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Regulation of Medical Devices to guide the development of a novel wound dressing

Dissertation thesis for the Master degree in Biotechnology

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October 2017

Declaração

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Nº do Cartão de Cidadão: 14433470

Título da dissertação:

Regulation of Medical Devices to guide the development of a novel wound dressing.

Orientadora:

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Ano de conclusão: 2017

Mestrado em Biotecnologia

Universidade do Minho, _____/_____/_____

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Joana Patrícia Ribeiro Aires Pereira

Agradecimentos

À minha orientadora, Professora Joana Azeredo, o meu especial agradecimento pela partilha de conhecimento, pelos conselhos, sugestões e sobretudo pela motivação e confiança que sempre demonstrou em mim.

Também gostaria de agradecer à Rute e à Rita, colegas do grupo de Biotecnologia de Bacteriófagos do Centro de Engenharia Biológica, pela valiosa ajuda na realização dos ensaios de citotoxicidade apresentados neste trabalho.

À minha família e amigos tenho a agradecer o apoio incondicional, o carinho e a compreensão, em especial aos meus pais e ao meu irmão. À Jéssica por sempre acreditar em mim e ao meu José por tudo.

Agradeço ainda a todas as pessoas que de alguma forma contribuíram para a realização desta dissertação e à Universidade do Minho por me proporcionar as condições necessárias durante todo este processo de aprendizagem.

Muito obrigada a todos.

Abstract

Chronic wounds represent a serious health condition that affects more than 50 million people worldwide, causing a devastating impact on the well-being of the patient as well as a serious problem for the economy. Thus, this work arises from the need to find solutions that can effectively combat this problem. Here is presented a comprehensive study of the regulatory framework of medical devices, focusing on the legal requirements applicable to the development of a novel wound dressing, incorporating antimicrobial agents to treat chronic wounds, named *BioMultiDress*. The main goal of this thesis was to understand what are the regulatory affairs that need to be considered in order to develop such a medical device. For that purpose, it was made an analysis on the regulations of the regulatory agencies, EMA and FDA, as well as an exhaustive collection of the technical documentation applicable to the *BioMultiDress*, attending that this device shall be classified as a short-term class III medical device, incorporating ancillary medicinal substances, which in this case are bacteriophages. It was also performed part of the biological evaluation of the *BioMultiDress*, by the assessment of the bacteriophages cytotoxicity, according to the international standard ISO 10993-5. The studied phages showed no potential cytotoxic effect on BALB 3T3 cells.

The knowledge and information herein compiled provides a guide to the medical devices manufacturers and regulatory authorities towards the development of such innovative medical devices as BioMultiDress. To assemble all the information, it was prepared a draft version of the *BioMultiDress Design Dossier*, containing the main achievements of this work.

Keywords

Chronic wounds, Medical devices, *BioMultiDress*, Standards, Bacteriophages, Design dossier

Resumo

As feridas crónicas representam uma condição de saúde grave que afeta mais de 50 milhões de pessoas por todo o mundo, causando um impacto devastador no bem-estar do doente, bem como um problema sério para a economia. Assim, este trabalho surge da necessidade de encontrar soluções que consigam efetivamente combater este problema.

Aqui é apresentado um estudo compreensivo do quadro regulamentar dos dispositivos médicos, com foco nos requisitos legais aplicáveis ao desenvolvimento de um novo penso que incorpora agentes antimicrobianos para o tratamento de feridas crónicas, designado *BioMultiDress*. O objetivo principal desta tese foi entender quais são as questões regulamentares que precisam ser consideradas com o intuito de desenvolver um dispositivo médico desta natureza. Para tal, foi feita uma análise dos regulamentos das entidades reguladoras, EMA e FDA, bem como uma recolha exaustiva da documentação técnica aplicável ao *BioMultiDress*, atendendo que este dispositivo deve ser classificado como um dispositivo médico de classe III de curto prazo, incorporando substâncias médicas auxiliares, neste caso, os bacteriófagos. Também foi efetuada parte da avaliação biológica do *BioMultiDress*, através da avaliação da citotoxicidade dos bacteriófagos, de acordo com a norma internacional ISO 10993-5. Os fagos estudados não apresentaram nenhum potencial efeito citotóxico em células BALB 3T3.

O conhecimento e a informação aqui compilados fornecem um guia aos fabricantes de dispositivos médicos e autoridades reguladoras para o desenvolvimento de dispositivos médicos inovadores como o *BioMultiDress*. Foi preparada uma versão preliminar do *Dossier de Design* do *BioMultiDress*, contendo os principais resultados deste trabalho.

Palavras-chave

Feridas crónicas, *BioMultiDress*, Dispositivos médicos, Normas, Bacteriófagos, Dossier de Design

Contents

List of Figures

List of Tables

Acronyms

510(k) - Section of the Food, Drug, and Cosmetic Act that deals with premarket notification

CA - Competent Authorities

CAB - Conformity Assessment Body

CE - of the French *Conformité Européene* meaning *European Conformity*

CFR - Code of Federal Regulations

CJEU - Court of Justice of the European Union

CTD – Common Technical Document

DHF - Design History File

DMEM - Dulbecco's Modified Eagle's Medium

DMSO - Dimethyl sulfoxide

EC - European Commission

EDTA - Ethylenediaminetetraacetic Acid

EEA - European Economic Area

EMA - European Medicines Agency

ER - Essential Requirements

ESOs - European Standardization Organizations

EU – European Union

FDA - Food and Drug Administration

HED - Humanitarian device exemption

HUD - Humanitarian Use Device

GHTF - Global Harmonization Task Force

IOS - International Organization for Standardization

ISO - derived from the Greek *isos* (equal)

MDD - Medical Devices Directive

MDRs - Medical Devices Regulations

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid

NANDO - New Approach Notified and Designated Organizations

NB - Notified Bodies

NB-MED - Notified Bodies Medical Devices

NR - Neutral red

PBS - phosphate-buffered saline

PEG - polyethylene glycol

PFU - plaque forming units

PMA - premarket approval application

PMN - premarket notification

QMS - Quality Management System

SDS - sodium dodecyl sulfate

STED - Summary Technical Documentation

UDI - unique device identifier

UPC - universal product code

US – United States

WHO - World Health Organization

XTT - 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl] -2H-tetrazolium hydroxide

1. Introduction

1.1. Motivation

Over the centuries, the human being has faced some epidemic diseases that endanger their health condition, most of them responsible for thousands or even millions of deaths worldwide. More recently, much has been said about diseases like diabetes and obesity, as well as cancer, that have emerged with an epidemic character, considered by many as the new epidemics of the 21st century. Thereby, it has been a concern of healthcare providers to raise awareness among the population about these serious healthcare issues, that are undoubtable huge, but not the single ones.

Growing silent, the problematic of non-healing chronic wounds has been taking epidemic proportions, representing a devastating impact on patient well-being and a great burden for the economy. Chronic wounds are open wounds on the surface of the skin which take long to heal and if left untreated, can progress to infections with hospitalization, amputation and death. This is a serious health problem that affect more than 50 million people worldwide, representing huge costs for the national healthcare systems. For example, in the United Kingdom (UK), the costs associated with wound care management (£5.3 billion) are higher than the one related to obesity (£5.1 billion) and quite close to the costs of cancer treatments (£5.6 billion) [1]. Likewise, some other studies conducted in Europe demonstrate the great economic burden associated with the costs of wound care treatment alone, compared to the overall other healthcare systems, representing 4% of the total costs supported by the National Healthcare Systems (Figure 1.1) [2-5].

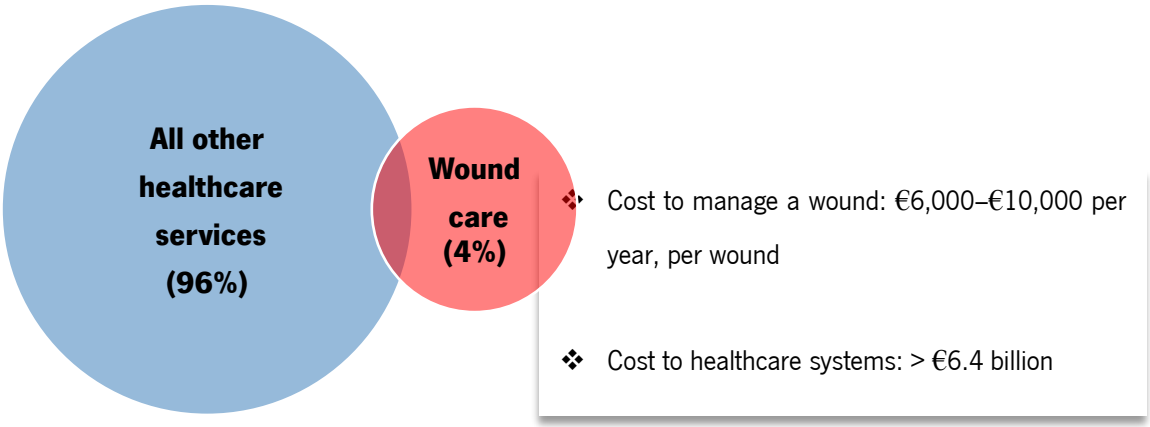


Figure 1.1. Wound care associated costs in Europe compared to all other healthcare services.

Nevertheless, more than the economic costs, the human costs must be the primary concern related to this disease, since chronic wounds seriously affect the quality of life not only of those affected, but also of family members and caregivers.

Finally, as one of the most frequent and serious problems that impairs the healing process of a chronic wound is the bacterial infection, it is extremely important to deal with this aspect. Currently, there are different types of wound dressings available on the market that incorporates antimicrobial agents such as antibiotics or silver-based antimicrobial agents. However, there is a great concern about the indiscriminate use of this type of agents, due to both the allergic reactions associated with its topical application, the risks of toxicity, the fact that they do not reach the desired penetration, the difficulty of absorption by the wounds and above all, the occurrence of bacterial resistance.

So, the motivation to embrace this study comes from the need to find solutions for this serious threaten which infected chronic wounds have become. A wound dressing that combines antimicrobial properties, such as the incorporation of bacteriophages, with healing factors could be an interesting approach. However, in order to develop such an innovative approach, it is important to address the regulatory aspects concerning medical devices. Thereby, the aim of this work is to provide a guide to assist the development of a novel wound dressing according to the current regulatory frames in Europe and USA.

1.2. Research Question, Aim and Objectives

From the current need for innovative therapeutic approaches that fulfill the gap of the treatment of chronic non-healing wounds infected with multi-drug resistant (MDR) bacteria, a novel wound dressing emerged. So, the primary research question that motivate the work herein presented is the following:

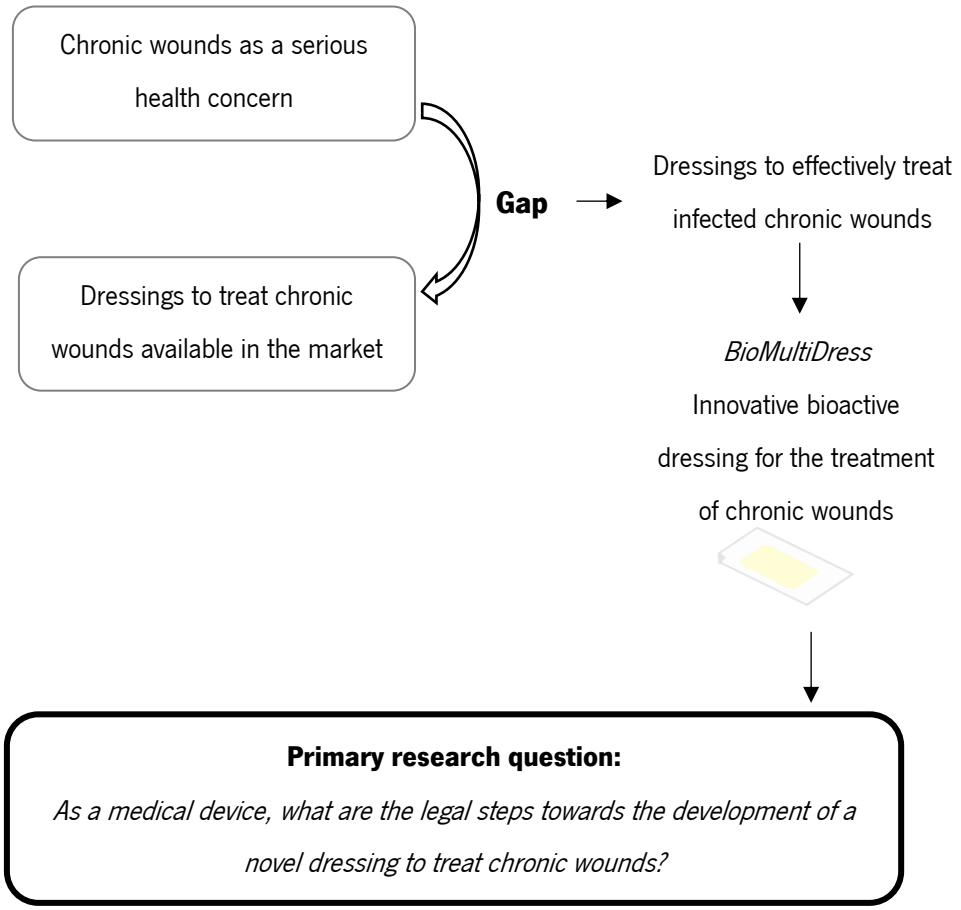


Figure 1.2. Primary research question that guides this master dissertation.

Hereupon, the aim of this research is to comprehend the regulation of medical devices to setting the legal requirements need to be taken in consideration in the development of a novel wound dressing for the treatment of chronic wounds. Subsequently, in order to accomplish the primary research question as well as the aim of this research, it was defined the following seven secondary objectives:

- To comprehend the legal framework of the medical devices industry, detailing how the sector are segmented under the supervision of the regulatory agencies of the two medical devices major markets, the European and the United States;

- To compare the European Medicines Agency (EMA) and The United States Food and Drug Administration (FDA) regulations, scrutinizing their similarities and dissimilarities as well as to understand their distinct pathways to market a medical device;
- To describe the technical documentation required to compile the information resulting of the development of a medical device attending both EMA and FDA regulations;
- To define the innovative wound dressing, *BioMultiDress*, addressing its therapeutic mechanisms and features;
- To classify the device and identify the applicable essential requirements and standards applied to the *BioMultiDress*;
- To evaluate the cytotoxicity of the medicinal substances (bacteriophages) which will be incorporating the *BioMultiDress*, according to the applicable International Standard ISO 10993 concerning Biological Evaluation of Medical Devices;
- To prepare a draft version of the *Design Dossier* for this novel wound dressing.

1.3. Dissertation Outline

This section presents the road map of this thesis, introducing the main topics of each chapter, and explaining the general structure of this research work. This dissertation is structured into 6 chapters as follows.

Chapter 1- *Introduction* - This chapter first introduces the problematic of chronic wounds as well as the motivation of the present research work, outlining the aim and secondary objectives to answer the primary research question that comes from the need to find solutions to tackle this critical public health concern. In this chapter is also defined the structure and the methodologies applied in this study.

