treatment that is the less invasive possible and, therefore, one surgery will be preferable when comparing to two surgeries. As before mentioned, the first generation of subperiosteal implants used to encompass two surgical procedures. With the constant technological developments, new strategies were implemented, and it became possible to perform this kind of subperiosteal dental restoration in one surgery only. To place an endosseous dental implant, one surgical intervention is usually enough.

Despite being possible to place an endosseous implant in only one surgical intervention, very aggressive techniques are associated with this type of dental restoration. Before placing the implant, the dentist needs to prepare and drill the bone, making it an extremely invasive procedure that causes a lot of discomfort to the patient. Additionally, the trauma caused by this type of surgical intervention, together with other factors such as occlusal overload and presence of micro-gaps may induce bone loss around the implant [51]. In its turn, the loss of bone in the peri-implant zone is expected to lead to the implant's exposure and inherent aesthetical issues and, ultimately, induce the implant's mobility and consequent failure [52].

To place a subperiosteal implant there is no need to drill the bone but, given the dimensions and geometry of the implant, big incisions have to be performed in the gingiva and high quantity of bone needs to be exposed for the settling of the implant. Furthermore, the strategies used to reduce the fit mismatch between the implant and bone make this procedure even more invasive and literature reports high levels of discomfort to the patient.

3.4.2. Materials, Aesthetics and Bacterial Affinity

As mentioned in the results section of this critical review, subperiosteal implants are made of metallic materials such as chromium-cobalt-molybdenum and titanium alloys. Authors believe that the use of metallic materials in subperiosteal implants is mainly due to their plastic deformation nature that makes it easier to adapt the implant to the bone surface geometry. Apart from the problems that may arise from the metallic ions release and bacterial adhesion, aesthetic issues are also commonly associated with the implementation of metallic materials in the dentistry field. This is one of the reasons why ceramic materials, such as zirconia, have been emerging as great alternatives in dental applications [53]. In fact, despite titanium and its alloys being currently the most used materials in endosseous dental implants, there are already some brands producing and implementing dental implants totally made of zirconia. Apart from its biocompatibility, low bacterial affinity, high mechanical strength, and excellent wear resistance, aesthetical issues are solved due to its tooth-like color [54].

3.4.3. Cost and Customization

Another two parameters that are extremely related and worth discussion are the implant cost and the customization. Subperiosteal implants are customized to each patient's bone geometry and therefore, high costs are associated with this technique. On the other hand, since an endosseous implant is not customized to each patient, quite lower costs are involved. An optimal solution would be the one able to produce completely customized dental implants, at a relatively low cost.

3.4.4. Optimal/Improved Solution

Given the limitations and advantages of each of these two restorative treatments (subperiosteal and endosseous implants), new approaches and solutions have been developed and implemented, as, for instance, root-analogue implants [49]. The authors of the present work believe that totally customized implants and minimally invasive procedures are the future of implant dentistry. In this sense, an optimal solution would encompass characteristics of both subperiosteal and endosseous implants.

3.4.4.1. Implant Customization

Today it is possible to easily obtain complete 3D information on the patient's bone geometry with techniques such as the Cone Beam Computed Tomography (CBCT). This technique generates cone-shaped beams and the images are obtained in one rotation resulting in considerably low levels of radiation. It provides small examination time, reduces the image unsharpness caused by the translation of the patient, and, consequently, reduces the image distortion. It is already reported in the literature that CBCT provides higher levels of accuracy when compared with conventional CT measurements. Al-Ekrish, [55] evaluated the accuracy of CBCT and found mean absolute errors of 0.49 mm for the overall data. Another study conducted by Suomalainen, A. [56] found measurement errors in the range of 2.3%-4.7% for CBCT scans. The information acquired from a CBCT scan allows the technicians and dentists to virtually reconstruct the patient's bone and posteriorly design a totally customized dental implant, tailored to the specific needs of the patient [38]. This approach of completely customizing a dental implant is expected to preserve more soft and hard tissues, as well as reduce the rehabilitation time and avoid the need for a second surgical procedure [57,58].

3.4.4.2. Surgical Procedure

The fact of not having the need to drill the bone is one of the advantages of the subperiosteal implants. The authors believe that an optimal solution would be the one that makes use of this concept, thus making the procedure less invasive. In this sense, the idea would be to customize the implant accordingly to the patient's cortical bone geometry at the time of implant design/implantation. This means that the implant would be designed to perfectly accommodate in the bone socket as it is, whether the tooth has been removed recently or months/years before. In the first situation, after tooth removal, the socket would just have to be properly cleaned and the implant would be designed accordingly to the post-extraction socket geometry. In the second situation (when the tooth has been removed a long time before), since the socket would probably already be filled with new bone, the implant would be designed and manufactured accordingly to the cortical bone new geometry. In both situations the implant would be placed above the available cortical bone, thus improving the implant's stability. This optimal solution would comprise a small surgery area since a good fitting of the implant to the bone is achieved and a relatively small area is enough for good anchorage. Eventually, some micro-screws can be used to fix this new implant. Additionally, both strategies have the advantage of preserving the natural cortical bone, and

consequently reduce the bone loss and inherent aesthetical issues due to improved stress distribution in resistant bone (cortical) [49]. In Figure 3. 2 it is possible to observe a schematic representation of the two aforementioned approaches, as well as an endosseous and a traditional subperiosteal implant.

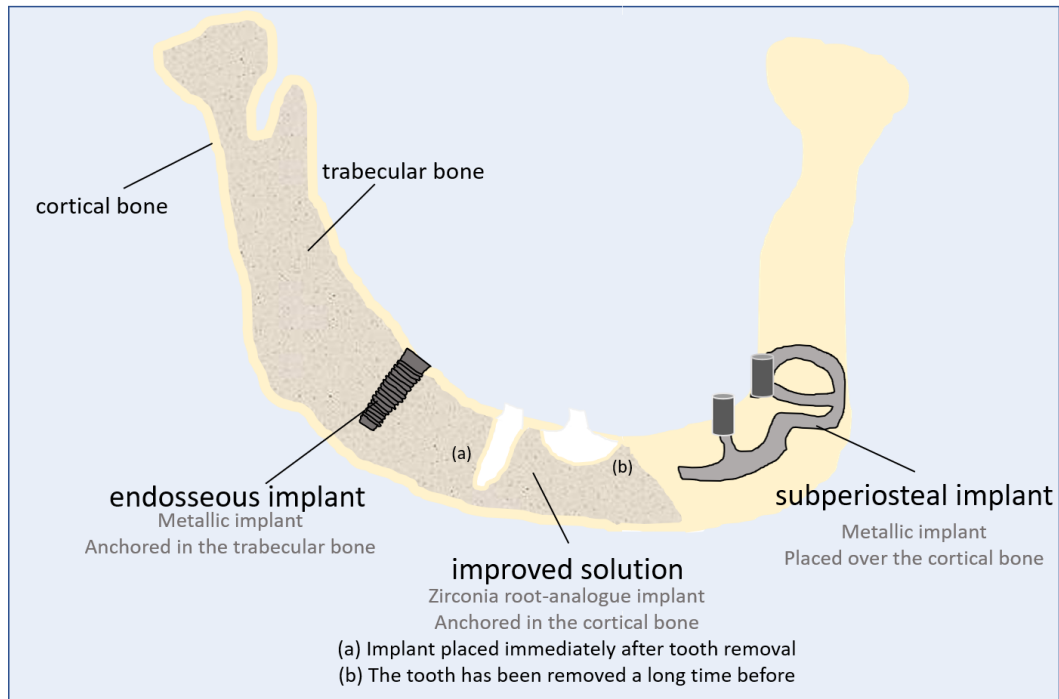


Figure 3. 2 Schematic representation of an endosseous implant, a subperiosteal implant and an improved solution.

Another advantage of placing a dental implant over the cortical bone (subperiosteal implants and optimal/improved solution) is that it will allow its functional use much more quickly than the endosseous implant because the latter is anchored on the trabecular bone and needs time for osseointegration. When the implant is anchored in the cortical bone, it can be loaded much more quickly since the implant mechanical stability will already be ensured.

3.4.4.3. Osseointegration and Anti-bacterial Strategies

Implant osseointegration is mandatory to achieve success and may be enhanced by implant-related factors such as material, geometry, and, as previously mentioned, by surface topography, surface treatments, and coatings. Other factors such as the mechanical stability and loading conditions, bone grafting, osteogenic biological coatings and, biophysical stimulation, and pharmacological agents such as simvastatin and bisphosphonates are also reported in the literature as good osseointegration promoters [59]. On the other hand, extreme implant mobility

and micromotions, inappropriate porosity, radiation therapy, certain pharmacological agents, and patient's related factors, such as osteoporosis, rheumatoid arthritis, age, smoking habits, and renal insufficiency are expected to inhibit osseointegration.

In this sense, biological phenomena such as osteoinduction, bone growth, cytocompatibility, and bacterial adhesion are extremely dependent on the interactions between the implant and the host bone [60]. Therefore, the implant's surface characteristics play a crucial role in the implant's osseointegration, and a proper implant surface is mandatory to promote osseointegration and avoid bacterial adhesion, critical factors for the implant's long-term success.

It is reported in the literature that rougher surfaces induce differentiation, growth, and attachment of bone cells, as well as increase mineralization [61]. Many techniques have been applied in order to create certain levels of roughness on the implant's surfaces. The most commonly reported in the literature are acid etching, sandblasting, plasma spraying, and the implementation of coatings, mainly hydroxyapatite coatings. The creation of micro and nano topographies has also been applied to induce surface roughness and improve the osseointegration [61].

On the other hand, smooth surfaces have been used to avoid bacterial adhesion. The accumulation of plaque around dental implants is extremely dependent on the implant's surface roughness [62,63]. Rougher surfaces are expected to induce a higher bacterial adherence due to the presence of grooves and pits since in these surface irregularities bacteria are protected from salivary flow or other biologic fluids and can more easily attach to the surfaces [64]. Given this information, the authors of the present review believe that an optimal implant solution should encompass a smooth surface in the peri-implant mucosa zone (the soft tissue that surrounds the dental implant), and a rough surface in the implant zone that is in direct contact with the bone.

Together with the implant roughness, the implant material also influences the bacterial adhesion process. Apart from the previously mentioned advantages of zirconia, this material has also been proved to have the ability to inhibit the level of bacterial adhesion, when compared to other materials used in dental applications, namely titanium [65-67].

The biofunctionalization of the implant's surface with the addition of different substances has also been reported by its positive effect on osseointegration. As mentioned in the previous section of this review, the addition of biomolecules, such as BMPs, to the implant's surface had been proved to drive multipotent cells into osteoblastic phenotype cultures. Other types of biomolecules are also referred in the literature: non-BMPs growth factors, peptides, and

extracellular matrix biomolecules [68].

Platelet-Rich Fibrin (PRF), a second-generation platelet concentrate, has also been applied in the dentistry field to accelerate tissue healing and bone regeneration [69]. It is reported in the literature that PRF improves tissue regeneration due to its effects on vascularization, capturing the circulating stem cells, immune control, and closure of the epithelium [70]. There are, in fact, some studies that indicate that PRF may increase the amount and rate of new bone formation during the early healing period and, consequently, provide faster implant osseointegration [69,71-73]. One of the main advantages of this procedure is that PRF can easily be prepared from autogenous non-anti-coagulated blood when centrifuged [71]. It is harvested from the patient's own venous blood without any additives and does not induce immune rejection [72]. Given that PRF is associated with a quite simple clinical technique and has been proved to promote faster osseointegration and consequently enhance the implant stability, the authors of this review suggest that implementing such strategy when placing a dental implant would be beneficial for the overall clinical success.

Finally, together with the PRF application, other strategies to promote the vascularization around the implant should be implemented. A rapid and efficient formation of a functional blood vasculature is mandatory for the success of wound healing and functional tissue regeneration [74]. Since the implant will be anchored in the cortical bone, poor blood supply is expected to occur.

In one study performed by Yu [75], multiple macro-channels were designed in porous β -TCP scaffolds, in a bone augmentation procedure previously to mandibular implant placement, and their effect on vascularization was assessed. Results revealed that channelled scaffolds significantly induced the new bone formation and increased the height of the mandible, indicating that the macro-channels facilitated the vascularization and bone formation. In another study [76], a review on the influence of the implementation of micro/macro channels on the vascularization was performed, and, although the size and number of channels found in the literature were different, all the studies showed that the channels played a crucial role in vascularization. The authors of this review are already evaluating and developing strategies that aim to induce an effective vascularization and consequently improve the osseointegration, by the implementation of micro-channels in the implant's surface. In fact, one study is already available in the literature [53]. The authors designed and produced zirconia surfaces with different micro-channels, and after hydrophilicity and capillarity analyses, authors concluded that the creation of inter-connected micro-channels, with 200 μm of width and 100 μm of depth, on the implant surface may be a promising solution for the promotion of the vascularization around the implants. However, the promotion of

a proper vascularization is not enough for achieving the implant osseointegration. In fact, the channels' size should be designed to allow not only the vascularization but also bone in-growth and consequent implant stability. Literature reports that scaffolds with pores with 100 μm of diameter are appropriate for cellular infiltration and consequent oxygen and nutrients supply [77]. However, for bone tissue ingrowth, pore sizes between 200 and 350 μm are preferable. In another study [78] the authors also suggest that the ideal pore size for bone in-growth range between 100 and 400 μm .

In this sense, the authors believe that an implant with micro-channels with the aforementioned dimensions (200 μm of width and 100 μm of depth) would be suitable for the promotion of both vascularization and bone in-growth. Nevertheless, further studies, namely in vitro studies, should be performed in order to corroborate these findings, since the optimal pore/channel size for bone regeneration is still not unanimous.

3.5. Conclusions

After a careful analysis on the main outcomes of subperiosteal implants and their comparison with endosseous implants, an improved and optimized solution for dental rehabilitation was purposed by the authors. This new approach should encompass characteristics of both subperiosteal and endosseous implants, overcome their limitations, and make use of the new technological advances:

- A completely customized process, tailored to each patient condition.
- Avoiding bone drilling and excessive bone exposure, as well as a single-step surgery, is required for the implementation of a minimally invasive procedure.
- A smooth surface in the peri-implant mucosa for antibacterial purposes and a rough surface in the implant zone that is in direct contact with the bone should be applied, aiming to promote the implant's osseointegration.
- The implant should be completely made in zirconia, avoiding both aesthetical issues and bacterial adherence.
- During the surgical intervention PRF should be administrated in order to promote a faster osseointegration; Proper vascularization around the implant should be achieved by the incorporation of micro-channels on its surface.

The authors truly believe that a dental implant encompassing the aforementioned features may overcome the limitations of the current strategies and be the future of implant dentistry.

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CHAPTER 4

Customized root-analogue implants: a meta-analysis review on outcomes from clinical trials and case reports

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Abstract

(1) It is estimated that 10% of the world's population will need a dental implant in their lifetime. Despite all the advances in the comprehension of dental implant designs, materials and techniques, traditional implants still have many limitations. Customized root-analogue implants are, therefore, gaining increased interest in dental rehabilitation and are expected to not only preserve more hard and soft tissues but also avoid a second surgery and improve patient overall satisfaction. In this sense, the aim of this review was to collect and analyse the clinical trials and case reports on customized root-analogue implants available in the literature; (2) This review was carried out according to the PRISMA Statement. An electronic database search was performed using five databases: PubMed, Google Scholar, Medline, Science Direct, and Scopus. The following keywords were used for gathering data: custom-made, dental implants, root-analogue, anatomical, customized and toothlike; (3) 15 articles meeting the inclusion criteria—articles reporting clinical trials, case reports or animal studies and articles with root-analogue implants and articles with totally customized implant geometries—were selected for the qualitative synthesis. The design and manufacturing techniques, implant material and surface treatments were assessed and discussed; (4) The performance of some root-analogue implants with specific features (i.e., macro-retentions) was successful, with no signs of infection, periodontitis nor bleeding during the follow-up periods.

Keywords: root-analogue implants; custom-made; CAD/CAM technology; zirconia; titanium; clinical trial

4.1. Introduction

Dental implants are an attractive option for replacing missing teeth, providing many advantages, reliability and comfort for improving quality of life [1]. There is a variety of different implants systems on the market [2,3] and some companies are already offering an implant selection system for their customers. However, the approaches aiming at implementation of completely customized dental implants are still uncommon [4].

Osseointegration has been defined as a direct and functional connection between bone and an artificial implant [5]. Traditional implants have a cylindrical or tapered geometry with threads along the screw length and over the placed abutment, followed by the crown (for a single tooth). Due to the geometry and design, they may only provide limited options for available implant

length, diameter, and thread parameters, and, therefore, cannot completely meet the personalized requirements of every patient [4,6]. This lack of proper congruency between the implant and the socket bone can eventually lead to implant failure due to stability loss and osseointegration [7,8]. To overcome this problem, novel approaches are being evaluated to manufacture customized root implants which are explicitly tailored to each patient's condition. This is expected to reduce the bone and soft-tissue trauma and promote a better primary stability, being thus a promising alternative for dental rehabilitation [6,7]. Additionally, the placement of such root-analogue implants (RAI) is a minimally invasive procedure, since they do not usually require bone drilling, sinus lifting, bone augmentation or other traumatic procedures [9].

Root-analogue implants were first described by Hodosh et al. back in 1969 [10]. A polymethacrylate implant was developed and clinically tested at that time, but outcomes were not satisfactory. Some years later, Lundgren D. et al. [11] reintroduced the topic by developing a titanium RAI and testing it in beagle dogs. Results revealed that the use of titanium instead of a polymeric material led to a success rate of 88% (28 of the 32 implants were successfully osseointegrated). Titanium remains still as “the gold standard” metallic material for dental implants. However, an increased concern with aesthetical issues has led to an increased interest in ceramic materials, namely zirconia, for such applications [12]. Apart from its toothlike colour, its high corrosion resistance, biocompatibility and high wear resistance make zirconia a promising material for dental implants [13]. This is being already deployed in dental practice with zirconia abutments [14,15].

Despite the recent developments in the design and implementation of totally customized root-analogue implants, reliable data on the long-term use of RAI in humans are still scarce. The present review aims on collection and analysis of the few clinical trials and case reports available in the literature on customized root-analogue implants. The objective is to compare their clinical performance together with additional parameters of these implants (materials used, surface treatments, design and manufacturing techniques). Also, aspects related to the surgical procedure, such as extraction of the teeth, the time between tooth extraction and implant placement, the time between placement and final reconstruction, and main biological outcomes were accessed.

4.2. Methods

4.2.1. Search Strategy

This review was carried out according to the PRISMA Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [16]. An electronic database search was performed using five databases: PubMed, Google Scholar, Medline, Science Direct, and Scopus. To conduct the search, Boolean operators such as “AND” and “OR” were used to correlate the keywords. The following keywords were explored and inserted with the field tag (Title/Abstract/Keywords): “custom-made”, “dental implants”, “root-analogue”, “anatomical”, “customized” and “tooth-like”. In addition, the references of review articles, as well as articles published in the International Journal of Oral and Maxillofacial Implants, International Journal of Oral and Maxillofacial Surgery, International Journal of Prosthodontics, Journal of Periodontology, Journal of Oral Implantology, Journal of prosthetic dentistry, and International Journal of Prosthodontics, were manually searched to include all the relevant articles available in the literature.

4.2.2. Study Selection

A screening process was conducted over the titles and abstracts retrieved by the databases search, to select the articles for full-text reading. From this screening process and after duplicates removal, 64 articles were selected for full-text reading. In order to assess their eligibility to be included in this review, the following inclusion criteria were applied: (1) articles written in English, (2) articles dated from 1990 to 2020, (3) articles reporting clinical trials, case reports or animal studies, (4) articles with root-analogue

implants, (5) articles with totally customized implant geometries. Articles not meeting these inclusion criteria were excluded from the review (articles comprising just a review were also excluded). Articles that met the inclusion criteria were entirely read and analysed considering the aim of this review.

4.2.3. Data Collection and Extraction

The information extracted from each article was divided into two main groups: the implant characterization and the aspects related to the clinical trial/case report. As far as the implant is concerned, a brief summary of the materials, design and manufacturing techniques, as well as surface modifications, were analysed. For the second, aspects related to the clinical procedure were also evaluated (the number of subjects, the number of implants per subject, the treated tooth,

the time between tooth extraction and implant placement, the time between the implant placement and final reconstruction, the follow-up periods, and the main technical or biological complications).

4.3. Results

4.3.1. Study Selection

Automatic and manual database searches resulted in a total of 348 records. After duplicate removal, 306 titles and abstracts were evaluated and a total of 64 full texts were selected to assess their eligibility to be included in this review. Of these 64 publications, 49 could not be included in the final analysis. These articles were excluded for one or more of the following reasons: (1) they did not present any clinical trial nor case data in the report; (2) the implant did not present an RAI geometry; (3) the implant was not actually customized to the specific patient; (4) they were purely review articles. The application of the exclusion criteria resulted, therefore, in 15 articles to be evaluated and analysed in detail for this work. In Figure 4. 1 the PRISMA flowchart used in this selection process is shown. Table 4. 1 lists these selected articles' titles, authors and year of publication. As can be seen, articles found in the literature and included in this review are dated from 1992 to 2018.

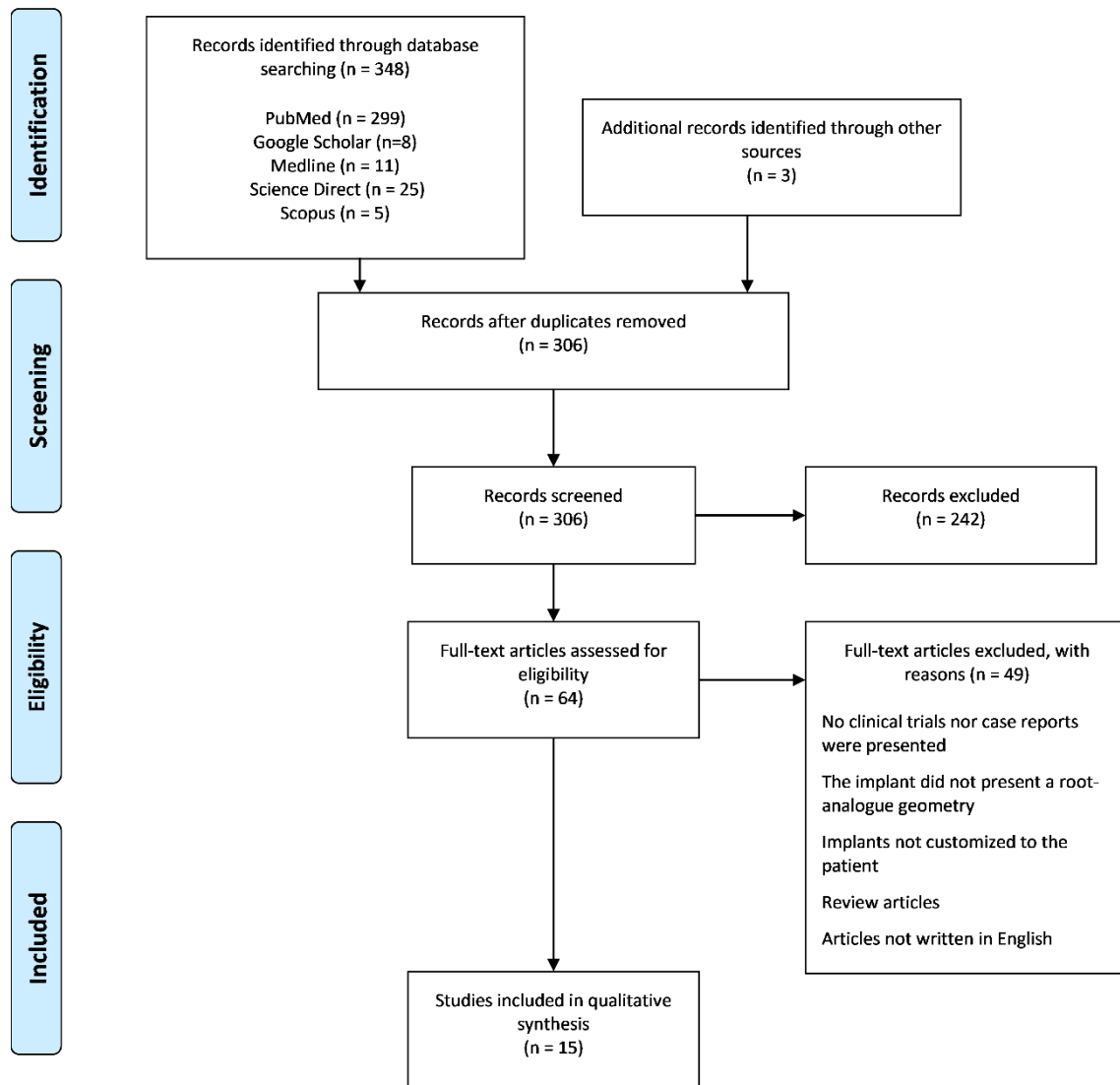


Figure 4. 1 Search strategy flowchart, adapted from [16].

Table 4. 1 Articles included in the review.

Author/Year	Title
(Lundgren, et al., 1992) [11]	Healing-in of root analogue titanium implants placed in extraction sockets. An experimental study in the beagle dog
(Kohal et al., 1997) [17]	Custom-made root analogue titanium implants placed into extraction sockets. An experimental study in monkeys
(Heydecke et al., 1999) [18]	Optimal aesthetics in single-tooth replacement with the Re-Implant system: a case report
(Pirker and Kocher 2008) [19]	Immediate, non-submerged, root-analogue zirconia implant in single tooth replacement

(Pirker and Kocher 2009a) [20]	Immediate, non-submerged, root-analogue zirconia implants placed into single-rooted extraction sockets: 2-year follow-up of a clinical study
(Pirker and Kocher 2009b) [21]	True anatomic immediate dental implant method a clinical case
(Pirker et al., 2011) [22]	Immediate, single stage, truly anatomic zirconia implant in lower molar replacement: A case report with 2.5 years follow-up
(Mangano et al., 2012) [23]	Custom-made, root-analogue direct laser metal forming implant: a case report
(Figliuzzi and Mangano 2012) [24]	A novel root analogue dental implant using CT scan and CAD/CAM: selective laser melting technology
(Mangano et al., 2014) [25]	Immediate, non-submerged, root-analogue direct laser metal sintering (DLMS) implants: a 1-year prospective study on 15 patients
(Pirker and Kocher 2015) [26]	Root analogue zirconia implants: true anatomical design for molar replacement—a case report
(Figliuzzi et al., 2016) [27]	A Direct Metal Laser Sintering (DMLS) root analogue implant placed in the anterior maxilla: case report
(Patankar et al., 2016) [28]	Immediate, non-submerged root analogue zirconia implant in single rooted tooth replacement: case report with 2 years follow-up
(Pour et al., 2017) [29]	Innovative single-tooth replacement with an individual root-analogue hybrid implant in the aesthetic zone: case report
(Moin et al., 2018) [30]	Immediate non-submerged custom root analogue implants: a prospective pilot clinical study

After a careful analysis of these fifteen articles, the authors were able to extract a lot of information to be analysed and compared. Below two main groups are shown in more detail: the data of the RAI itself (used materials, design and manufacturing techniques, surface treatments) and the parameters of the clinical trials or report cases (number of patients, number of implants, treated teeth, clinical procedures, follow-up periods, etc.).

4.3.2. Design and Manufacturing Techniques, Implant Material and Surface Treatments

The dental implants developed in the scope of these investigations present different materials and design/manufacturing techniques. Also, different surface treatments, to promote stability and osseointegration are reported. In Table 4. 2 it is possible to observe a summary of the root-analogue implants' characteristics.

Table 4. 2 Summary of the selected articles: implant material, design and manufacturing techniques and implant surface treatments.

Author, Year [Reference]	Material	Design Technique	Manufacturing Technique	Surface Treatment
Lundgren, et al., 1992 [11]	Commercial purity (CP) titanium	Copying the original tooth, with the help of a detection needle [31]	Milling	Polishing
Kohal et al., 1997 [17]	Grade II titanium	Laser scanning of the original tooth	Milling	Sandblasting with Al_2O_3 (500 μm)—intraosseous Polishing-extraosseous
Heydecke et al., 1999 [18]	Grade II titanium	A root model was created using a silicon putty material, based on the extraction socket. Its geometry was modified to perfectly fit in the alveolus and posteriorly laser scanned. The surface of the root analogue implant has a honey-comb pattern [32]33	Milling	Sandblasting with Al_2O_3 (500 μm)—intraosseous Polishing-extraosseous
Pirker and Kocher 2008 [19]	Zirconia	Laser scanning of the original tooth root. Macro-retentions were designed on the implant surface. A crown stump was designed for later connection to the crown	Milling	Sandblasting
Pirker and Kocher 2009a [20]		Laser scanning of the original tooth root (Group A) and laser scanning of the original tooth root plus macro-retentions on the surface (Group B)		
Pirker and Kocher 2009b [21]		Laser scanning of the original tooth root. Macro-retentions were designed on the implant surface. A crown stump was designed for later connection to the crown		
Pirker et al., 2011 [22]		Laser scanning of the original tooth root. Macro-retentions were designed on the implant surface. A crown stump was designed for later connection to the crown		
Mangano et al., 2012 [23]	Ti6Al4V	DICOM datasets sent to a 3D reconstruction software, following by segmentation and a 3D reconstruction of the non-restorable root. A “virtual extraction” was performed, isolating the root as an STL file sent to a reverse-engineering software where the root was processed, and the prosthetic abutment was added. The diameter of the implant neck was reduced in the area in contact with the thin buccal bone	Direct Laser Metal Forming (DLMF)	Acid etching (50% oxalic acid and 50% maleic acid)
Figliuzzi and Mangano 2012 [24]				
Mangano et al., 2014 [25]				
Pirker and Kocher 2015 [26]	Zirconia	Laser scanning of the original tooth root. Macro-retentions were designed on the implant surface. A crown stump was designed for later connection to the crown	Milling	Sandblasting
Figliuzzi et al., 2016 [27]	Ti6Al4V	DICOM datasets were sent to a 3D reconstruction software, following by segmentation and a 3D reconstruction of the non-restorable root. A “virtual extraction” was performed, isolating the root as an STL file sent to a reverse-engineering software where the root was processed, and the prosthetic abutment was added. The diameter of the implant neck was reduced in the area in contact with the thin buccal bone	Direct Metal Laser Sintering (DMLS)	No information
Patankar et al., 2016 [28]	Zirconia	After the extraction, the tooth was modified with light cured composite material to receive the crown afterward. Macro-retentions were designed on the root surface only on the	Milling	Sandblasting

mesial and distal surface with light cured flowable composite material. The modified root was laser scanned				
Pour et al., 2017 [29]	Titanium (root)	Impressions of the maxilla and a digital volume tomography (DVT) were taken. The DVT data with the impression used to determine the exact dimensions of the implant, were sent to Natural Dental Implants (NDI). Utilizing the 3D data derived from the DVT and the digitized casts, NDI designed and fabricated a patient-specific root-analogue immediate implant with a predesigned abutment. Additionally, microretentions were designed on the implant surface	Milling Materials bonded with a glass solder for sealing the interface between the implant and abutment	Radiation
	Zirconia (abutment)			Acid etching Sandblasting (on the titanium part only)
Moin et al., 2018 [30]	Grade IV titanium (root)	After patient DICOM files acquisition and STL files of stone casts and bite registrations, a 3D envelope was created for the selected tooth representing the extension of the root, alveolar bone, marginal bone level, gingival margins, adjacent and antagonist dental structures, and anatomical structures. Within this 3D envelope, CAD designs of the root analogue implant were made consisting of root/implant portion and an abutment portion	Milling The two parts were bonded with a glass solder	Sandblasting Acid-etching
	Zirconia (abutment)			(on the titanium part only)

Almost half of the implants used in these works (7 out of 15) are totally made of titanium or its alloys, namely commercial purity (CP) titanium [11], grade II titanium [17,18] and Ti6Al4V [23–25,27]. On the other hand, six of the analysed articles aimed to develop dental implants 100% made of zirconia [19–22,26,28]. Additionally, two of the publications found in the literature reported dental implants consisting of both titanium and zirconia [29,30]. These two RAI have a titanium root with a zirconia abutment on the top.