Chapter 2 – *Medical Devices* – This chapter insights the main characteristics of medical devices, by the understanding how the medical devices sector is regulated in the two major markets of the industry. Simultaneously, the characteristics that are common or distinct to the legal framework of the European and United States regulatory agencies are identified. The distinct pathways to the market that may be followed by the product in accordance with EMA or FDA regulations are also identified and described.

Chapter 3 – *Technical Documentation* – In this chapter, it is explained what is the documentation that needs to be collected to prove conformity of the device under development, with the regulatory requirements for both EMA and FDA. This chapter also describes two document guidelines that will help

in the organization of that information, the *Recommendation NB-MED / 2.5.1 / Rec5 on technical documentation* and the *Summary Technical Documentation (STED).*

Chapter 4 - *Study Case: BioMultiDress- Innovative bioactive dressing for the treatment of chronic wounds* – This chapter could be divided in two parts: one that reviews the state of the art of the problem of chronic wounds and the bacteriophages, viruses that combat bacteria, as a potential therapy approach to treat infected non-healing wounds; and the second part where the therapeutic solution is presented, consisting in a wound dressing with antimicrobial and regenerative properties to treat chronic infected wounds, named *BioMultiDress.* Besides, it is also presented the classification of this new product according to the European legal framework, including the applicable requirements and standards of compliance.

Chapter 5 - *Biological Evaluation of Medical Devices* – This chapter overviews how the biological evaluation is performed in the case of medical devices in general, highlighting the particular case of the *BioMultiDress*. Thus, in this chapter, the biological evaluation tests applicable to *BioMultiDress* are identified according to the International Standard ISO 10993, concerning Biological Evaluation of Medical Devices. Also in this chapter, it is included one experimental assay, performed to assess the cytotoxicity effect of one of the materials that will be incorporated in the dressing, the bacteriophages.

Chapter 6 –*Conclusions and Final Considerations* – This final chapter summarized the main conclusions of this work by answering to the primary research question and highlighting some final considerations on further research to improve the *BioMultiDress*.

1.4. Research Approach

In order to address the primary research question that motivated this study, which is, to know what are the steps that must be followed to get the legal approval to develop a novel wound dressing for the treatment of chronic wounds, it has to be defined a methodology approach, guided by the aim of this research, that lead to the answer of the initial question proposed.

Thus, the research approach that was applied in this study could be divided into two components that accomplished the overall methodology of investigation: a theoretical investigation and an experimental investigation. In the theoretical part, which constitutes the major component of the herein present investigation, a bibliographical and documentary revision of the published legislation, norms, guidances, press releases, articles and publications on the subject under analysis was carried out. The bibliographical and documentary review was supported by the following main sources of information:

- Consultation of the *European Commission* website: (http://ec.europa.eu/health/home_en);
- Consultation of the *Press Release Database of the European Commission* website: (http://europa.eu/rapid/search.htm);
- Consultation of the *European Medicines Agency* website: (http://www.ema.europa.eu/ema/)
- Consultation of the *U.S. Food & Drug Administration* website: (https://www.fda.gov/);
- Consultation of *International Medical Device Regulators Forum* website: (http://www.imdrf.org/);
- Consultation of the *International Organization for Standardization – ISO* website: (https://www.iso.org/home.html?=);
- Consultation of the *European Association Medical devices of Notified Bodies* website: (http://www.team-nb.org/about-us/);
- Consultation of the *Competent National Authority INFARMED I.P.* website: (http://www.infarmed.pt/web/infarmed/infarmed);
- Consultation of the notified body *TÜV SÜD* website: (http://www.tuv-sud.com/home_com).

The experimental investigation was carry out within the scope of the Chapter 5 - *Biological Evaluation of Medical Devices,* consisting in the cytotoxicity evaluation of the bacteriophages, as one of the materials integrating the final product. The experimental methodology was based on the test protocol described in *Annex A - Neutral Red Uptake cytotoxicity test – of the ISO 10993- 5:2009*, fully detailed in the Annex II of the section *Annexes*.

2. Medical Devices

2.1. Introduction

Medical devices have been an integral part of human health care since antiquity. As early as Neolithic times (7000 BC), medical devices had been used to treat and diagnose diseases, being that there was found surgical instruments used in cranial trepanations dating back that period [6]. Medical devices have undergone a huge evolution over time, performing a crucial role on the increase of life quality insofar as they provide better quality, safety and efficacy of the healthcare. The innovation allied to the rapid advancement of technologies became the major drivers of growth for medical devices industry, making this highly innovative sector a potential market expected to reach around €323 billion by 2021, being the major opportunities in cardiovascular, surgical and infection control segments [7].

According to the World Health Organization (WHO), medical devices can be defined as:

"an article, instrument, apparatus or machine that is used in the prevention, diagnosis or treatment of illness or disease, or for detecting, measuring, restoring, correcting or modifying the structure or function of the body for some health purpose. Typically, the purpose of a medical device is not achieved by pharmacological, immunological or metabolic means"[8].

As seen from the broad definition given to medical devices, it is perceptible that the range of products included in the category is also very large and variable in complexity and applications, resulting in 1.5 million different medical devices and over 10 000 types of devices available worldwide. For example, the term "medical device" include from therapeutic devices with local applications, like simple bandages, to highly sophisticated computerized medical equipment , like auxiliary life support machines or even artificial bones [8].

Not only the diversity of devices but also the different definitions resulting from distinct legal frameworks, makes this area of medical device technology very complex and constitute a challenge to the regulation of innovative products. Therefore, the main differences and converging points between the two major entities of medical device regulatory systems worldwide, European Medicines Agency (EMA) and Food and Drug Administration (FDA), will be scrutinized in terms of definitions, classification, regulatory framework and the pathway to the market. But before that, it would be interesting to understand in functional terms what distinguishes the two agencies. Briefly, EMA is a decentralized agency of the European Union (EU), responsible for the scientific evaluation, supervision and safety monitoring of medicines developed by pharmaceutical companies for use in 28 EU Member States, and FDA is the agency responsible for protecting the public health by overseeing medical products in all 50 states of the United States (US) [9, 10].

Finally, it is important to underline that the medical devices sector is now experiencing a period of many changes. After several years of profound review of the regulatory framework, on May 25th, 2017, the new European Medical Device Regulations (MDRs) became effective. These new regulations, including the Regulation (EU) 2017/745 on medical devices and Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices, were published by the European Union legislator in the Official Journal of the European Union, replacing the current EU Medical Devices Directives, Council Directive 93/42/EEC on medical devices and Directive 98/79/EC on *in vitro* diagnostic medical devices. The Regulations will bring an updated and more robust EU legislative framework to ensure better protection of public health and patient safety [11].

Regulation (EU) 2017/745 is expected to be applied from 26 May 2020, while Regulation (EU) 2017/746 is expected to arrive two years later from 26 May 2022. Therefore, the research work presented herein will consider the legislation currently applied, which means that the Medical Devices Directives will guideline this study case.

2.2. Definitions

The complexity of medical devices technology is soon denoted by the absence of an universally accepted definition. Nevertheless, attempts have been made to achieve standardization among national medical device regulatory systems, more specifically, the creation of Global Harmonization Task Force (GHTF), resulted from a partnership between regulatory authorities and regulated industry. GHTF was incepted in 1992, comprising representatives from five founding members: European Union, United States, Canada, Australia and Japan, and its major goal was to promote convergence in regulatory practices, increasing access to safe, effective and clinically beneficial medical technologies, encouraging technological innovation and facilitating international trade around the world, throughout the publication and dissemination of harmonized guidance documents on basic regulatory practices. Although this organization has been extinguished because of the lack of consensus between the entities, its mission has been taken over in 2011 by the International Medical Device Regulators' Forum (IMDRF), a new organization whose purpose is precisely to reinforce the work previously left by the GTHF [12, 13]. Despite many challenges have dictate the end of GHTF, some of the work accomplished is still available in IMDRF website and the guidelines created are still widely used as model documents for the industry [14].

Thus, since there is no single definition for the term "medical device" it is important to understand how different definitions are currently in the main regulatory systems and what in practice these differences may represent. Table 2.1. shows the definitions presently accepted in Europe and in the

United States of America, given by the respectively regulatory authorities European Medicines Agency (EMA) and Food and Drug Administration (FDA). The GHTF definition is also presented since it is adopted by countries that are still trying to develop their own medical device regulatory system.

Table 2.1.EMA, FDA and GHTF medical devices definitions.

Regulatory System	Definition
EMA[1]	Any instrument, appliance, apparatus, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: - diagnosis, prevention, monitoring, treatment or alleviation of disease; - diagnosis, monitoring, alleviation of or compensation for an injury or handicap; - investigation, replacement or modification of the anatomy or of physiological process; - control of conception; and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but that may be assisted in its function by such means
FDA[2]	An instrument, apparatus, implement, machine, contrivance, implant, *in vitro* reagent, or other similar or related article, including a component part, or accessory which is: - recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them, - intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or - intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and that is not dependent upon being metabolized for the achievement of any of its primary intended purposes.
GHTF[3]	Any instrument, apparatus, implement, machine, appliance, implant, *in vitro* reagent, software, material or other similar or related article: a) intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purpose(s) of: - diagnosis, prevention, monitoring, treatment or alleviation of disease, - diagnosis, monitoring, treatment, alleviation of or compensation for an injury, - investigation, replacement, modification, or support of the anatomy or of a physiological process, - supporting or sustaining life, - control of conception, - disinfection of medical devices, - providing information for medical or diagnostic purposes by means of *in vitro* examination of specimens derived from the human body; and b) which does not achieve its primary intended action in or on the human body by pharmacological, immunological or metabolic means, but that may be assisted in its intended function by such means.

[1] In Medical Devices Directive 93/42/EEC; [2] In section 201(h) of the Food Drug & Cosmetic Act; [3] In GHTF proposed document SG1(PD)/N071R04 - Definition of the Term 'Medical Device' published in March 28, 2011.

Considering the definitions presented in the table above, the similarities between them are quite evident, however a more detailed analysis allows to find some particularities that can affect the way the device is regulated and consequently mislead manufacturers in terms of the requirements that the product must accomplish. For example, unlikely EMA and GHTF definitions, the FDA's does not include the word *software* which may lead to the misconception that these products are not regulated by the FDA, however they do, and some guidance documents have been recently released in order to standardized this particular type of devices, namely, "Medical Device Accessories – Describing Accessories and Classification Pathway for New Accessory Types- Guidance for Industry and Food and Drug Administration Staff", released on last December [15].

Another difference that should be noted is that both EMA and GHTF definitions, distinctly to FDA's, assign the task of defining the device's purpose use specifically to the manufacturers: "intended by the manufacturer to be used", while in the FDA's definition such delegation is not specified. However, under FDA 21 Code of Federal Regulations 801.4 regulation, the words "intended uses" refers to "...the objective intent of the persons legally responsible for the labeling of devices. The intent is determined by such persons' expressions or may be shown by the circumstances surrounding the distribution of the article.". The manufactures play an essential role in the definition of intended use of a device, prove of this is the resolution by the Court of Justice of the European Union (CJEU), in 2012, after a product, *ActiveTwo*, used as a modular system capable of recording electrical signals from the brain, heart and muscles has not been considered a medical device. Despite of the competitor company, *Brain Products GmbH*, had claimed that the product was a medical device because it was capable of being used in a medical diagnostic context and are not being sold as so, the court decided that products which fall within the definition of a medical device but are not intended, by their manufacturer, to be used for a "medical purpose", are not medical devices covered by the European *Conformité Européene* (CE) certification requirements for medical devices under the Medical Devices Directive (MDD) [16]. This decision demonstrated the fragility of the expression "medical purpose" because even if a product falls within the definition of medical device it is up to its manufacturer to decide whether the product has a medical purpose or not.

There is also another definition present on EMA, FDA and GHTF medical device definition that requires a clear interpretation, namely as regards to the primary/principal intended purposes/action of the device. Currently, the number of products having both a device and drug component and medical devices incorporating a medicinal substance are increasing due to the innovative emerging technologies in the medical industry, making the differentiation of the principal mode of action between a medical

device function and medicinal product action more difficult. These products that fall into the borderline area between medical and medicinal products are called "borderline products" or "combination products", depending if they are regulated in Europe or in the United States, respectively (Table 2.2) [17, 18].

Table 2.2. Borderline and Combination Product Definition.

Borderline Product[1] (Europe)	Borderline cases are considered to be those cases where it is not clear from the outset whether a given product is a medical device, an *in vitro* diagnostic medical device, an active implantable medical device or not. Or alternatively, borderline cases are those cases where the product falls within the definition of a medical device but is excluded from the Directives by their scope. Where a given product does not fall within the definition of medical device or is excluded by the scope of the Directives, other Community and/or national legislation may be applicable.
Combination Product[2] (US)	(1) A product comprised of two or more regulated components, i.e., drug/device, biologic/device, drug/biologic, or drug/device/biologic, that are physically, chemically, or otherwise combined or mixed and produced as a single entity; (2) Two or more separate products packaged together in a single package or as a unit and comprised of drug and device products, device and biological products, or biological and drug products; (3) A drug, device, or biological product packaged separately that according to its investigational plan or proposed labeling is intended for use only with an approved individually specified drug, device, or biological product where both are required to achieve the intended use, indication, or effect and where upon approval of the proposed product the labeling of the approved product would need to be changed, e.g., to reflect a change in intended use, dosage form, strength, route of administration, or significant change in dose; or (4) Any investigational drug, device, or biological product packaged separately that according to its proposed labeling is for use only with another individually specified investigational drug, device, or biological product where both are required to achieve the intended use, indication, or effect.

[1] In Manual on borderline and classification in the community Regulatory framework for medical devices Version 1.17 (09-2015); [2] In 21 CFR 3.2(e) [17,18].

As shown in the table above, while in the US there is a clear definition for a "combination product", published in the Federal Register by the Executive departments and agencies of the Federal Government, Europe lacks a clear definition of what is a "combined" product. As so, the regulatory route will be defined by the principal intended action of the combination, which could follow either on a medical device or on a medicinal product classification, depending on its primary purpose. Besides the distinction by the principal mode of action, there are some other aspects that could be considered to better define

on which classification the product falls like, for example, the assessment of both medical device and medicinal product definitions, determining which one better covers the product characteristics. Some of the examples of combination products include biologic wound-care products containing antimicrobial agents, antimicrobial catheters or antibacterial-releasing dental restorative materials [19].

Some peculiarities have been discussed throughout this section regarding the definition of "medical device", being clear that there are some different interpretations of both Europe and United States definitions. The fact that a product being classified as a medical device in the US by the FDA does not mean that it is necessarily classified the same way in Europe. For example, condoms are considered a medical device both by US and Europa regulatory agencies. Conversely, a toothbrush is considered a medical device in the US while in Europe it is classified as a personal hygienic and cosmetic product, unless the manufacturer makes a medical claim [20]. This represents a key point of divergence on the way Europe and the US FDA classify which will be discussed on the next section *2.3- Classification.*

2.3. Classification

Medical devices can be classified under several criteria. Classification attending the risk to both patients and users constitutes one of the most important classification systems, being that system defined by the rules and regulations established by the regulatory agencies and it will determine what are the pathways for the approval process so that a product can reach the market under the proper conformity assessment route. Therefore, product classification is essential to establish the necessary requirements during product development and design controls, as well as represent an important tool in terms of the determination of the costs and time taking to bring the device to the market [21, 22].

Both agencies, EMA and FDA, demonstrate similarities when it comes to medical device classification related to the perceived risk of the product type. The European classification are ruled by the Medical Device Directive (93/42/EEC as modified by 2007/47/EC), setting out 18 rules presented on Annex IX (submitting further to other Directive annexes, depending on the regulatory control applied to the device), which are additional explained in the guidance document MEDDEV 2.4 of June 2010. The classification process could be subcategorized and, for each of the broad categories, there are certain rules which apply according to several factors such as duration of contact with the body, whether or not the device is invasive or surgically invasive, whether the device is implantable or active or whether or not the device contains a substance, which in its own right is considered to be a medicinal substance and has action ancillary to that of the device. Thus, as a result of the interpretation of these rules and depending on the intended purpose, a medical device may be classified as Class I, Class IIa, IIb and III,

and the higher the risk classification, the higher the level of assessment required [22]. Similar to European risk classification system, FDA also categorize medical devices into one of three regulatory categories based on the level of control necessary to assure the safety and effectiveness of the device —Class I, Class II, and Class III, with regulatory controls increased along the risk class (Table 2.3.).

Table 2.3. Medical device's risk categorization under EMA and FDA regulations. *Adapted from [20, 23].*

Regulatory System	Risk level	Device Class	Examples
EMA	Low	I I Sterile I Measuring	Bed pans Sterile plasters Thermometers
	Medium	IIa	Hearing aids Powered wheelchairs
	High	IIb	Ventilators, Infusion pumps
	High	III	Silicone gel-filled breast implants Vascular replacement heart valves
FDA	Low	I	Examination gloves Elastic bandages
	Moderate	II	Bone fixation screw Infusion pumps
	High	III	Heart valves Pacemakers

However, the categorization process is quite different when compared to the one regulated by EMA, instead of rules, FDA pre-define classifications for approximately 1 700 different generic types of devices and grouped them into 20 medical specialties (e.g., general hospital, immunology, orthopedic, dental, molecular genetics), named panels [15, 24]. In order to access the device class and also the existing exemptions, FDA provides an online classification database so that one can find the regulation number which contains the classification regulation for the respective device. To accomplish this, there are two possible procedures: go directly to the classification database and search for a part of the device name, or, knowing the device panel to which the device belongs, go directly to the panels list and identify the device and the corresponding regulation. The definition of the device class will clarify the type of premarketing submission required by FDA, which could range from a premarket notification called PMN

or 510K for Class I or II, not exempt, to a premarket approval application (PMA) for Class III devices. As already mentioned, this classification is risk based, which means that it attends to the risk the device poses to the patient and/or the user, but also will depend on both intended use and indications for use [15].

Medical devices risk assessment represents the regulatory classification ruled by regulatory agencies, however, there are other criteria by which different medical devices can be distinguished (Figure 2.1).

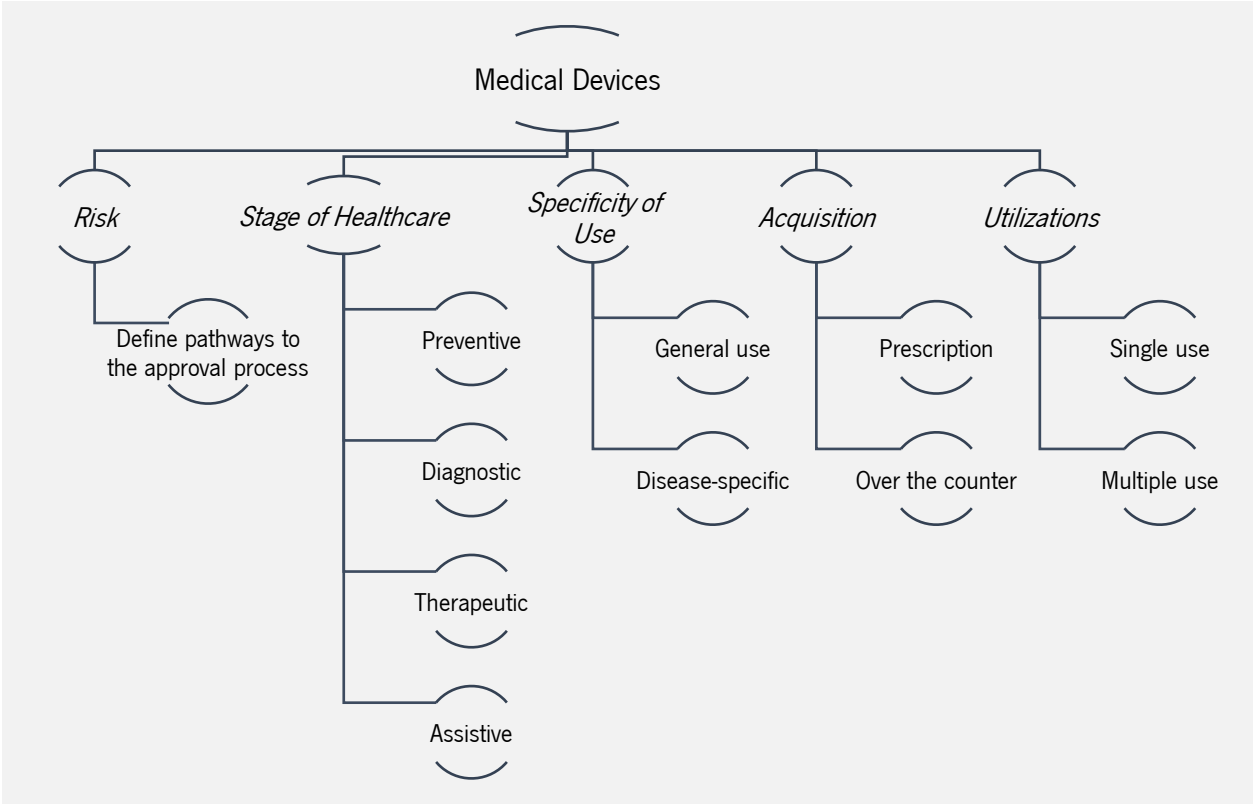


Figure 2.1. Different criteria for classification of medical devices. Adapted from [19, 25].

Thus, considering the specificity of use, one can divide into medical devices for general use (e.g. thermometer) and disease-specific (e.g. implants). The number of utilizations is also a distinguish criteria between devices for single use (e.g. needles) and multiple use (e.g. blood glucose tests). When it comes to the source of acquisition, it is possible to categorize according to restrictive character into medical devices that require a prescription (e.g. insulin pump) and those that are sold directly to the consumer, also called 'over the counter' products (e.g. condoms). Finally, depending on the stage of healthcare in which they are used, medical devices can be also differentiated into preventive (e.g. sterilization equipment), diagnostic (e.g. endoscopes), therapeutic (e.g. sterile dressings,) and assistive (e.g. hospital beds) [20, 25].

Finally, it can be considered other classification criteria, the nomenclature system created by the international agency called Global Medical Device Nomenclature (GMDN). The GMDN list, created by the worldwide medical devices sector specialists in accordance with the international standard ISO 15225, comprises the generic names used to identify all the medical devices products, divided into the ones used for diagnostic, prevention, monitoring and the ones intended to be used in the treatment or alleviation of disease in human beings. This list aims to provide a designation system that can be used for the exchange information between the healthcare authorities, regulators or providers and the manufacturers, in order to support patient's safety. Thus, this nomenclature system categorizes medical devices attending three criteria, device category, generic device group and collective terms, which are increasing in specificity and resulting in a 5-digit GMDN Code cross- referenced to a specific Term Name and Definition that are common for all medical devices with substantially similar generic features (e.g. GMDN code: [47569]; GMDN Term Name: "Scalpel, single use"). Currently, the use of this codification is subject to the payment of fees, and despite being recommended by the IMDRF, it is not mandatory [26].

2.4. Regulatory framework

Regulatory authorities have the essential role to ensure the safety, quality and efficacy of all drugs and medical devices circulating in their country as well as mediating the process of passing innovative therapies from the field of research to public use, as soon as possible. Although there are some regulatory agencies worldwide, from Japan, Brazil, Russia, among others, the leading regulators in the medical device sector are EMA, responsible for the legislation of all its 28 member countries, and the FDA, remaining the entity responsible for the largest medical device market in the world. Each one of them has its own definitions and classifications concerning medical devices, as shown in the previous sections, and both have their regulatory dissimilarities that will be discussed in this section.

2.4.1. United States Medical Devices Regulation

The Food and Drug Administration is an agency within the Department of Health and Human Services of the United States, responsible not only for the safety of nation's food supply, cosmetics, tobacco and products that emit radiation but also responsible for the protection of the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices. With the emerging public health threats, FDA plays a key role in the promotion of advancing the public health by helping to speed innovations that make medical products more effective, safer, and more affordable and by fostering development of new medical products [10]. As a government agency, FDA is

the regulatory authority responsible for the monitoring of, among other products, medical devices. The origins of Food and Drug Administration can be traced to the early 19th century, although it was not known by its current name until 1930, FDA's modern regulatory functions began with the passage of the 1906 Pure Food and Drugs Act. In 1938, the Federal Food, Drug and Cosmetics Act set out several laws that gave Food and Drug Administration more tools to supervised the safety of food and cosmetics and to ensure that drugs are not only effective but safe [27, 28].

Since the 1976 Medical Device Amendments to the Federal Food, Drug, and Cosmetic Act (FDCA), FDA regulates medical devices, meaning that, in order to legally sold a medical device in the US, the person or company that wants to sell it must seek approval from the FDA, as so they must present evidence that the device is reasonably safe and effective for a particular use [29]. Currently, the FDA's division responsible for regulating companies who manufacture, repackage, relabel, and/or import medical devices sold in the US is the Center for Devices and Radiological Health (CDRH). In order to receive FDA's approval, there are some basic regulatory requirements that manufacturers of medical devices distributed in the US must comply with (Table 2.4), set out in Title 21 Code of Federal Regulations (CFR) (codification of the general and permanent rules that were published in the Federal Register by the Executive departments and agencies of the Federal Government) Part 800 to Part 1299, covering various aspects of design, clinical evaluation, manufacturing, packaging, labeling and post market surveillance concerning medical devices products.

Thus, to fulfil the regulatory requirements presented on table 2.4., the compliance with standards is very important. Standards are documents which give not only international specifications for products, services and systems, to ensure quality, safety and efficiency but are also essential to facilitate international trade. Therefore, the creation of organizations which develop such documents, adopted and recognized in various regulatory systems, as for example, the International Organization for Standardization (IOS), commonly designated by ISO, has facilitated the task of medical devices manufacturers, since the knowledge and the use of these documents contribute to ensure product's conformity with the legal specifications [30]. For example, ISO 9001 and ISO 13485 establishes the requirements for a quality management system for both the design and manufacture of medical devices, covering aspects including risk management, design control during product development, and verification and validation systems. As so, these standards should be used to ensure that the quality requirement-Quality System Regulation (QSR)/Good Manufacturing Practices (GMP) – is completely in conformance with the regulations.

Table 2.4. Regulatory requirements set out by 21 Code of Federal Register that manufacturers of medical devices distributed in the US must comply.

Regulatory Requirement	21 CFR	Description
Establishment Registration	807	Owners or operators of places of business that are involved in the production and distribution of medical devices intended for use in the US are required to register annually with the FDA.
Medical Device Listing	807	Manufacturers must list their devices with the FDA.
Premarket Notification 510 (k)	807 subpart E	Demonstrate that the device is substantially equivalent to one legally in commercial distribution in US: (1) before May 28, 1976; or (2) to a device that has been determined by FDA to be substantially equivalent.
Premarket Approval (PMA)	814	Required for Class III devices which pose a significant risk of illness or injury, or devices found not substantially equivalent to Class I and II predicate through the 510(k) processes. Includes the submission of clinical data to support claims made for the device.
Investigational Device Exemption (IDE)	812	Allows the investigational device to be used in a clinical study in order to collect safety and effectiveness data required to support a PMA application or a 510(k) submission to FDA.
Quality System Regulation (QS)/Good Manufacturing Practices (GMP)	820	Requirements related to the methods used in and the facilities and controls used for: designing, purchasing, manufacturing, packaging, labeling, storing, installing and servicing of medical devices.
Labeling	801	Includes labels on the device as well as descriptive and informational literature that accompanies the device.
Medical Device Reporting	803	Identify and monitor significant adverse events involving medical devices.

2.4.2. Europe Medical Devices Regulation

The European Medicines Agency was founded in 1995 with the major purpose of ensuring efficacy and safety of human and veterinary medicines across Europe by assessing them to rigorous scientific standards, and promoting research and innovation in the development of medicines, however, medical devices have been regulated in Europe before the creation of the regulatory agency with each country having its own legislation [31]. In fact, Medical Devices Directive intended to harmonize the laws relating to medical devices within the European Union was first released in 1993, imposing rules on the manufacture, placing on the market and monitoring of medical devices. Currently, the core medical devices legal framework consists of three directives: the European Council Directive 93/42/EEC, covering most of the medical devices, the European Council Directive 90/385/EEC on active implantable medical devices, and the European Council Directive 98/79/EC on *in vitro* diagnostic medical devices. These Directives, called "New Approach Directives", were drafted in May 1985 by the Council of Ministers in accordance with the "New Approach to technical harmonization and standards" model. This resolution represented a major achievement in the development of the Single Market, delegating the responsibility for working on technical rules and providing European standards to the European Standardization Organizations (ESOs), like CEN, bringing together the National Standardization Bodies of European countries. Thus, medical devices directives aim to ensure a high level of protection for human health and safety and as well as a good functioning of the Single Market. Although the central regulatory framework is based on these three main directives, several technical revisions have been modifying and implementing directives over the time [32, 33].

Therefore, to ensure safety and performance, all medical devices must fulfil the Essential Requirements (ER) set out in the Directives mentioned above, for example, in the Medical Devices Directive 93/42/EEC, these requirements are set in the Annex I (Figure 2.2). The compliance of these requirements is crucial to obtain the Conformité Européene (CE) mark, which is the legal requirement for the placing of the medical device on the European market and declares the conformity of the product with EU legislation, enabling free movement within the European Economic Area (EEA).

Generally, to accomplish a harmonized application of the directives, the construction of a checklist demonstrating compliance of the device with the essential requirements based on consultation of standards, guidance documents MEDDEV, consensus statements, and interpretative documents published in the Official Journal of European Commission is a key procedure for a product to comply with legal specifications [34].

Essential Requirements	General Description of Quality System	Reference Documents
10.1 Devices with a measuring function must be designed and manufactured in such a way as to provide sufficient accuracy and stability within appropriate limits of accuracy and taking account of the intended purpose of the device. The limits of accuracy and taking account of the intended purpose of the device. The limits of accuracy must be indicated by the manufacturer.		
10.2 The measurement monitoring and display scale must be designed in line with ergonomic principles, taking account of the intended purpose of the device.		
10.3 The measurements made by devices with a measuring function must be expressed in legal units conforming to the provisions of council directive 80/181/EEC		

Figure 2.2. Example of an Essential Requirement Checklist.