For the design of RAI two main differences were detected in techniques. Part of the studies described RAI design after the tooth removal, for example, by laser scanning of the original root [11,17–22,26,28]. In that approach [19–22,26,28] macro-retentions were designed on the implant surface, to ensure the implants' stability. Additionally, a crown stump was designed for a posterior connection to the final restoration. In the second technique, RAI was designed before tooth extraction [23–25,27,29,30], aiming to reduce the surgical interventions. This strategy comprises the acquisition of the 3D CT (Computed Tomography) images of the patient with posterior image treatment with the help of 3D software.

Milling of the pre-designed models presents the most common manufacturing method [11,17–22,26,28–30]. Four clinical studies reported manufacturing with Direct Laser Metal Sintering (DLMS), also referred to as Direct Laser Metal Forming (DLMF) [23–25,27]. For the implants consisting of two different materials (such as titanium fixture and zirconia abutment), a biocompatible glass solder was used to create a permanent bond between the implant root and

abutment [29,30].

Surface treatment techniques usually involve sandblasting [17–22,26,28–30] but some also report other complementary surface treatments to sandblasting, namely polishing and acid etching [17,18,29,30]. Three studies included exclusively acid etching on their implant surface [23–25], which correspond to the Ti6Al4V implants manufactured by DLMS, and one simply polished the implant surface [11].

In Figure 4. 2 It is possible to observe a schematic representation that summarizes the aforementioned characteristics of the selected studies, for a better understanding.

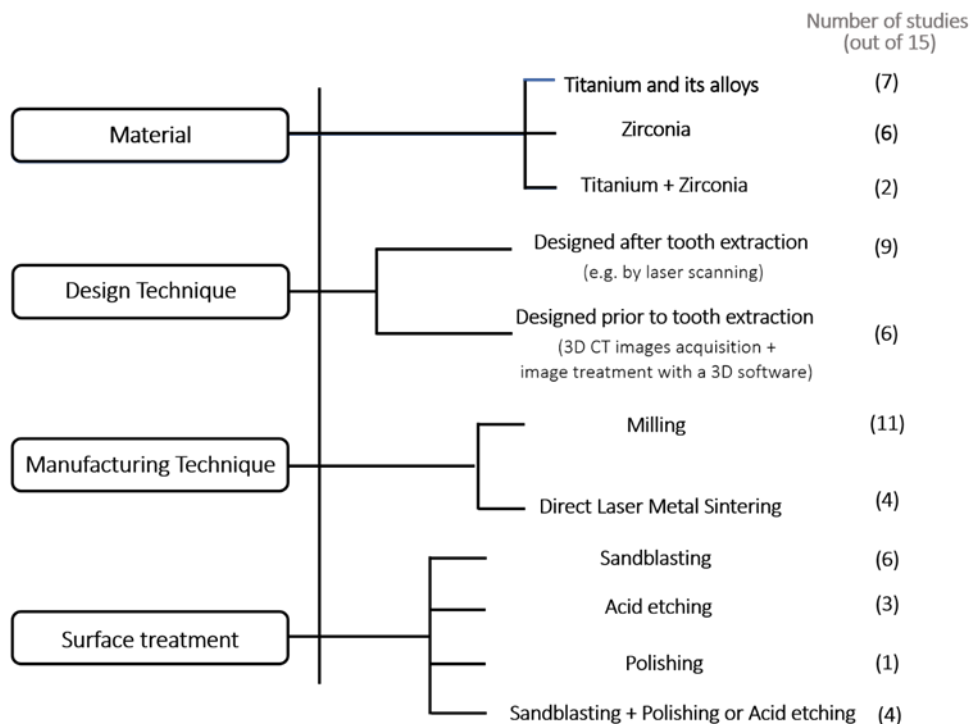


Figure 4. 2 Schematic representation of the materials, design and manufacturing techniques, and surface treatments of the selected and analysed studies.

4.3.3. Clinical Trials/Case Reports Summary

In this section of the work, the authors summarized the main parameters and procedures of the clinical trials/case reports (Table 4. 3). Among the selected articles, two-thirds (10 out of 15) correspond to a case report study, meaning that only one patient and one tooth were evaluated [18,19,21–24,26–29]. As far as the clinical trials are concerned [11,17,20,25,30], two of the articles correspond to pre-clinical trials [11,17], which means that the studies were performed in animals, namely in beagle dogs (4 subjects, with eight implants each) and monkeys (three subjects, with four implants each). The other clinical trials were performed in eighteen [20], fifteen [25], and

five subjects [30]. The treated tooth varied from article to article.

After a careful analysis of the clinical procedures carried out in these studies, it was possible to conclude that almost half of the strategies (in 7 articles) perform the implant placement immediately after tooth extraction, in the same surgical procedure [17,23–25,27,29,30]. In the pre-clinical trial performed in beagle dogs [11], four teeth were extracted on the same day of the implant placement, whereas the other twenty-eight teeth had been extracted two weeks before. In the remaining articles, implants were placed between 1–8 days after the tooth extraction. All authors mention that the implants were placed into the socket under finger pressure and subsequent gentle tapping with a hammer and a mallet. Additionally, primary stability was commonly checked by palpation and percussion. As far as the final restoration is concerned, three to four months is the most reported time to wait between the placement of the implant and the crown, being reported in ten articles [19–25,27,28,30]. The follow-up periods reported in these studies range from six months to three years, being one year the most common period of time [23–25,27,30]. In the results section of the analysed articles, it is possible to find which were the main clinical and/or biological complications that occurred during the follow-up period, as well as the overall performance of the implant and whether success was achieved or not.

Table 4. 3 Summary of the clinical trials/case reports parameters and results.

Author/Year	Type of Study	Subjects *	Implant Per Patient	Tooth	Time between Extraction and Implantation	Implant Placement Details	Time between Placement and Reconstruction	Follow-Up	Technical and/or Biological Complications; Overall Performance
(Lundgren, et al., 1992) [11]	Pre-clinical trial (beagle dogs)	4 (2–3-years-old)	8	Variable	Dog 1–3 (2 weeks) Dog 4 (0 day)	Intra-alveolar soft tissue was removed, and the bone walls were not scraped. The implants were immediately placed. The mucoperiosteal flaps were repositioned and sutured	2 months	3 years	2 implants were lost in the first week. 30 implants were retained in the socket (of these, 2 were denuded because of late mucosal perforation but remained clinically stable). The remaining 28 implants fulfilled the clinical criteria for osseointegration

(Kohal et al., 1997) [17]	Pre-clinical trial (Macaca fascicularis)	3	4	Upper central and lateral incisors	0 day	The implants were tapped into their respective socket. The mucoperiosteal flaps were repositioned and sutured.	-	6 months	Buccal bone fracture at the time of implantation. Some of the implants could not be inserted to the intended depth. Four implant exposures (6 days, 8 days, 9 days and 2.5 months). None of the implants was surrounded by soft connective tissue. None of the implants were lost and all were clinically stable at the end of the experiment.
(Heydecke et al., 1999) [18]	Case report (human)	1 (45, gender not specified)	1	Max. Sn. lateral incisor	1 day	The implant was placed into the socket using the attached insertion bar under finger pressure and subsequent tapping with a hammer and a mallet. Primary stability was checked with the handles of 2 dental mirrors. The insertion bar was removed and a custom-made healing cap was placed.	6 months	No data	Bony resorption and buccal soft tissue recession. The final result was compromised by metal shining through the thin gingiva.
(Pirker and Kocher 2008) [19]		1 (63, gender not specified)	1	1st max. Dx premolar	4 days		4 months	2 years	Stable implant. No changes on the peri-implant marginal bone level. No bleeding. No signs of periodontitis nor bone resorption.
(Pirker and Kocher 2009a) [20]	Clinical trial (human)	18 Group A (4 F, 2 M, 27–60-years-old) Group B (4 F, 8 M, 27–65-years-old)	1	Variable	Group A 1–4 days Group B 1–8 days	The implant was placed into the socket under finger pressure and subsequent gentle tapping with a hammer and a mallet. Primary stability was achieved as checked by palpation and percussion.	Group A No restoration Group B 3–13 months	2 years	Primary implant stability was achieved in all patients and no complications, such as swelling, inflammation, bleeding and pain (Group A). 5 implants were lost within 26–128 days (Group A). Implants were lost suddenly without prior pain or infection (Group A). Implant lost after 624 days. However, the lack of osseointegration was already observed on day 18 (Group B). All 11 remaining implants healed uneventfully with no complications (Group B). Soft tissue retraction ranged from 0–1.5 mm within the first year and remained stable thereafter. Many implants (58%) had no observed soft tissue retraction and maintained an aesthetic gingival architecture (Group B). There was no wound infection, no signs of periodontitis, and no implant mobility/dislocation (Group B).

(Pirker and Kocher 2009b) [21]	Case report	1 (27 M)	1	Dx. lateral maxillary incisor	7 days	3 months	15 months	Stable implant, unchanged peri-implant marginal bone level. No bleeding. Excellent aesthetic result. No signs of periodontitis nor bone or soft tissue recession	
(Pirker et al., 2011) [22]	Case report	1 (50 F)	1	1st mand. Sn. molar		4 months	2.5 years	Stable implant, unchanged peri-implant marginal bone level. Complete apical peri-implant ossification with no signs of peri-implantitis	
(Mangano et al., 2012) [23]	Case report	1 (55 M)	1	1st max. Dx. premolar		3 months	1 year	Primary stability was achieved, due to the perfect correspondence between the implant and the post-extraction socket The implant was still in function after a one-year follow-up The implant was stable, with no signs of infection, unchanged peri-implant marginal bone level and no peri-implant radiolucency The radiographic profile of the implant–crown complex was very similar to that of a natural tooth No prosthetic complications. The prosthetic restoration showed optimal functional and aesthetic integration	
(Figliuzzi and Mangano 2012) [24]	Case report	1 (50 F)	1	2nd max. Dx. premolar	0 day	The implant was placed into the socket under finger pressure and subsequent gentle tapping with a hammer and a mallet. Primary stability was achieved as checked by palpation and percussion	3 months	1 year	Implant in function after one year. The implant was stable with no signs of infection. Good conditions of the peri-implant tissues. Unchanged peri-implant marginal bone level and no peri-implant radiolucency. No prosthetic complications
(Mangano et al., 2014) [25]	Clinical trial	15 (8 M, 7 F, 39–55-years-old)	1	Premolars (8 max; 7 mand)	0 day				No implants were lost, leading to a survival rate of 100%. All implants were stable with no signs of infection. Unchanged peri-implant marginal bone level and no peri-implant radiolucency. The radiographic profile of the implant–crown complex was very similar to that of natural teeth. No prosthetic complications
(Pirker and Kocher 2015) [26]	Case report	1 (41 F)	1	Maxillary 2nd Sn. molar	6 day				7 months

(Figliuzzi et al., 2016) [27]	Case report	1 (45 M)	1	Lateral Dx. max. incisor	0 days	The implant was gently inserted in the socket using a little percussion hammer. Primary stability was achieved, as a consequence of the congruence between the implant and the socket. Then, sutures were positioned	3 months	1 year	After one year, the implant was still in function. No biological complications were reported. The peri-implant tissues were mature and stable. Little or no peri-implant bone loss, and no soft tissue recession
(Patankar et al., 2016) [28]	Case report	1 (22 F)	1	Dx. mand. 1st premolar	3 days	The implant was placed into the socket under finger pressure and subsequent gentle tapping with a hammer and a mallet. Primary stability was achieved as checked by palpation and percussion	4 months	18 months	Stable implant. Unchanged peri-implant marginal bone level and complete apical peri-implant ossification. No signs of peri-implantitis and no bleeding
(Pour et al., 2017) [29]	Case report	1 (35 F)	1	Max. Sn. central incisor	0 day	The implant was inserted and seated with cautious tapping into the socket and buccal augmentation was achieved with Bio-Oss for stabilizing the tissue architecture. The relief cut was sewn up with three single button sutures	6 months	16 months	Satisfactory aesthetics and stability of the surrounding tissues. Stability of the bone and implant functionality observed
(Moin et al., 2018) [30]	Clinical trial	5	1	Premolars	0 day	The implant was placed into the socket under finger pressure and subsequent gentle tapping with a hammer and a mallet. Primary stability was achieved as checked by palpation and percussion	3 months	1 year	In one patient, the implant showed mobility and symptoms of peri-implant infection after 4 weeks. The implant was removed at the 12 months evaluation, all remaining implants were successful. Two patients showed an absence of buccal bone around the implant. Healthy mucosal appearance in all remaining implants

* Remarks: 00A refers to age (years) and gender of patients (e.g., 45F is a 45 year old female).

After evaluating the performance of the implants of the case reports [18,19,21–24,26–29], it is possible to observe that outcomes of the different studies are however rather similar (Table 4. 3). In fact, none of the implants was lost after the follow-up period. All the authors, except Heydecke et al. reported good implant stability, no bleeding, no signs of periodontitis nor bone recession. In study [18], bony resorption and soft tissue recession led to a slight discoloration of the marginal peri-implant mucosa. Excellent aesthetic results were reported in the other case reports, mainly in the ceramic-based implants. In general, all authors mentioned a quite satisfactory implant performance during the follow-up period of the case reports. This is an interesting observation especially for the peri-implantitis appearance. It is well-known that the acid etching or electrochemical treatment of titanium aimed to generate an anatase layer is very beneficial for the prevention of biofilm formation [33,34]. However, this is not straight possible for zirconia and there have been concerns that full zirconia implants might be not so resistant to biofilm formation. This issue was recently demonstrated to be possible to solve with a thin coating of zirconia with TiO₂-forming formulations [35], but no such treatment was used in the reported cases. It looks that this matter might need an additional investigation.

For the less satisfactory clinical trials the outcomes were mainly limited due to the higher number of tested implants (Table 4. 3). In the pre-clinical trial performed in beagle dogs [11], 2 of the 32 implants were lost in the first week. Other two implants were not clinically stable after one year. The remaining 28 implants fulfilled the clinical criteria for osseointegration. In the other pre-clinical trial [17], performed in monkeys, some complications were also observed. Despite 4 of the 12 implants being exposed after a certain period of time, none of the implants was lost. However, the follow-up period of this study was too short (6 months), which may be influencing these results. In the clinical trial performed by [20] two different implants were evaluated: one designed by laser scanning of the original tooth root (Group A) and other designed also by laser scanning and with the incorporation of macro-retentions in its surface (Group B). The results of the two tested groups are very different. In Group A, five (out of six) implants were lost. The incorporation of the macro-retentions (Group B) led to more satisfactory results since only one implant was lost (out of twelve). The other eleven implants presented no signs of infection nor mobility. F. Mangano et al. [25] carried out a clinical trial in fifteen patients. After a one-year follow-up, none of the implants was lost and all of them were stable with no signs of infection. In the last analysed clinical trial [30] one (out of five) implant was lost after four weeks, due to implant mobility. The other four implants were considered successful after a one-year follow-up.

4.4. Discussion

This review has evaluated the clinical performance of customized root-analogue implants (RAI). The most differentiating aspects found in the selected literature were the type of clinical study (case reports, clinical trials or pre-clinical trials), the implant material(s) and the designing techniques.

Two materials-titanium alloys) and zirconia-were the only materials found in RAI used in these studies. The excellent mechanical properties of titanium, its biocompatibility, high corrosion resistance and low weight, are well known for this material as a solution for dental implants [36]. However, its colour together with the possible long-term corrosion and release of ions to the body environment has led to an increase in the interest in zirconia as an alternative to this metallic material [37].

Zirconia is characterized by its biocompatibility, sufficiently low bacterial affinity (yet higher than treated titanium), high mechanical flexural and compressive strengths, excellent wear resistance, and adjustable white colour, being a promising solution to overcome the aesthetic issues caused by metallic dental implants [38]. The implants' surface finishing, namely their roughness has been proved to have a huge influence on the implant osseointegration. Some published studies indicate that rough surfaces promote a faster osseointegration comparing to smooth ones [39–41], and many techniques have been applied for the creation of the desired implant roughness. The most common found in literature, and also in the clinical studies of this review are sandblasting and acid etching [42,43]. Sandblasting followed by an acid etching treatment might be considered as the gold standard surface modification in the dental implants market world [44]. On the other hand, it has been shown [34,45,46] that using simple roughness value as a single parameter is a significant oversimplification, as other factors together with porosity, hydrophilicity, nano- and macrotopology are important for implant success (at least, for metallic titanium materials).

One of the biggest differences found in the evaluated literature was the implant designing technique. Some authors opted to scan the original tooth by laser, others designed the implant before tooth extraction, using the patient radiographic (CT) images, followed by 3D image manipulation, and posterior implant milling. This approach, known as computer-aided design/computer-aided manufacturing (CAD/CAM) technology, has become increasingly popular in the dentistry field over the past years [47]. In fact, many dental offices worldwide have been

trying to implement modern IT solutions in their daily practice in order to reduce costs, work more efficiently, and increase patient satisfaction [48]. Modern CAD/CAM solutions seem to be the future of the dentistry field, namely for the customization of dental implants.

The success of an implant is known to be directly dependent on its osseointegration process. This process requires an initial interlocking between the alveolar bone and the implant (primary stability) and later, biological fixation through continuous bone remodelling toward the implant (secondary stability) [49]. There are key factors that influence implant stability: surface roughness (as previously mentioned), the congruity between the implant and bone, the period of time between tooth extraction and implant placement, bacterial adhesion, among others [50]. The majority of the studies that are being analysed reported ideal primary stability, where a perfect correspondence between the implant and the post-extraction socket was observed. However, in one study [17] some implants could not be inserted to the intended depth leading to implant exposures. Nevertheless, none of those implants was lost.

For the latency time (between tooth extraction and implant placement), a healing period of 6–9 months was previously recommended (a late implant placement). Later, insertion of implants after 2–3 months was suggested (a delayed implant placement), and more recently, immediate implantation has been clinically tested too [7]. Despite new interest in immediate implant insertion, the literature reports it encompassing two main problems: (a) maintaining the implant primary stability and (b) preventing soft tissue ingrowth during the healing period [7]. Additionally, it is might to higher infection risks, flap dehiscence over the extraction site, and incongruity between the socket wall and the implant [3,14,33]. The articles evaluated in the scope of this review performed the implant placement immediately after tooth extraction or a few days later. However, and contrarily to what was reported in the literature, it seems that this strategy did not trigger any specific side effects nor biological complications. In fact, primary stability was achieved in most of the tested implants.

Another aspect that is worth discussing is the incorporation of macro-retentions on the implants' surface. Some authors mention the incorporation of these protrusions on the implant surface aiming to promote an improved attachment to the bone and consequently improved mechanical stability. Pirker et al. [20] have even compared the performance of RAI with and without macro-retentions, and results were clearly conclusive: RAI without the macro-retentions ($n = 5$) were suddenly lost, without prior pain or infection, in the first 128 days. On the other hand, among the RAI with macro-retentions ($n = 12$), only one was lost. These results were later corroborated in

one study performed by Moin D. et al. [51] that analysed, by means of FEA (Finite Element Analysis), the influence of 5 custom root-analogue implant designs on the stress distribution of peri-implant bone.

The results of this study revealed that the addition of macro-retentions to an RAI standard design would have a positive effect on the stress distribution, reduce the concentration of bone stress, and provide a better primary stability. Despite being a promising alternative, the authors believe that the macro-retentions alone may not be enough to ensure implant mechanical stability. Since the RAI geometry is characterized by its conical shape, there is a risk that the implants may tend to be expelled and hence other strategies to avoid this risk should be further explored and developed. It is also notable, that FEA analysis conventionally used in dentistry usually suffers from an oversimplification of tissue properties, which are not well known (especially for soft tissues), and where anisotropy is seldom considered [52,53]. The advantage of the linear elastic models is of course in the provision of simple and direct prediction of the tissues properties for the sake of the computational efficiency but the usefulness of such data is very questionable (e.g., “elastic modulus of mucosa” ranging from 0.1 to 680 MPa [54]). FEA outcomes should be considered as a complement to the clinical studies, aiming to better understand the influence of some variables on the implants’ clinical performance.

Together with the aforementioned mechanical features, there are also some mechanobiological aspects expected to improve the implants’ osseointegration. Some reported techniques are being developed and evaluated aiming to promote the infiltration and supply of nutrients and fluids around dental implants, consequently inducing vascularization at the implant’s surface [55]. However, in this review not much information regarding these biological stimuli has been found: only one article referred that small perforations were created in the palatal tissue of the socket to stimulate bleeding [29]. Other techniques, such as the creation of hydrophilic surfaces or the incorporation of micro-channels on the implant’s surface would also have a positive impact on the implant’s vascularization [56], and eventually needed to be further explored to reveal their potential clinical benefits.

Despite the satisfactory clinical results observed in the selected articles, none of these solutions is widely available on the market—the manufacturing companies and dentists tend to prefer standard products with lower associated costs; most of the dental clinics do not have the necessary equipment (CBCT) for the design of such customized solution; despite its weaknesses, conventional dental implants have been reported with success rates of 90–95% for 10 years follow

up periods [57] based on current definitions of success, which are questionable in the opinion of the authors of this paper.. Authors believe that these factors may be hindering the worldwide practice of such dental treatment and studies should proceed, focusing on the implementation of RAI in the global dental market.

The main findings of this review show that:

- Titanium and zirconia are the selected materials for the manufacturing of RAI.
- CAD/CAM technology followed by surface treatments such as sandblasting has been the preferred manufacturing technique for such applications.
- The clinical outcomes of the analysed studies suggest that further investigations should be performed aiming to evaluate whether RAI may be considered a promising solution for the replacement of missing teeth or not.

The limitations of this study rely, mainly, on the reduced number of articles meeting the inclusion criteria.

4.5. Conclusions

This review includes 15 identified clinical studies and cases with RAI. These studies have a high heterogeneity of follow-up periods, sites, techniques and thus it is difficult to compare results and draw definitive conclusions about validated RAI outcomes. Nevertheless, some general considerations and trends in RAI application can be made:

- Clinically tested RAI are made of titanium and/or zirconia; no other materials were reported for the analysed period.
- CAD/CAM is an effective technology to design and manufacture RAI and it is being implemented in the dental practice.
- Sandblasting and/or acid etching on the implant surface seems to be effective in promoting the implant osseointegration.
- The addition of macro-retentions on the implant surface induces a positive effect on the stress distribution in the bone surrounding the implant. However, this strategy alone may not be enough to promote the implant mechanical stability, due to its conical geometry.

- Immediate implant placement may be considered successful in RAI, unless there are no clinical contraindications.
- The performance of some RAI with specific features on its surface, namely the incorporation of macro-retentions, was proved to be successful, with no signs of infection, periodontitis nor bleeding during the follow-up periods.

Given the results of the evaluated clinical studies, customized root-analogue implants (cRAI) may be the future of dental rehabilitation. However, and as expected, the literature on the clinical performance of these implants is still scarce, and further well-designed clinical studies, namely long-term randomized controlled trials, are required to corroborate these findings.

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CHAPTER 5

In silico evaluation of the stress fields on the cortical bone surrounding dental implants: comparing root-analogue and screwed implants

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Abstract

Tooth loss is a problem that affects both old and young people. It may be caused by several conditions, such as poor oral hygiene, lifestyle choices or even diseases like periodontal disease, tooth grinding or diabetes. Nowadays, replacing a missing tooth by an implant is a very common process. However, many limitations regarding the actual strategies can be enumerated. Conventional screwed implants tend to induce high levels of stress in the peri-implant bone area, leading to bone loss, bacterial bio-film formation, and subsequent implant failure. In this sense, root-analogue dental implants are becoming promising solutions for immediate implantation due to their minimally invasive nature, improved bone stress distribution and because they do not require bone drilling, sinus lift, bone augmentation nor other traumatic procedures. The aim of this study was to analyse and compare, by means of FEA, the stress fields of peri-implant bone around root-analogue and screwed conventional zirconia implants. For that purpose, one root-analogue implant, one root-analogue implant with flaps, two conventional implants (with different threads) and a replica of a natural tooth were modelled. COMSOL was used to perform the analysis and implants were subjected to two simultaneous loads: 100 N axially and 100 N oblique (45°). *Results:* revealed that root-analogue implants, namely with flaps, should be considered as promising alternatives for dental implant solutions since they promote a better stress distribution in the cortical bone when compared with conventional implants.

Keywords: Peri-implant bone loss; Root-analogue implants; Finite Element Analysis; Minimally invasive approach; Zirconia; Immediate load

5.1. Introduction

Nowadays, there are several implant options for edentulous people. However, and despite being a widespread treatment, the success of a dental implant depends on many factors and may be compromised as a result of postplacement complications. The loss of the supporting bone, and consequent loss of stability, is, probably, the main reason why dental implants fail (John et al., 2016). Surgical trauma, occlusal overload, presence of micro gaps, peri-implantitis, allergic reactions, foreign body rejection, the material and design of the implant or even poor oral hygiene may lead to this bone loss (Levin, 2008; Macedo et al., 2017). The presence and maintenance of surrounding bone is what dictates whether an implant succeeds or not (Dantas et al., 2019). In this sense, one of the biggest challenges in implantology nowadays is the process of bone

resorption around the implant immediately after insertion and during its use. There are many theories regarding the relation between bone adaptation and strain rate and magnitude. The Frost mechanostat theory (Frost, 1987; Mahnama et al., 2013) classifies bone behaviour on the basis of the mechanical strain. This theory describes four micro-strain zones, that correspond to certain strain intervals, and their relationship with the bone mechanical adaptation (Vidyasagar and Apse, 2003). The extreme zones-disuse atrophy and pathologic overload - are expected to lead to bone loss. Disuse atrophy occurs for strain magnitudes below 50–200 μ -strain, whereas pathologic overload tends to occur when the magnitude strain values overcome 4000 μ -strain. Between these two zones, the frost mechanostat theory describes two other μ -strain zones: the steady state zone (100–2000 μ -strain) that is thought to enable a favourable bone reaction and the physiologic overload zone (2000–4000 μ -strain) that is expected to lead to an increased bone mass (Vidyasagar and Apse, 2003). In the past, it was believed that before implant insertion, healing periods of 6–9 months were required (late implant placement). A few years later, 2–3 months was proposed as the most appropriate time to wait between tooth removal and implantation (Regish et al., 2011). Lately, immediate implant insertion after tooth extraction has emerged as a great alternative, due to a reduction in the treatment time and surgical interventions, consequently leading to a reduction of costs and a quality of life improvement (Pirker and Kocher, 2008; Regish et al., 2011). Additionally, immediate implant insertion is expected to avoid alveolar bone resorption and soft-tissue regression as a consequence of early load (Pirker and Kocher, 2008). Standard implants have a cylindrical or tapered geometry with threads along their length and their main limitation is that they only provide limited options for implant length, diameter and thread parameters. Consequently, they cannot completely meet the requirements of every patient (Chen et al., 2014, 2012; Didier et al., 2017). On the other hand, the incongruence between the socket wall and the implant shape leads to the need of using barrier membranes or bone augmentation to avoid the formation of connective tissue between the implant and socket (He et al., 2016; Rodrigues et al., 2017). In this sense, customizing immediate load dental implants tailored to each patient condition would, not only preserve more hard and soft tissues, but also reduce rehabilitation time and avoid a second surgical intervention (Chen et al., 2014; He et al., 2016). Customized root-analogue implants were first introduced by Hodosh et al. (1969). This novel approach is becoming a promising solution for immediate single stage replacement of teeth and is acquiring a special interest due to its minimally invasive nature, and also because it does not require bone drilling, sinus lift, bone augmentation nor other traumatic procedures (Pirker and Kocher, 2011).

Additionally, it respects the natural anatomy of the socket, preserving it and is expected to promote an excellent primary stability as well as osseointegration (Rodrigues et al., 2017). Root-analogue titanium implants were developed by Lundgren (Prithviraj et al., 2011) and proved to be effective and to promote osseointegration in the first-month follow up period. However, studies indicate that after 9 months of the insertion, failure rates of approximately 48% were observed, which is not acceptable for clinical purposes (Kohal et al., 2002). Pirker et al. (Pirker and Kocher, 2011, 2008), developed a root analogue implant completely made of zirconia, due to its good mechanical properties, biocompatibility and aesthetical advantages (Inokoshi et al., 2014; Teng et al., 2001). Other features were implemented in this implant: micro and macro retentions, reduced diameter of the implant next to the cortical bone and a single-stage implantation process. Authors found that this new strategy leads to better results when comparing to root-analogue titanium implants with a uniform surface (Pirker and Kocher, 2008). Finite Element Analysis (FEA) has been applied as an effective method to investigate the biomechanical behaviour of dental implants, namely the bone stress/strain around implants (dos Santos Marsico et al., 2017; van Staden et al., 2006). Studies regarding the biomechanical response of standard dental implants are abundant, however, when it comes to root-analogue dental implants studies are scarce. Moin et al. (2016), performed a FEA analysis on five different root-analogue implants and evaluated the stress distribution of peri-implant bone. The five press-fit designs with a standard identical abutment, had some different macro-retentions on their surface and were compared to a control implant (without any features). Results revealed that the addition of targeted press-fit geometry to the standard surface results in a positive response on the stress distribution and lower bone stresses, providing a better primary stability. Chen et al. (Chen et al., 2017), evaluated the stress distribution and micro-motion (for both immediate and delayed loading protocols) of two custom-made dental implant designs: one with the geometry of the natural root and another with a root-analogue design accompanied by a rotational symmetric body with a threaded characteristic. These approaches were compared to standard cylindrical dental implants. Results of this study suggest that the use of immediate root-analogue implants with a threaded characteristic, lead to smaller micro-motion in the bone-implant interface and a favourable stress distribution was observed. Given what was mentioned before, the main goal of this research was to evaluate the stress fields in the peri-implant bone area of four different implant designs: a conventional Semi-Threaded Implant (STI), a conventional Threaded Implant (TI) a Root-Analogue Implant (RAI) and a Root-Analogue Implant with Flaps (RAIF). Additionally, a replication of a Natural Tooth (NT), surrounding bone and periodontal ligament (PDL)

geometry were modelled, to act as reference. FEA was performed in all models and zirconia was used as the implants' base material.