In Europe, the regulatory system is managed by three organizations that guarantee that all the legislation process occurs in conformity as well as the compliance of the requirements set by the European Commission (EC) directives: manufactures, Notified Bodies (NB) and Competent Authorities (CA). Eventually, other authorized representatives and distributors may be involved as long as the devices are manufactured outside the EU. Manufactures are defined in the European Council Directive 93/42/EEC as the "(...) legal person with responsibility for the design, manufacture, packaging and labelling of a device before it is placed on the market under his own name, regardless of whether these operations are carried out by that person himself or on his behalf by a third party (...)"; generally, manufacturer itself can assure compliance and applies a CE mark, if the device are classified as low risk Class I, also referred as "self-market". Nevertheless, for devices with increasing risk classes, namely Class I Sterile / Measuring, Class IIa and IIb or Class III, the device must undergo to a more complex review process, as so Notified Bodies are called [35].

Notified Bodies are private for-profit entities established and accredited by a Member State whose main function is to provide the services necessary to assess whether a product meets specific standards so it can obtain the CE marking. Each NB has an identification number composed of 4 digits, corresponding to its identification number, which appears after the marking symbol CE (Figure 2.3). The manufacturer may choose any of the NBs designated under the Directive applicable to the product, irrespective of the Member State in which it is based.

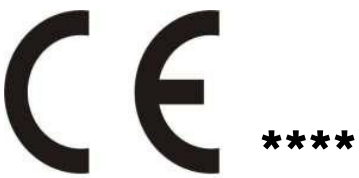


Figure 2.3. Representation of a CE mark with "****" representing the identification number attributed to the notified body.

The European Commission has a regularly updated database, called NANDO (New Approach Notified and Designated Organizations) Information System, including lists with information concerning national NBs, like the identification number of each NB, as well as the list of tasks for which they were notified [36]. Additionally, NBs are supervised by Competent Authorities which assess the compliance with the requirements of existing legislation and verifying their ability to carry out conformity assessment procedures, evidence of their competence, independence, impartiality, integrity and professional secrecy. The procedure for designation and notification of a Conformity Assessment Body (CAB) applicant for NB shall take place in the Member State where the NB is based. In Portugal, the function of Designation Authority and Notification Authority of national NBs is assumed by INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde I.P. [37].

Competent Authorities are governmental organizations responsible for the transposition and application of the MDD requirements into National Law. In addition, CA are responsible for post-approval surveillance and, as previously discussed, the supervision of notified bodies. Each country has their own CA, consequently there are some differences among CAs in terms of the structure, staffing, funding, and functions between nations, resulting in some variability between the procedures they consider necessary for fulfill the directives' requirements [32].

Generally, the regulatory framework constitutes the driven force for the development of harmonized procedures, concerning the manufacturing, labeling, clinical data required, among others. Therefore, there are some components, previously discussed, that allows the achievement of this conformity and constitutes the regulatory framework itself, such as the **regulatory rules**, a **government-approved regulatory authority**, **conformity assessment bodies**, a **classification scheme** based on potential risk for the user, a **Quality Management System (QMS)**, a **system for evaluating the clinical safety and performance** of a device, a **system for granting marketing approval** and a **surveillance system** for the device in the market. Table 2.5 compiles the key elements of the regulatory framework in the United States and Europe, mentioning the regulations, standards and guidelines as well as the regulatory authorities responsible for compliance with each of the regulatory components crucial to the conformity of medical devices.

Table 2.5. Comparison between medical devices regulations applicable for United States and Europe.

Regulatory component	United States	Europe
Regulatory rules	21 Code of Federal Regulations – 801, 803, 807, 812, 814, 820	Medical Device Directive - 93/42/EEC
Government-approved regulatory authority	FDA	Competent Authorities
Conformity assessment bodies	FDA	Competent Authorities
Classification scheme	Risk class: I, II, III	Risk Class: I, IIa, IIb, III
Quality management system	ISO 9001 and ISO 13485	Annex II and Annex V
System for evaluating the clinical safety and performance	Investigational Device Exemptions (IDEs)	MEDDEV 2.7/1 revision 4
System for granting marketing approval	FDA	Competent Authorities/ Notified Bodies
Surveillance system	FDA	Competent Authorities/ Notified Bodies

2.5. Medical Devices Approval Processes

The pathways for the approval process concerning the market entrance of a medical device are defined by the device's risk classification. Therefore, classification constitutes an important step when it comes to delineate the proper conformity assessment route for the intended device and this criterion is valid to both Europe and US regulatory approval processes.

In Europe, any medical device commercially available needs to have the CE mark. The CE marking is a legal requirement for the sale of devices in European market, ensuring that the manufacturer declares compliance with the mandatory requirements imposed by the medical devices directives. Therefore, in order to proper achieve this mandatory marking, legal procedures need to be followed and they are as complex as the greater is the risk class of the intended medical device. First, it is necessary to define which Medical Device Directive applies to the intended device. Figure 2.4 illustrates the different pathways that a device can follow undergoing the Directive 93/42/EEC, which covers most of the devices. However, if a particular device is covered by another directive, like 90/385/EEC on active medical

devices, the route is usually the same as per the Class III shown on figure 2.4 for the Directive 93/42/EEC.

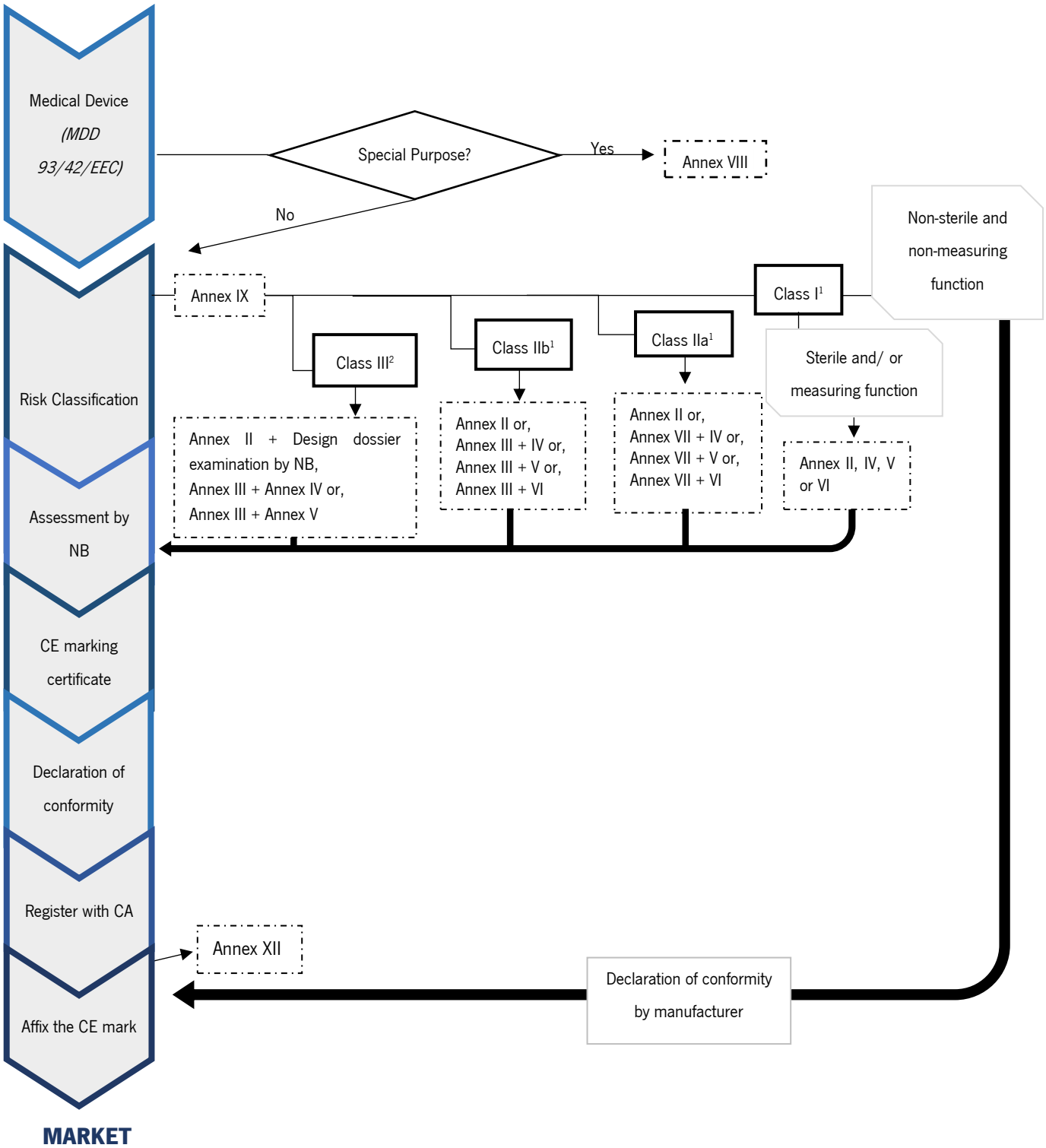


Figure 2.4. Medical devices pathway to the market in Europe. [1] Prepare a Technical File; [2] Prepare a Design Dossier. *Annex II* – EC Declaration of conformity (Full quality assurance system); *Annex III* – EC Type-examination; *Annex IV* – EC Verification; *Annex V* – EC Declaration of conformity (Production quality assurance); *Annex VI* – EC Declaration of conformity (Product quality assurance); *Annex VII* – EC Declaration of conformity; *Annex VIII* – Devices for special purposes; *Annex IX* – Classification criteria; *Annex XII* – CE marking of conformity. *NB*- Notified Body; CE- *Conformité Européene*; CA- Competent Authority.

Once the Directive is defined, which is in the present MDD 93/42/EEC, the next step is to determine if the device has or not a special purpose. If so, Annex VIII should be consulted regarding if the product is custom-made or intended for the clinical investigations, following the procedures according to the information presented on the referenced Annex. On the other hand, if the device does not fit in any special purpose, occurring in most of the cases, risk classification, based on Annex IX of the MDD, is the next stage. The classification according the risk class represents not only a point of divergence but also establishes the guiding thread for the rest of the device's regulatory path. At that stage, it is necessary to implement the Quality Management System in accordance with the proper Annexes of 93/42/EEC, based on ISO 13485 standard, to achieve QMS compliance for each risk class, as also demonstrate in Figure 2.4. Additionally, it is necessary to prepare a Technical File for Class I, IIA and IIb and a Design Dossier to Class III devices, providing detailed information on the product and one more time demonstrates compliance with 93/42/EEC [38].

Once the QMS is implemented and the technical documentation prepared, there are two different pathways to the market: if the intended device is Class I non-sterile and non-measuring, there is no need of an audit by a notified body, therefore the following step is the preparation of a declaration of conformity by the manufacturer, which is a legally document which claims that the device is in conformity with the applicable directive, and affix the CE marking; if the device is a Class I with sterile and/or measuring function, Class IIa, Class IIb or Class III, the QMS and the documentation must be audit by a notified body, aimed the issued of a CE Marking certificate for the device (generally valid for 3 years) and an ISO 13485 certificate for the facility (valid for 1 year). Hereupon, it must be prepared a declaration of conformity and, once the device is registered with the Competent Authority, the CE mark could be affixed [38].

Generally, the medical device approval processes in Europe could last from few days to few years, depending clearly on the device's risk class but also on the manufacturer capacity and, for example, the need of clinical data.

Furthermore, all the devices information collected by CA is exchanged with the European Commission through a databank called Eudamed - European Database for Medical Devices – and only these two parties have access to it under the current European law. Although, with the new proposed device regulations, this data will be expanded to other players involved in the medical devices sector, like for example, notified bodies, manufacturers and medical public institutions, attempting to overcome the transparency issue that is widely associated with the current European procedures.

Although the risk classification systems are similar, the regulatory approval process for a device is considerably different in United States compared to Europe. The fact that in US there is no Notified Bodies systems, all the information is centralized in FDA, which contributes to the observed distinct pathways to the market in Europe and US. Figure 2.5 presents the different routes that a device could follow in order to be commercialized in the US market.

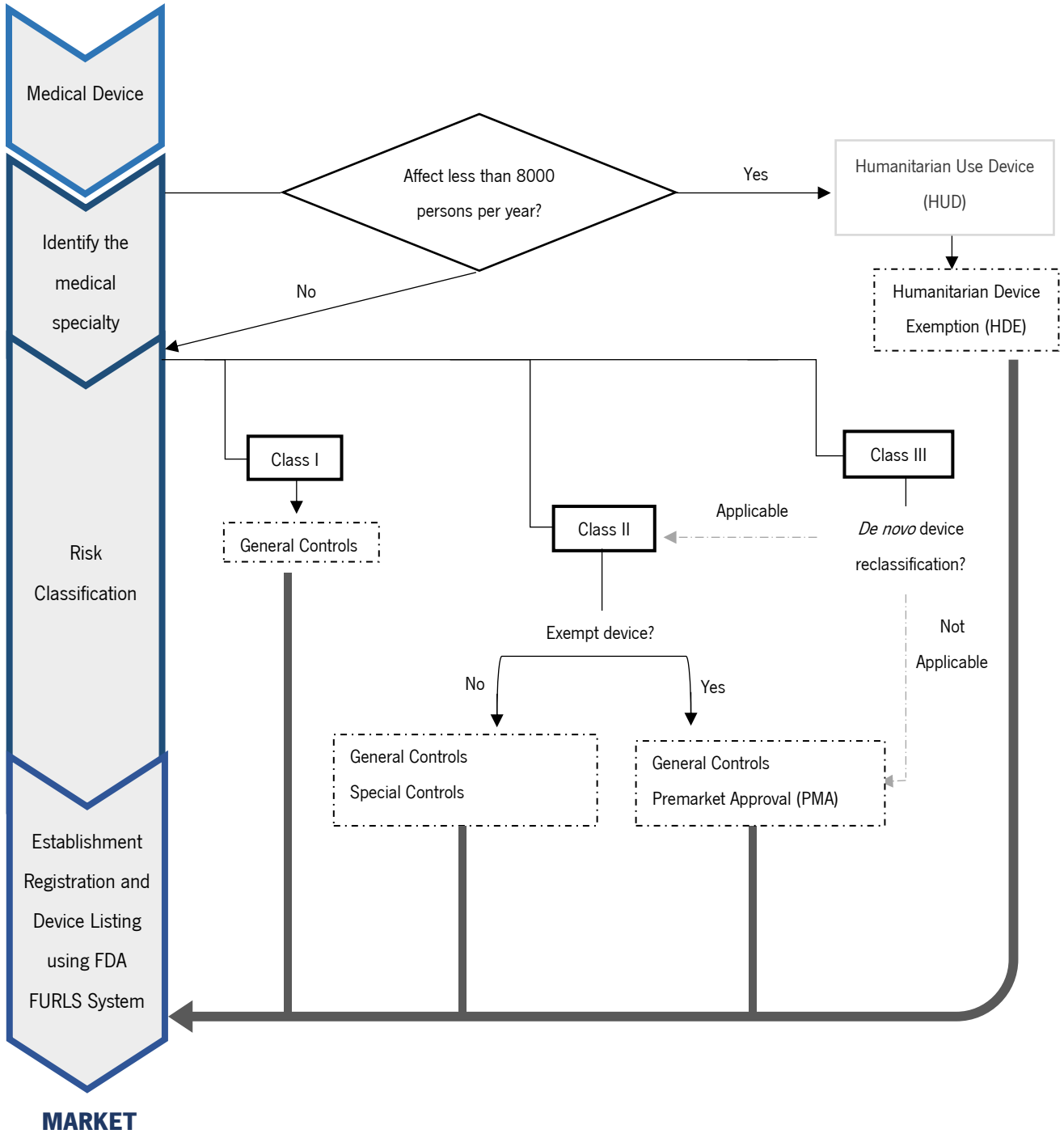


Figure 2.5. Medical devices pathway to the market in US.

The first step targeting the selling of a device in US market goes through the identification of the medical specialty "panel" and the associated three letter Product Code and seven-digit Regulation Number, consulting the FDA classification database. If a particular device doesn't fit any of the 20 pre-defined panels, one can use 513 (g) FD&C Act (21 U.S.C. 360c(g)) to request a new classification to the FDA. Once this identification is done, the next step is to define if the rare disease or condition for the intended use of the device affects, or not, less than 8000 persons/year. In affirmative case, the medical device is classified as a "Humanitarian Use Device", a special Class III case, and consequently follows the FDA marketing application called Humanitarian Device Exemption (HDE), exempted the device from the effectiveness requirements of Articles 514 and 515 of the FD & C Act. Otherwise, if the problem affects more than 8000 individuals, risk classification constitutes the subsequent step.

Therefore, reached the stage of risk-based classification, there are three main classes that will guide the next steps: Class I, Class II and Class III. If a device belongs to the lowest risk class, it goes under the least stringent regulatory process, in fact required to all device's classes – General Controls. This regulatory process consists on basic provisions to ensure the safety and effectiveness of the device, aimed the compliance with Quality System Regulation/ Good Manufacturing Practices. On the other hand, there are the Class II devices, which, although in some cases could be exempt from premarket notification 510(k), most of them are submitted to the 510 (k) process. This process is implemented when it is claimed that the device is substantially equivalent to an already legally marketed. Additionally to this premarket submission and the general controls, no exempted class II devices must fulfill special controls like for example, premarket data requirements, guidelines, patient registries, special labelling requirements and post-market surveillance.

Furthermore, most of devices included in the highest risk class, plus innovative class II devices resulting by the de novo classification, require clinical studies which imply the application of Investigational Device Exemption (IDE) regulation, a system to evaluate the safety and performance of the device and which will subsequently support the premarket approval application (PMA), the most rigorous path that these devices should follow until the market entrance.

The last and common step that all devices from all risk classes must undergo in order to be legally sold in US, is the company registration and device listing using FDA FURLS System.

Overall, as it exposed throughout this section, Europe and United States present quite different medical devices approval processes, however both have their strengths and fragilities. While in Europe the complex regulatory system composed by government agencies (CA) and private for- profit companies (NB), may turn the entire development more complicated and ambiguous, in US the centralized authority system makes the process more straightforward. Still, Europe process may be more flexible, shorter and shipper. Another difference lies in the availability of the information resulted by the approval processes, which in US is public unlike in Europe. Finally, in terms of timelines to device approval, the consensus is that in Europe the process is faster than in the US, however this is not as simple since some studies have shown that the US's get to the market faster. Additionally, the lack of available data, due to the European confidentiality regulations, can mislead this assessment.

3. Medical Devices Technical Documentation

This chapter makes a parallelism between the regulatory controls governed by the two major markets for medical devices, accordingly, the documentation required by the regulatory agencies of both Europe and the United States will be discussed. The chapter also describes the two guidelines available for structuring the technical documentation of a medical device, one issued by the Coordination of Notified Bodies Medical Devices (NB-MED), considered the *European format* and the other issued by the Global Task Force Study Group, the format "STED", recognized by US, European, Canadian, Australian and Japanese regulators, as well as in other markets.

3.1. Technical Documentation

The technical documentation constitutes a documented evidence that a medical device complies with the requirements of the applicable regulations. It is the responsibility of the manufacturers to collect the information that supports the performance of the device in a structured, objective and scientific-evidence based manner.

The importance of the technical documentation is easily understandable by the fact that this comprehensive description of the medical device will be assessed by regulatory agencies in order to grant or not to grant the marketing approval. Therefore, the data provided by the manufacturer should demonstrate how the design and manufacture of the medical device comply with the Essential Requirements of safety and effectiveness and/or other applicable regulations and guidelines. It should be also proved that the medical device was produced under a quality management system which comply with the applicable requirements, as the ones described by the FDA 21 CFR 820, Quality System Regulation (QSR), or the ones that follow the EN ISO 13485 standard, Medical devices: Quality management systems – Requirements for regulatory purposes.

Generally, as higher is the risk classification of a medical device, the more exhaustive is the documentation required, as a result from a more complex process of development. This is also consistent with the way that information flows between regulatory authorities and manufacturers. While for higher-level risk devices the technical documentation concerning product design, labelling and manufacturing processes is submitted as part of applications for marketing approval (design examinations in the EU and 510(k)s and PMAs in the US). Conversely, for Class I devices, documentation does not have to be delivered but must be always available and updated for consultation by the regulators at any time. To facilitate this process, there are regulations and guidelines that contains the specifications required for the applications submissions next to the main jurisdictions (described in the next sections).

Overall, it can be concluded that the success of the medical device market approval process relies in the quality level of the technical documentation. A poorly structured dossier or inconsistent documentation can seriously reduce the chances of getting the product to market, on the other hand, when a document is clear, well structured, objective and precise, the probability of obtaining approval in a short time is higher and still facilitates post-marketing surveillance activities. Moreover, it should be noted that the dossier of the technical documentation is a dynamic and controlled document, which needs to be updated whenever necessary, that is, if any relevant alteration was done to the medical device.

3.1.1. Technical Documentation by EU Regulations

As already mentioned, the CE marking is a legal requirement for the placing of medical devices on the European market. Considering that the compiling of the technical documentation is key to obtaining such mark, it is logical that this process represents a critical step for the success and speed of the product's market entrance (Figure 3.1), intervening in two essential phases, in its own design throughout the development of the product and also in the process of auditing the product by the NB.

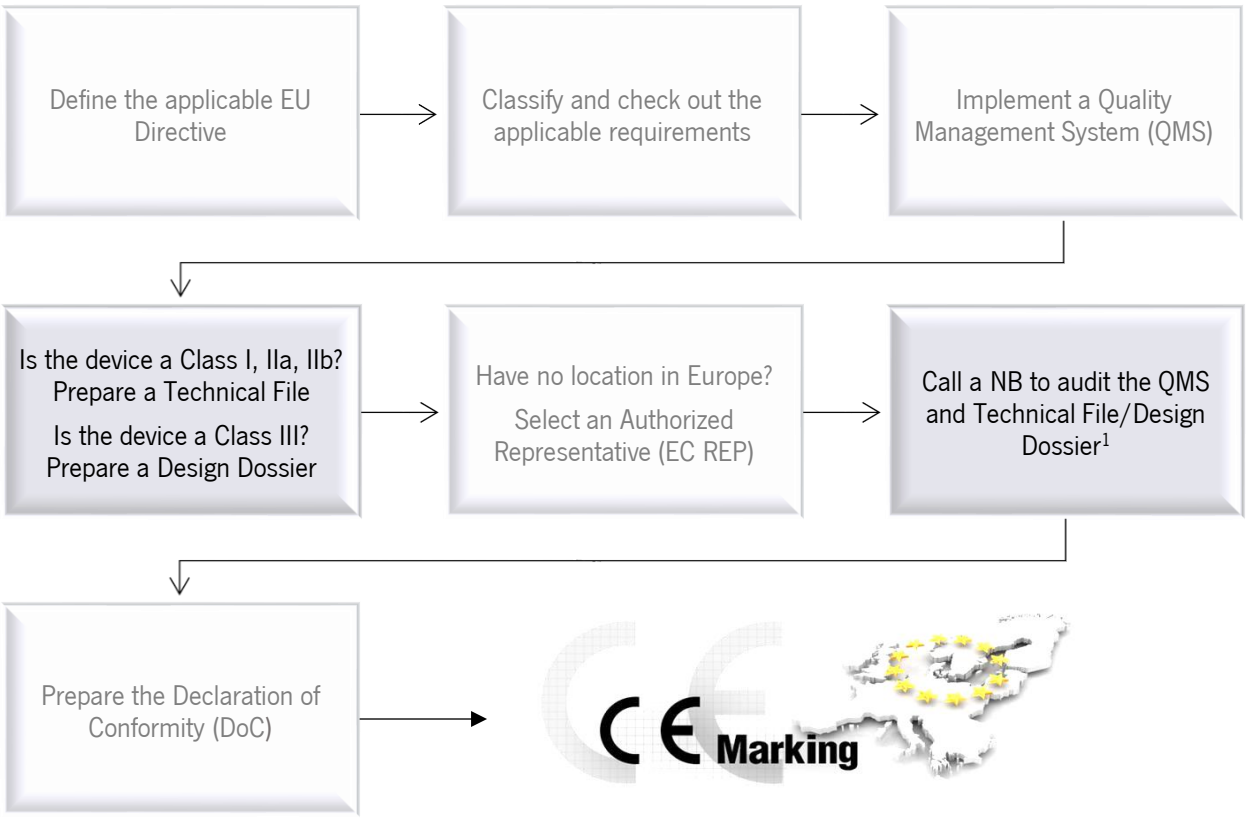


Figure 3.1. Basic steps in the CE marking process (the technical file role is shaded). [1]Unless the device is a Class I non-sterile, non-measuring.

In Europe, medical devices directives constitute not only the support for all the devices regulatory questions but also constitute the guideline for the content of the technical documentation, therefore all products, comprised in any of these directives, require the existence of a Technical Dossier or a Design Dossier.

The “Technical File” designation is widely used to name the compilation of technical documentation regarding the medical devices belonging to the Class I, Class I with sterile and/or measuring function, Class IIa and Class IIb. As regards the complexity of this documentation, a greater detail is expected for the devices belonging to higher classes IIa and IIb, on the other hand, for class I devices, the file may be less complex, insofar as the device-associated risk is lower. The "Design Dossier" designation generally refers to the technical documentation collected for the higher risk class of medical devices, Class III, consequently requiring a more complex and detailed dossier [39].

According to the Directive 93/42/CEE, the Class I sterile and/or measuring devices, as well as those belonging to the Classes IIa, IIb and III require the intervention of a third party, the Notified Body, for the assessment of their conformity. On the other hand, this assessment procedure does not occur in the same way if the devices belong to Class I. The affixing of the CE marking for these low risk devices shall be totally of the manufacturer responsibility, who is obliged to prepare a Declaration of Conformity, notify the Competent Authority and subject to supervision by the said competent authority. Notwithstanding the need of a notified body intervention for the device conformity assessment, it should be always necessary a complete compilation of technical documentation, despite the device risk classification, in order to be prepared for possible inspection or review by the Competent Authority or an unannounced audit by the notified bodies. Thus, it is perceptible the need of keeping the documentation constantly updated during the device life cycle and, in the specific cases that the manufacturer is not located in Europe, that information should always be available in the EU Authorized Representative [40].

Currently, there are some guidelines that assist on technical documentation. For instance, the Notified Body Operations Group (NBOG) is a working group set up by Member States and the European Commission precisely to improve the overall performance of Notified Bodies in the medical devices sector by the production of written supporting documents and guidelines. The *NBOG BPG 20010-2, Guidance on Audit Report Content (Mar 2010)* and the *NBOG CL 2010-1: Checklist for audit of Notified Body´s review of Clinical Data/Clinical Evaluation (Mar 2010)* are two examples of these documents and, despite not being properly design for the manufactures, they should consult these guidance files in order to predict the points at which the audit of the NB will focus [41]. Additionally, there are another guideline - *NB-MED Recommendation 2.5.1 Technical Documentation*- that is consider the *European format* for the

generation of the technical documentation and will be detailed in section 3.2.1, “Recommendation NB-MED/2.5.1/Rec5 Technical Documentation”.

Finally, it is important to keep the technical documentation for a period of not less than 5 years after the last product has been manufactured.

3.1.2. Documentation Required by US Regulations

In the United States, medical devices must receive the FDA approval in order to be marketed. Hence, there are some regulations and guidelines that document the necessary requirements for the preparation of the technical documentation which in turn will prove the compliance of the device.

The 21 Code of Federal Regulations, namely the *21 CFR Part 807 Subpart E, Premarket Notification Procedures (510k)* and the *21 CFR Part 814 Subpart B, Premarket Approval (PMA) Application* are two examples of regulatory requirements describing different submissions processes, according to the device specifications. However, there are other regulatory requirements set out by 21 Code of Federal Register that manufacturers of medical devices distributed in the US must demonstrate compliance targeting the FDA approval, as previously discussed in Section 2.4.1, “United States Medical Devices Regulation”.

Consequently, the information resulting from the medical device product development, in conformance with the regulatory requirements, must be compiled in the technical documentation, such as the Design History File (DHF), Device Master Record (DMR) and the Device History Record (DHR). The DHF is mandatory in US and it is intended to compile information, regarding the design controls and required by the quality system regulation, to demonstrate that the finished device was developed in accordance with the approved design plan. Moreover, the Device Master Record includes records on device specifications which may be drawings, components, formulation or software, the production process specifications, including the equipment, production procedures, methods and environment, the quality assurance procedures, regarding acceptance criteria and equipment, also comprise detailed information about the packaging, labeling and the installation, maintenance, and servicing procedures for a finished device. While the Design History File should be prepared according to 21 CFR Part 820.30, the Device Master Record should follow the 21 CFR Part 820.40 [42].

Lastly, the Device History Record (DHR) is the history of the device and everything necessary to build it, in other words, it records the production history of a finished device. This file contains the batch records for each lot, the date of manufacture, the quantity manufactured and released for distribution, the records which prove the device is manufactured in accordance with the DMR, the primary identification label and labeling used for each production unit and any device identification(s) and control

number(s) used, such as unique device identifier (UDI) or universal product code (UPC) [42]. Figure 3.2, shows the FDA Quality Systems Regulations Records, including the contents that should be addressed in each file.