5.2. Materials and Methods

5.2.1. 3D Modelling

All five models addressed in the scope of this research work were designed in a CAD software (SolidWorks, 2018, Dassault Systems SolidWorks Corp., Concord, MA, USA). For simplification, all implants were modelled as a single body structure, with a simplified geometry (perfectly rounded), and placed in a bone model composed by trabecular bone and 2 mm of cortical bone. As seen in Figure 5. 1(a), in the STI model the thread is at the subcrestal level, whereas in the TI (Figure 5. 1(b)) the thread extends to the cortical bone. On the other hand, in the RAI, RAIF and NT models (Figure 5. 1(c), (d) and (e), respectively), a thin layer (1 mm) of cortical bone was designed (lamina dura), mimicking the tooth socket (Huynh-Ba et al., 2010), once, in these three strategies, this thin layer is expected to be preserved. The flaps observed in the RAIF model were designed to promote a better mechanical stability, as well as to improve the stress distribution along the implant. Additionally, in the NT geometry (Figure 5. 1(e)) it is possible to observe the periodontal ligament between the cortical bone and tooth.

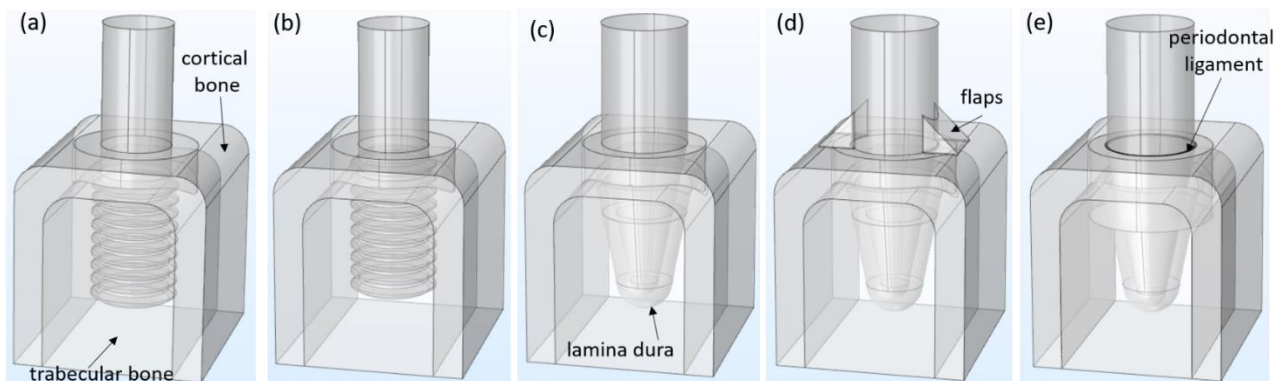


Figure 5. 1 Schematic representation of the studied models: (a) STI - Semi-Threaded Implant; (b) TI - Threaded Implant; (c) RAI - Root-Analogue Implant; (d) RAIF - Root-Analogue Implant with Flaps; and (e) NT - Natural Tooth.

5.2.2. Finite Element Modelling

The five models described above were then imported into COMSOL Multiphysics 5.3, COMSOL Inc. Sweden, to generate the FE models. The mechanical properties of the materials were provided to the software based on previous studies and literature (Table 5. 1). All materials were

considered isotropic and homogeneous. Additionally, the PDL was considered as a non-linear elastic material.

Table 5. 1 Material properties in FEA.

	Poisson's ratio	Young's modulus (GPa)
Cortical bone	0.3 (van Staden et al., 2006)	13.7 (van Staden et al., 2006)
Trabecular bone	0.3 (Zhang et al., 2016)	0.7 (Dorogoy et al., 2017)
Zirconia	0.3 (Fuh et al., 2013)	210 (Fuh et al., 2013)
Dentin	0.31 (van Staden et al., 2006)	18.6 (van Staden et al., 2006)
PDL	0.45 (Yan et al., 2012)	-

To simplify the simulation, the interfaces between zirconia and trabecular or cortical bone, cortical bone and trabecular bone, dentin and PDL, and between the PDL and cortical bone were treated as perfectly bonded connections. The number of nodes and tetrahedrons of each FE mesh can be found in Table 5. 2. Average element qualities are also described, according to the skewness parameter (in which values closer to 1 represent higher element quality).

Table 5. 2 Average element quality, and number of nodes and tetrahedrons of the mesh of the different models.

	Tetrahedral elements	Nodes	Average element quality
STI	1368886	229928	0.6619
TI	1479941	248406	0.6616
RAI	141095	24309	0.6562
RAIF	180003	31169	0.6541
NT	339593	61003	0.6586

Once the problem was defined, two external loads of 100 N were applied, at both axial and oblique directions. Oblique loading was performed from the buccal side at 45° relatively to the implant/tooth axis (Figure 5. 2(a)). A fixed constraint was implemented in the surfaces of the cutting section and the bottom of both the trabecular and cortical bone. The fixed constraint was applied to all the degrees of freedom of the nodes belonging to the highlighted surfaces (Figure 5. 2(b)). Mesh convergence was achieved, and its analysis was performed in function of the implant/tooth displacement.

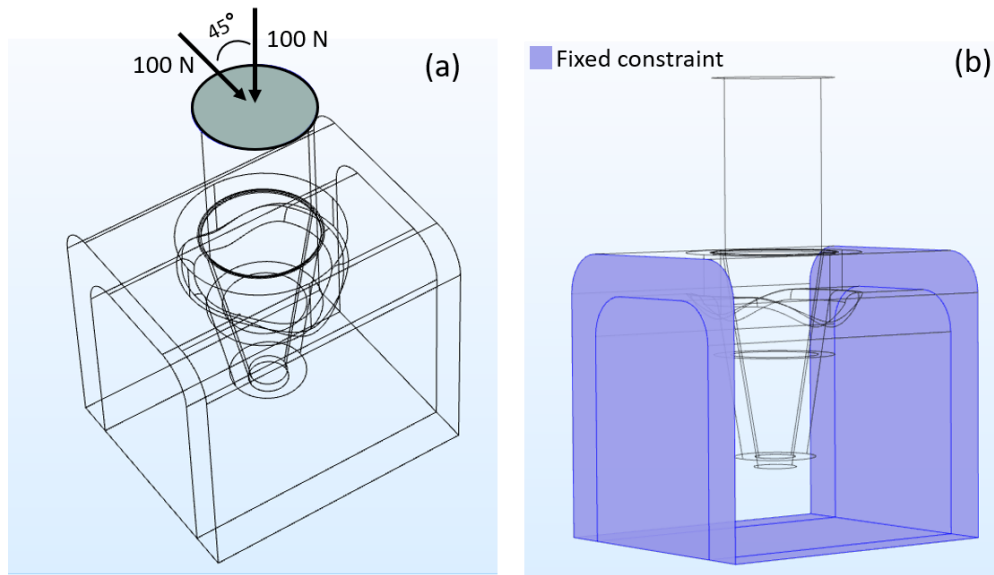


Figure 5. 2 Boundary conditions: (a) Applied loads; (b) Fixed constraint.

5.2.3. Results Analysis

The Von Mises equivalent stresses, the hydrostatic pressures and the octahedral shear strain values in the cortical bone caused by the loading were analysed. To better evaluate the response of the peri-implant bone, authors limited the results analysis to a circular section (hereafter referred as partition) of cortical bone around the implant/tooth with 8.6 mm of diameter and 5 mm height, as seen in Figure 5. 3.

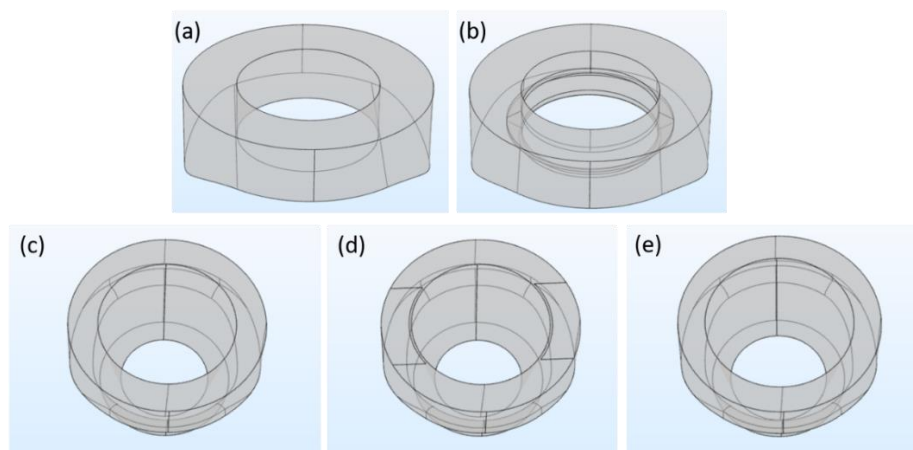


Figure 5. 3 Analysed cortical bone volume (partition) for the different models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.

Since this region is considered the most relevant in terms of loss of the implant stability due to bone resorption, it is crucial to understand and predict whether it is being correctly loaded or not. To determine the hydrostatic pressures in the partition, equation (1) was used:

$$p = -\sigma_m = -\left(\frac{\sigma_{xx} + \sigma_{yy} + \sigma_{zz}}{3}\right), \quad (\text{Eq.1})$$

where, σ_{xx} , σ_{yy} , σ_{zz} are the normal stress tensors.

In order to compare results with literature (Frost mechanostat theory), equation (2) was used to determine the octahedral shear strain values. Implant and tooth displacements were also evaluated and compared.

$$\gamma_{oct} = \frac{2}{3} \sqrt{(\varepsilon_1 - \varepsilon_2)^2 + (\varepsilon_2 - \varepsilon_3)^2 + (\varepsilon_3 - \varepsilon_1)^2}, \quad (\text{Eq.2})$$

where, ε_1 , ε_2 and ε_3 are the principal strains.

5.3. Results and Discussion

5.3.1. Stress Distribution and Hydrostatic Pressure in the Peri-implant Cortical Bone

Figure 5. 4 displays the numerically determined stress fields in terms of the equivalent von Mises stresses at the cortical bone, for the five models. As can be seen, the higher values are observed in the peri-implant bone for all models, except for the NT. In this specific geometry, it is possible to observe that the stresses are better distributed, due to the colour gradient along the tooth socket. Also, the maximum value observed in the NT model (18 MPa) is quite lower than the ones observed for the other four geometries. The STI and TI models were the ones with the highest maximum stresses, around 70 MPa and 60 MPa, for the semi-threaded and threaded implant, respectively. Additionally, the incorporation of flaps in the root-analogue implant induced a decrease in the von Mises stresses in the peri-implant bone, transferring them to the bone adjacent to the flaps. Since this graphical analysis might be misleading and since these maximum values may only affect a neglectable volume of bone and might not be representative of the significant peri-implant stress states, results from volume-based analysis of peri-implant bone on von Mises stress and hydrostatic pressure are shown posteriorly. In this analysis, authors determined which quantity of the model volume was subjected to a certain von Mises or hydrostatic pressure value, thus, neglecting residual volume results.

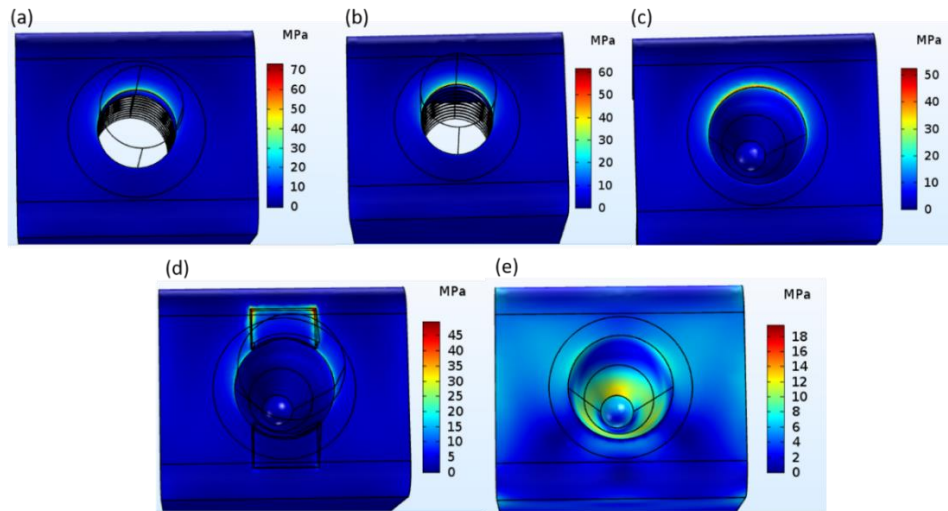


Figure 5. 4 Von Mises stress distribution in the cortical bone for the studied models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.

In Figure 5. 5 it is possible to observe the von Mises stress distribution (on pre-defined von Mises intervals) in the studied circular partition, as a function of the volume fraction. Analysing the two threaded implants, despite having a similar behaviour, results showed that placing an implant at the crest level (semi-threaded implant) increases the maximum stresses in the peri-implant bone. In fact, some similar studies have already suggested that placing an implant in the trabecular bone would negatively affect the stress distribution along the bone (León et al., 2014; Rismanchian et al., 2013). On the other hand, placing a threaded implant in the cortical bone has already been proved to have some advantages regarding the implant stability (De Castro et al., 2014). Comparing the threaded implants with the root-analogue ones, it is possible to observe that the last ones present a better stress distribution once, for the same von Mises stresses, higher volume fractions are observed. Additionally, the threaded implants tend to induce some hot spots, evidenced by the high values of von Mises for very small volume fractions.

The addition of flaps in the root-analogue model reduced the maximum von Mises stresses values and, as can graphically be seen, it was the geometry that presented the most similar behaviour to the natural tooth. The natural tooth model, in turn, was the one that presented the best stress distribution, as should be expected. In a natural tooth, the PDL absorbs the loads placed on the tooth during mastication and distributes them to the surrounding bone (Chu et al., 2013), leading to a more uniform stress distribution.

A further refinement in the analysis reveals that the volume percentages with von Mises stresses higher than 10 MPa were quite small, for all models: 7.2% for the STI model, 7.5% for the TI, 2.9% for the root-analogue implant, 0.2% for the RAIF and the natural tooth presented 0.7% of

the studied volume with stresses higher than 10 MPa. The maximum values of the von Mises stresses are not reported in this analysis once, as previously stated, they are associated with vestigial volume fractions and might result from the geometrical process of the model construction and highly local effect of the FE mesh, without any physical meaning.

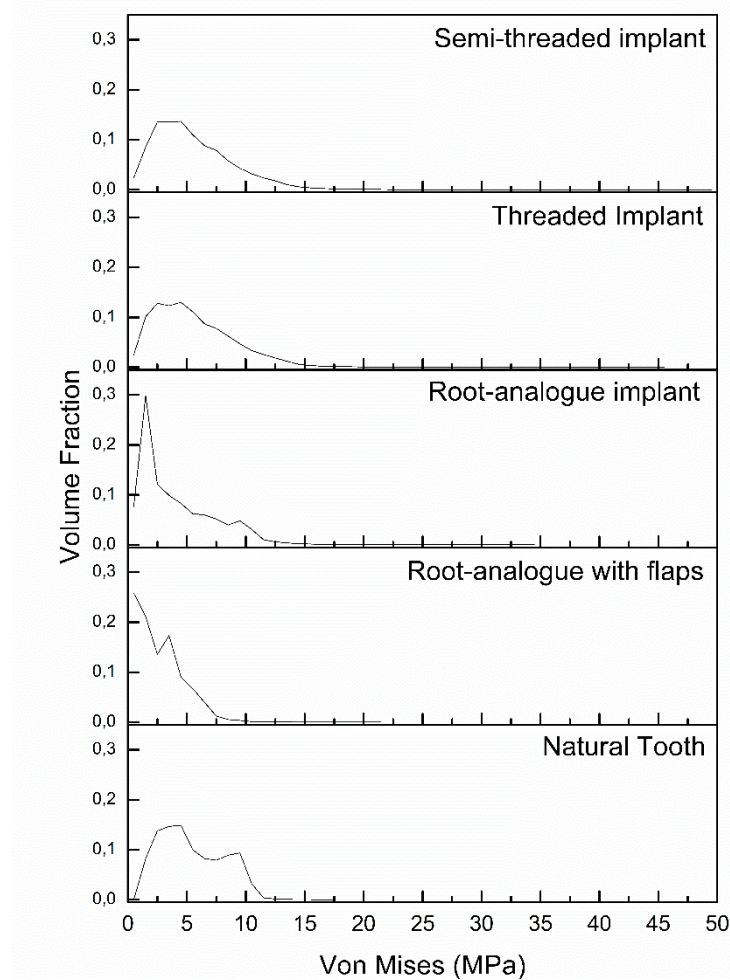


Figure 5. 5 Von Mises stress distribution in the circular partition, as a function of the volume, for the different models.

The hydrostatic pressure in the partition volume was also analysed in the scope of this study, once, with this evaluation, it is possible to understand which volume fractions of the partition were subjected to tensile or compressive mean stresses, through the state of tension at each gauss point.

It is possible to observe, in Figure 5. 6, that the hydrostatic pressure values in the threaded implants reached higher compressive values, associated with quite small volume fractions, which indicates that these models tend to induce hydrostatic pressure hot spots and consequent stress

concentration. On the other hand, the root-analogue (with and without flaps) and the natural tooth models exhibited higher volume fractions for hydrostatic values around 0 MPa. Regarding the volume fraction subjected to traction (negative values of hydrostatic pressure), the percentages were as follows: 52.95% for the STI model, 52.52% for the TI, 69.42% for the RAI, 67.05% for the RAIF and 69.98% for the NT model. These values indicate that the threaded implants were equally subjected to tensile and compressive stresses whereas the other three models were mainly under tensile mean stresses. Literature states that the periodontal ligament cells are repeatedly stretched and relaxed by tensile mechanical forces under normal conditions *in vivo* (Tsuji et al., 2004), which suggests that the PDL in the NT model used in the FE analysis was successfully designed. Additionally, and in agreement with the von Mises results, the incorporation of flaps on the root-analogue implant led to a better stress distribution and may be considered as a promising approach for dental implants design. Also, the fact that the root-analogue implants presented higher bone volumes under tensile stresses, when compared with threaded implants, make them great candidates for such applications once tensile stresses are expected to induce bone formation (IKEGAME et al., 2016).

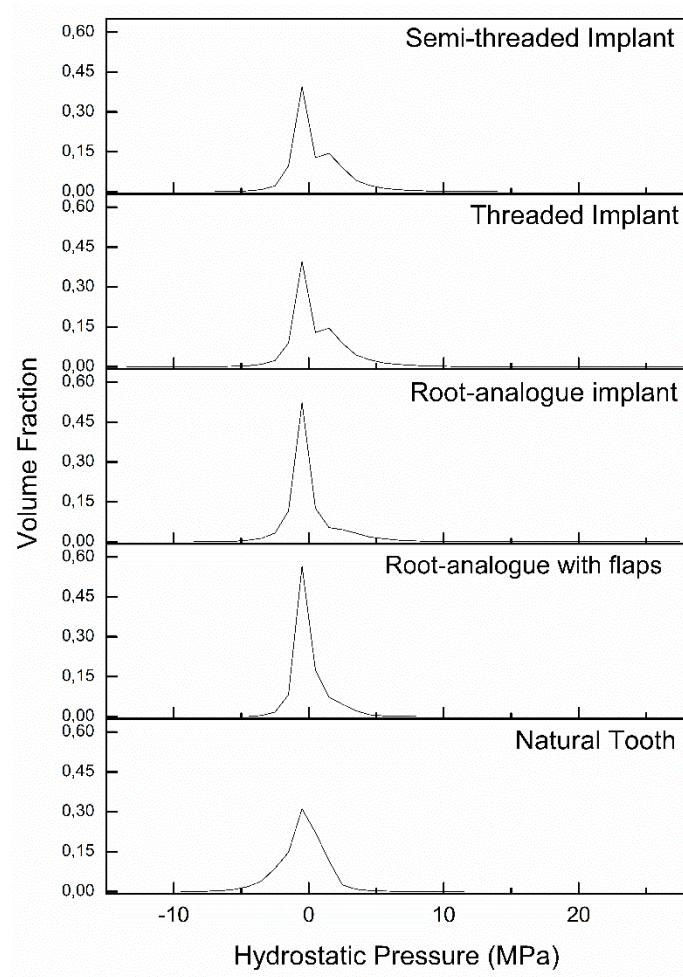


Figure 5. 6 Hydrostatic pressure in the circular partition, as a function of the volume, for the studied models.

5.3.2. Octahedral Shear Strain in the Peri-implant Cortical Bone

The octahedral shear strain in the partition was analysed, in order to understand if bone around implant was being correctly stimulated, in order to avoid bone loss and promote its remodelling around implant, since mechanical strain is known to be one of the stimuli for alveolar bone remodelling. Table 5. 3 displays the average μ -strain values obtained in this study. The five models presented comparable mean values of shear strain and similar results were obtained in other studies found in literature (de Abreu et al., 2013; De Vasconcellos et al., 2013).

Table 5. 3 Average μ -strain values for the five models.

Model	Average μ -strain
STI	643.3
TI	513.5
RAI	367.5
RAIF	221.6
NT	509.5

The frost mechanostat theory was used to complement this evaluation. In this sense, the values obtained in the FE analysis were divided in the four μ -strain zones: disuse atrophy, steady state, physiologic overload and pathologic overload. Results can be seen in Figure 5. 7.

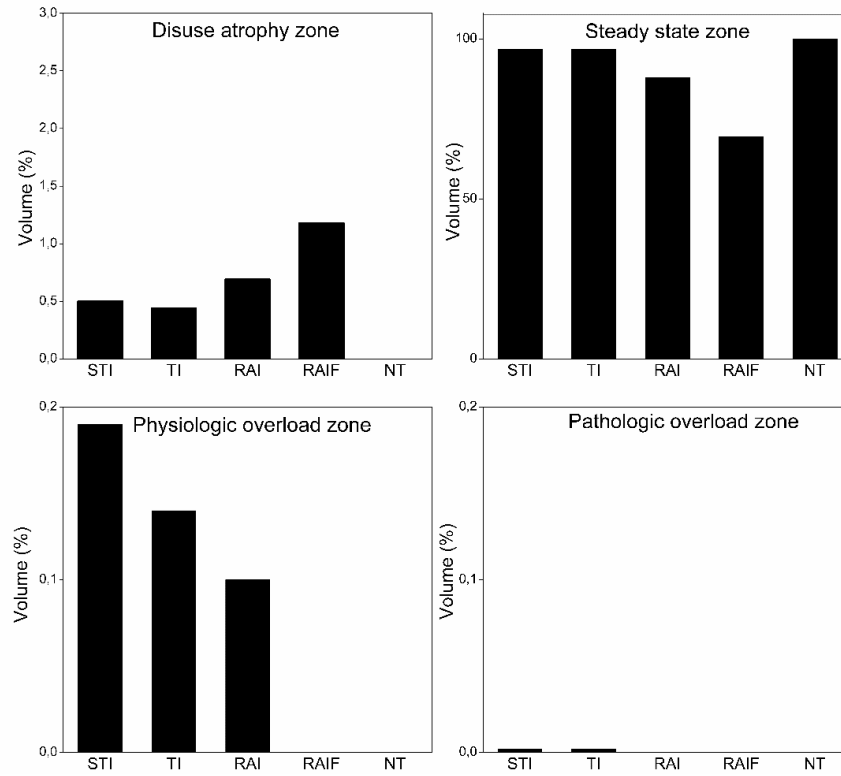


Figure 5. 7 Percentage of the circular partition volume in the four μ -strain zones: Disuse atrophy ($< 50 \mu$ -strain); Steady state (100 - 2000 μ -strain); Physiologic overload (2000 - 4000 μ -strain); and Pathologic overload ($> 4000 \mu$ -strain).

Disuse atrophy values are related with shear strains below 50 μ -strain. The steady state zone was defined for values between 100 and 2000 μ -strain, whereas the physiologic overload zone corresponds to strains between 2000 and 4000 μ -strain. Finally, pathologic overload was considered for values above 4000 μ -strain. As can easily be seen, the five tested models were mainly subjected to shear strain values in the steady state zone: approximately 100% of the volume of the STI, TI, RAI and NT models were subjected to μ -strain values between 100 and 2000, whereas the RAI model only presented approximately 86% of its volume in the steady state μ -strain zone.

Regarding the disuse atrophy zone, as expected, the natural tooth was not subjected to strain values below 50 μ -strain (van Ruijven et al., 2007). The RAI model was the one that presented the highest volume percentage in the aforementioned strain range, which may indicate that the flaps are hindering bone to be enough stimulated. However, it is important to mention that

all percentages were below 1.5%, meaning no significant differences were found for the five models.

The threaded implants were those that presented the highest volume percentage in the physiologic overload zone, the zone that is expected to lead to an increased bone mass. Despite the graphical differences displayed in the figure, percentage values were below 0.2%, which indicates, again, that no significant differences were observed for the five models, in the referred zone.

Finally, results revealed that only the threaded implants reached strain values above 4000 μ -strain. The volume percentages associated with these results are quite low, which may indicate that these are not representative values.

Despite no huge differences were found between the five models in the scope of this analysis, it allowed authors to conclude that the finite element analysis was correctly designed and performed, once real values were obtained and bone-deformation values are within physiological parameters (Santiago et al., 2016).

5.3.3. Implant Displacement

Total implant/tooth displacements were also evaluated in the scope of this research work. In Figure 5. 8 it is possible to observe these results, for the five models. Both threaded implants presented a maximum of 0.0134 mm of displacement. The root-analogue implant showed 0.0107 mm of displacement whereas the one with flaps exhibited a displacement of only 0.008 mm. The natural tooth, in turn, reached the highest displacement value, 0.97 mm.

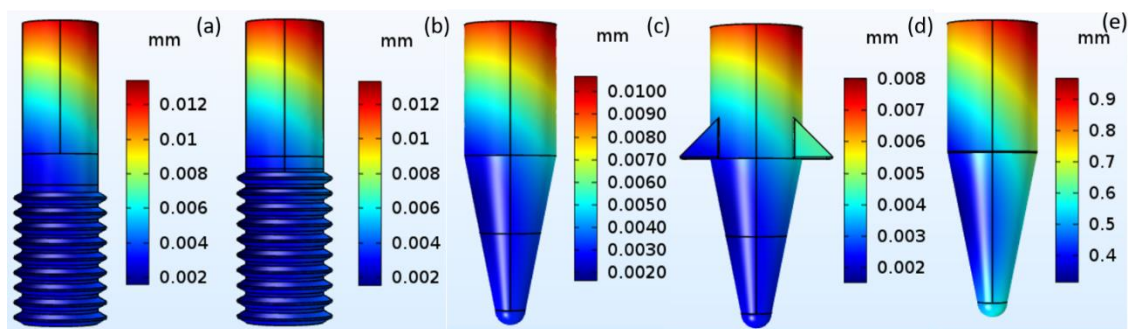


Figure 5. 8 Implant/Tooth total displacement for the studied models: (a) STI; (b) TI; (c) RAI; (d) RAIF; and (e) NT.

The values obtained in this results section are in accordance with the expectations. First, the two threaded implants presented the same displacement, which was expected due to the similar model geometry. The root-analogue implant model presented a lower displacement, when compared with the threaded ones. This may be related with the fact that, in this model, the implant is in contact with cortical bone, rather than trabecular bone. Additionally, the incorporation of flaps decreased the implant displacement substantially, indicating an improved implant stability. Finally, the natural tooth model was the one that presented the highest displacement, which is related with the elastic nature of the PDL present in this geometry.

5.4. Conclusions

Within the limitations of this study, the following conclusions can be drawn:

- Root-analogue implants induce a better distribution of the von Mises stresses along the cortical bone partition when compared with threaded implants;
- Root-analogue implants and the natural tooth model were mainly subjected to tensile stresses in the circular partition. In this sense, root-analogue implants seem to be a much-improved alternative for dental implants once tensile stresses are expected to induce bone formation;
- The five tested models were mainly subjected to shear strain values in the steady state zone, which indicates that the μ -strain results are within the physiological parameters.
- The incorporation of flaps in the root-analogue model reduced the implant displacement, indicating a better implant stability. On the other hand, as expected, the PDL led to higher displacement values, associated with its elastic nature.
- In general, given the results obtained in this study, root-analogue implants seem to be a promising solution for dental implant options, overcoming some limitations of the threaded conventional implants. However, results could be further improved by taking into consideration pre-tension of PDL, that was not considered in the FE analysis. The flaps design could also be improved in order to avoid μ -strain values in the disuse atrophy zone.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmbbm.2020.103667>.

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CHAPTER 6

Design and optimization of zirconia functional surfaces for dental implants applications

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Abstract

Zirconia is becoming a promising solution for biomedical applications, namely for dental implants, due to its biocompatibility, and mechanical and aesthetical properties. Despite the constant developments in the dentistry field, strategies to promote an effective vascularization at the implant's surface and consequently improved osseointegration are still not enough. In this sense, with the aim of promoting the vascularization at the implant's surface, zirconia surfaces with micro-channels were designed and evaluated regarding their hydrophilicity and capillarity. A CAD/CAM system was used to design and produce the specimens and different techniques were used to characterize the surfaces. The obtained average surface roughnesses are in accordance with the literature for similar materials. Results revealed that the produced materials present high levels of hydrophilicity, whether in contact with water or FBS - Fetal Bovine Serum. Additionally, micro-channels with 200 μm of width and 100 μm of depth were the ones that presented higher capillarity, thus being promising solutions for the promotion of implants vascularization, and consequently improved osseointegration.

Keywords: Zirconia dental implants; Implant vascularization; Surface hydrophilicity; Capillarity; CAD/CAM technology

6.1. Introduction

Zirconia, namely Yttria stabilized zirconia (YSZ), a ceramic material, has been widely used in biomedical applications, namely in the dentistry field [1]. Its biocompatibility, low bacterial affinity [2,3], good chemical and dimensional stability, high mechanical strength, excellent wear resistance, and its white color are the main features that make zirconia a promising solution for such applications [4,5]. Despite its advantages, the physical and mechanical properties of zirconia tend to degrade as a result of a low-temperature aging process [6]. This process is characterized by a slow transformation of the tetragonal zirconia phase into the monoclinic, associated with an increase in the volume of the ceramic that stresses the particles and results in subcritical crack growth, offering a way for water to penetrate inside the material [6,7]. This phenomenon is strongly affected by the material grain size [8]. Above critical grain sizes, Y-TZP is less stable and, consequently, more susceptible to tetragonal-monoclinic phase transformation. On the other hand, for grain sizes below 0.2 μm , the transformation does not occur, leading to a reduced fracture toughness [8]. In this sense, the sintering conditions have a huge impact on the stability and

mechanical properties of zirconia, through the control of the grain size. In order to avoid the stress-induced transformation phase, sintering temperatures between 1350 and 1550 °C have been applied in the dentistry field [9]. Additionally, some authors believe that to avoid the tetragonal-monoclinic phase transformation, zirconia should not be grinded nor sandblasted.

Regardless of all the efforts that have been carried out in the last few years, long-term dental implants survival and osseointegration is still an issue.

The term “osseointegration” was first introduced and developed by Dr. Par-Ingvar Branemark in 1985, to define the direct structural and functional connection between a living bone and the surface of a loadcarrying artificial implant [10,11]. This process requires an initial interlocking between the alveolar bone and the implant (primary stability) and later, the biological fixation through continuous bone remodeling towards the implant (secondary stability). Osseointegration is a very complex process and there are many factors influencing the formation and maintenance of bone at the implant surface [10]. Additionally, some authors believe that the success of a dental implant is highly dependent on the interaction between the implant and intraoral tissues [12]. Surface properties such as roughness have a huge impact on the implant's osseointegration once they will influence the adhesion and proliferation of the osteoblasts on the implant's surface. Bone regeneration involves a sequence of cellular and extracellular biologic events in the interface bone-implant, regulated by growth and differentiation factors released by blood cells [13]. In this sense, one of the biggest challenges in implantology nowadays is controlling the process of bone resorption around the implant immediately after insertion and during its function (after 12 months) [12,14].

Some investigations have been carried out to improve the zirconia implant-bone interface interactions, and promote effective osseointegration [6,15]. Techniques such as sandblasting [16], coatings of zirconia, spray drying of a bioactive material [17] and chemical etching [18] have been applied in this field to promote an increased implant surface roughness and microporosity, and, consequently, induce the implant osseointegration. All these approaches are based on the mechanical interlocking between the host bone and the implant but do not take into consideration biological mechanisms such as vascularization.

Osteoconduction, the process by which bone grows on a surface is crucial to achieving the implant osseointegration and consequent long-term stability [19]. On the other hand, to achieve osteoconduction, bone growth factors together with a proper blood supply are mandatory. This blood supply, responsible for providing nutrition is achieved through angiogenesis, which

corresponds to the formation of new blood vessels from the pre-existing vascular network [20–22].

As far as the bone regeneration is concerned, angiogenesis plays a crucial role in bone remodeling by providing the functional connection between the implanted material and the surrounding host tissues [20], by assisting and accelerating the regenerative process. To achieve so, it is suggested to decorticate the surrounding bone to assist the connection between blood vessels in the bone and the implant surface [23,24].

Some studies have been conducted to evaluate the role of dental implant surface characteristics, such as topography, hydrophilicity, surface charge, and other treatments, on the angiogenesis process surrounding the bone after dental implant insertion. Mohammad-Ali Saghiri et al. [25], concluded that the surface topography and hydrophilicity are the main features that an implant should encompass to promote angiogenesis and osteogenesis, once they induce the production of many pro-angiogenic growth factors. Schwarz et al. [26] reported that the hydrophilicity of a dental implant surface is more efficient for soft and hard tissue integration than the surface microtopography. In another study [27], it was concluded that hydrophilic dental implant surfaces tend to promote faster growth of human umbilical vein endothelial cells and osteoblast-like cells when compared with smooth hydrophobic implant surfaces. Also, the growth and function of undifferentiated endothelial progenitor cells that produce pro-angiogenic factors, were proved to be promoted by hydrophilic implant surfaces [28]. Lang N. et al. [29], also evaluated and compared the osseointegration rate and degree of hydrophilic and hydrophobic implant surfaces during the early phases of healing in a human model. Results showed that the degree of osseointegration after four weeks was higher for the hydrophilic when comparing with the hydrophobic surface.

On the other hand, capillarity, a spontaneous movement of a fluid resulting from the cohesive forces between the fluid and the surrounding surface, is also required to achieve an efficient vascularization [30]. An implant surface with capillary properties will allow fluids, namely blood, to flow against gravity and spread, promoting, consequently, the cell infiltration and the proper supply of nutrients [31]. In this sense, strategies such as the production of scaffolds with interconnected pores or micro-channels have been investigated for biomedical applications to find an optimal design to facilitate these cell and nutrient flows. Results have shown that scaffolds or implant surfaces with capillary action may be a promising solution to improve bone regeneration through the flow of fluids into narrow spaces, such as pores or micro-channels [31–33].

Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) has revolutionized the dental implants world and its impact in society has been increasing lately [34]. This technique encompasses three big components: the digitalization scanner that transforms geometry into digital data to be processed by the computer; the software that processes the data and produces a data set for the fabrication of the product; and production technology that transforms the data into the final product [35]. After image acquisition and 3D modeling, the CAD model is converted into milling strips for the CAM-processing and loaded into the milling device, that can have different numbers of axis (3, 4 or 5 axes) [34,35]. This manufacturing process can be performed by a computer numerical control (CNC) machining system, that uses power-driven tools to mechanically cut the material into the desired geometry [36]. This subtractive manufacturing technique has been proved to reduce the production time of the implants and to produce very complex geometries that would be difficult to create with conventional techniques [35,36].

Targeting dental implants applications, this research work aimed to design and assess the hydrophilicity and capillarity of zirconia substrates with micro-channels on the surface, machined by a computer numerical control system. To find an optimal solution, micro-channels with different widths and depths were evaluated.

6.2. Materials and Methods

6.2.1. Specimen Design and Manufacturing

Regarding specimen manufacturing, as previously stated, a CAD/CAM system was used. To perform this manufacturing process, the 3D CAD models were firstly drawn according to the desired geometries (*SolidWorks Corporation, Dassault Systèmes S.A, USA*). That models were posteriorly implemented in the CAM software that, in turn, created a code to be understood by the machine firmware (*DWX – 50 by Roland, Serbia*). This process was performed in a zirconia green compact disc (High Strength Zirconia – 3Y-TZP, with a scaling factor of 1.249) from *Dental Direkt, Germany*, and had to follow some specifications, according to the manufacturer (Table 6. 1).

Table 6. 1 Guide for cutting Zirconia with DWX – 50 [37].

Tool	Process	X & Y direction cutting	Z direction cutting	Spindle speed (rpm)	Path Interval (mm)	Cut-in amount (mm)	Finish margin (mm)
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		speed (mm/min)	speed (mm/min)				
R1 ball	Rough cutting	2100	1200	25000	1	0.8	0.15
R0.5 ball	Finish cutting	900	900	25000	0.1	0.1	-

After the green machining process, specimens were sintered in a furnace (*Zirkonofen 700 Ultra-Vakuum from Zirkonzahn - Zirkonofen*) at 1500 °C, with a heating/cooling rate of 8 °C/min and a holding time of 2 h. This equipment allows the sintering of zirconia substrates in a hermetically sealed chamber, thus guaranteeing no contamination. In a first attempt, twelve different micro-channels with different dimensions were designed in a zirconia surface (Table 6. 2), as referred to as P1.

Table 6. 2 P1 micro-channels dimensions and respective designation.

		Milling tool diameter (μm)	Micro-channel designation
Micro-channel depth (μm)	100	100	C1
		200	C2
		300	C3
		500	C4
	80	100	C5
		200	C6
		300	C7
		500	C8
	50	100	C9
		200	C10
		300	C11
		500	C12

After wettability and capillarity analysis, the channels with the most promising results (C2 and C3) were designed in another zirconia surface (hereafter referred to P2). In this second specimen, micro-channels were designed in both horizontal and vertical orientations. Finally, given the results obtained in P2, a third specimen was designed. This third specimen - P3- has a cylindrical shape, that intends to mimic a dental implant geometry. To sum up, three zirconia specimens were produced. The dimensions of the micro-channels on their surfaces resulted from an iterative process that evolved according to the wettability and capillarity results. The first

specimen is characterized by twelve different micro-channels; the second specimen has on its surface two different micro-channels, in both horizontal and vertical orientations (the ones with the best results in the previous specimen); and the third specimen has an implant-like geometry with micro-channels with the same dimensions (the one with the best wettability and capillarity results in the previous specimen) around the entire geometry. In Figure 6. 1 it is possible to observe the CAD models of the three specimens, according to the sequence above explained, as well as their dimensions after sintering (taking into consideration a sintering shrinkage of $\approx 20\%$).

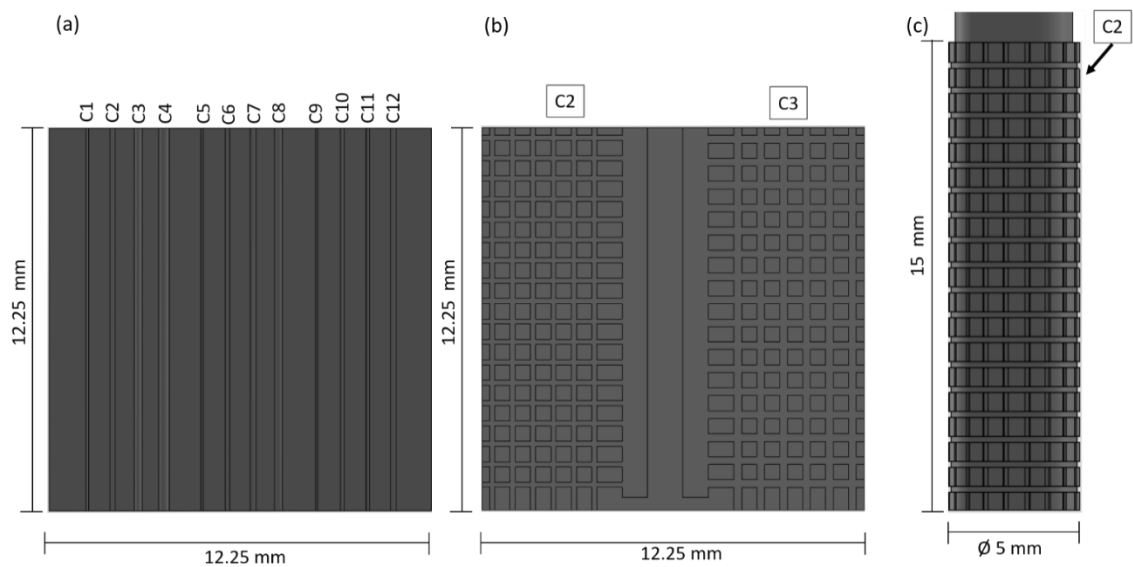


Figure 6. 1 Specimens design and final dimensions: (a) P1; (b) P2; (c) P3.

6.2.2. Surface Analysis

After the manufacturing process and prior to any analysis, all specimens were carefully brushed with an RBSTM 50 solution (*Sigma-Aldrich, EUA*) and ultrasonically cleaned with isopropanol for 5 min to remove any loose debris or surface contamination, followed by a drying procedure with a vacuum system (*Alcatel Adixen Rotary Vane Vacuum Pump, France*).

6.2.2.1. Scanning Electron Microscopy Analysis and Profilometry

To evaluate the manufacturing process and to better analyze the micro-channels dimensions and topography, Scanning Electron Microscopy (SEM) and profilometry analysis were carried out. The topography of the micro-channels was assessed by field emission guns scanning electron microscopy (*JSM-6390LV, JEOL, Japan*).

As far as the micro-channels' depth, width and roughness are concerned, a profilometry analysis was performed. Data was acquired from an optical 3D profilometer (*Profilom 3D*) from *Filmetrics – A KLA Company*. Surface roughness was measured at both the micro-channels bottom and at the specimens' top surface. Three measurements with 300 μm of length were performed at randomly selected areas and R_a -average roughness-was the selected surface roughness parameter. Before any SEM and profilometry observations, specimens were gold coated by a sputtering process (*Model 108A, Cressington, UK*).

6.2.2.2. Surface Wettability

The surface wettability of P1, P2, and P3 specimens was assessed through the contact angle measurement method. The sessile drop technique was used in an optical goniometer *OCA 15 Plus (Dataphysics, Germany)*. Two different fluids were used in this analysis: deionized water (at 18.2 Ohm), to compare with literature results, and Fetal Bovine Serum (FBS), representing a biologic fluid. FBS is the liquid fraction of clotted blood from foetal calves, without cells, clotting factors or fibrin, but containing a large amount of nutritional and macromolecular factors that promote cell growth. This liquid has been applied to provide nutrients supply in many tissue engineering applications [38]. In this specific research work, FBS (*Gibco, Spain*) was used as a blood substitute, to extrapolate how these specimens would behave when in contact with blood.

Three droplets of each fluid were applied in the surface of each of the three tested specimens, and contact angles were measured immediately after the droplet reaches the surface (0 s), and 10 s later. A volume of 5 μL and a dosing rate of 1 $\mu\text{L/s}$ were applied for P1 and P2. Due to its geometrical configuration, in the P3 specimen, only 1 μL of fluid was dropped, at a dosing rate of 1 $\mu\text{L/s}$.

6.2.2.3. Surface Capillarity

The capillarity of the micro-channels was assessed through a test in which the specimens were placed in contact with a fluid and the fluid rise time was measured by assessing its level inside the channels. Since the specimens are characterized by a high opacity and white color, these tests could not be performed with water. Instead, two different fluids were used. In a first approach a red color pigment was used, and, in a second stage of the tests, capillarity with FBS was assessed. Despite being a reddish fluid, it was not possible to observe the fluid rise in the FBS

solution. In this sense, a droplet of pigment was also added to this second fluid, to facilitate the posterior image analysis. The fluid rise in the channels was recorded and posteriorly analyzed in a media player program followed by *ImageJ* software. Before each test, specimens were properly cleaned and dried, as above mentioned.

6.3. Results and Discussion

6.3.1. Specimens Characterization

The different zirconia specimens produced by CNC machining were inspected by scanning electron microscopy and the top views are presented in Figure 6. 2. In Figure 6. 2(a) and (b), that correspond to the P1 specimen, it is possible to observe four representative micro-channels (a), and a high magnification image of a micro-channel bottom (b). It is possible to observe that the channels' bottom is characterized by an irregular topography, that resulted from the milling process.

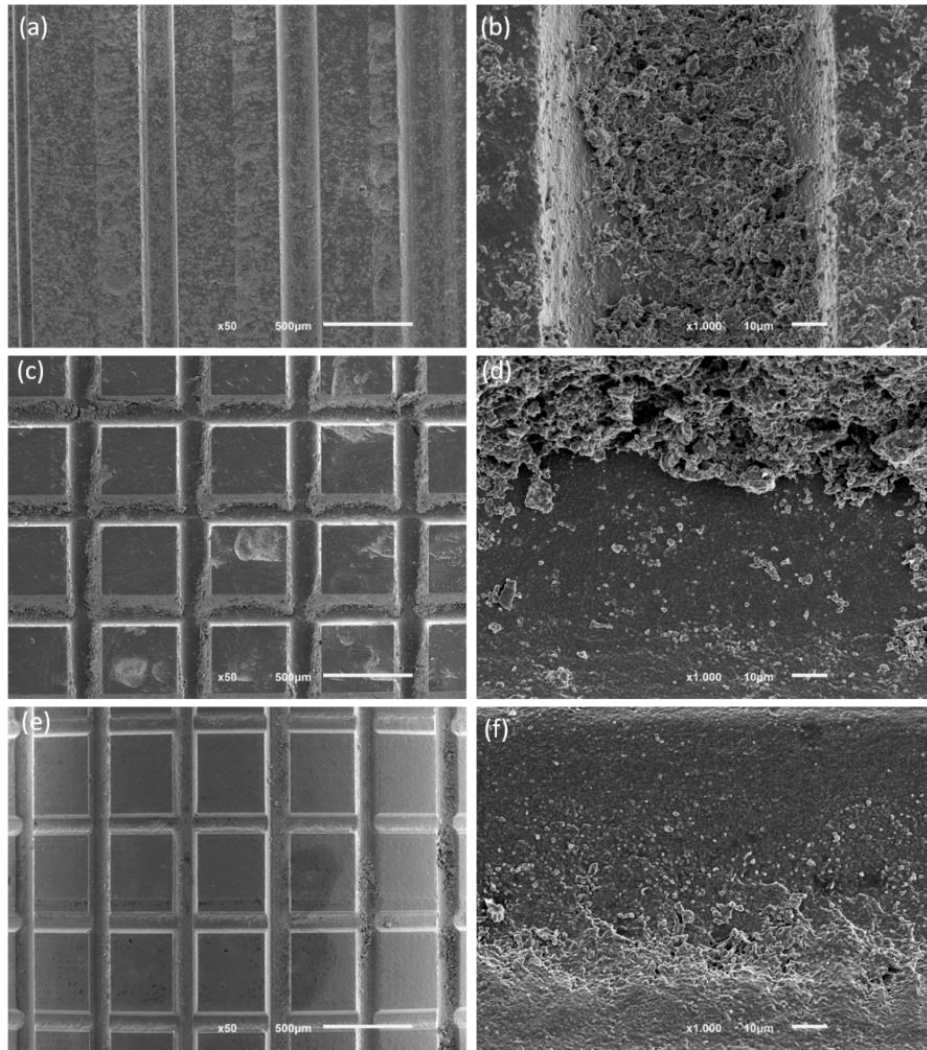


Figure 6. 2 SEM micrographs of the surface topography of the three specimens with the respective micro-channels: (a), (b) P1 specimen; (c), (d) P2 specimen; (e), (f) P3 specimen.

In Figure 6. 2(c) and (d), the P2 surface specimen is represented. The micro-channels on the surface of the P2 specimen are characterized by 100 μm of depth and were machined with a milling tool of 200 μm of diameter. It is possible to observe, in the channels' borders, the accumulation of material that resulted from the milling process and were not well sintered nor removed during the cleaning procedure. That accumulation of material is transversal to all produced specimen and may have influenced the subsequent experimental results. However, in Figure 6. 2(e) and (f), (P3 specimen) the accumulation of material in the micro-channels is not so evident, which indicates the sintering process and cleaning procedure were more effective. In Figure 6. 3 it is possible to observe two representative images of the profilometry analysis outputs, corresponding to a C2 channel. In Figure 6. 3(a) the dimensions (depth and width) of the micro-channel are represented, whereas in Figure 6. 3(b) it is possible to depict the topography of the

channel. As before mentioned, the real dimensions of the channels were acquired by a profilometry analysis in three distinct sites of the micro-channel and detailed results can be seen in Table 6. 3.

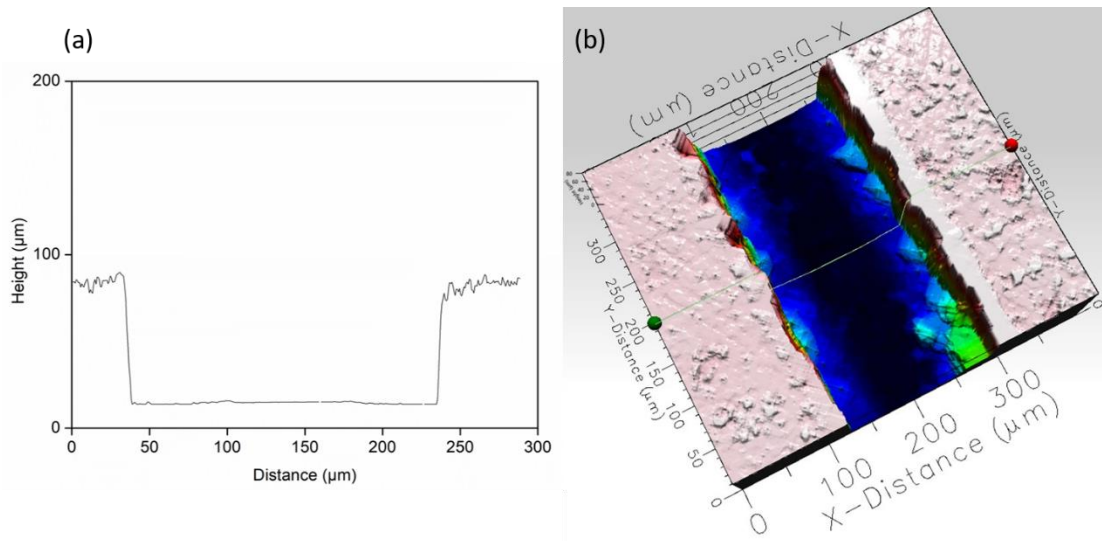


Figure 6. 3 Representative figures of the profilometry analysis software outputs: (a) C2 micro-channel depth and width; (b) C2 micro-channel topography.

As expected, due to the shrinkage of the zirconia compact during the sintering process, the real dimension observed in the specimen is approximately 20% smaller than what was designed in the CAD model.

Table 6. 3 Micro-channels depths and widths acquired by profilometry: CAD model vs. real dimensions.

Micro-channel designation	CAD model micro-channel depth (μm)	Real micro-channel depth (μm)	CAD model micro-channel width (μm)	Real micro-channel width (μm)
C1	100	50*	100	85.3
C2	100	75	200	180.9
C3	100	80	300	232.9
C4	100	82	500	328.2
C5	80	38*	100	85.1
C6	80	58	200	176.9
C7	80	72	300	230.7
C8	80	68	500	292.1
C9	50	37	100	84.6
C10	50	38	200	188.2
C11	50	38	300	206.9
C12	50	40	500	238.9

It is important to mention that some measurements, highlighted in the table with an asterisk, were quite below what was expected. These unexpected measurements, namely the C1

and C5 micro-channels depths, may be a result of the material accumulation in the bottom of the channel, observed in the SEM images. Also, the micro-channels that were machined with a milling tool of 500 μm (C4, C8, and C12) presented width values quite lower than what was expected which may indicate that material was accumulated in the channel borders, reducing its width.

In the following figure (Figure 6. 4) it is denoted the roughness measurement results and the respective standard deviations. As before mentioned, surface roughness was measured at the specimens' top surfaces and the micro-channels bottom.

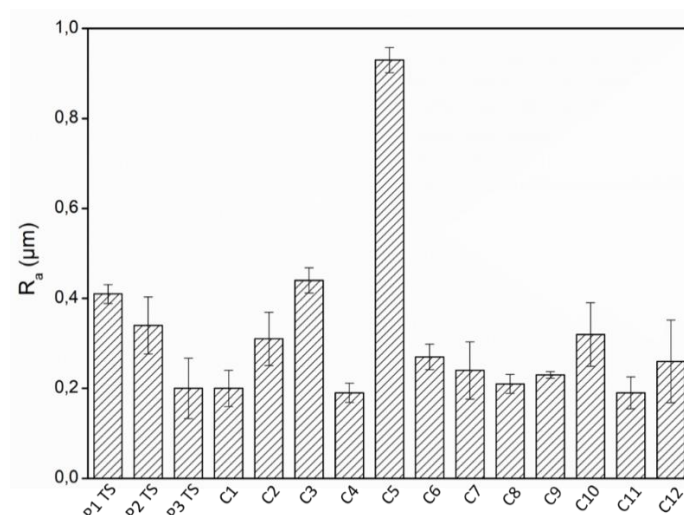


Figure 6. 4 Roughness (mean $\pm$ SD) of the specimens' top surface (P1 TS, P2 TS and P3 TS) and in the bottom of the micro-channels.

As far as the specimens' top surfaces are concerned, it is possible to conclude that, despite having been manufactured with the same procedure, minimal differences in the average surface roughness were observed. The P1 top surface was the one with a higher average roughness ($0.41 \pm 0.02 \mu\text{m}$), followed by the P2 surface ($0.34 \pm 0.06 \mu\text{m}$) and the P3 top surface presented the lowest average roughness ($0.2 \pm 0.06 \mu\text{m}$). These results are in accordance with the SEM images (Figure 6. 2(a), (c) and (e)), where the P3 surface appeared to be smoother than the other two. The results found in this study are comparable to those found in literature for similar materials. One study performed by Hamid et al. [39] evaluated the surface roughness of zirconia manufactured by a CNC milling machine, for dental applications, and results revealed similar values for surface roughness. Additionally, literature reports that the average surface roughness for zirconia implants ranges from $0.13 \mu\text{m}$ to $0.36 \mu\text{m}$ [40,41]. Regarding the roughness measurements in the micro-channels bottom, it is possible to conclude that there are no significant

differences between them, except for the C5 channel, which exhibited the highest average roughness ($0.93 \pm 0.03 \mu\text{m}$). This discrepancy in the results may be, again, related to the accumulation of material in the micro-channel, inherent to the manufacturing process.

6.3.2. Wettability Analysis

Hydrophilic implant surfaces have already been considered as a key factor in the biological response of dental implants. To assess the hydrophilicity of these zirconia surfaces, wettability tests were performed. In Figure 6. 5 it is possible to observe a representative contact angle measurement when the droplet touched the surface (a), and 10 s after (b).

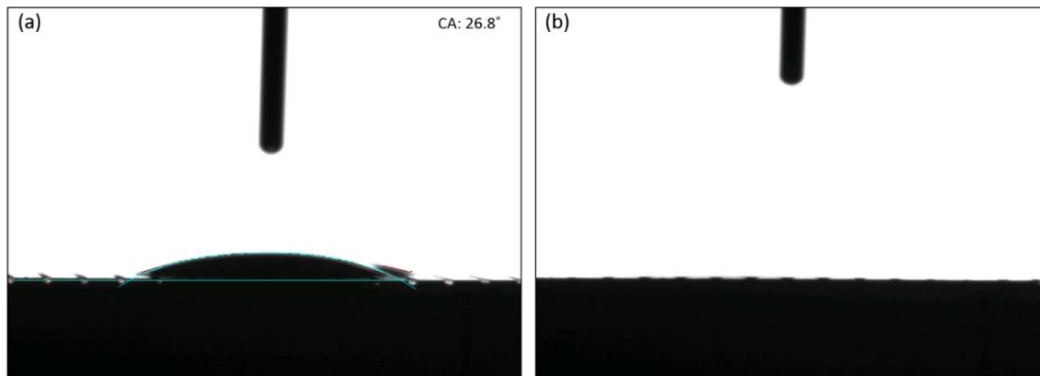


Figure 6. 5 Representative images of a water droplet and measured contact angle: (a) immediately after reaching the surface; (b) 10 s later.

Additionally, in Figure 6. 6, it is possible to observe the water and FBS contact angle mean values (and standard deviations) for the three tested surfaces, at the moment the droplet touched the surface, and 10 s later.

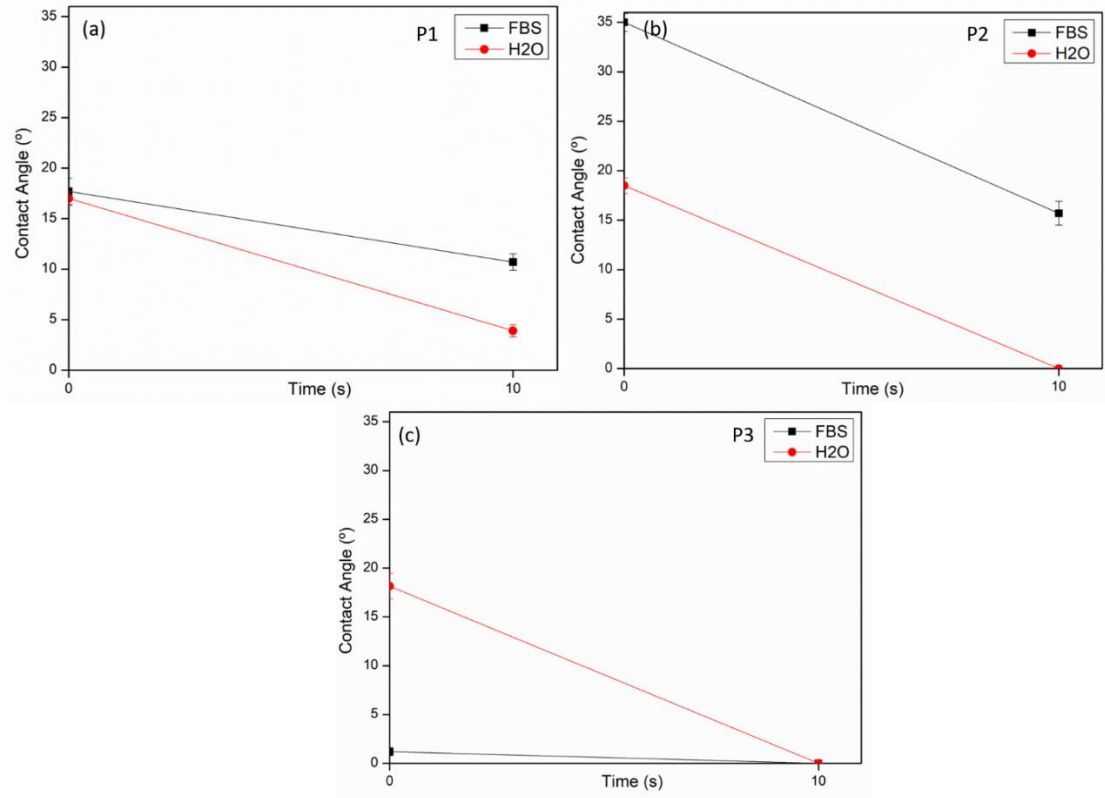


Figure 6. 6 Water and FBS contact angles (mean $\pm$ SD) of P1 (a), P2 (b) and P3 (c) specimens, for 0 s and 10 s.

Analyzing the results presented above, it is possible to observe that the contact angles measured with FBS are quite higher than those measured with water, for the P1 and P2 specimens. The same did not occur with the SP3 specimen, where the FBS contact angles were slightly lower than those measured with water. However, the differences between the two fluids are not representative, since the maximum value obtained for the FBS was 35° (for the P2 specimen, at the moment 0 s) and for the water was 18.5° (also for the P2 specimen in the initial moment).

Another conclusion that can be drawn from this analysis is that, after 10 s, the droplets contact angles decreased substantially, for all tested conditions, and, in specific cases, the measured contact angles after 10 s were approximately 0° (P2 (water) and P3 specimens).

As established in the literature [42], contact angles below 90° indicate that a surface is hydrophilic, whereas those with contact angles above 90° are designated as hydrophobic surfaces. Given this criterion, all measurements performed in the scope of this analysis led to contact angles below 90° which indicates that the produced specimens are hydrophilic. Additionally, when 0° were obtained, surfaces may be considered super-hydrophilic [42]. In general, despite no significant differences were observed, P3 specimen presented lower contact angles when

comparing with the other two specimens, which may be related to the lower surface roughness.

These results are in accordance with a study performed by Himma et al. [43] where an increased surface roughness led to higher water contact angles. The results obtained in this section of the work, especially regarding the FBS fluid, suggest that these surfaces are promising solutions for dental implant applications since their hydrophilic nature is expected to facilitate the proper blood supply around the implant and consequent osseointegration of implant adjacent tissues.

6.3.3. Capillarity Analysis

The micro-channels designed on the zirconia surfaces had the main goal of promoting the infiltration and supply of nutrients and fluids around dental implants, inducing the vascularization at the implant's surface. In this sense, the capillarity of the micro-channels was assessed by means of a qualitative analysis, where two different fluids were evaluated: a red pigment and FBS. With this analysis authors intended to find out which combination of micro-channels depth and width presented the highest capillarity effect. In Figure 6. 7 it is possible to depict the results for the P1 specimen, where the variation of the fluid height with time is represented in intervals of 3 s. To ensure clarity of results analysis, standard deviation values have been neglected and therefore are not represented in the figure.