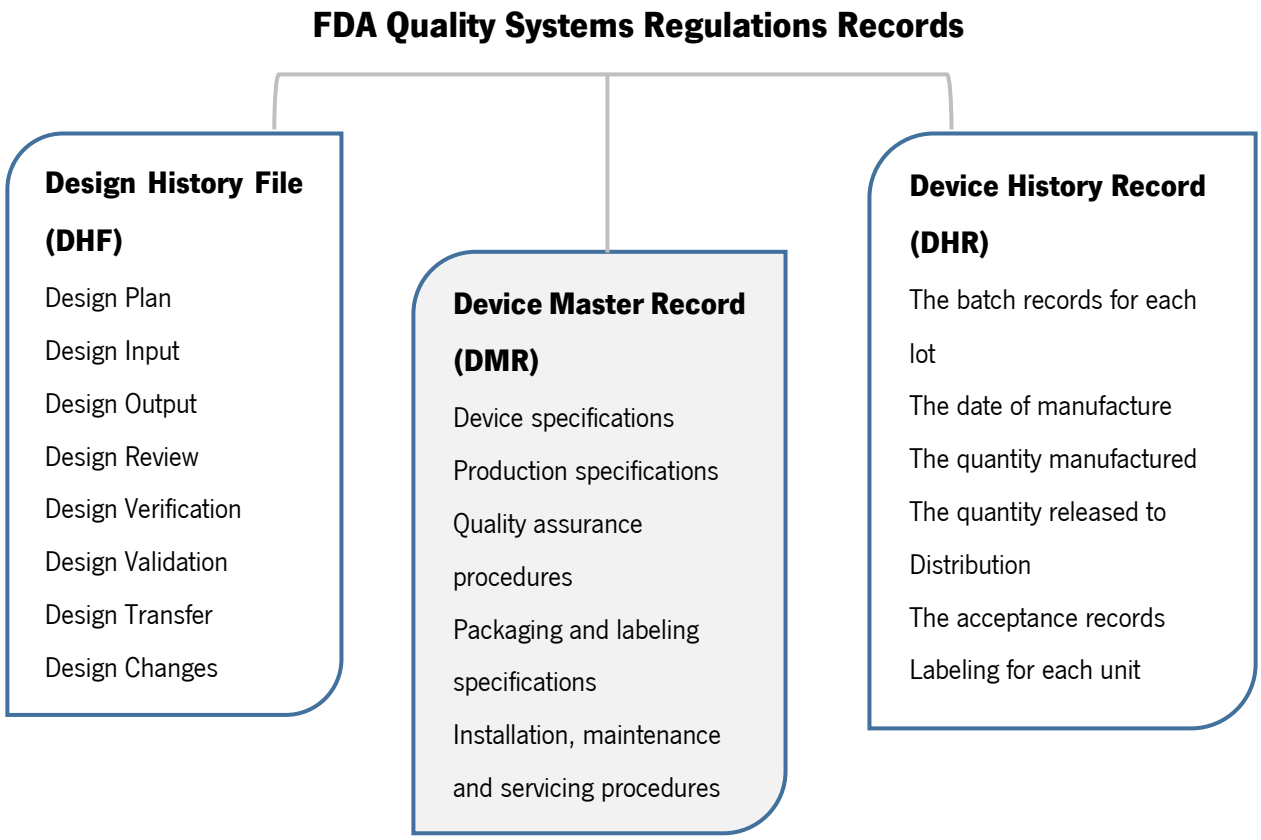


Figure 3.2. FDA Quality Systems Regulations Records: the Design History File, the Device Master Record and the Device History Record.

Despite sound quite similar and being related somehow, these files respond to different requirements, follow distinct subparts of the 21 CFR Part 820 - Quality System Regulation and, consequently, output the information in a different way. One can distinguish the three terms and establishing at the same time a relationship between them, by consider the Design History File as the collection of the design records from beginning to launch as well as any changes over time, considering too that the Device Master Record result from the output of the specifications previously developed in the DHF during the design process. At the end, the Device History Record appears as the proof that the product was manufactured in conformance with the DMR [43].

Overall, the documentation must be complete as well as compliant because during an FDA audit, it could be assessed the consistency of the generated documentation by the comparison between the DMR

and the corresponding DHR. Concluding, this supporting documentation constitute the evaluation route for the medical device approval process according to FDA regulatory requirements and so this could be considered the US equivalents to the technical file/dossier design in Europe.

3.2. Guidelines for technical documentation preparation

As shown earlier, organizing the technical documentation can be laborious because there is a lot of information and details that could not be missed or misunderstood in order not to undermine the review process and subsequently the approval next to the competent authorities. Despite the information required by both Europe and US jurisdictions be fundamentally the same, the level of complexity will be mostly dependent on the risk classification of the medical device but could also be conditioned by the type of submission if the process target a FDA approval, for instance.

Notwithstanding, several entities have issued guidance on the topic of technical documentation, namely the Notified Body Operations Group, Global Task Force Study Group, International Medical Device Regulators Forum, the International Organization for Standardization or the Co-ordination of Notified Bodies Medical Devices. The framework released by these organizations aims to support and help in the preparation of concise, organized and coherent technical documentation to guarantee the success of the medical device market entrance. In particular, if a manufacturer has already identified an NB to carry out its device revision, it is recommended to that manufacturer also request guidance to that NB concerned the content and format that they expected to evaluate attending their own checklist.

Regarding the technical documentation structure, it should be noted that, although there are no specific requirements for the manufacturer to structure the technical documentation in a specific way, to be aware of how the notified body will perform the revision constitutes by itself a definition of structure. Furthermore, there are two available orientations that will be addressed in the next sub-sections which represent the currently used structure models, the Recommendation NB-MED/2.5.1/Rec5 and the "STED" guidance document.

3.2.1. Recommendation NB-MED/2.5.1/Rec5 Technical Documentation

The NB-MED group issued *Recommendation NB-MED / 2.5.1 / Rec5 on technical documentation* to provide guidance to manufacturers, notified bodies and competent authorities on the technical documentation needed to meet the requirements of the medical devices directives [44].

Generally, the information that should be comprised in a Technical File or Dossier Design includes these items present on the recommendation and represented on Figure 3.3.

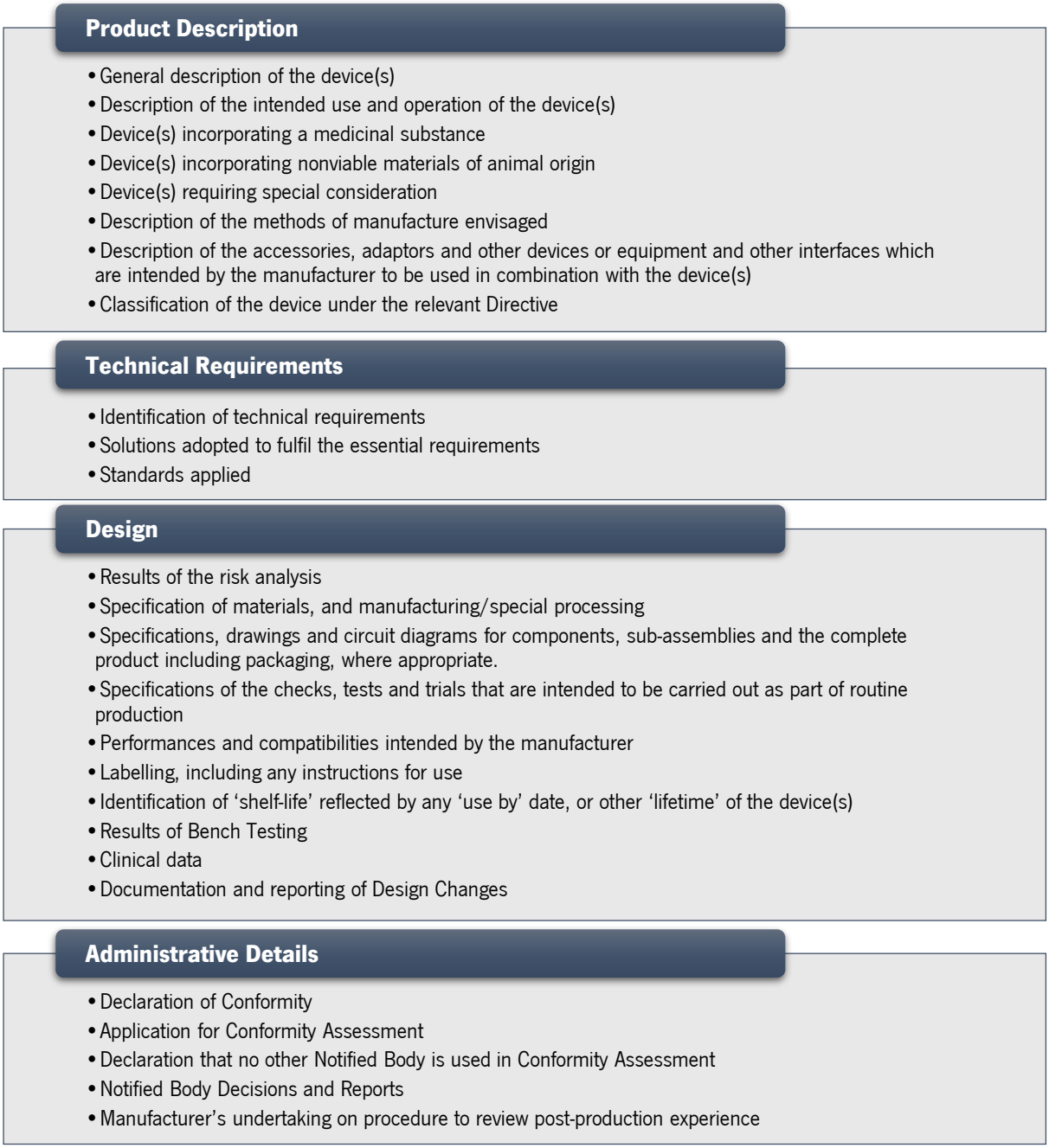


Figure 3.3. Descriptive information of the *Recommendation NB-MED/2.5.1/Rec5 Technical Documentation*.

Therefore, considering that the technical documentation should be as concise as complete, it is important to manage the information in the most effectively way possible to facilitate the device placing on the market and market surveillance activities. To accomplish this, the NB-MED Recommendation suggests that the structure of the dossier should be divided into two parts, Part A and Part B (Figure 3.4) [44].

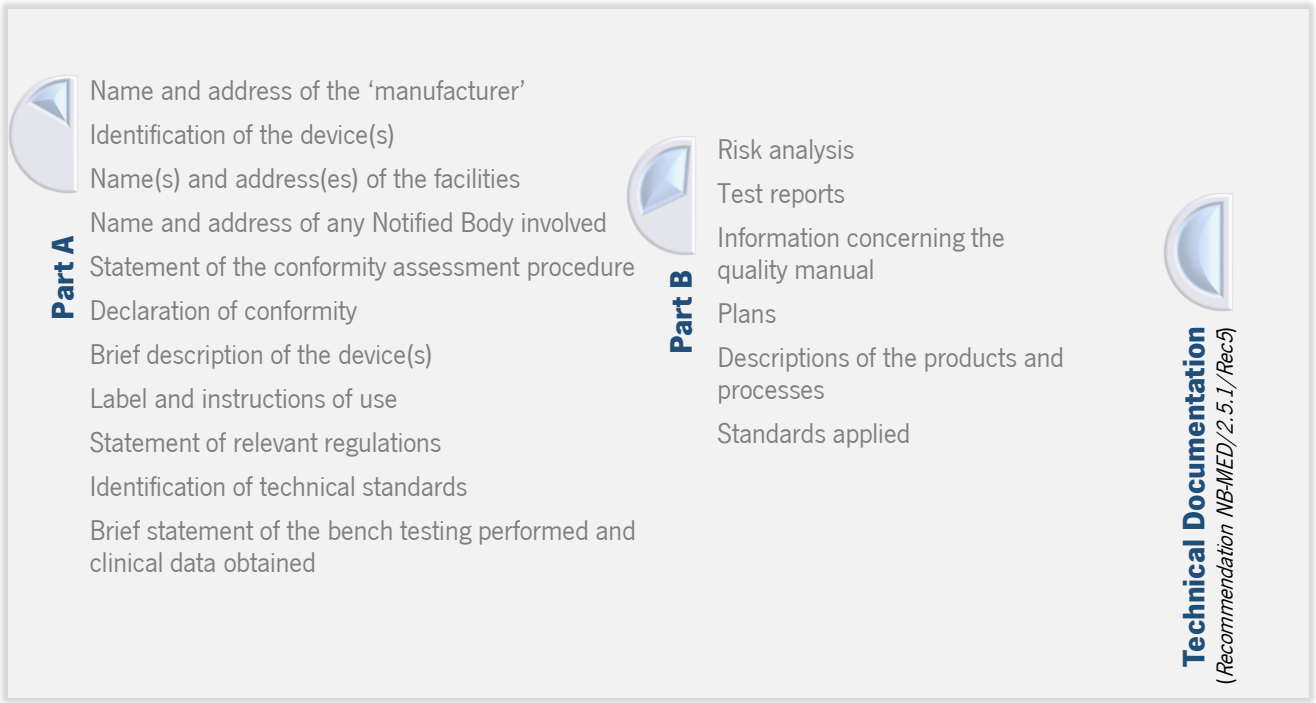


Figure 3.4. Recommended structure for the technical documentation according to the *Recommendation NB-MED/2.5.1/Rec5 Technical Documentation.*

Essentially, the Part A contains a summary of the essential technical data relevant to the conformity assessment procedures like the name and address of the 'manufacturer, facilities or any notified body involved, a statement of the conformity assessment procedure being followed, a declaration of conformity, a brief description of the device, relevant standards and regulations with which compliance is claimed, label and instructions of use as well as a brief statement of the bench testing performed and clinical data obtained. On the other hand, the Part B details the remaining technical documentation derived from the risk analysis, the test reports, the descriptions of the products and processes, information concerning the quality manual and standards applied [44].

In general, this format, sometimes also referred as *EU Technical File*, may be maintained in hardcopy or electronic format. Finally, all the elements set out in the Annexes to the Directives must be present and the relevant essential requirements described in Annex I must be complied with.

3.2.2. Summary Technical Documentation (STED) Guidance Document

The Summary Technical Documentation (STED) guidance was created by the former Global Harmonization Task Force (GHTF). As already explained in section *2.2. Definitions*, GHTF, the precursor to the current International Medical Device Regulators Forum (IMDRF), emerges in attempts to standardize medical device regulatory submissions across different jurisdictions. Therefore, it is in this context that some guidelines were created, precisely to be used as non-binding reference documents for the medical devices industry, decreasing differences among markets and allowing patients earlier access to new treatments [45].

Basically, the STED GHTF Guidance Document provides recommendations on the content and structure of summary technical documentation to be assembled and submitted to a Regulatory Authority or Conformity Assessment Body for premarket review as well as to assess post-market continuing conformity, thus allowing the manufacturer to prepare a dossier which demonstrates that its product complies with the essential safety and performance requirements [45].

The GHTF member countries regulators from US, Canada, Australia and Japan recognized and implemented the STED format, just like other markets such as the Brazilian medical devices market. In Europe, the scenario is a little different, as a member of GHTF, the STED is also recognized, still it has not yet officially adopted, being the formats based on the NB-MED Coordination Group Recommendation the most used ones. Nevertheless, in 2013 for the first time it was made a subtle reference to the STED in an official European Commission document, named *Commission Recommendation (2013/473 / EU) of 24 September 2013 on the audits and assessments performed by notified bodies in the field of medical devices.* Therefore, in this recommendation it is stated that the technical documentation should cover the items listed in the STED in order to be complete as well as the additional items required by European legislation (Figure 3.5).