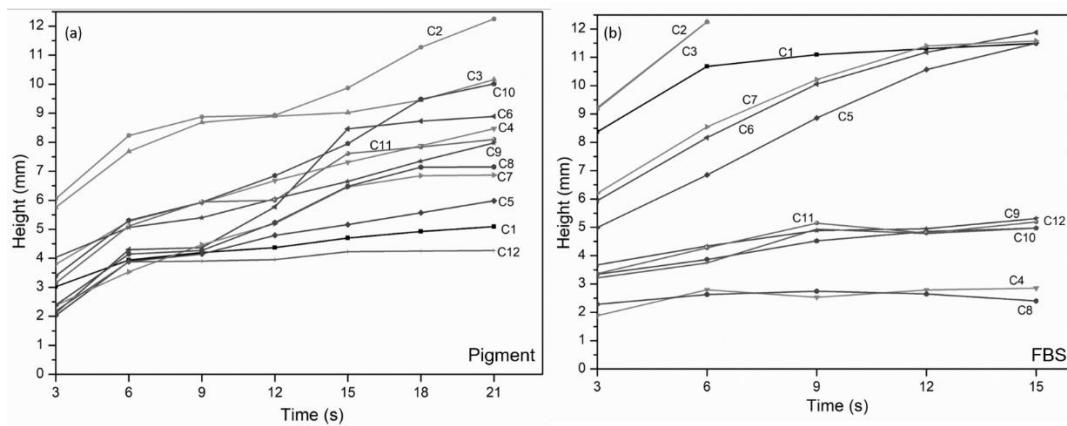


Figure 6. 7 Fluid height variation with time, in the P1 micro-channels, during the capillarity test: (a) pigment; (b) FBS.

After a careful analysis of the previous figure, it is possible to observe that in the tests with the pigment (Figure 6. 7(a)), the fluid only reached the top of the channel - 12.5 mm of height-in the microchannel C2, whereas with the FBS (Figure 6. 7(b)), both C2 and C3 channels were completely filled by the fluid. These two channels presented a similar behavior with the FBS (the

fluid reached the top of the channel after approximately 6 s). It is possible to observe that the fluids height variation is higher in the first seconds, and, as time passes, the height variation is lower. This behavior may be explained by the fact that the rise against gravity in narrow cavities tends to become more difficult with height [31,44]. In general, the FBS fluid, when comparing with the pigment, tended to rise faster in the channels, and stagnated after approximately 15 s, for all the tested micro-channels. On the other hand, the pigment took about 21 s to stagnate in the micro-channels. These results suggest that the dimensions (depth and width) of the microchannels C1, C4, C5, C6, C7, C8, C9, C10, C11, and C12 are not the most appropriate for the promotion of the capillarity effect since the fluids were not able to rise until the top of the channel.

In Figure 6. 8, the capillarity results of P2 are presented. This specimen is characterized by C2 and C3 channels, disposed in both vertical and horizontal directions, resulting in a checkered geometry.

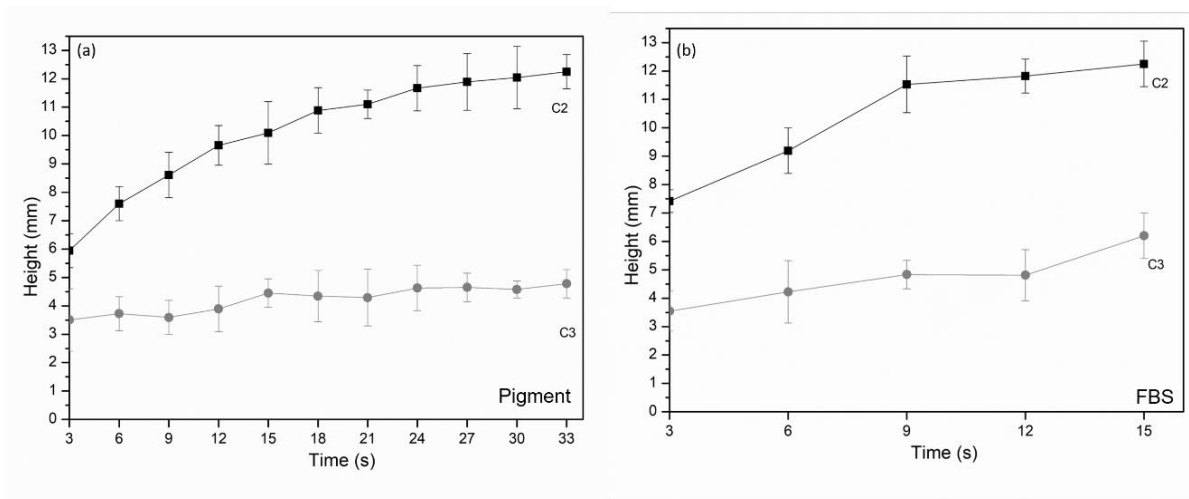


Figure 6. 8 Fluid height variation with time (mean $\pm$ SD), in the P2 micro-channels, during the capillarity test: (a) pigment; (b) FBS.

Comparing the results of the tests performed with the pigment (Figure 6. 8(a)) and with the FBS (Figure 6. 8(b)), despite presenting a very similar tendency, the FBS tended to rise faster in the channels, and after 15 s, C2 channels were completely filled with FBS. On the other hand, the pigment took about 33 s to rise until the top of the C2 channels. It is also possible to observe that none of the fluids was able to reach the top of the C3 channels: the pigment height in these channels was about 4.7 mm and the FBS was able to rise 6.2 mm on the C3 channels. These results indicate that, for the designed geometry, C3 channels are not promoting the desired capillarity effect, needed for the promotion of vascularization, since the fluids were not able to reach

the top of the channels.

Given the results presented above, C2 channels (in both vertical and horizontal directions) were designed in a cylindrical geometry to mimic a dental implant - P3 specimen. Capillarity analysis was also performed in this specimen, with the two previously mentioned fluids. The results are depicted in Figure 6. 9. In accordance with the previous capillarity tests, both fluids were able to reach the top of the C2 channels, indicating that the dimensions of these channels are appropriate for the induction of capillarity. Again, the FBS tended to rise faster in the micro-channels, and, after approximately 27 s, the entire geometry was covered by the fluid. The pigment was also able to rise and infiltrate in the C2 channels but took about 51 s to surround the geometry.

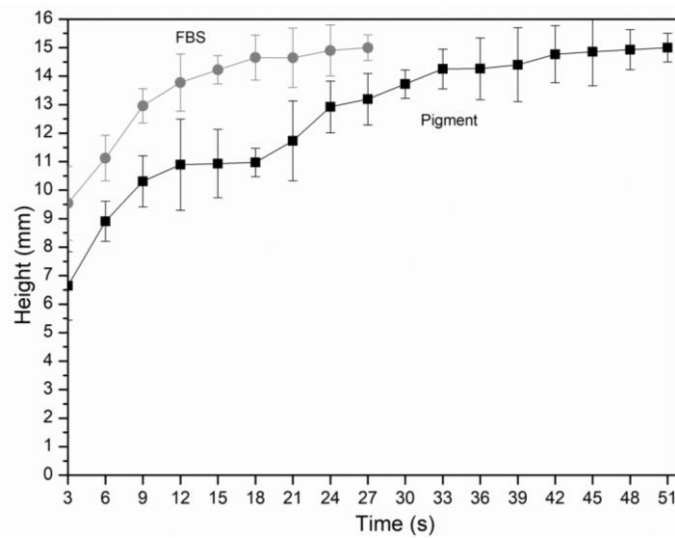


Figure 6. 9 Fluid height variation with time (mean $\pm$ SD), in the P3 micro-channels, during the capillarity test, for both the pigment and FBS fluids.

Finally, in Figure 6. 10 it is possible to observe some representative images of the capillarity tests with FBS, resulting from the video recordings.

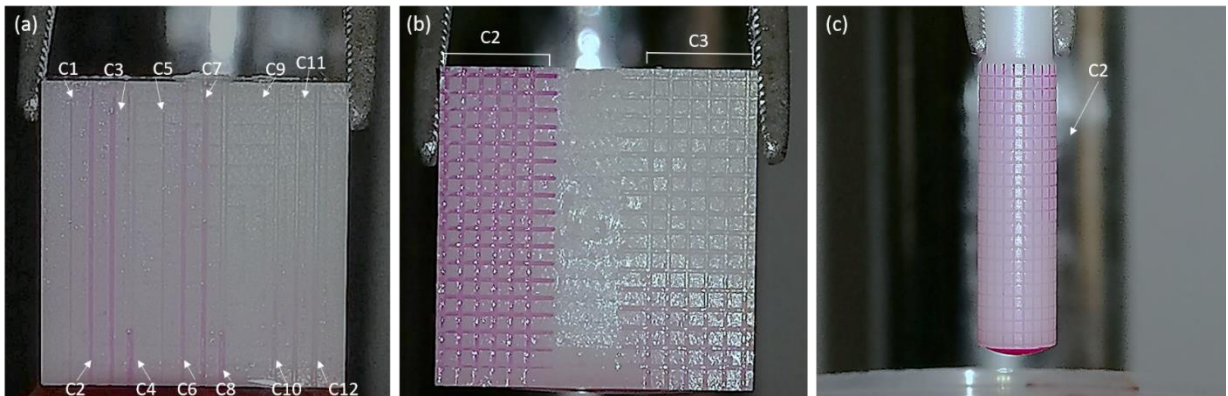


Figure 6. 10 Representative images of the fluid rise in the capillarity tests: (a) P1; (b) P2; and (c) P3.

Figure 6. 10(a) corresponds to the twelve micro-channels that were designed in the P1 specimen. It is possible to verify that the C2 channel was the only one completely filled with FBS, followed by C3, which was nearly filled. In Figure 6. 10(b) – P2 specimen-it is clear the filling of the C2 channels, whereas the fluid on the C3 channels only reached 6.2 mm of height. The last figure (Figure 6. 10(c)), corresponds to the cylindrical geometry with C2 channels on the surface - P3 specimen. It is completely covered in FBS, proving the capillarity effect of these channels.

Together with the channel dimensions and the hydrophilicity of zirconia, there are other mechanisms behind this capillarity effect, that have already been reported [33]. When the specimens contact the fluid surface, the fluid molecules adhere to the surface and cohere to each other, pulling each other through narrow cavities, namely the designed micro-channels

6.4. Conclusions

Specimens of 3Y-TZP zirconia with different micro-channels on the surface were successfully produced by a CAD/CAM system and their hydrophilicity and capillarity were assessed. Within the limitations of this study, some conclusions can be drawn:

- The manufacturing process proved to be effective in creating complex geometries with different dimensions. Nevertheless, the accumulation of material on the micro-channels' borders and bottom led to the creation of slightly different surface topographies, due to the different milling tools and the cleaning process;
- The average roughness values of the as-produced specimens are comparable to those found in the literature for zirconia implants;
- All specimens revealed a hydrophilic nature (contact angles bellow 90°), with both water and FBS fluids, with no significant differences in contact angles between the tested groups;

Capillarity analysis revealed an extreme capillary effect for micro-channels with 100 μm of depth and 200 μm of width.

This work presents a novel zirconia design for dental implants applications, with inter-connected micro-channels on its surface. The hydrophilicity and capillarity nature of the developed approach is expected to induce and facilitate crucial biological responses such as proper blood and nutrients supply (vascularization). These biological functions are key factors in the promotion of early and long-term implant osseointegration and stability.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 7

Bacteria co-culture adhesion on different texturized zirconia surfaces

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Abstract

Zirconia is becoming reckoned as a promising solution for different applications, in particular those within the dental implant investigation field. It has been proved to successfully overcome important limitations of the commonly used titanium implants. The adhesion of microorganisms to the implants, in particular of bacteria, may govern the success or the failure of a dental implant, as the accumulation of bacteria on the peri-implant bone may rapidly evolve into periodontitis. However, bacterial adhesion on different zirconia architectures is still considerably unknown. Therefore, the adhesion of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* to zirconia surfaces with different finishings was evaluated and compared to a titanium surface. The adhesion interaction between *S. aureus* and *P. aeruginosa* was also evaluated using a co-culture since these bacteria are infamous due to their common presence in chronic wound infections. Results showed that different bacterium species possess different properties which influence their propensity to adhere to different roughness levels and architectures. *E. coli* revealed a higher propensity to adhere to zirconia channelled surfaces (7.15×10^6 CFU/mL), whereas *S. aureus* and *P. aeruginosa* adhered more to the titanium control group (1.07×10^5 CFU/mL and 8.43×10^6 CFU/mL, respectively). Moreover, the co-culture denoted significant differences on the adhesion behaviour of bacteria. Despite not having shown an especially better behaviour regarding bacterial adhesion, zirconia surfaces with micro-channels are expected to improve the vascularization around the implants and ultimately enhance osseointegration, thus being a promising solution for dental implants.

Keywords: Bacterial adhesion; Dental implants; Zirconia; Surface characteristics

7.1. Introduction

Dental implants are a common practice in the dentistry field and research on their design, materials and techniques has gained a special interest over the past few years (Gaviria et al., 2014). Commercially available dental implants are mostly composed of titanium or its alloys, since this material has demonstrated excellent mechanical properties and biocompatibility (Cionca et al., 2000). However, it possesses poor wear resistance, and its appearance comprises a major aesthetic drawback. In addition, it releases metallic ions to the physiologic environment, and thus the development of novel materials and fabrication strategies to overcome these limitations are

warranted (Sikora et al., 2018; Souza et al., 2015).

Zirconia, namely tetragonal polycrystalline zirconia stabilized with 3 mol% yttrium oxide (Y-TZP), is being applied in dental applications as an alternative to metallic solutions (Kelly and Denry, 2008; Dantas et al., 2019). This ceramic material is a promising option for such applications mainly due to its opaque white color, low bacterial affinity and good biocompatibility (Cionca et al., 2000). Furthermore, zirconia presents low thermal conductivity, good fracture resistance, suitable flexural strength, high corrosion and wear resistance (Yin et al., 2017). Despite all of these advantages, zirconia is a bioinert material, which means that it does not spontaneously induce osseointegration and bone formation in the vicinity of the implant (Faria et al., 2019). In this sense, efforts are being carried out aiming to improve the zirconia-bone interactions and, consequently, improve the implant osseointegration (Chevalier et al., 2011; Moura et al., 2017). Apart from dental applications, zirconia has also been applied in a wide variety of purposes and industries, namely in the cutlery field. Zirconia knives have been proved to present numerous advantages over common stainless-steel ones: higher sharpness, higher wear resistance, anti-bacterial properties, and also an improved corrosion resistance (Bi et al., 2018).

Surface roughness and its influence on the implant osseointegration has been a subject of study over the past few years. A recent review performed by Pesce P. et al. evaluated the influence of abutment surface roughness on peri-implant soft tissue behaviour. Results revealed an enhanced connective tissue fibre attachment next to rougher Ti abutments, when compared to machined ones (Pesce et al., 2019). On the other hand, Rezk F., et al. mentioned that abutments with rougher surfaces (above 0.4 μm) increase the affinity of microorganisms and the risk of peri-implant diseases (Al Rezk et al., 2018). Many techniques are already being implemented to control the implants or abutments surface roughness. Sandblasting, acid etching and spray drying represent some examples of these techniques (Depprich et al., 2008; Sennerby et al., 2005). Sandblasting has been applied in dentistry as one of the most effective strategies to increase the bond strength between resin luting materials and restorative materials (Nishigawa et al., 2016). On the other hand, sandblasting followed by an acid etching procedure (SLA) is considered the gold standard surface treatment in the worldwide dental implants market (Roccuzzo et al., 2014; Buser et al., 2012; Costa et al., 2019). Despite being considered a successful surface treatment, the clinical survival of zirconia-based implants with this surface finishing may be compromised as a result of the surface contamination due to residual ceramic particles or even bacterial colonization due to the increased roughness (Faria et al., 2020). A promising alternative to SLA is treating zirconia with

purely chemical treatments, namely with hydrofluoric (HF) acid. In fact, zirconia implants treated with this chemical approach are already available in the market and their clinical performance has been proved to be as successful as titanium implants (Bergemann et al., 2015; Oliva and Oliva J. D, 2010).

In addition to the surface roughness, there are other features and parameters that may influence the implant osseointegration and overall function. An increased surface wettability, for instance, is expected to increase the interactions between the implant surface and the biologic environment (Bornstein et al., 2008). Also, capillarity, a spontaneous movement of a fluid resulting from the cohesive forces between the fluid and the surrounding surface, is crucial to achieve an efficient vascularization around the implant (Jokinen and Franssila, 2008). An implant surface with capillary properties is expected to improve the flow of fluids, namely blood, and thus promote, cell infiltration and proper nutrient supply (Marques et al., 2019). With the aim of enhancing cell and nutrient flow around dental implants, surfaces with interconnected pores and/or micro-channels are also being designed and developed (Marques et al., 2019; Oh et al., 2014). One study performed by the authors research group concluded that the implementation of micro-channels on zirconia surfaces led to high capillarity and hydrophilic behaviours. These behaviours are expected to facilitate biological responses, such as vascularization around the implant, leading to an improved osseointegration and stability (Dantas et al., 2020).

The lack of osseointegration is a well-identified problem. Nevertheless, the adhesion of microorganisms to the implant surface and consequent plaque formation is considered one of the most important factors that influence the primary stability of implants (Costa de Medeiros Dantas et al., 2016). It is known that the quality and quantity of bacterial plaque on the implant surface will be detrimental in dictating whether an implant succeeds or fails (Costa de Medeiros Dantas et al., 2016). This plaque formation is commonly associated with lack of proper oral hygiene but also with the characteristics of the implant components used in the restoration, namely the implant-abutment interface (doNascimento et al., 2014). In fact, the accumulation of bacteria in the implant-abutment assembly is still one of the most critical challenges in dental implantology. An inaccurate fit between these two components may induce the colonization of bacteria that can, in turn, lead to inflammatory reactions with consequent bone resorption (Pedrazzi et al., 2014).

Zirconia is known to have the ability to inhibit the level of bacterial adhesion on its surface when compared to other materials commonly used in dental implant applications (Yin et al., 2017; Manicone et al., 2007). One study performed by Cassio do Nascimento and co-workers evaluated

the bacterial adhesion on titanium and zirconia abutment surfaces, depicting a significantly higher total bacterial count in titanium specimen (do Nascimento et al., 2014). In another study, bacterial adhesion was investigated on three different materials, under the same surface polishing: resin, titanium and zirconia. Resin specimens exhibited the highest susceptibility to bacteria adhesion when compared to the other two materials (Lee et al., 2011). On the other hand, there was no significant difference between titanium and zirconia specimens. Afya Sahib Diab Al-Radha et al. (2012) also assessed the surface properties of titanium and zirconia surfaces and their influence on bacterial adhesion. Four different surfaces were tested: polished zirconia (PZ), titanium blasted with zirconia (TBZ), titanium blasted with zirconia then acid etched (TBZA), and polished titanium (PT). The overall results suggest that zirconia and titanium blasted with zirconia are more effective in reducing the bacterial adhesion.

There are several studies that indicate that the accumulation of plaque around dental implants is extremely dependent on the surface roughness (Truong et al., 2010; Kang et al., 2017; Riedewald, 2006; Schubert et al., 2019). In fact, rougher surfaces, due to the presence of grooves and pits, are expected to induce a higher bacterial adherence, since, in these surface irregularities, bacteria are protected from the shear stress of salivary flow or other biologic fluids (Costa de Medeiros Dantas et al., 2016). As far as zirconia dental implants are concerned, literature reports that a surface roughness ranging from 130 to 360 nm is crucial to avoid bacterial adhesion (Yin et al., 2017). Apart from the surface roughness, other surface characteristics, such as wettability and surface charge, may also influence the bacterial adhesion to the implants surface (Costa de Medeiros Dantas et al., 2016; Azam et al., 2015). Understanding the phenomenon of bacterial adhesion to zirconia surfaces may also become a useful tool to address the potential microbial cross-contamination that may be induced by the improper use of certain food contact surfaces, including utensils and zirconia cutlery, such as knives and forks (Fink et al., 2017). Many consumers are not aware that domestic food contact surfaces and utensils are considered an important mean of transmission of foodborne diseases (Erickson et al., 2015).

Despite being already in the market, the microbiological response of zirconia implants, in particular when specific geometrical features are intended, such as micro-grooves, for improved osseointegration, still needs to be further investigated. Therefore, in this study, the adhesion of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* on different zirconia surfaces was assessed. Moreover, a co-culture of *S. aureus* and *P. aeruginosa* was performed aiming to evaluate the interactions between these two bacteria, since they are the most common

cause of chronic wound infections and are frequently found together (Alves et al., 2018). The tested specimens were as-sintered zirconia (AS), sandblasted and acid etched zirconia (SbE), zirconia with micro-channels on its surface (MC), zirconia with micro-channels followed by an acid etching treatment (MCE) and titanium with SLA (Ti) as control group, representing the surfaces of commonly used dental implants. Surface parameters such as roughness, wettability and capillarity were also evaluated and discussed.

7.2. Materials and Methods

7.2.1. Production of Titanium Specimens

Titanium specimens were cut from a biomedical grade V (Ti–6Al–4V alloy) rod, resulting in specimens with 8 mm diameter and 2 mm of height. Posteriorly, a surface treatment was applied to the specimens' surfaces, aiming to mimic the surface finishing of the commercially available dental implants. This surface modification, referred to as SLA, consists of a sandblasting treatment followed by acid etching, and the procedure is explained in section 7.2.3 (Scientific Review for Str, 2020; Stafford et al., 2014).

7.2.2. Production of Zirconia Green Compacts

In the scope of this research work, zirconia green compacts were produced by cold press technique. A 3 mol% yttria-stabilized zirconia spray-dried powder (TZ-3YSBE, Tosoh Corporation, Japan), with a theoretical density of 6.05 g/cm³ and high purity (99%) was used as starting material. This powder is characterized by spherical agglomerates with an average particle size of 60 µm and crystallites with an average diameter of 36 nm.

A cylindrical steel mold with an internal diameter of 10 mm was used for the compaction of the powder. In a first step, the zirconia powder was introduced in the mold and 200 MPa of pressure was applied, during 30 s. In the second step of this manufacturing technique, the pressure was evenly released and the obtained green compact (with 10 mm of diameter and 5 mm of height) was carefully removed from the mold.

7.2.3. Surface Texturing

Four zirconia surfaces and a titanium surface were tested regarding their propensity for bacterial adhesion. The sintering process to which all zirconia specimens were subjected will be explained in section 7.2.4 of this work.

Titanium specimens were subjected to an SLA treatment, aiming to mimic the roughness values commonly found in dental implants. White corundum angular particles with a maximum size of 250 μm were projected by a blasting gun with an air pressure of 0.6 MPa and a distance of 10 cm between the nozzle and the treated surface. After being properly cleaned with isopropanol, the specimens were subjected to a 5 min acid-etching process (with 32% HCl, 96% H₂SO₄ and H₂O (2,1,1) (v/v)) at 65 ± 3 °C.

The second group of specimens – AS – correspond to as-sintered zirconia surfaces, which means that these specimens were not subjected to any surface treatment.

The third group of specimens – SbE – was developed mainly for comparison purposes since authors mimicked the treatment used in conventional implants – sandblasting followed by an acid-etching process. This surface treatment was performed after the sintering process, and it encompass two steps. In the first step - the sandblasting - white corundum spherical particles with a maximum particle size of 250 μm were projected, during 30 s, by a blasting gun. A constant air pressure of 0.6 MPa was applied and a distance of 10 cm was kept between the nozzle and the treated surface. After this procedure, specimens were ultrasonically cleaned in isopropanol for 5 min. In the second step, the same specimens were subjected to an acid etching treatment. In this stage, the sandblasted surfaces were immersed, for 30 min, in hydrofluoric acid (48% (v/v)), followed by a cleaning procedure in an ultrasonic bath of isopropanol for 5 min.

The fourth group of specimens – MC – correspond to specimens with micro-channels on their surface, aiming to improve the vascularization around the implant. These micro-channels were designed in the pre-sintered zirconia specimens by a Computer Numerical Control (CNC) machining technology. The micro-channels were firstly designed in a CAD software (SolidWorks Corporation, Dassault Systemes S.A, USA), followed by the development of a file with the code to be read by the machine firmware (DWX – 50 by Roland, Serbia). After this machining process, specimens were sintered. The micro-channels dimensions (200 μm of width and 100 μm of depth) were previously discussed in another study from the authors (Dantas et al., 2020) that aimed to assess which was the best combination of dimensions to promote a proper vascularization.

The fifth and last group of specimens – MCE – correspond to MC specimens with a

posterior etching treatment. Following the micro-channels design and the sintering process, specimens were immersed in hydrofluoric acid (48% (v/v)) during 4 h. This fourth group of specimens was developed to assess the influence of the etching treatment on the micro-channels surface roughness and bacterial adhesion. For a better understanding, in Figure 7. 1 it is provided a schematic representation of the five different surfaces developed.

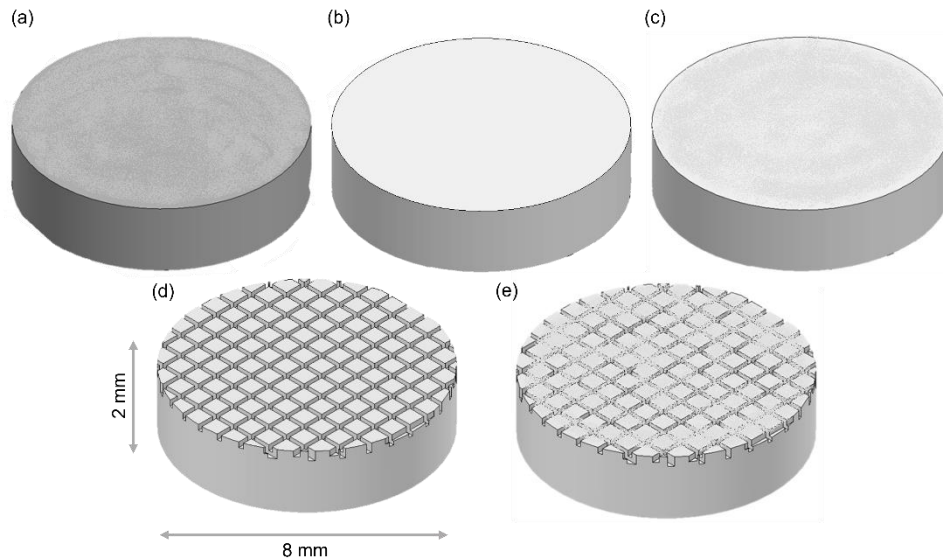


Figure 7. 1 Schematic representation of the produced specimens: (a) Ti- titanium with SLA; (b) AS- zirconia as-sintered; (c) SbE- zirconia with sandblasting and acid etching; (d) MC- zirconia with micro-channels on the surface; (e) MCE- zirconia with micro-channels and acid etching.

7.2.4. Zirconia Green Compacts Sintering

To sinter the zirconia green compacts developed in the scope of this research work, a high-temperature furnace - Zirkonofen 700, Zirkonzahn, Italy - was used. This process was carried out at 1500 °C with heating and cooling rates of 8 °C/min and a holding time of 2 h. After the manufacturing process, all specimens were carefully brushed with an RBSTM 50 solution (Sigma-Aldrich, USA) and ultrasonically cleaned with isopropanol for 5 min to remove any loose debris or surface contamination.

7.2.5. Specimens Cleaning Procedure

Before the *in vitro* tests, all specimens were subjected to a careful cleaning procedure. First, they were manually cleaned with ethanol for 2 min to remove any grease they could contain. Then, the specimens were sprayed with distilled water and subjected to an ultrasonic bath with

isopropanol and RBSTM 50 solution (Sigma-Aldrich, USA) during 30 min. Posteriorly they were rinsed with distilled water and set in an ultrapure water bath for 20 min. Finally, all specimens were subjected to 20 min of UV-C in a laminar flow cabinet.

7.2.6. Surface Analysis

In order to assess whether the applied surface treatments were successful in achieving different surface topographies, surface roughness was evaluated, using a profilometer by Mitutoyo (SJ 210, Japan).

The surface wettability of the as-produced specimens was also assessed, by means of contact angle measurements. An optical goniometer (OCA 15 Plus, Dataphysics, Germany) was used to perform the sessile drop technique. Three droplets of deionized water (at 18.2 Ohm) with a volume of 5 μL and a dosing rate of 1 $\mu\text{L/s}$ were applied to the surface of each tested specimen, and contact angles were measured immediately after the droplet reached the surface (0 s), and after 10 s.

The capillarity of the different tested conditions was assessed through a test in which the specimens were placed in contact with a fluid (a commercial aqueous solution containing phenol red ($\text{C}_{19}\text{H}_{14}\text{O}_5$)) and the fluid rise was observed and recorded by means of photography. This red color pigment was used for the performance of this experimental procedure since the tested materials are characterized by a high opacity and white color.

Scanning electron microscopy (SEM) was performed on the as-produced specimens to analyse their topography and the manufacturing process (JSM- 6390LV; JEOL, Japan). SEM was also used to observe the bacterial interactions with the produced specimens. Only two surface conditions were selected for this analysis – SLA titanium (Ti) and zirconia with micro-channels (MC). The bacterial fixation on the surfaces followed a procedure previously described in the literature (Padrão et al., 2016). Briefly, after removing the culture media, 1 mL of 2.5% (v/v) glutaraldehyde in phosphate buffered saline (PBS) was added to each specimen for 1 h at room temperature to induce the bacteria fixation. Subsequently, a dehydration process with serial ethanol dilutions with increasing ethanol percentage (55, 70, 80, 90, 95, 100% (v/v)) was performed. Specimens were immersed in each solution for 30 min at room temperature and, in the end, the remaining ethanol was left to evaporate. Prior to any SEM analysis specimens were sputter-coated with gold (Model 108 A, Cressington, UK).

7.2.7. Bacterial Adhesion Protocol

The bacteria used for the tests were *E. coli* (CIP 110067) and *S. aureus* (CIP 76.25) and *P. aeruginosa* (CIP 76.110). Pre-inocula were prepared by incubation of each bacterium at 37 °C and 120 rpm. *E. coli* and *S. aureus* were incubated in tryptone soya broth (HiMedia Laboratories Pvt. Ltd., India), whereas *P. aeruginosa* and *S. aureus* (when in co-culture) were cultured in nutrient broth (Condalab, Laboratorios Conda S.A., Spain). In each pre-inocula, bacterial concentration was adjusted by densitometry (Epoch Microplate Spectrophotometer, Biotek, USA) at 600 nm to a McFarland standard optical density corresponding to a bacterial concentration of approximately 1×10^7 colony forming units (CFU)/mL.

Three specimens of each condition were inserted into 24-well plates and incubated with each bacterium for 24 h at 37 °C and 120 rpm. As previously mentioned, the produced specimens have 8 mm diameter and 2 mm of height which corresponds to an apparent exposed area of 100.53 mm² (resulting from the sum of the specimen top and lateral areas). Subsequently, the broth was aseptically removed, and each specimen was put into a sterile 15 mL falcon tube with 3 mL PBS solution. To detach the adhered bacterium from the specimens surfaces, each falcon tube was subjected to an ultrasonic bath (50/60 Hz, J.P. Selecta, Spain) for 10 min and then vortexed for 1 min. Afterwards, the CFU/mL of each sample was estimated in Petri dishes incubated at 37 °C for approximately 12 h. This protocol was also performed using a co-culture of *S. aureus* and *P. aeruginosa* containing approximately 1×10^7 CFU/mL of each bacterium for 24 h at 37 °C and 120 rpm. In Figure 7. 2 it is possible to observe a schematic representation of the developed surfaces and tested bacteria, for a better comprehension.