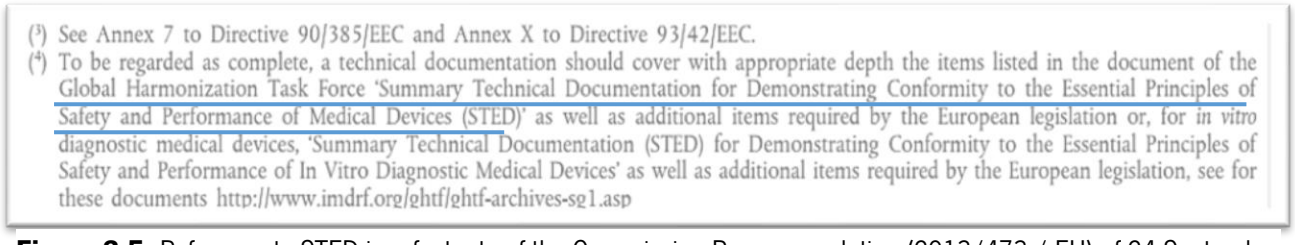

(3) See Annex 7 to Directive 90/385/EEC and Annex X to Directive 93/42/EEC.
(4) To be regarded as complete, a technical documentation should cover with appropriate depth the items listed in the document of the Global Harmonization Task Force 'Summary Technical Documentation for Demonstrating Conformity to the Essential Principles of Safety and Performance of Medical Devices (STED)' as well as additional items required by the European legislation or, for *in vitro* diagnostic medical devices, 'Summary Technical Documentation (STED) for Demonstrating Conformity to the Essential Principles of Safety and Performance of In Vitro Diagnostic Medical Devices' as well as additional items required by the European legislation, see for these documents http://www.imdrf.org/ghtf/ghtf-archives-sg1.asp

Figure 3.5. Reference to STED in a footnote of the Commission Recommendation (2013/473 / EU) of 24 September 2013 on the audits and assessments performed by notified bodies in the field of medical devices.

Briefly, *GHTF/SG1/N011:2008 - Summary Technical Documentation for Demonstrating Conformity to the Essential Principles of Safety and Performance of Medical Devices (STED),* recommends the following template for the STED dossier (Figure 3.6).

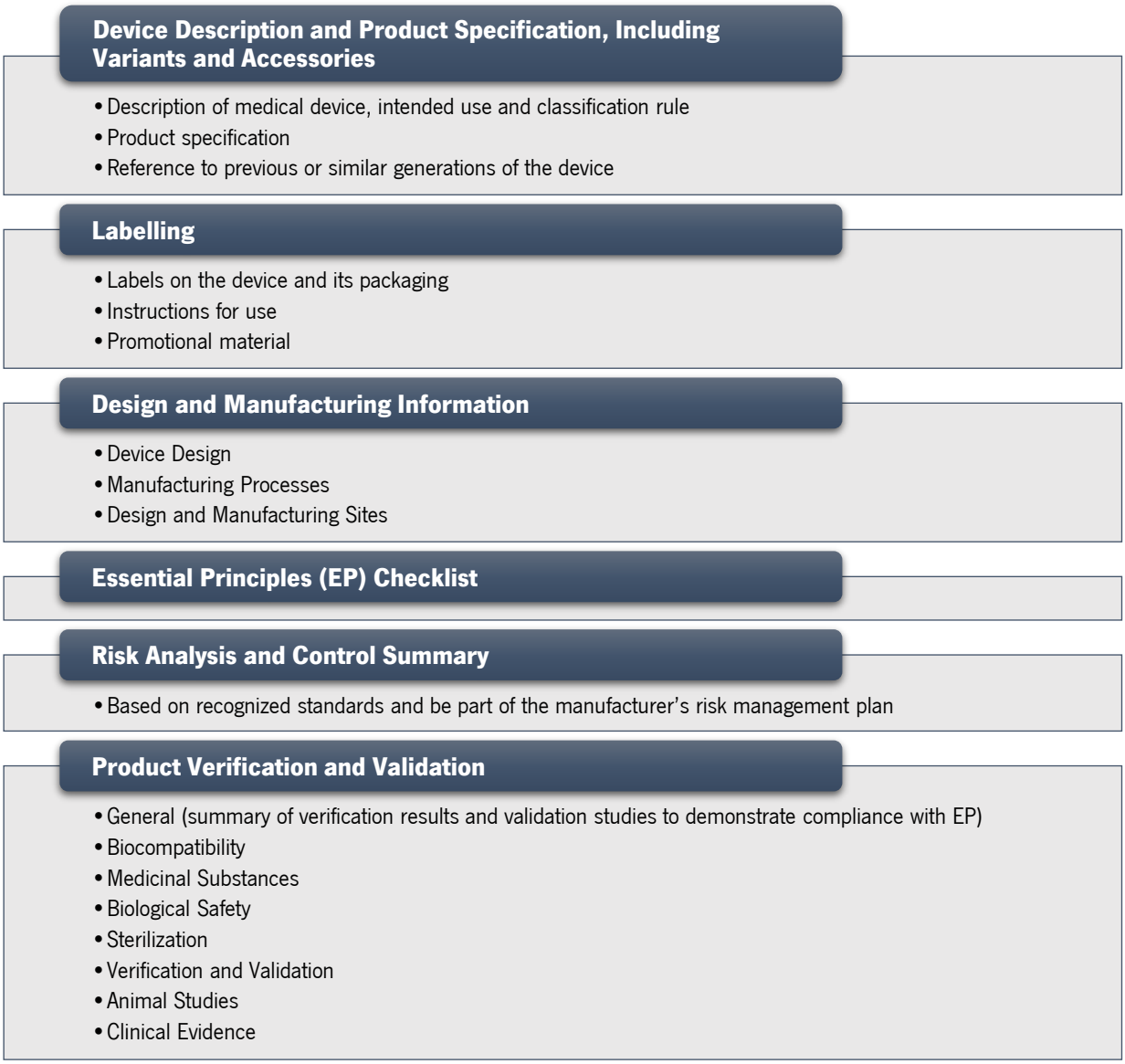


Figure 3.6. Descriptive information of the STED dossier.

Of course, the information included in the STED will vary depending on the level of complexity of the product concerned, thus depending on features included in the device such as the presence of new or potentially harmful materials, incorporation of new technologies or even the change in intended use for a device that is already marketed.

Regarding the dossier format, in the STED guidance there is no specific recommendation addressing this topic, however it is mentioned that it would be helpful for both manufacturers and reviewers to follow the order that the STED dossier items are listed, that is, first the *Device Description* and *Product Specification*, then *Labelling* and so on.

Overall, the core information needed to prepare a STED dossier is very similar to the information required to build the dossier in the European format. Concluding, the use of STED is a value-added tool especially for multinational companies that wish to generate a single document, accepted in the largest markets of the medical device sector, thus facilitating the documentation management and updating.

3.3. Elements of a Technical File/Dossier Design

So far, it has been explained what is it the technical documentation of a medical device, what is the documentation required by the regulators of the two largest markets in the sector, as well as the existence of models that help organize all this necessary information, whose preparation represents an extremely important procedure for conformity assessment and consequent product approval.

Herein, it will be scrutinized some of the elements included in technical documentation, more specifically in Technical Files or Dossier Design. Then, the next topics following presented intend to list the essential elements comprised in technical documentation, giving a brief description and characterization of general items that constitute the different sections of the overall dossier, as previously defined. So, these are some of the recommended elements which shall appear in the documentation:

i. Description of the medical device

This section should include a general description of the device, its design and characteristics, also should comprise representative images and describe the target population as well as include any intended range of variants (e.g. size, number, name).

ii. Classification of the device under the relevant Directive

In this part, it should be included the device classification along with a brief rationale for that and the rule number(s) applied under the Directive. The guidance document, *MEDDEV 2.4/1 rev9 – Classification of medical devices (June 2010),* could assist in this topic [22].

iii. Conformity assessment procedure follow under the applicable Directive

To compile the information necessary in this section, it must be considered the previous step of classification because, as explained earlier, the choice of the conformity assessment procedure is

dependent on the classification of the device and the different procedures are described in Annexes II to VIII of the Directive 93/42/EEC (see also section *2.5. Medical Devices Approval Process*, Figure 2.4).

iv. Essential Requirements (ER) Checklist

This segment is of extreme importance in the general documentation, as has been explained before, because through this checklist, the manufacturer defines the technical requirements and specifications that must be satisfied to ensure that each requirement of the applicable directive is met, as so it could be seen as a guidance for the overall design and product development. In this checklist, it should be addressed the applicability of each ER to the device (if a particular ER not apply, a brief rationale should be given), the applicable standard that was used, the demonstration of compliance with the ER and the location of the documentation evidencing the demonstration of conformity. According to the Article 5 of Directive 93/42/EEC, the compliance with the European Harmonized Standards published in the EU Official Journal presumes compliance with the essential requirements [44].

v. Standards and guidelines applied

As in the essential requirements, also the standards and guidelines used should be listed as part of the technical documentation. There are several standards and guidelines that could oversee different parts of the device development, like for example the *EN ISO 14971: Medical devices – Application of risk management to medical devices, 2012* or the *MEDDEV 2.7.1. Evaluation of Clinical Data: A Guide for Manufacturers and Notified Bodies. 04.2003.* It is useful and recommended that along with the standards list, it is included the complete title of the standard, the corresponding identification numbers, including the revision number of the standard likewise the organization responsible for its preparation [46].

vi. Description of the intended use of the device

In this section, it should be detailed the intended use of the device, including information on the intended purpose/application, the intended patient population or medical condition(s), the intended user(s) and the administration route.

vii. Relevant regulatory information

The technical documentation must contain information (name, address, contacts, certifications, ...) and details about the manufacturer, suppliers, subcontractors, authorized representative (if applicable) and other relevant entities. Additionally, other details could be presented here like the date of first placing on the market or countries where the medical device is marketed [46].

viii. Manufacturing process

This part shall include a description of the manufacturing process, including a flowchart of the manufacturing process, a brief description of the method and manufacturing conditions and inspections, environmental monitoring, labeling control, traceability, criteria for release of the finished product, among others [46].

ix. Declaration of Conformity

The EC Declaration of Conformity is a formal statement issued by the manufacturer declaring that the device concerned meets the provisions of the Directive which apply to it. This document set out several information, as demonstrated in Figure 3.7 [39, 46].

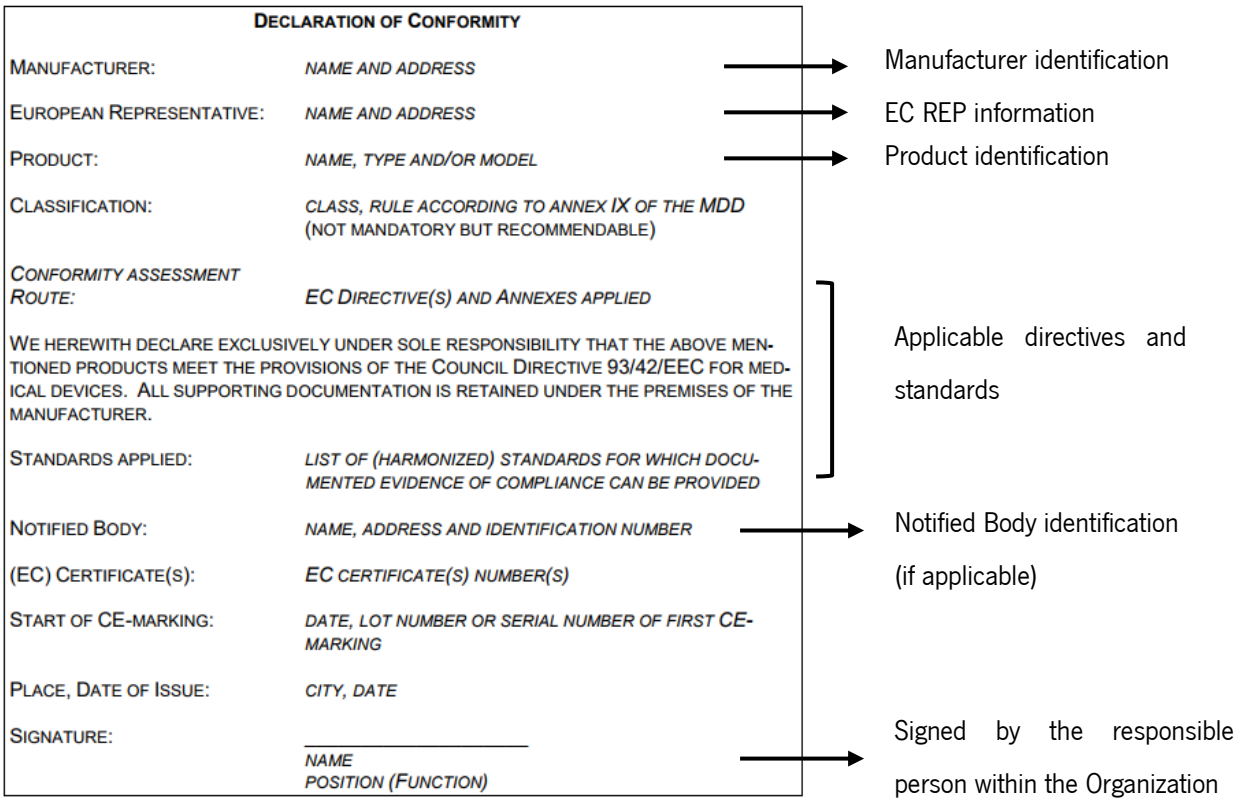


Figure 3.7. Template of a EC Declaration of Conformity.

x. *Risk Analysis and Risk Management Dossier*

From this segment of the technical documentation should result a risk management dossier outcoming from the risk analysis, the risk assessment activities as well as the measures to minimize the risks (if applied). This is an exhaustive process that intend to demonstrate whether there are risks associated with the use of the product, whether it is compatible with a high level of protection of health and safety, and whether these risks are acceptable when counterbalanced with the expected benefits to

the patient or user. The process should be compliant with the standard *EN ISO 14971:2012 – Application of risk management to medical devices [47]*.

Clearly, the previous items are just few of the elements that a complete technical documentation includes, being the full list detailed in guidance documents as the ones earlier shown in *3.2. Guidelines for technical documentation preparation.*

4. Study Case: *BioMultiDress*, the innovative bioactive dressing for the treatment of chronic wounds

Recalling the primary objective of this study, to identify the legal procedures in order to develop a novel medical device for the treatment of chronic wounds, so far it has been analysed the medical devices sector under the regulations of the EMA and FDA agencies. Hence, once the specifications involved in the regulation of this sector were already scrutinized, in this chapter it will be presented all the details of the therapeutic mechanism involved in the device as well as the technical requirements of the intended wound dressing, the *BioMultiDress.* But first, it will be done a briefly overview of the problematic of chronic wounds, the existing different types of wound dressings available on the market, as well as the introduction of bacteriophages as antimicrobial agents.