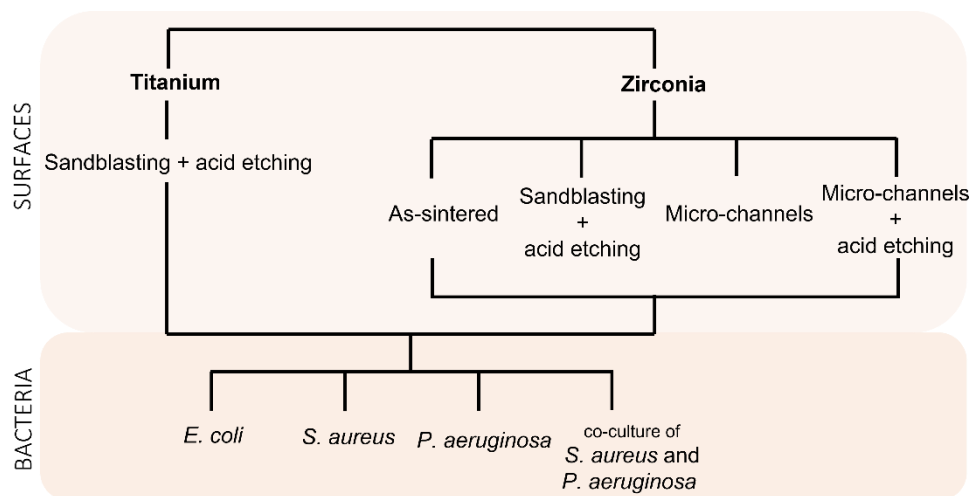


Figure 7. 2 Schematic representation of the developed surfaces and tested bacteria.

7.2.8. Statistical Analysis

The bacterial adhesion data was subjected to statistical analysis. Results are presented as the mean $\pm$ standard error of the mean. The assumption of normality was verified, and t-tests or one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests were performed. The level of significance was set at $\alpha = 0.05$. This analysis was carried out using GraphPad 6 software (Prism, California).

7.3. Results and Discussion

7.3.1. Specimens Characterization

The different produced specimens were inspected by scanning electron microscopy and the top views are presented in Figure 7. 3. In Figure 7. 3(a), which corresponds to the titanium specimen (Ti), it is possible to observe an irregular surface, resulting from the SLA treatment. Figure 7. 3(b) and (c) correspond to as-sintered zirconia (AS) and zirconia with sandblasting and acid etching (SbE), respectively. By comparing the two micrographs it is easily denotable that the applied surface treatment, as expected, leads to the formation of a more irregular surface, characterized by an increased roughness. As far as the machined surfaces are concerned (Figure 7. 3(d) and (e) for MC and MCE, respectively) it is possible to observe that the micro-channels bottom is characterized by an irregular topography, that resulted from the milling process. Additionally, in the borders of the channels, it is possible to denote the accumulation of material from the milling process that was not effectively sintered nor removed through the cleaning protocol. This material accumulation, observed in all specimens that were subjected to the machining process, may have influenced the subsequent *in vitro* testing. Again, the surface treatment performed on the MCE specimen seems to have induced an increase in the surface roughness. This increase is not so clear as in the SbE condition that was subjected to both sandblasting and acid etching, whereas MCE underwent only an acid etching treatment.

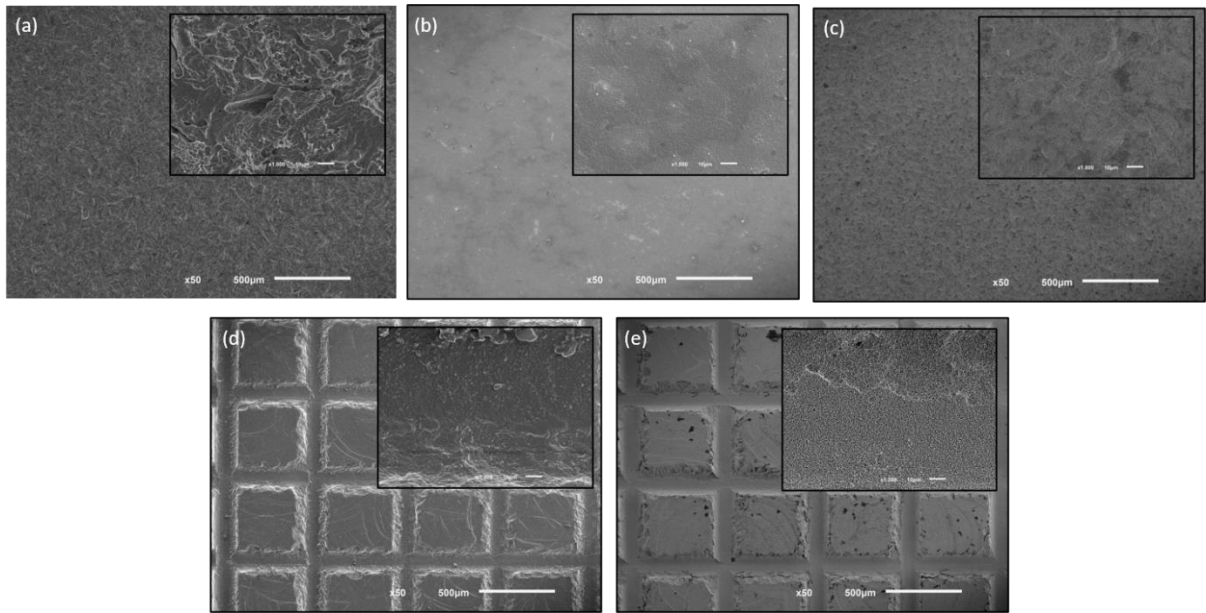


Figure 7. 3 SEM micrographs of the surface topography of the produced specimens: (a) Ti; (b) AS; (c) SbE; (d) MC; (e) MCE.

The results observed in the previous images are corroborated by the roughness measurements present in Table 7. 1.

Table 7. 1 Mean (SD) of surface roughness of the produced specimens. Roughness of MC and MCE specimens was measured on the top surface.

Specimen	Ti		AS	SbE		MC	MCE
Surface roughness, Ra (μm)	After sandblasting	After etching	0.29 (±0.04)	After sandblasting	After etching	0.28 (±0.01)	0.73 (±0.07)
	2.38 (±0.50)	1.71 (±0.40)		2.09 (±0.23)	1.59 (±0.19)		

As seen in Table 7. 1, titanium specimens treated with sandblasting and etching present a mean surface roughness of $1.71 \pm 0.4 \mu\text{m}$. These results are in accordance with similar studies found in literature, where SLA titanium specimens were characterized (Costa et al., 2019; Chen et al., 2016). On the other hand, and as expected, the average roughness decreased after the acid etching treatment, when compared with sandblasted titanium (Kim et al., 2008). As far as the zirconia specimens are concerned, the obtained average roughness measurements are also in accordance with the literature (He et al., 2014; Ewais et al., 2014). In addition, the sandblasting treatment induced a considerable increase in the average roughness when compared with as-sintered zirconia ($2.09 \pm 0.23 \mu\text{m}$ vs. $0.29 \pm 0.04 \mu\text{m}$, respectively) (He et al., 2014; Kirmali et al., 2014). Surface roughness decreased when an acid etching treatment was applied over the sandblasted surface. When comparing MC with MCE specimens, the surface roughness of the

zirconia was greater when the surface was treated with etching, as reported in analogous studies where rough zirconia surfaces were desired for improving the shear bond strength of dental resin cements to zirconia (Lee et al., 2019).

7.3.2. Wettability and Capillarity Analysis

Together with surface topography, its hydrophilicity is also expected to have a huge impact on the promotion of proper implant osseointegration. In fact, hydrophilic implant surfaces were proved to promote a faster growth of osteoblast-like cells, when compared with hydrophobic surfaces (Shi et al., 2014). To assess the wettability of the produced specimens, water contact angles (WCA) were measured in static (at the moment the droplet touched the surface) and dynamic mode after 10 s (Figure 7. 4). Literature reports that a surface is considered hydrophilic if a WCA below 90° is obtained. On the other hand, surfaces with WCA above 90° are designated as hydrophobic. After analysing the results, it is possible to conclude that all specimens present WCA below 90° , denoting a hydrophilic nature (Himma et al., 2019). Additionally, the Ti specimen presents considerably higher WCA when compared with all the zirconia specimens. The WCA obtained for this condition ($86.3 \pm 4.9^\circ$ and $70.1 \pm 4^\circ$, for 0 and 10 s, respectively) are in accordance with previous studies found in literature, which report titanium WCA between 70 and 90° regardless of surface roughness (Ma et al., 2017). Despite these results, there are some studies in the literature that state that the WCA of SLA titanium can raise up to 150° (Gittens et al., 2013). These higher WCA found for the SLA condition may be explained by the Wenzel's theory, that states that as the surface becomes rougher, it gets harder for the water to penetrate through the grooves due to the trapped packets of air (de Leon and Advincula, 2015). As far as the zirconia specimens are concerned, despite not existing significant differences between them, an increased surface roughness (SbE and MCE) seems to induce higher WCA when compared to smooth surfaces (AS and MC). These results are, again, corroborated with results found in similar studies that indicate that higher surface roughness led to higher WCA (Dantas et al., 2020; Himma et al., 2017).

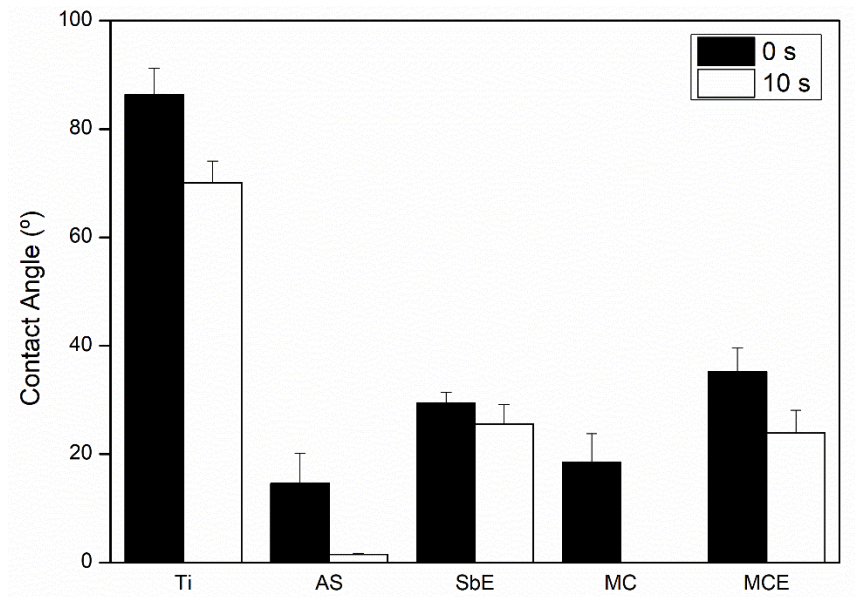


Figure 7. 4 Contact angles (mean $\pm$ SD) of tested materials, for 0 s and 10 s.

Furthermore, it is also possible to conclude that, for all tested specimens, WCA after 10 s is substantially lower than in the initial moment. For the smooth surfaces (AS and MC), after 10 s, the measured CA was approximately 0° , confirming the hydrophilic nature for these specimen conditions.

The main goal of designing micro-channels on the zirconia surface was to induce and facilitate the infiltration and supply of nutrients and fluids around dental implants, consequently improving the vascularization at the implants surface. In Figure 7. 5 it is possible to observe the tested specimens surface after contact with the red pigment.

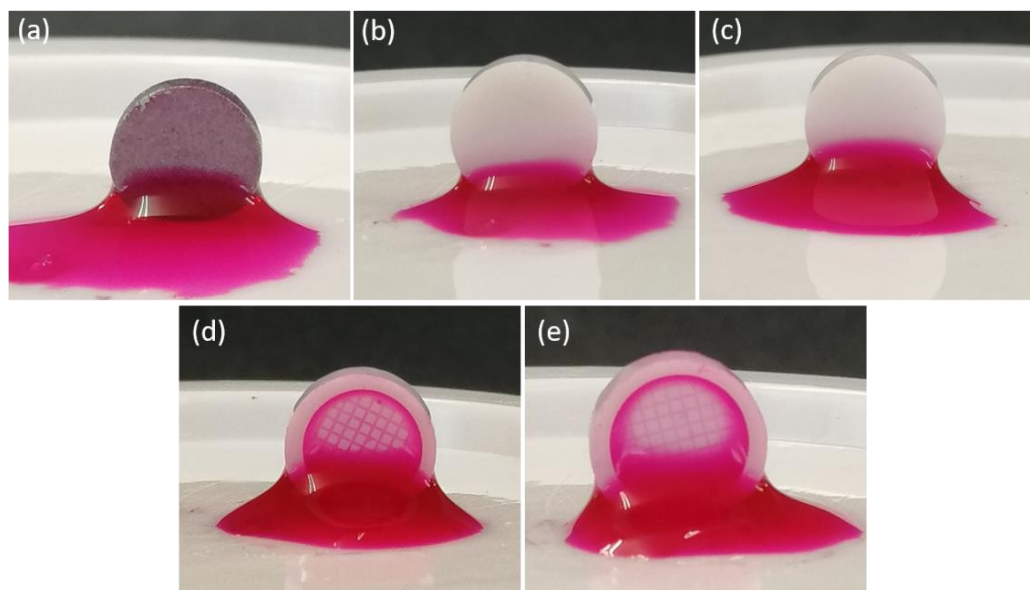


Figure 7. 5 Representative images of the fluid rise in the capillarity tests: (a) Ti; (b) AS; (c) SbE; (d) MC; (e) MCE.

In Figure 7. 5(a), that corresponds to the SLA Ti, it is possible to denote the wetting of the entire surface, which is in accordance with other studies found in the literature that report a capillary nature for this type of material (Bartolomeu et al., 2020; Ou et al., 2016). Figure 7. 5(b) and (c) - AS and SbE, respectively-indicate that, as expected, zirconia specimens without any microfeature on the surface were not able to promote the desired capillary effect. On the other hand, the incorporation of micro-channels on the zirconia substrate effectively induced the fluid rise and spread. This is evidenced by the reddish color of the entire specimens at the end of the test (Figure 7. 5(d) and (e)). In fact, this behaviour was already found in another study performed by the same authors of this article, where different micro-channels dimensions were evaluated aiming to find the best combination of depth and width for the promotion of proper vascularization (Dantas et al., 2020). These results suggest that the fluid molecules adhere to the zirconia surface, cohere, and then pull each other through narrow cavities such as the designed channels.

7.3.3. Bacterial Adhesion

7.3.3.1. Mono-cultures

As previously mentioned, in the scope of this research work, three bacteria were tested regarding their adhesion on different surfaces. In Figure 7. 6 it is possible to observe the obtained results determined from the viable counts. In Figure 7. 6(a), which corresponds to *E. coli*, it is

obvious that the zirconia specimen with micro-channels (MC) was the one that promoted the greater bacterial adhesion, being the results statistically different from all the other tested conditions. One possible reason for an increased *E. coli* adhesion to the MC specimen could be related to the bacteria shape and charge, to the increased area of exposure, and the rough topography that characterizes the channels walls, as a consequence of the machining process. It also seems that applying an acid etching treatment over the micro-channels surface (MCE) considerably reduced the adhered bacteria, when comparing with the previous specimen.

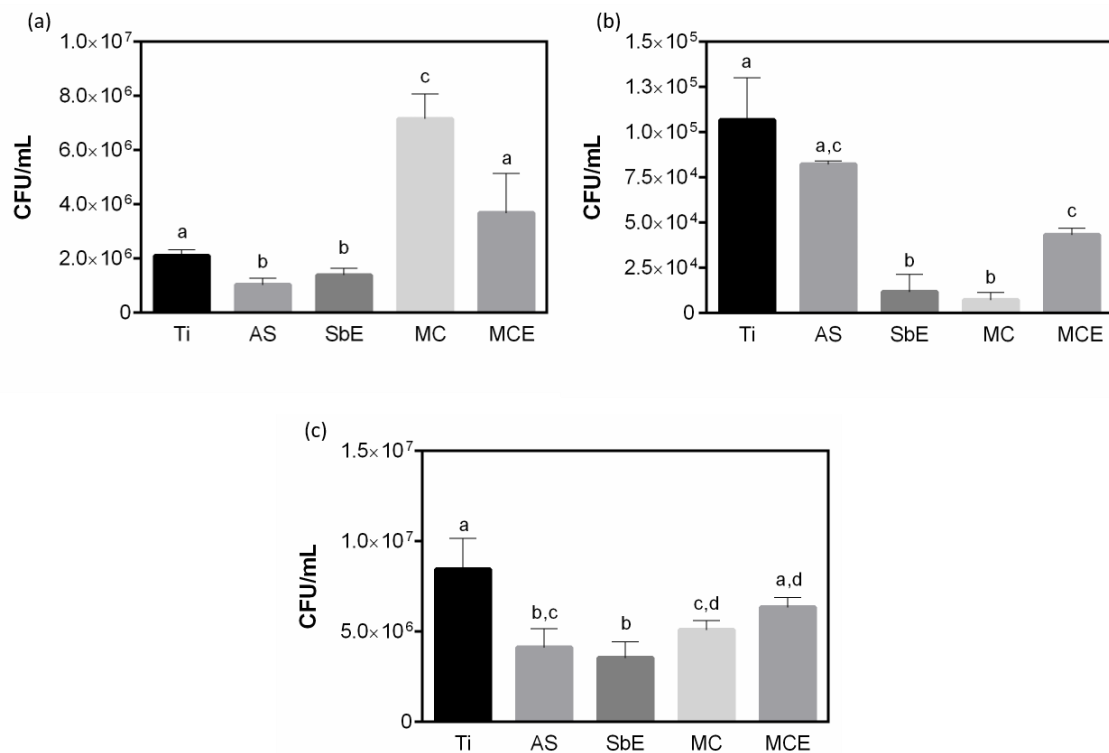


Figure 7. 6 Bacterial adhesion (CFU/mL) on the tested surfaces: (a) *Escherichia coli*; (b) *Staphylococcus aureus*; (c) *Pseudomonas aeruginosa*. The error bars represent the standard deviation. Different letters between distinct columns denote significant differences using one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

In addition, these results concurred with the findings of Chen, C. et al., where no correlation between surface roughness and *E. coli* bacterial adhesion was found (Chen et al., 2016). Figure 7. 6(b) shows that *S. aureus* presents a higher propensity to adhere to the titanium control group (Ti), being the results statistically different from all the other zirconia specimens, except AS. Contrarily to *E. coli*, the micro-channels walls did not favour adhesion of *S. aureus*. Moreover, the etching treatment (MCE) led to an increased bacterial adhesion when comparing to the MC specimen, eventually due to the increased surface roughness. Figure 7. 6(c) displays the *P.*

aeruginosa results in which, similarly to *S. aureus*, this bacterium seems to adhere more to the SLA Ti specimen when comparing to zirconia surfaces. However, no significant differences are observed between the control group and MCE. When comparing zirconia specimens with and without micro-channels it seems that *P. aeruginosa* shows a higher adherence to channelled surfaces, which may be related to factors inherent to the machining process or the increased exposed area.

Literature reports contradictory outcomes when discussing the influence of surface roughness on bacterial adhesion. While some studies found that there is a clear relationship between these two parameters (Medilanski et al., 2002; Arnold and Bailey, 2000), others did not observe such a correlation (Chen et al., 2016; Verran and Boyd, 2001). In fact, and according to the results found in the present study, no correlation can be established. This lack of consensus regarding the ideal surface roughness is observed in different implant parts, from the implant itself to the implant abutment. Pesce P. et al. in a systematic review mentioned that some authors are in favour of rougher abutment surfaces since they improve the creation of the connective tissue and reduce the epithelial down-growth into the bone-implant interface; on the other hand, others claim that smoother surfaces are expected to reduce plaque formation and, consequently, reduce the risk of periimplantitis (Pesce et al., 2019). Many factors can contribute to the different levels of bacterial adhesion apart from its surface roughness and material. Physiological differences between the tested bacteria, the surface physicochemical properties, and surface wettability are some examples (Cheng et al., 2019), and further studies should be carried out in order to better understand these results.

7.3.3.2. Co-culture of *S. aureus* and *P. aeruginosa*

S. aureus and *P. aeruginosa* are the most common bacteria present in chronic wounds and their virulence and surface proteins have been proved to affect wound healing, namely around dental implants. When these two bacteria co-exist in the same environment, their virulence and antibiotic resistance are even more pronounced, making the infection control a real challenge (Serra et al., 2015). Therefore, the assessment of the influence of the interactions between these two bacteria on the adhesion to the studied specimens is paramount. Figure 7. 7 displays the adhesion of *S. aureus* and *P. aeruginosa* separately and in co-culture. When comparing *S. aureus* adhesion to the different tested surfaces alone and in co-culture with *P. aeruginosa* (Figure 7. 7(a)) it is possible to conclude that when in co-culture this bacterium shows significantly higher levels of adhesion for all tested conditions, which indicates that the presence of *P. aeruginosa* stimulates

the growth and adhesion of *S. aureus* and eventually promoted biofilm formation. On the other hand, *P. aeruginosa* adhesion shows a slight decrease when this bacterium is in co-culture with *S. aureus* (Figure 7. 7(b)). These results suggest that the presence of *S. aureus* somehow inhibits the adhesion of *P. aeruginosa*, despite no significant differences were observed for the micro-channelled specimens (MC and MCE).

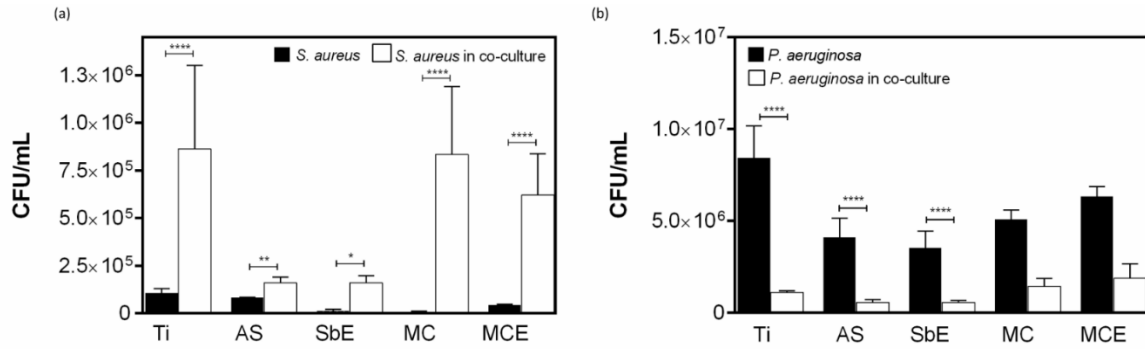


Figure 7. 7 Bacterial adhesion (CFU/mL) in co-culture: (a) *Staphylococcus aureus*; (b) *Pseudomonas aeruginosa*. The error bars represent the standard deviation and (*) indicates statistically significant differences ($p < 0.05$).

While there are several studies in the literature reporting the adhesion of these bacteria to implant surfaces inoculated separately, studies reporting the influence of their interaction on the adhesion to implants surfaces are scarce. In fact, most studies found in literature concerning the interactions between *S. aureus* and *P. aeruginosa* comprise studies regarding the biofilm physiology, the treatment of biofilm-related infectious diseases, and their susceptibility to antibiotics (Alves et al., 2018; Trizna et al., 2020). Alves P. and colleagues, for instance, assessed the interaction between *S. aureus* and *P. aeruginosa* in biofilm cultured for 24–72 h, and bacterial aggregates analogous to those observed in early biofilm formation, and interaction with human keratinocytes (Alves et al., 2018). Results showed that *S. aureus* predominated in biofilm and non-attached bacterial aggregates, acting as a pioneer for the attachment of *P. aeruginosa*. The *in vivo* and *in vitro* interactions between *P. aeruginosa* and *S. aureus* were analysed by Hotterbeekx A. and co-workers and, among other conclusions, authors found that *S. aureus* showed an increased virulence in the presence of *P. aeruginosa* (Hotterbeekx et al., 2017). Authors also state that certain proteins secreted by *S. aureus* inhibit the surface attachment of some *P. aeruginosa* clinical isolates.

Wijesinghe G. and colleagues evaluated the influence of the culture media on *in vitro*

growth, adhesion, and biofilm formation of *S. aureus* and *P. aeruginosa* (Wijesinghe et al., 2019). Results depict the huge impact of nutrient composition of the culture media on the biofilm and planktonic growth kinetics of the two bacteria, both in mono and co-culture. Furthermore, the same authors found that *P. aeruginosa* facilitates the microcolony formation of *S. aureus* and outcompetes *S. aureus* in co-culture biofilms. Yang L. and co-workers also evaluated the interactions between these two bacteria and concluded that the extracellular polymeric substances (EPS) secreted by the bacteria when in co-culture facilitates the interspecies interactions by the formation of mixed compact microcolony structures during biofilm formation (Yang et al., 2011). The authors also stated that when in co-culture *P. aeruginosa* is able to protect *S. aureus* from phagocytosis.

To better understand these interactions and the propensity for biofilm formation, specimens with adhered bacteria were inspected by scanning electron microscopy and results can be observed in the next section.

7.3.3.3. Scanning Electron Microscopy Analysis

In Figure 7. 8 it is possible to observe the SEM images of the adhered bacteria for two different specimens: Ti and MC. Figure 7. 8(a) and (b) correspond to *E. coli* adhered to Ti and MC, respectively. The surface topography of the Ti specimen makes it difficult to clearly observe the adhered bacteria. On the other hand, on the zirconia micro-channel, it is easily detectable a great amount of adhered *Escherichia coli*, both in the bottom and walls of the channel. These results are in accordance with the bacterial adhesion count (Figure 7. 6(a)), where this bacterium showed a high value of CFU/mL for this specimen condition.

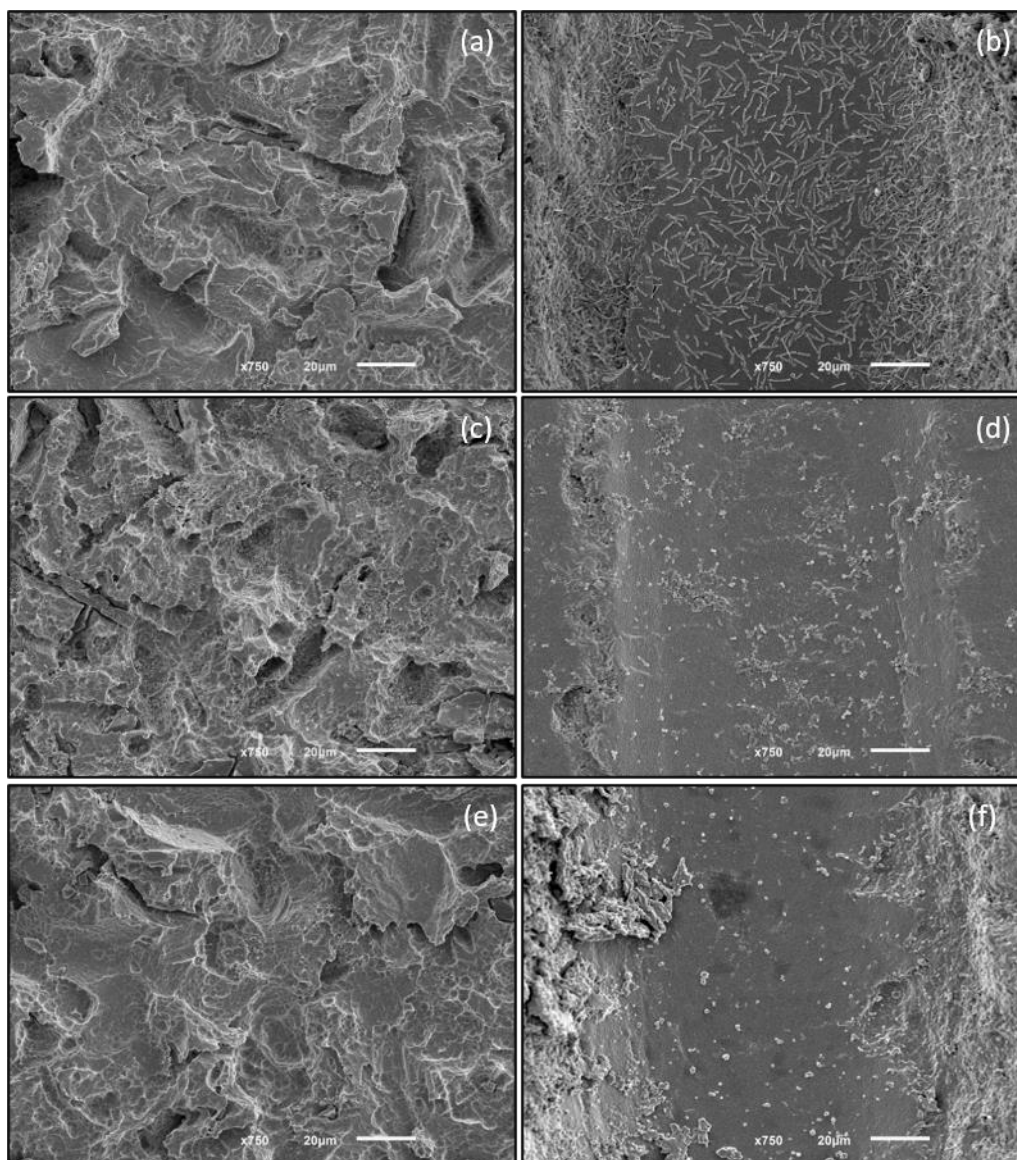


Figure 7. 8 Representative micrographs of the adhered bacteria: (a) and (b) *E. coli* on Ti and MC, respectively; (c) and (d) *S. aureus* on Ti and MC, respectively; (e) and (f) *P. aeruginosa* on Ti and MC, respectively.

As observed in Figure 7. 8(b), the irregular topography of the channel walls seems to facilitate *E. coli* adhesion. In Figure 7. 8(c) and (d), it is observed *S. aureus* adhered to the specimens. Again, it is perceivable the zirconia surface, due to its smoother nature. It is even possible to observe some biofilm formation, characterized by small groups of microorganisms that stick to each other.

Figure 7. 8(e) and (f), in turn, refer to the adhesion of *P. aeruginosa* to the tested surfaces. Despite being very difficult to detect this bacterium by SEM, after a careful analysis, it was possible to observe some bacteria in both specimens, with no significant differences between them. Authors believe that this type of bacterium adheres more to irregular surfaces and may, probably, be hidden

beneath the asperities of the Ti surface.

In Figure 7. 9 it is shown SEM micrographs of the adhered bacteria when in co-culture (*S. aureus* and *P. aeruginosa*) for the Ti specimen (Figure 7. 9(a)) and for the zirconia specimen with micro-channels – MC (Figure 7. 9(b)).

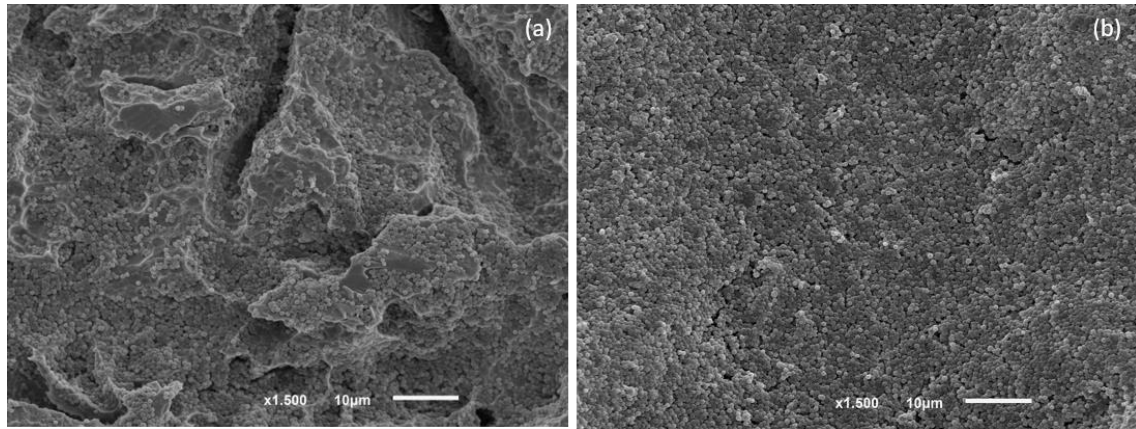


Figure 7. 9 Representative micrographs of the adhered bacteria in co-culture: (a) Ti; (b) MC.

As expected, and as observed in Figure 7. 7 and Figure 7. 9, *S. aureus* in co-culture revealed a considerably higher number of adhered bacteria, when comparing to the mono-culture. In fact, when analysing the previous images, it is possible to conclude that both surfaces are completely covered by this bacterium, which corroborates the findings of other authors mentioned before. There are several factors that may be on the basis of these results: the increased virulence of the *S. aureus* when in co-culture with *P. aeruginosa*, the presence of EPS, as well as the fact that *P. aeruginosa* may have facilitated the formation of *S. aureus* microcolonies. On the other hand, and despite being very difficult to detect *P. aeruginosa* in the previous micrographs, according to Figure 7. 7, the CFU/ mL of each bacterium in co-culture are very similar. This suggests that *P. aeruginosa* may have adhered to the surfaces and be hidden underneath *S. aureus*, making it difficult to detect them in the previous images.

7.4. Conclusions

Different zirconia surface architectures were successfully produced and tested regarding their bacterial adhesion propensity. Surface roughness and wettability of the zirconia specimens were also assessed. SLA titanium was used as control material, due to its common use in conventional dental implants. The present study supports several conclusions:

- All specimens presented a hydrophilic behaviour, in which an increased surface roughness (Ti, SbE and MCE) led to higher WCA, when comparing to smooth surfaces (AS and MC). After 10 s, WCA decreased substantially for all the tested conditions.

- *E. coli* exhibited a clear preference to adhere to the MC specimen. This behaviour seems to be related to the bacteria shape and charge, as well as the rough topography of the micro-channels walls. Nevertheless, changing the surface with an acid etching treatment considerably reduced *E. coli* adhesion. In addition, this bacterium is not very often found in the mouth environment and was used in this study mainly for comparison purposes, since it is a common model for Gram negative bacterium.

- *S. aureus* revealed a higher propensity to adhere to the SLA Ti specimen. Contrarily to *E. coli*, when a surface treatment is applied to the zirconia channelled specimen, *S. aureus* adhesion increased. This seems to indicate that *S. aureus* prefers rougher surfaces.

- *P. aeruginosa* exhibited an increased adhesion to the Ti control group. When comparing between different zirconia specimens, this bacterium adhered more to channelled surfaces (specially MCE), which may be related to the machining process and surface roughness.

- When in co-culture, the presence of *P. aeruginosa* seems to stimulate the adhesion of *S. aureus* and eventually promote biofilm formation. This is evidenced by the significant increase of bacterial adhesion for all tested conditions (in comparison with the mono-culture). On the other hand, *S. aureus* seems to prevent an extensive adhesion of *P. aeruginosa*.

- Channelled zirconia surfaces did not reveal particular better performance regarding the bacteria adhesion when comparing to the other tested specimens. However, these structures are expected to promote an improved vascularization around the implants and consequent enhanced osseointegration, evidenced by the capillarity analysis. Therefore, combining this effect with the quite satisfactory results found in this research work, channelled surfaces seem to be a promising alternative for dental implants applications.

Given the results obtained in the scope of this study, it is clear that the adhesion of bacteria to a specific surface is a multifactorial mechanism. The interactions between the bacteria and the surface seem to be influenced by the bacteria present, the surface material and roughness, as well as the environment around them, namely the presence or not of other bacteria. This is the major limitation of the present study: no correlation can be established between the adhesion of the

different tested bacteria on the developed surfaces. Future studies will comprise 3D dental implants and bacterial communities to assess their efficacy to mitigate bacterial adhesion.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Author statement

Telma Dantas: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization, Funding acquisition.

Jorge Padrão: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing, Visualization.

Mariana Rodrigues da Silva: Methodology, Investigation, Writing - Review & Editing. Paulo Pinto: Conceptualization, Methodology, Investigation, Writing - Review & Editing.

Sara Madeira: Methodology, Investigation, Writing - Review & Editing.

Paula Vaz: Supervision.

Andrea Zille: Conceptualization, Methodology, Validation, Writing - Review & Editing, Visualization, Supervision.

Filipe Silva: Conceptualization, Methodology, Validation, Writing - Review & Editing, Visualization, Supervision Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 8

Customized root-analogue dental implants - Procedure and errors associated with image acquisition and treatment, and manufacturing technology in an experimental study on a cadaver dog mandible

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Abstract

The global market for dental implants is constantly evolving. To overcome the limitations of conventional strategies, new approaches, namely the development of CAD/CAM customized implants, are emerging as promising solutions for replacing missing teeth, thus avoiding destruction of bone by drilling it. Despite the efforts that are being carried out, the designing, including image acquisition, and manufacturing efficacy of such rehabilitation therapy is not easily found in the literature. In this sense, this research work aimed to assess the errors associated with the image acquisition, treatment and manufacturing of Customized Root-Analogue Implants (CRAI). The experimental procedure, carried out in a dog cadaveric mandible, encompassed the image acquisition and treatment and the subsequent manufacturing of four different CRAI. Two Image acquisition methods (CBCT and X-ray), and different software for their treatment were used, and the final CRAI were manufactured by CNC.

Results revealed the CBCT scan allowed to properly visualize the original teeth contours. However, all the obtained teeth geometries presented smaller surface areas when compared to the original teeth indicating however a systematic predictable error. On the other hand, when comparing the surface area of the digital and the manufactured teeth, very small differences were observed, which indicates that the CNC manufacturing technique is a promising solution for such applications. When placing the produced CRAI in the dog mandible it was possible to observe a lack of congruency between the CRAI and bone, mainly due to image acquisition process errors and the destruction of the periodontal ligament during the soft tissues removal process. Thus, CBCT images seem to be a feasible route to design root-analogue implants, and, if needed, a compensation strategy for the systematic image acquisition error may be implemented.

Keywords: Customized Root-Analogue Implant (CRAI); CAD/CAM; Cone-Beam Computed Tomography; Zirconia.

8.1. Introduction

Dental implants are the gold standard treatment for the rehabilitation of missing teeth [1]. The success or failure of a dental implant is extremely related to the preservation of soft and hard tissues around it, which, in turn, may be influenced by factors such as the clinician's experience, the surgical protocol, loading time, the insertion torque, the presence of oral biofilm, among many

others [2–5]. On the other hand, during mastication, teeth perform a series of tensile and compression forces that are transmitted to the surrounding bone [6]. These forces are necessary for the maintenance of the shape and density of the bone, and, when a tooth is missing for a long time, the available bone density and volume may not be enough to properly anchor the implant and consequently compromise its performance [7]. In addition, it is known that increasing age is associated with a progressive loss of mandibular bone, which will also play an important role in the achievement of the implant's imprisonment [8]. To overcome this lack of supporting bone, some strategies have been used, namely the placement of short implants or bone grafts [9]. However, while short implants do not seem to present any better outcomes when compared to standard ones, bone grafts are associated with an increased postoperative morbidity, higher costs, and higher risk of complications [9,10]. Additionally, some limitations are associated with conventional dental implants, namely the invasiveness of the surgical procedure, due to the need for bone drilling and the crestal bone loss around the implant [10]. One study performed by Sravani U., et al [11] reports crestal bone loss of about 2.23 to 2.75 mm after 12 months of implant insertion. In another study, authors found an average peri-implant bone loss at 3 years of follow-up of approximately 1.02 mm [12]. The latter believes that this bone loss, in turn, facilitates the plaque accumulation in the implant surrounding bone, leading to peri-implantitis and induces the implant exposure with consequent aesthetical problems [13].

In this sense, aiming to find better solutions for the replacement of a missing tooth, new strategies and techniques have been developed.

Customizing dental implants according to each patient's oral condition has been investigated as a promising solution to overcome the limitations of conventional implants, namely the incongruency between the implant and the socket wall [14]. Root-Analogue Implants (RAI) are designed to fit in the extraction socket instead of adapting the bone to a standardized screw-shaped implant [15]. There are not many studies reporting the clinical outcomes of this type of dental restoration yet [16] but some advantages may be enumerated. Root-analogue implants are associated with a minimally invasive surgical procedure which is expected to reduce the bone and soft-tissue trauma and consequently induce an improved implant primary stability [17,18]. By mimicking the geometry of the original teeth, RAI will re-establish the physiological peri-implant conditions, occlusal loading, and aesthetics and promote a proper stress distribution in the alveolar bone, thus avoiding implant surrounding bone bone loss and their consequences as plaque accumulation, peri-implantitis, and aesthetical issues [19].

The constant technological advances, namely in digital technology have led to the development of novel treatment modalities in the dentistry field [20]. CAD/CAM (Computer-Aided Design/ Computer-Aided Manufacturing) has been applied for the production of different implant components, such as abutments and crowns but also the production of surgical guide templates for guided implant placement [21]. Medical computed tomography (CT) has been used to assess and measure the mandibular anatomy with high accuracy [22]. However, this technique is associated with high x-ray doses and high costs, which led to the introduction of low-dose and low-cost cone-beam CT (CBCT). This technology allows the visualisation of structures from different angles thus enabling a more accurate evaluation of the architecture and dimensions of bone [22,23]. It also allows a more clear visualisation of the contour and density of the bone, the cortex, the trabecular pattern, and the surrounding anatomical structures, namely the periodontal ligament, when compared to conventional imaging [23].

Combining the CBCT scan technology with CAD/CAM has been investigated to design and produce customized root-analogue implants [21]. Moin D., et al [21] created a single-rooted titanium RAI by the combination of CBCT data acquisition with CAD/CAM technology. Despite lacking clinical evidence, the authors concluded that this approach is very feasible to effectively create customized titanium RAI. Evans Z., et al [24] also developed a protocol for analysing and developing customized root-analogue implants from the patients' cone-beam data. The implants were placed at the time of extraction and were later radiographically and mechanically evaluated, to assess their fit and stability. The results of this study represent a proof of concept that RAI can be produced from cone-beam data and are expected to improve implant fit, safety, aesthetics, and restorability when comparing to conventional protocols. Ludlow J., et al [25] aimed to assess the accuracy of 3D measurements of the mandibular anatomy through the analysis of CBCT scans of multiple positions skulls. Results revealed that the skull orientation influenced the CBCT volumes. Average measurement errors between 0.2 and 2.1 mm were obtained, being in line with the literature regarding errors of both conventional and cone-beam CT. Additionally, operator experience is also expected to influence measurement accuracy and reproducibility.

CNC (Computer Numerical Control) has been applied in many sectors such as mold making, laser cutting, 3D printing, milling, and drilling, for different types of industry [26]. By adjusting parameters such as feed, depth of cut, and angulation, this technique allows the production of very complex customer-specific products and eliminates intermediate time-consuming steps, such as the manufacturing of molds [27]. In this technique, the models produced

by the CAD software are converted into milling strips for the CAM-processing and then loaded into the milling device [28]. As far as the dentistry field is concerned, CNC milling technique has been used in a wide range of products, that go from complete dentures, crowns, implant bridges, abutments among many others [27,29,30]. Customized root-analogue implants have also already been fabricated by this manufacturing technique [16]. Pirker W., et al [31,32] designed, milled, and clinically tested zirconia root-analogue implants. Results revealed high success rates, with no signs of periodontitis, bone resorption nor bleeding. Additionally, excellent aesthetic outcomes are reported. Patankar A., et al [33] also fabricated root-analogue implants by CNC milling, and good functional and aesthetic results were achieved.

Despite its increasing interest, the errors associated with the overall process of customizing root-analogue implants by CAD/CAM are not commonly reported in the literature. In this sense, in this study, the authors aimed to analyse the procedure and errors associated with the image acquisition and treatment, and manufacturing of customized root-analogue dental implants. A cadaveric dog mandible was used and the errors inherent to the CBCT scan, the implant's design, and the CNC manufacturing process were evaluated.

8.2. Materials and Methods

8.2.1. Study Design

To evaluate the errors associated with the image acquisition, treatment and manufacturing of root-analogue dental implants, a cadaveric dog skull was used. The first step of this study was the extraction of one incisor tooth, hereafter referred to as In1. After tooth extraction a CBCT- CBCT1- was performed in the dog head, followed by the removal of its soft tissues. Two molar and one incisor teeth were then extracted (M1, M2, and In2, respectively), and another CBCT – CBCT2- scan was performed. By comparing CBCT1 and CBCT2 authors aimed to assess the influence of the soft tissues in image acquisition. Based on the CBCT2, the geometry of the extracted teeth was assessed resulting in three different STL files (STL files A) to be later manufactured. The extracted teeth were also assessed by means of a 3D scanner to obtain their geometry (STL files B) and results were compared with the teeth designed based on the CBCT (STL files A). These last files were then milled by CNC and the final products were compared with the real extracted teeth. In addition, an implant was designed and fabricated based on the In1 socket geometry. The four final products were manufactured by CNC were then inserted in the dog mandible. The fit of the teeth to the dog mandible was evaluated by means of X-ray.

For a better understanding, Figure 8.1 schematically represents the performed steps.

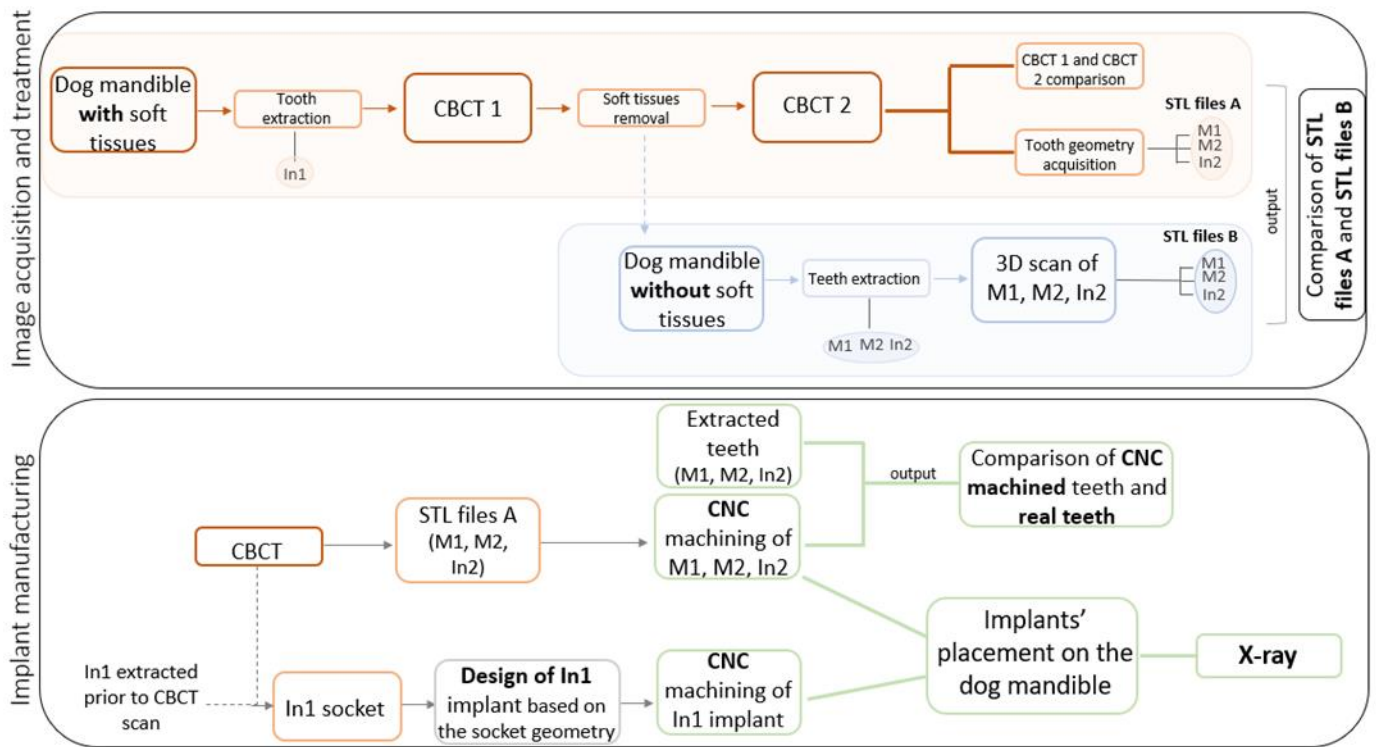


Figure 8.1 Flowchart representing the performed procedure.

In the following sections of this manuscript, detailed information regarding the overall process is depicted.

8.2.2. Image acquisition

Dental CBCT scans were acquired using Planmeca ProMax 3D equipment (Planmeca USA, Inc.), in Centro Médico Dentário (Porto, Portugal), at two different moments, as previously mentioned. The dog skull was positioned on a flat horizontal platform in the CBCT device and images were acquired at a tube voltage of 90 kV, a tube current of 6.3 mA, and an exposure cycle of 12 seconds. The number of basis images acquired in each scan was 401, and the reconstruction isotropic voxel size was 0.2 mm. The CBCT data set for each performed scan was exported in digital imaging and communication in medicine (DICOM) format.

For the acquisition of the geometry of the original extracted teeth (M1, M2, and In2), a 3D extraoral scanner was used – Shining 3D DS300 Autoscan3B (Shining 3D Tech Co., Ltd.). This equipment is characterized by an accuracy of < 15 micron and the acquired data was extracted in STL format – STL files B (Figure 8.1). To evaluate the congruency between the produced teeth and the dog mandible bone, a X-ray system (53Kv; 12.5 mAs) - Intech's Veterinary Digital DR

System- was used and the distances between the teeth and bone were measured in RadiAnt (RadiAnt DICOM Viewer 2020.2, USA).

8.2.3. Soft Tissues Removal

For the removal of the skull soft tissues, an empiric method was used. The dog skull was placed in a pressure cooker and boiled in hydrogen peroxide for about 4 hours. Then, the remaining tissues were carefully removed with the help of a sharp paring knife and a scalpel. This procedure was carried out in Hospital Veterinário do Bom Jesus (Braga, Portugal).

8.2.4. Image Treatment

The DICOM volumes exported from the CBCT scan were then imported into image analysis software RadiAnt (RadiAnt DICOM Viewer 2020.2, USA), to create 3D surface models of the teeth. For the segmentation of the teeth, a region of interest limited to the teeth and surrounding tissues was selected. To separate the tooth from the surrounding tissues a threshold value based on the histogram analysis, the local grey level value, and image gradient was selected. The resulting STL files were subsequently imported into Materialise Magics (Materialise NV, Belgium) to remove artefacts and imperfections of the geometries.

On the other hand, for the development of In1, the tooth that was designed based on the socket geometry, after segmenting the region of interest in RadiAnt, Autodesk Meshmixer was used to manipulate the 3D mesh to reconstruct the socket contours. Materialise Magics was used to invert the obtained mesh and also to smoothen the surface.

8.2.5. Implant Manufacturing

After the image acquisition and treatment of the selected teeth, hyperDENT® (FOLLOW-ME! Technology, Germany) was used for the definition of the milling strategy, and the resulting protocol was subsequently uploaded in the CAD/CAM milling machine - CORiTEC 250i (imes-icore®, Germany). The parameters used in the milling process are described in Table 8.1.

Table 8.1 Milling parameters.

Tool	Process	X & Y direction cutting speed (mm/min)	Z direction cutting speed (mm/min)	Spindle speed (rpm)	Path Interval (mm)	Finish margin (mm)
R1.25 ball	Rough cutting	1200	600	19000	0.5	0.1
R0.5 ball	Finish cutting	800	400	22000	0.05	-

This process was performed in a zirconia green compact disc (High Strength Zirconia – 3Y-TZP, Prettau Zirkon 98H10), with a scaling factor of 1.25 from Zirkonzahn, Italy. After this green machining process, the resulting models were sintered at 1500 °C, with a heating/cooling rate of 8 °C/min and a holding time of 2 h, in a specific furnace (Zirkonofen 700 Ultra-Vakuum from Zirkonzahn - Zirkonofen).

8.2.6. Image Analysis

For the analysis of the results, different software were used.

Aiming to evaluate the CBCT scan acquisition errors, a comparison between the STL file resulting from the CBCT scan of the original teeth (placed in the mandible) – STL files A- and the 3D scanner of the teeth after extraction – STL files B- was performed, in CloudCompare software. This software was also used to assess the milling process errors, by comparing the 3D scanner of the machined teeth with the designed ones.

On the other hand, RadiAnt (RadiAnt DICOM Viewer 2020.2, USA), software was used to compare the scans before and after soft tissues removal (CBCT1 and CBCT2), to segment regions of interest in the image treatment process and to measure the distances between the produced teeth and the bone, when placed in the mandible.

8.3. Results and Discussion

8.3.1. Soft Tissues Influence on Image Quality

After CBCT1 and 2 scans acquisition, DICOM data was imported in RadiAnt and 3D surface models were developed, mimicking the anatomy of the dog skull. In Figure 8.2(a) it is depicted the model representing the skull before soft tissues removal (CBCT1), whereas in Figure 8.2(b) and

Figure 8.2(c) it is possible to observe the dog maxilla and mandible after soft tissues removal, respectively (CBCT2).

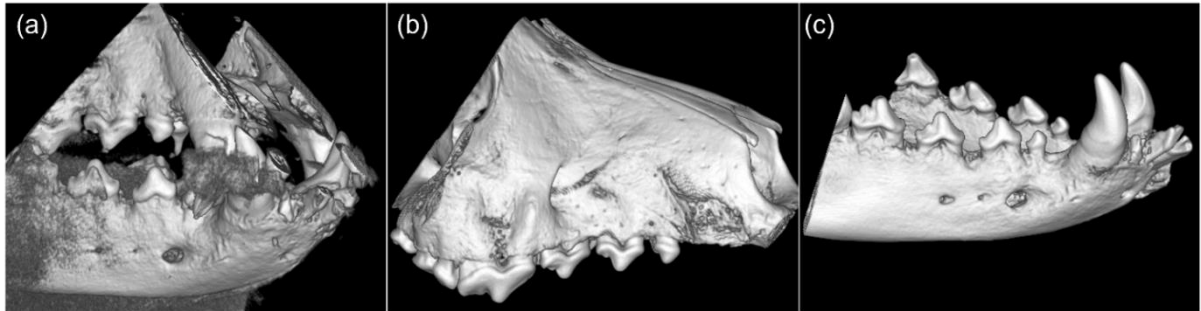


Figure 8.2 3D reconstructions of the Cone-Beam Computed Tomographies: (a) dog skull with soft tissues- CBCT1; (b) dog maxilla after soft tissues removal- CBCT2; and (c) dog mandible after soft tissues removal- CBCT2.

By comparing Figure 8.2(a) with Figure 8.2(b) and Figure 8.2(c) it is possible to conclude that the first one is characterized by higher image unsharpness due to the presence of the soft tissues. The teeth and bone contours are much more evident in CBCT2, which makes it easier to extract tooth geometries. However, and by applying appropriate thresholds, authors were able to remove the influence of the soft tissues on image quality and accurate 3D models of the bone and teeth were also achievable with this CBCT scan. This indicates that the presence of soft tissues does not compromise the developed strategy for tooth geometry acquisition.

8.3.2. Implant Development

Four teeth were selected for segmentation, image treatment, and CNC manufacturing in the scope of this research work. The selected teeth were one upper molar, one lower molar, and two lower incisors (Figure 8.3). Incisor 1 (In1) was extracted before CBCT scan acquisition, and its design was performed based on the remaining tooth socket. The designing of the two molars (M1 and M2) and the second incisor (In2) was based on the 3D model acquired from the CBCT scan.

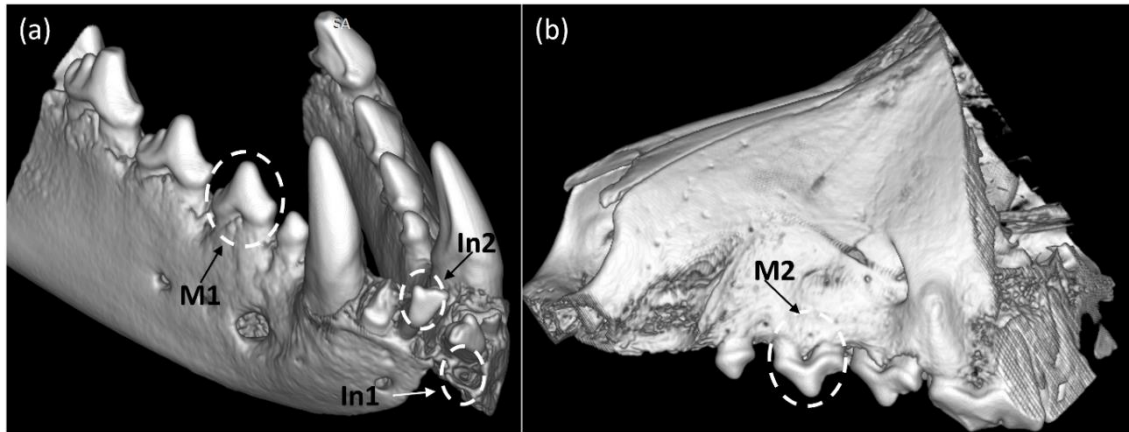


Figure 8.3 Selected teeth: (a) selected teeth from the mandible; and (b) selected tooth from the maxilla.

In Figure 8.4 it is possible to observe the representative strategy used to segment and extract M1 geometry. The same strategy was followed for the extraction of M2 and In2 geometries. In Figure 8.4(a) it is represented the dog mandible after application of some filters that allow to properly visualize the tooth contours. Then, a region of interest was defined, encompassing the select tooth and some adjacent tissues (Figure 8.4(b)). After some adjustments and image manipulation, it was possible to extract an approximate geometry of the selected tooth (Figure 8.4(c)).

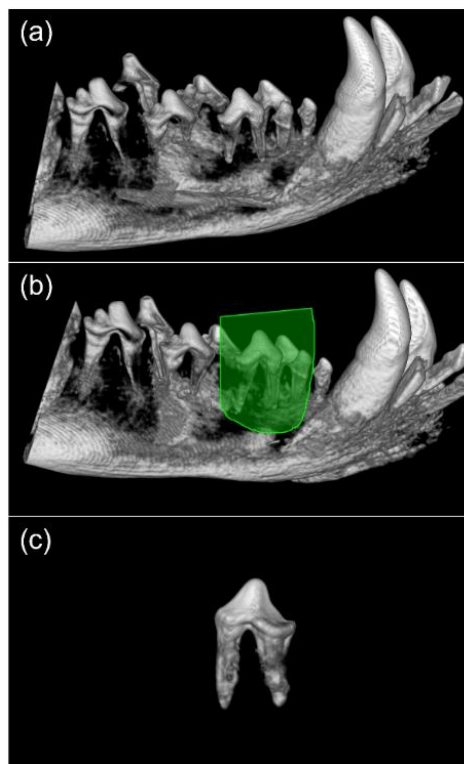


Figure 8.4 M1 tooth segmentation in *RadiAnt*. (a) application of a threshold based on the image grey levels; (b) selection of the region of interest; and (c) M1 model resulting from the software.

The model obtained in the previous step was then imported into *Materialise Magics* as an STL file for further surface improvements, represented in Figure 8.5(a).

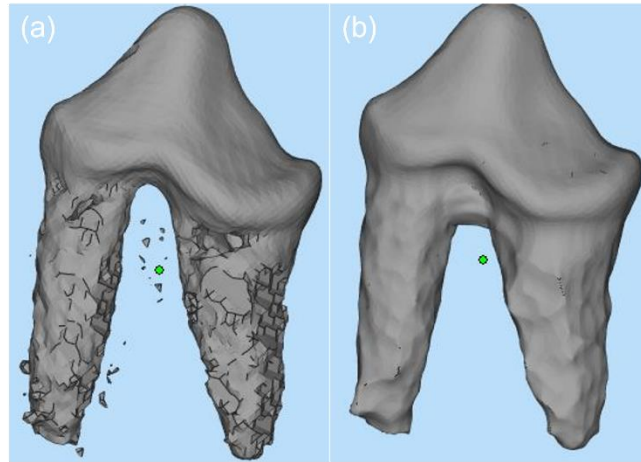


Figure 8.5 M1 treatment in *Materialise Magics*: (a) tooth before artefacts removal; and (b) tooth after artefacts removal and surface smoothing.

This software was used to remove some design inaccuracies and artefacts, and also to smoothen the tooth surface, as can be seen in Figure 8.5(b). As previously mentioned, the process described above was performed for M1, M2, and In2. The resulting models represent the STL files A (see Figure 8.1) and were then manufactured by CNC.

As far as the treatment of In1 is concerned, as previously mentioned, it was developed based on the socket geometry.

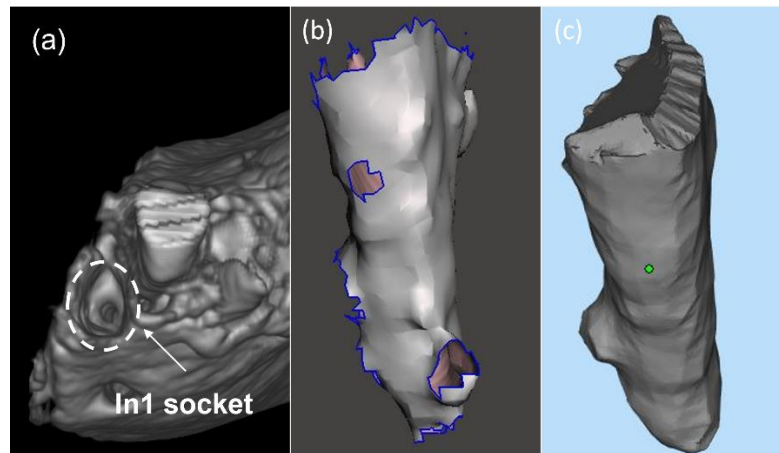


Figure 8.6 Designing In1 based on the socket geometry: (a) region of interest segmentation; (b) 3D mesh creation in *AutoDesk Meshmixer*, and (c) final model.

After segmenting the region of interest in RadiAnt (Figure 8.6(a)), the STL file was imported into AutoDesk Meshmixer (Figure 8.6(b)) to create a 3D mesh of the socket hole. Materialise Magics was then used to invert the mesh. Finally, AutoDesk Meshmixer was used again to create a solid body that would correspond to the tooth geometry, as seen in Figure 8.6(c).

8.3.3. Geometric Differences and Errors

One of the main purposes of this research work was to assess the geometric errors associated with the image acquisition and treatment of a root-analogue implant. The errors inherent to the image treatment may be somehow dependent on the accuracy of the software, as well as the operator, not discriminated in this study. To assess these errors, the teeth geometries resulting from the CBCT scans (STL files A) were compared to the 3D scans of the original teeth after extraction (STL files B) in *CloudCompare* software. This process was performed for the three teeth referenced above: M1, M2, and I_n2. The two STL files of each tooth were imported into the software simultaneously and were then superimposed, as seen in Figure 8.7. The green translucent geometry corresponds to the original tooth 3D scan whereas the orange one corresponds to the one extracted from the CBCT scan. The green solid parts correspond to the superimposition of the two teeth.

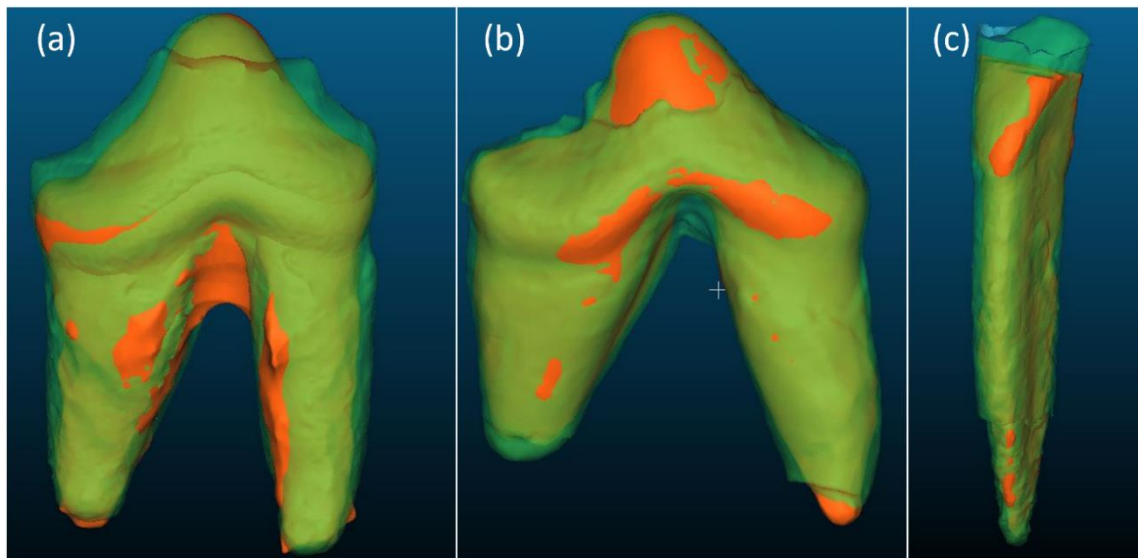


Figure 8.7 Superimposition of the SLT files resulting from the 3D scan (green) and CBCT scan (orange): (a) M1; (b) M2; and (c) I_n2.

When observing Figure 8.7, it is possible to conclude that the developed teeth have less material than the original ones, evidenced by the higher representativity of the green translucent color when compared to the orange one, for all the geometries. This may indicate that the drawn geometries are smaller than the original teeth, which may be related to the errors inherent to the CBCT scan acquisition [34] and will be later further explored. In addition, it is also possible to observe some specific areas where the developed geometry presents more material than the original tooth. After teeth superimposition, the distances between the two geometries were

calculated by the software, and the corresponding errors were determined. The 3D scan of the original tooth was considered the reference surface and therefore, a positive value corresponds to a decrease in surface for the CRAI and vice versa.

Figure 8.8 displays the deviations, in millimetres, obtained after superimposing the two surfaces of each tooth. The M1 tooth (Figure 8.8(a)) presents a maximum positive deviation of 0.73 mm and a maximum negative deviation of 0.51 mm. M2 (Figure 8.8(b)), in turn, exhibits a maximum positive and negative deviations of 0.34 and 0.76 mm, respectively. Finally, In2 presents a maximum positive deviation of 0.20 mm and a maximum negative deviation of 0.80 mm.

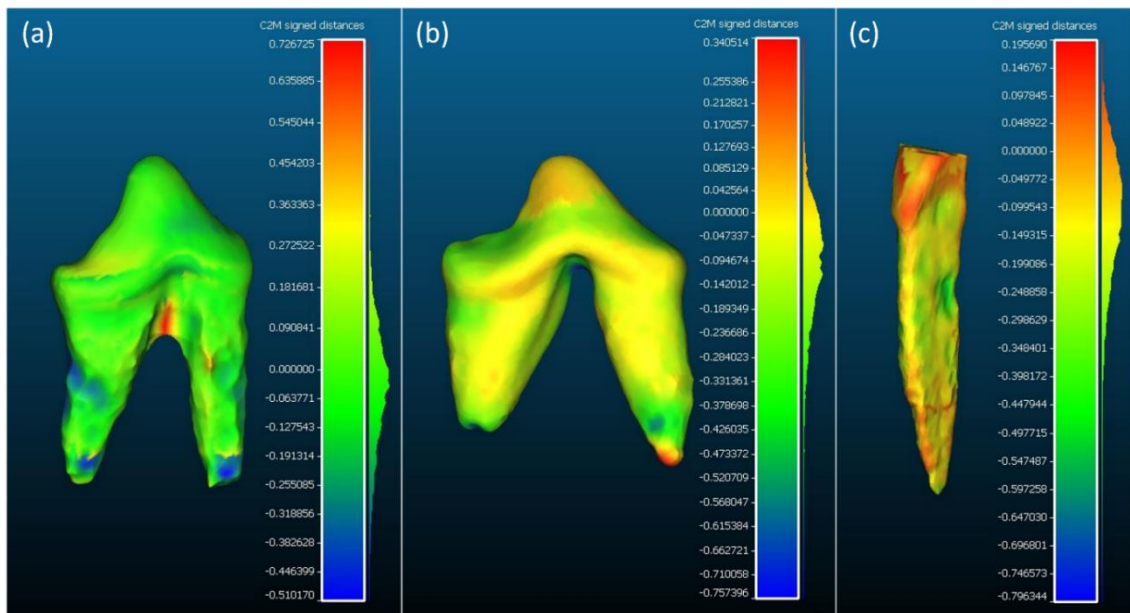


Figure 8.8 Deviations of the superimposed surfaces (measurement in millimetres): (a) M1; (b) M2; and (c) In2.

Despite being quite high deviations, as seen in Figure 8.8, these values correspond to punctual sites (represented in red or blue) that may be a result of some limitations in the image treatment process, and their representativeness may be neglected. In fact, given the color gradient present in the three figures it is denotable that most of the geometry surface has deviations close to 0 mm.

For a better understanding, in Table 8.2 it is possible to observe the deviations, in percentage, of the treated geometries when compared to the original teeth. As previously mentioned, negative deviations mean that the developed teeth have less material than the original ones, whereas positive deviations indicate an excess of material. The three treated surfaces present higher negative deviations than positive ones which indicate that, in general, they have less material than the original ones.

Table 8.2 Deviations between the two compared surfaces - 3D scan of the original tooth and developed tooth.

Tooth	Negative deviations (%)	Positive deviations (%)	Deviations between -0.2 and 0.2 mm (%)	Deviations between -0.1 and 0.1 mm (%)
M1	59.47	40.53	78.22	45.10
M2	82.61	17.39	75.53	46.51
In2	85.78	14.22	76.29	45.09

Literature reports that the success of dental implants is determined by the biomechanical quality of bone tissue located at a distance smaller than 0.2 mm from the implant surface and, therefore, deviations between -0.2 mm and 0.2 mm were considered acceptable by the authors [35]. In addition, it is stated in the literature that, to prevent soft-tissue ingrowth, gaps between the bone and implant over 0.5-1 mm should be avoided. For gaps smaller than these values, osseointegration is expected to occur [36]. From Table 8.2, it is possible to conclude that the three developed teeth exhibit approximately 75-80% of their geometry with deviations below 0.2 mm. Additionally, percentages of deviations between -0.1 mm and 0.1 mm were also determined. Despite all the geometries presenting almost 50% of their geometries with deviations between -0.1 and 0.1 mm, authors believe that these values are quite low and higher percentages should be achieved in order to produce even more realistic teeth. There are many factors that may be inducing these differences between the developed and the original teeth, namely the 3D scanner and CBCT equipment accuracies, and also the operator learning curve.

In Table 8.3 it is possible to observe the surface areas of both the developed and the original teeth. The surface areas of the developed teeth are smaller than the original ones, for the three teeth, which corroborates the previous results. The developed M1 presented a surface area change of -10.40 % when compared with the original tooth, M2 exhibited a difference of -8.36% and In2 was the one with the highest surface area change (-16.62%).

Table 8.3 Comparison of surface area change between the 3D scan of the original teeth and the 3D surface models developed from the CBCT.

Tooth	3D scan original tooth (mm²)	CBCT design (mm²)	Surface area change (%)
M1	286.223	256.448	-10.40
M2	304.115	278.680	-8.36
In2	200.458	167.132	-16.62

These results indicate that the developed geometries are smaller than the original ones. Apart from the influence of the operator and the errors inherent to the image treatment software,

the image acquisition process may also be leading to such differences. There are some studies in the literature reporting the accuracy and reliability of CBCT for dental applications. Behnia H., et al [34] measured the thickness of the maxillary bone on a CBCT scan and compared these measurements with direct clinical measurements. Mean relative errors of 0.60 ± 0.84 mm were observed. In another study, a maximum mean measurements error of 1.27 ± 1.43 mm was reported [37]. Baumgaertel S., et al [38] also investigated the reliability and accuracy of dental measurements made on CBCT scan reconstructions and concluded that the CBCT measurements tended to slightly underestimate the anatomic truth.

Despite these differences between the developed and the original teeth, the treated surfaces were fabricated by CNC, aiming to assess the errors associated with the manufacturing process. The produced models were then 3D scanned in order to compare them with the digital models, in CloudCompare. In Table 8.4 it is possible to observe the obtained surface area changes. This comparison was performed for the three aforementioned teeth and also for the In2, the tooth developed based on the socket geometry. By analysing the values in the following table, the authors are able to conclude that the milling of the teeth is a quite feasible process. In fact, when comparing the manufactured teeth with the digital ones, all the geometries presented surface area changes below 2%. These values are in accordance with literature that reports this manufacturing technique as a very accurate and precise technology for the fabrication of dental components [39,40].

Table 8.4 Comparison of surface area change between the 3D digital surface models and the fabricated teeth.

Tooth	Digital models (mm²)	Manufactured tooth (mm²)	Surface area change (%)
M1	256.448	255.182	-0.49
M2	278.680	274.506	-1.49
In1	63.665	62.708	-1.50
In2	167.132	170.233	+1.86

In Figure 8.9 it is possible to observe the original and the manufactured teeth. When performing the original In1 tooth extraction, it was not possible to preserve its integrity and therefore it was not considered in this work. However, since the goal was to develop this tooth based on the socket geometry it didn't interfere with the results.



Figure 8.9 Original vs. manufactured teeth: (a) M1; (b) M2; (c) In1; and (d) In2.

From Figure 8.9 it is easy to observe that the manufactured geometries are slightly smaller than the original ones, which may be related to the image acquisition and treatment errors previously mentioned. Apart from the overall size, there are also some features that differ from the original teeth. In Figure 8.9(a) and Figure 8.9(b), which correspond to M1 and M2, respectively, it is denotable that the narrow gap between the two roots of the manufactured teeth is slightly different from the original one. This difference is again related to the image treatment process since the available software made it difficult to properly mimic these specific narrow features. As far as In1 is concerned (Figure 8.9(c)), since it was developed based on the tooth socket, it does not encompass the crown, it only encompasses the tooth root. Finally, in Figure 8.9(d), which corresponds to In2, it is possible to observe that the upper part of the crown is not perfectly mimicking the original tooth, probably due to its sharp appearance. These results are in accordance with Table 8.3, where this tooth exhibits the highest surface area change, of about 16.62%.

To validate this research study, the produced models were placed in the dog mandible and X-rays were performed to assess their fit into the teeth sockets. Results can be seen in the following section.

8.3.4. Teeth Placement and Final Validation

In Figure 8.10 it is possible to observe the X-ray scans of the manufactured teeth when placed in the dog mandible.

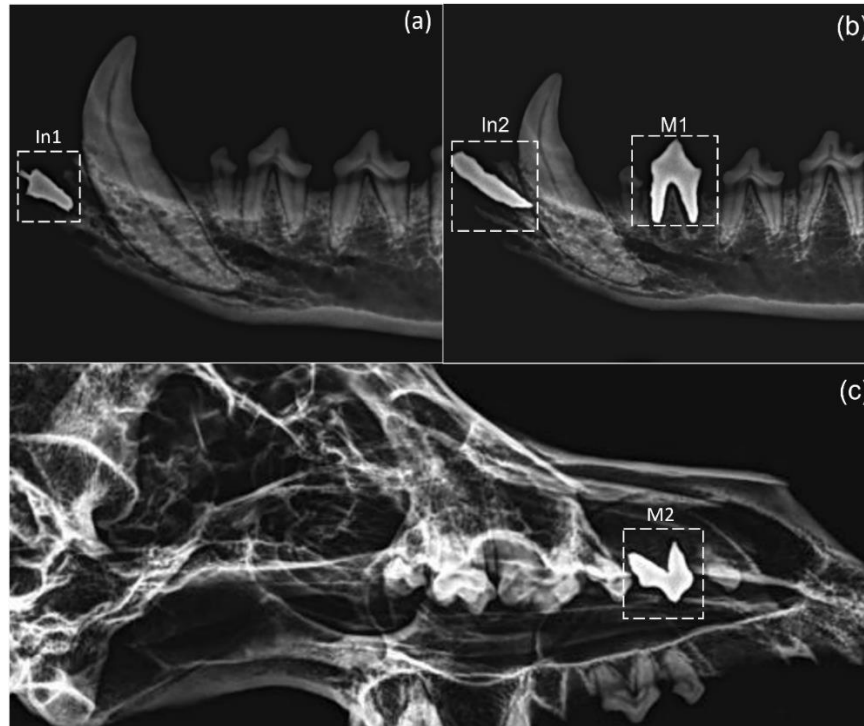


Figure 8.10 X-ray scans after teeth placement: (a) In1; (b) In2 and M1; and (c) M2.

After placing the teeth in the respective socket, it was verified that the congruency between them and the bone was not successfully achieved, since none of them presented proper imprisonment. In fact, these results were expected since, as previously mentioned, the manufactured teeth exhibit smaller surface areas when compared with the original ones. Additionally, when removing the soft tissues, the periodontal ligament (PDL) was also removed and, a gap of about 0.15-0.30 mm was created, increasing the socket dimensions [41–43].

In Figure 8.11 it is possible to observe the measured distances between the manufactured teeth and the bone, according to the RadiAnt software.

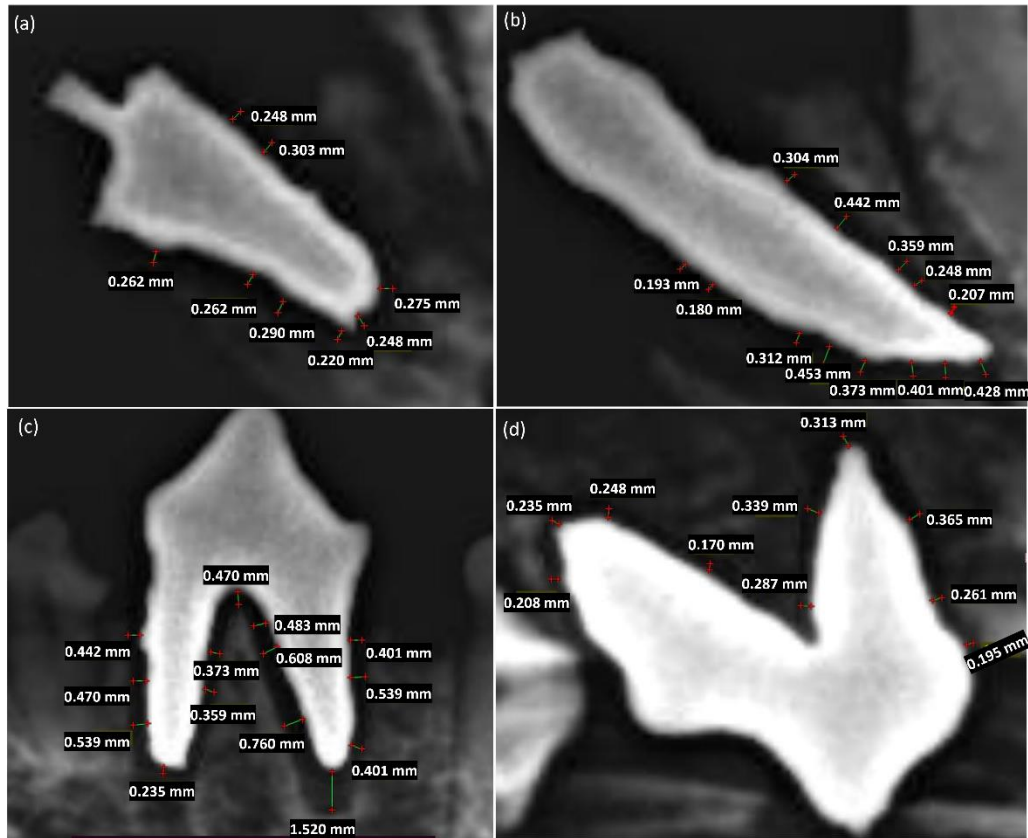


Figure 8.11 Distances between the manufactured tooth and the bone, measured in *RadiAnt*: (a) In1; (b) In2; (c) M1; and (d) M2.

The distances between the tooth and the bone varied along the tooth geometry and values between 0.170 and 1.520 mm were obtained. These values correspond to both the PDL thickness (that was removed in the soft tissues removal process) and also to the errors inherent to the image acquisition and treatment processes. These values also allow corroborating the results presented in Figure 8.8, regarding the difficulties in mimicking some specific features, namely the narrow cavity between the two roots of M1 (Figure 8.11(c)).

Some distances between the original teeth and bone were also measured in order to better understand the influence of the soft tissues' removal process. In the following figure (Figure 8.12) two original teeth and the respective measured distances are represented.

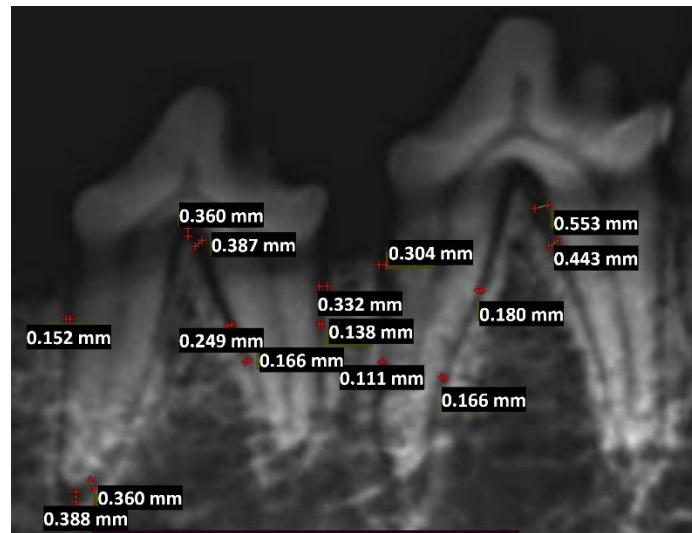


Figure 8.12 Distances between the original tooth and bone, measured in *RadiAnt*.

As can be seen, a clear gap between the teeth and bone is also observed in the original teeth, which indicates that the soft tissues removal process led to the destruction of the PDL, and consequently induced this gap between the teeth and bone. This indicates that, in a real situation, where there is no destruction of the PDL, the distances measured in Figure 8.11 would only result from the image acquisition and treatment processes and consequently be much smaller.

8.4. Conclusions

Within the limitations of this study, some conclusions can be drawn:

- The soft tissues around the bone have a negligible interference with the CBCT scan image analysis. It is possible to successfully develop the intended teeth based on these data.
- The developed teeth are slightly smaller than the original ones. Surface area changes ranging from 8 to 16 % were observed, which seems to be related to both the CBCT scan acquisition and image treatment errors.
- The selected technology for the manufacturing of the teeth – CNC milling- seems to be a very feasible process, since when comparing the developed and the manufactured teeth, surface area changes below 2% were obtained.
- When placing the manufactured teeth on the dog mandible, a lack of imprisonment was observed. These results were expected since authors already knew the manufactured teeth are smaller than the original ones. Additionally, when removing the skull soft tissues, there was a destruction of the PDL which led to an increased lack of congruency between the teeth and bone.

Given the previous outcomes, authors are aware that studies should proceed, aiming to minimize the obtained geometric errors. However, a systematic error is obtained and the design of root analogue implants, if needed, may incorporate some compensation strategies as, for instance, the incorporation of micro/macro retentions on the models' surface or even scaling the models up for the promotion of a better fit onto the tooth socket and thus promote the mechanical retention of the customized RAI.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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Compliance with ethics guidelines:

Telma Dantas, Flávio Rodrigues, João Araújo, Paula Vaz, Filipe Silva declare that they have no conflict of interest. This article does not contain any studies with human or living animal subjects performed by any of the authors

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CHAPTER 9

Conclusions

Considering the work developed in the scope of this PhD work, this chapter presents a suggestion of an implant prototype and the main conclusions that can be drawn. Some suggestions for future work are also presented.

9.1. General Discussion and Conclusions

This PhD focused on a strategy to design and develop a tooth-like implant tailored to each patient's condition. Techniques such as the acquisition of the CBCT scan of the real tooth or socket, implant 3D modelling, and CNC manufacturing are the basis of the developed strategy. Different approaches and features were evaluated to be incorporated in the implant. A final implant prototype designed based on the main conclusions of the developed work is presented in the end of this section.

In chapter 3, a critical review on subperiosteal implants was carried out, together with a comparison with conventional ones. Given the main conclusions that could be drawn, an optimized solution, making use of characteristics of both subperiosteal and endosseous implants was purposed. That strategy would encompass a customized and minimally invasive process, performed in a single-step surgery. Authors also suggest that an ideal implant would be made of zirconia, aiming to avoid aesthetical issues and bacterial adhesion. In addition, features such as the incorporation of surface micro-channels and administration of PRF were also suggested as promising strategies, for the promotion of implant osseointegration and vascularization.

Given the main goal of this PhD work - the development of a customized root-analogue implant - chapter 4 corresponds to a meta-analysis review on outcomes from clinical trials and case reports of customized root-analogue implants. Despite the scarce literature on this matter, some conclusions could be drawn. Titanium and zirconia allied to CAD/CAM technology were the most reported materials/technique for the manufacturing of such medical devices. Sandblasting and acid etching treatments were reported as effective on the promotion of the implant osseointegration, and the incorporation of surface macro-retentions seemed to induce a positive effect on the stress distribution on the bone around the implant, with no signs of infection, periodontitis nor bleeding, during the follow-up periods.

Following the aforementioned conclusions, chapter 5 aimed to assess the stress fields on the cortical bone surrounding root-analogue and screwed conventional implants, through FEA. This study allowed us to conclude that, as expected, root-analogue implants induce a better stress distribution along the cortical bone surrounding the implant, when compared to threaded ones. The different tested models were mainly subjected to shear strain values in the steady-state zone, which indicates that the μ -strain results are within the physiological parameters, and the incorporation of flaps in the root-analogue implant may be a promising strategy to improve the implant's stability. In general, the results of this *in-silico* study indicate that root-analogue implants overcome some limitations of the threaded conventional implants. However, the study design could be further improved

by, for instance, taking into consideration the pre-tension of the periodontal ligament, for a more realistic approach.

Chapter 6 aimed to design and develop by CAD/CAM different zirconia surfaces with micro-channels. These surfaces were then characterized by different parameters, namely their roughness, hydrophilicity, and capillarity. Results revealed that the average surface roughness of the produced specimens is comparable to those found in the literature for zirconia implants. All specimens revealed a hydrophilic nature, with no significant differences in contact angles between the tested groups, and capillarity analysis revealed an extreme capillary effect for micro-channels with 100 μm of depth and 200 μm of width. The main conclusion that could be drawn from this chapter is that the hydrophilicity and capillarity nature of the developed surfaces is expected to induce and facilitate crucial biological responses such as proper blood and nutrient supply (vascularization), and consequent implant osseointegration and stability.

In chapter 7 zirconia surfaces with micro-channels with 100 μm of depth and 200 μm , were tested regarding their bacterial adhesion propensity. These micro-channels dimensions were the ones that presented the most promising results in the previous chapter and were, therefore, selected for further studies. In this sense, this study aimed to assess the adhesion of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* to different zirconia surfaces (channeled, non-channeled, with sandblasting and/or acid etching) and compare the results to a titanium surface. The main conclusion of this work is that the adhesion of bacteria to a specific surface is a multifactorial mechanism, that may be influenced by the bacteria, the surface material, and roughness, as well as the environment around them. In fact, results revealed that the tested bacteria adhere differently to the surfaces, depending on their roughness and architecture. While *e.coli* adhered more to zirconia channelled surfaces, *S. aureus* and *P. aeruginosa* presented a higher propensity to adhere to the titanium control group. As previously mentioned, zirconia surfaces with micro-channels are expected to improve the vascularization around implants and, therefore, despite not having shown improved antibacterial properties, these are still promising solutions for dental implants' surfaces.

The last experimental chapter of this PhD work, chapter 8, aimed to assess the procedure and errors associated with image acquisition, treatment, and manufacturing technology of customized root-analogue implants. This study, performed on a cadaver dog mandible, allowed us to conclude that it is possible to successfully reproduce the teeth/socket geometry from a CBCT scan image. However, results showed that the developed teeth presented slightly lower surface

areas when compared to the original ones, which may be a result of both the CBCT errors and the image treatment procedure. As far as the manufacturing technique is concerned- CNC milling- it has been proved to be a very feasible process, exhibiting errors below 2%. Finally, when placing the manufactured teeth on the dog mandible, a lack of stability was observed, which may be related to both the CBCT acquisition errors and the destruction of the PDL during the soft tissues' removal process.

9.1.1. Implant prototype

According to the outcomes of the experimental work developed in the scope of this PhD project, a metal-free implant prototype is presented in this section. The purposed customized root-analogue implant is composed of three main parts and a mini-screw, as seen in Figure 9.1.

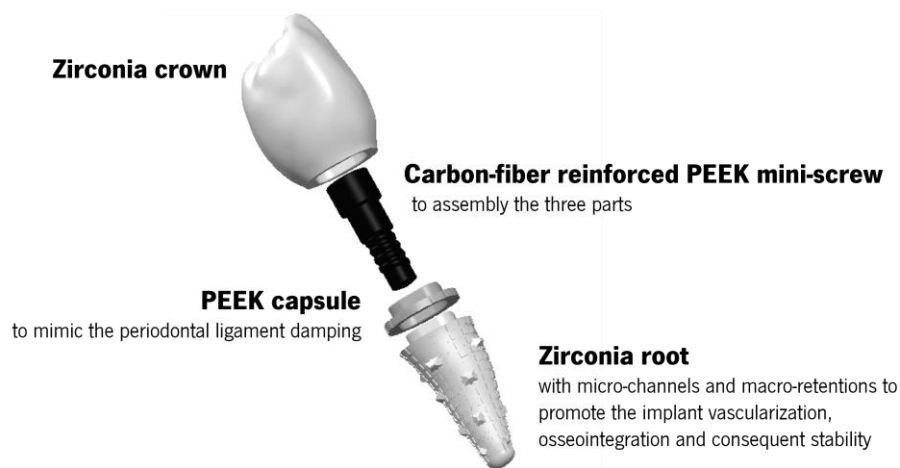


Figure 9.1 Implant prototype.

The first part, the crown, is made of zirconia and produced by a CAD/CAM technology, that has been extensively applied in the dentistry field – customized design and posterior CNC machining.

The implant itself, which replaces the tooth root, is also made of zirconia and manufactured by the same technique- designed based on the patient's CBCT and manufactured by CNC. The root surface encompasses micro-channels and some macro-retentions to promote the implant's vascularization, imprisonment, and consequent osseointegration.

The third component, a PEEK capsule, was designed to mimic the periodontal ligament

damping, once it has already been proved that the PDL has a huge impact on the stress distribution in the cortical bone. This part is also produced by CNC milling.

Finally, to assembly the three main components, a carbon-fiber-reinforced PEEK mini-screw was designed and manufactured by a hot-pressing technique. This material has already been successfully used in other medical applications (such as orthopedics), and, apart from being responsible for the implant assembly, the screw is also expected to withstand tensile forces, due to the longitudinal carbon fibers.

9.2. Suggestions for Future Works

Based on the findings of this PhD work together with the implant proposal above presented, some suggestions for future works can be enumerated:

- Perform *in vitro* experiments on the implant prototype to assess its bacterial adhesion propensity;
- Repeat the experiments on the dog mandible to assess the influence of the incorporation of the macro-retentions on the implant imprisonment. Scale the implants models up, to check if the systemic error associated with the CBCT image acquisition and treatment can be eliminated;
- Evaluate the fatigue resistance of the PEEK capsule under physiologic conditions;
- Evaluate the resistance to fracture and fatigue of the implant, according to ISO 14801;
- Perform pre-clinical trials in animal to validate the implant.

9.3. Further Contributions

In addition to the studies developed and described in this thesis, some conferences/workshops attendances can also be appointed:

- T.A.Dantas. Natural-inspired tooth replacement. International Workshop on Innovating (IWI), 11-16 June 2018, Massachusetts Institute of Technology, Boston – United States.

- T.A.Dantas. From the biomedical engineering to the development of a product. XIV Jornadas de Engenharia Biomédica. 4 February 2019, University of Minho, Braga - Portugal.
- T.A. Dantas, J.P. Carneiro Neto, J.L. Alves, Paula C.S. Vaz, F.S. Silva. Evaluation of the stress distribution of the cortical bone surrounding root-analogue implants in comparison with screwed implants: a finite element analysis. 8th International Conference on Mechanics of Biomaterials and Tissues, 15-19 December 2019, Hawaii-United States