4.1. Chronic Wound: a serious health concern

4.1.1. Chronic wound as a global problem

Wound is an injury that cause a defect or a disruption in body tissue, usually involving the skin. Depending on the nature of the healing process, wounds can be two types: acute or chronic. The main difference between them two relies in the nature of repair process and the time taken for a wound to heal; as such acute wounds (like surgical wounds, traumatic wounds or burns) heal quickly, about 8-12 weeks [48, 49].

Chronic wound can be defined as a break of the anatomic and functional integrity of the skin, which reparative process does not occur or that is slow, taking beyond 12 weeks. Chronic wounds are classified as typical and atypical, where about ninety-five percent (95%) of wounds are typical one, which include leg ulcers, frequently caused by venous or arterial deficiencies [venous or arterial leg ulcer (VLU or ALU, respectively)], diabetic foot ulcers (DFUs) or pressure ulcers (PUs) (Figure 4.1) [50, 51].

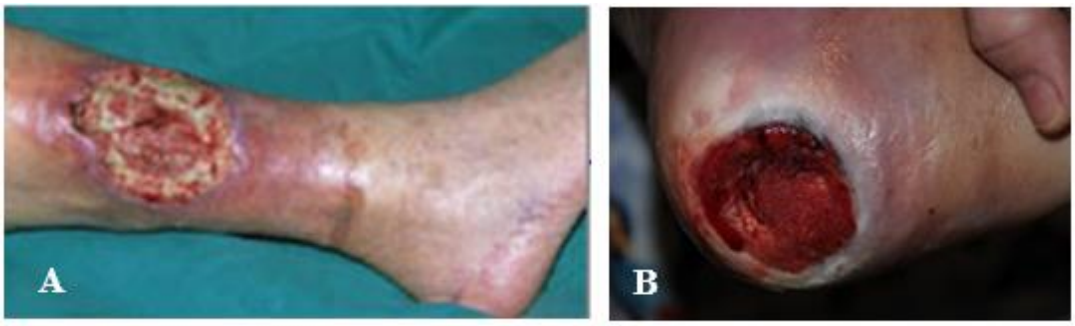


Figure 4.1. Representation of two different types of typical chronic wounds. (A) Leg ulcer. *Adapted from [50].* (B) Pressure ulcer. *Adapted from [51].*

On the other hand, the atypical chronic wounds can be a result of autoimmune disorders, infectious diseases, vascular diseases and vasculopathies, metabolic and genetic diseases or drug related reactions [52, 53].

The impaired tissue repair in chronic wounds due to the essential phases of homeostasis, inflammation, proliferation, and tissue remodeling does not occur in the proper sequence, at a specific time. There are some factors that affect the healing process: the systemic factors, related to the overall health or disease state of the individual, like age, stress, diabetes, obesity, alcoholism, smoking, immunocompromised conditions (cancer, AIDS) and nutrition, and the local factors, related to the characteristics of the wound itself, like oxygenation, infection (caused by *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp, Enterococcus spp.*), foreign body and venous sufficiency [54, 55].

Chronic, non-healing wounds affect significantly the quality of life of patients and the families that care for them, also place a massive financial burden on healthcare systems, as well as morbidity and mortality for afflicted individuals [49]. It is estimated that in worldwide, chronic wounds occur in 50 million people, with costs that exceeds the billion dollars. In the United States for example, chronic wounds affect around 5 to 7 million patients and the associated costs exceed 25 billion dollars a year [49, 56]. In Portugal, the information available about the epidemiology of wounds is limited and the economic impacts are poorly described.

This disease represents a challenging problem that affects the entire world since it is expected that in developed countries, 1-2% of the population experience a chronic wound during their lifetime [49]. This is even more alarming because of the considerable increased of population aging and increased susceptibility to infections and other diseases.

4.1.2. Dressings for chronic wounds

A dressing can be defined as a wound covering. Since the Egyptian times, the practice of treating wounds with dressings remains functional, obviously with some improvements brought by the advances in molecular biology, biotechnology (techniques, methods, equipment) and the inherent knowledge of the mechanisms behind the injury and inflammation process [57]. An ideal wound dress should have some characteristics that optimizes the healing process, assuring optimal moisture wound environment, such as: i) capacity to provide thermal insulation, gaseous exchange, and to help drainage and debris removal thus promoting re-epithelialization by stimulating collagen synthesis; ii) biocompatible and not provoke

any allergic or immune response reaction; iii) protect the wound from secondary infections; iv) and should be easily removed without causing trauma [58, 59].

A large variety of wound dressings are available in the market and some of the most common types are shown in the table 4.1. As it can be seen, there are few wound care products that effectively combine the essential steps of tissue removal, infection control and moisture balance. Thus, it would be important to develop new devices that optimize the healing process and simultaneously provide maximum comfort for the patient, since the treatment of chronic wound is usually a painful procedure for the afflicted individuals.

Table 4.1. Types of dressings available in the market for chronic wounds. +++, most effective, ++, more effective; +, effective; +/−, variable effectiveness; −, Not effective. *Adapted from [60].*

Type	Characteristics	Tissue removal	Infection control	Moisture balance
Films and membranes	Thin polyurethane membrane coated with a layer of acrylic adhesive.	+	-	-
Nonadherent	Foam-based. Low adherence to tissue.	-	-	-
Hydrogels	High water content (80%-90%) allows for easier debridement.	++	+/-	++
Hydrocolloids	Contains polymers, proteins, polysaccharides and adhesives.	+++	+/-	++
Acrylics	Allow uptake of the wound fluid by the acrylic pad.	+++	+/-	++
Calcium alginates	Absorb exudate and form a hydrophilic gel. Hemostatic qualities.	++	+	+++
Composite dressings	Semi or nonadherent pad with adhesive borders.	+	-	+++
Foams	Absorb large amounts of exudate. Highly conformable.	-	-	+++
Hypertonic	Draw debris out of the wound by osmosis. Contains sodium chloride.	+	+	++
Hydrophilic fibers	Form a gel, when in contacted by exudate.	+	-	+++
Antimicrobials	Silver, honey, iodine or chlorhexidine type. Available in many forms.	+	+++	+
Negative-pressure devices	Create a localized controlled subatmospheric-pressure environment. Removal of wound exudate and bacterial reduction by wound contraction.	-	+	+++

4.1.3. The challenges of infected chronic wound therapy

Infection is one of the main reasons why wound healing may stop, leading to increased risks of patient morbidity, discomfort, prolonged hospitalization and, in the worst-case scenario, mortality [55, 61]. Thus, systemic antibiotic therapy and topical antimicrobial dressings are usually requiring for the treatment of infected wounds [62].

As previously shown (Table 4.1), there are some dressings that incorporate antimicrobials, such as silver, honey, iodine or chlorhexidine, that seems to control release at the wound surface and promote infection control. However, the usage of these dressings lacks robust evidence for their benefit, and also could present some negative effects on patients that include cytotoxicity, allergenicity and, in the case of indiscriminate use, bacterial resistance [62, 63].

Furthermore, some studies have reported complications associated to the antibiotics application that threaten the efficacy of this therapy, such as the deficient vascularization and insufficient local antibiotic concentrations as well as an increase in the incidence of multi-drug resistant (MDR) organisms, namely methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing gram-negative bacteria [64, 65]. So, it seems quite urgent to find solutions, that is for example, other antibacterial agents to effectively tackle this problem of bacterial infections.

4.2. Bacteriophages: a potential solution for the antibiotic resistance

4.2.1. Bacteriophage Therapy

Since the antibiotic resistance has become a global concern to public health, the need for the new approaches to deal with bacterial infections is urgent. Bacteriophages or phages are viruses that allow the treatment of a target bacterial infection with no damage for the normal host microflora [66]. After infecting the bacteria, phages can progress either to a lytic or lysogenic phase. A lytic phase will cause cell lysis whereas in lysogenic phase the phage becomes integrated into the host genome, becoming a prophage [67].

The application of bacteriophages as antibacterial agents is known as bacteriophage therapy. Phages were first discovered in 1915 and attempts to evaluate their potential to the treatment of human bacterial infections began few years later. In fact, bacteriophage therapy has been applied for decades in Eastern Europe and the former USSR States, with the Eliava Institute in Tbilisi in Georgia as one of the main centers. Otherwise, in Western medicine this practice was pushed to the

background by the arrival of antibiotics, which could be produced in a commercial more cost-effective way and revealed a broader spectrum of activity. Recently, more research on therapeutic use of bacteriophages has been recommended due to the problems associated with antibiotics usage such as the increasing risk of drug-resistant bacteria, the restricted choice of effective treatments and the lack of novel antibiotics [66, 68].

Obligated lytic phages, also called virulent phages, or even more specific non-transducing lytic phages, follow the lytic phase and have been used for phage therapy because of their capacity to kill their bacterial host by lysing the infected cell (Figure 4.2). Therefore, several phages characteristics make them compelling alternative to the antibiotics, for example, they are self-replicating as long as the target bacteria are present, they are highly specific and do not disrupt the normal microflora as well as minimized the unwanted side effects and the development of resistance [69, 70].

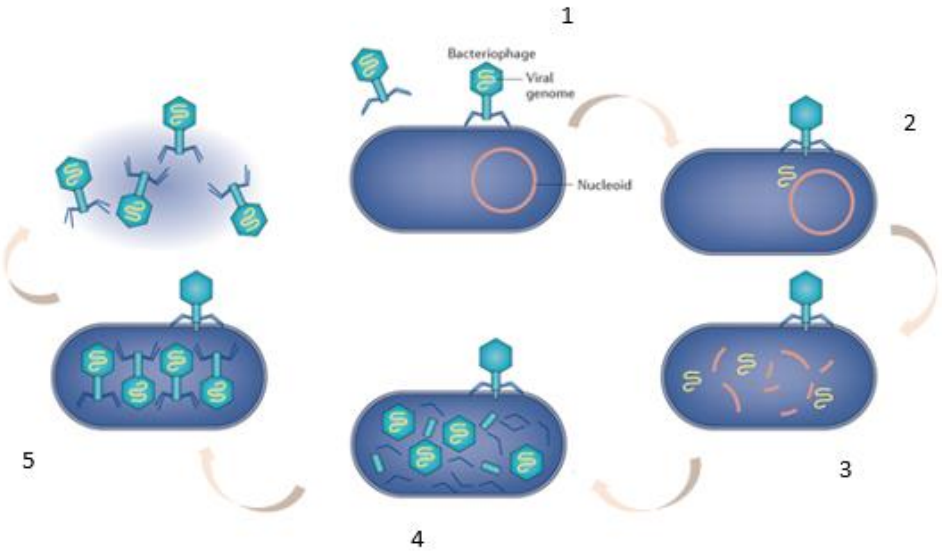


Figure 4.2. Lytic life cycle of bacteriophages. *(1) Attachment to the bacterial cell; (2) Injection of viral DNA; (3) Breakdown of bacterial chromosome; (4) Synthesis and assembly of new phages, using bacterial materials and phage enzymes; (5) Bacterial cell lysis and phage release. Adapted from [71].*

Some studies have demonstrated that this form of biological therapy is considerable promising. In 2009, a phase I clinical trial demonstrated the safety of a bacteriophage-based preparation in participants with chronic wounds [72]. Also, the results of a recent investigation suggest that topically administered phage treatment could be effective against chronic infections, mostly when applied with wound debridement [73].

Nowadays, the therapeutic use of phages is again put forward as a potential way to address the antibiotic crisis, however, it is still necessary to develop more reliable data to answer the fundamental clinical questions and outline the safety and efficacy requirements for phage therapy.

4.2.2. Legal framework for phage therapy

In the United States, bacteriophages applications are supervised by the Division of Vaccines and Related Product Applications of the Center for Biologicals Evaluation and Research (CBER), because there is no guideline for the therapeutic use of bacteriophages in humans published by the Food and Drug Administration [74].

Similarly, in Europe, bacteriophages are classified by European Medicines Agency as biological agents and thus phage therapy falls under the scope of the existing European regulatory framework on biological medicinal products, as outlined in Directive 2001/ 83/EC and with this classification, the phage-based products only can be used in patients after a marketing authorization, based on pharmaceutical, preclinical and clinical documentation [75].

Despite of these classifications represent a starting point, phage therapy lacks of a well-defined regulatory framework that fully regarded the clinical trials for its application, thus, a provisional solution has been used in Europe for the sporadic practice of this therapeutic with the supervision of medical ethical committees under the World Medical Association Declaration of Helsinki [76].

4.2.3. Phage-based products in the market

In the last decade, some non-human phage-based products with application in the agricultural and diagnostic sector, animal husbandry and veterinary have becoming available in the market [77].

Phage companies provide or seek to provide phage-based products commercially, and some of them already have the approval of FDA and EMA regulatory authorities. The first product that received the approval of FDA was AgriPhage™, in 2005, to treat crop diseases [78]. The biotechnology company *Intralytix Inc*, produced the first approved food safety-related bacteriophage product, the ListShield™, that consists in a phage cocktail that targets *Listeria monocytogenes* contaminants on ready to eat foods containing poultry and meat products [79]. These and other ongoing phage-based products are shown in Table 4.2 and them approval may be important to the acceptance of the application of bacteriophage therapeutics for humans.

Table 4.2. Active companies in the commercialization of phage products. *Adapted from [70, 77].*

Phage Products for human use

Company	Product/ Primary product area	Phase of development
AMPLIPHI	AB-SA01: *S. aureus* in Chronic Rhinosinusitis	Phase I trial completed
	AB-SA01: *S. aureus* infections in topical wounds	Phase I trial completed
	AB-PA01: *P. aeruginosa* lung infections	Pre-clinical
	AB-PA01: *P. aeruginosa* in Chronic Rhinosinusitis	Pre-clinical
ENBIOTIX	EBX-003 for infected Prosthetic Joints	Pre-clinical
PHICO THERAPEUTICS	Anti MRSA products	Pre-clinical
TECHNOPHAGE	TP-102 for Chronic Ulcers	Phase I trial
PHERECYDES PHARMA	PP0121 for *E. coli* infections	Phase I trial
	PP1131 *for P. aeruginosa* infections	Phase II trial

Phage Products for agricultural use

Company	Product/ Primary product area	Phase of development
INTRALYTIX	ListShield™	Product available
	EcoShield™	Product available
	SalmoFresh™	Product available
OMNILYTICS	AgriPhage™	Product available
EBI FOOD SAFETY	LISTEX P100™	Product available

4.3. *BioMultiDress:* Innovative bioactive dressing for the treatment of chronic wounds

4.3.1. General description

The *BioMultiDress* is a novel multifunctional dressing for the treatment of chronic wounds that combine regenerative and antimicrobial agents, released under control throughout the wound healing process.

This device will be comprised by two major regions, a surface adhesive region, polyurethane film, and a central absorbent region, polyurethane foam. In the central absorbent region, it will be incorporated the alginate hydrogel with two therapeutic agents, hyaluronic acid and bacteriophages.

The product will be supplied as sterile single-use device and the composition of the *BioMultiDress: Innovative bioactive dressing for the treatment of chronic wounds* is shown on Figure 4.3.

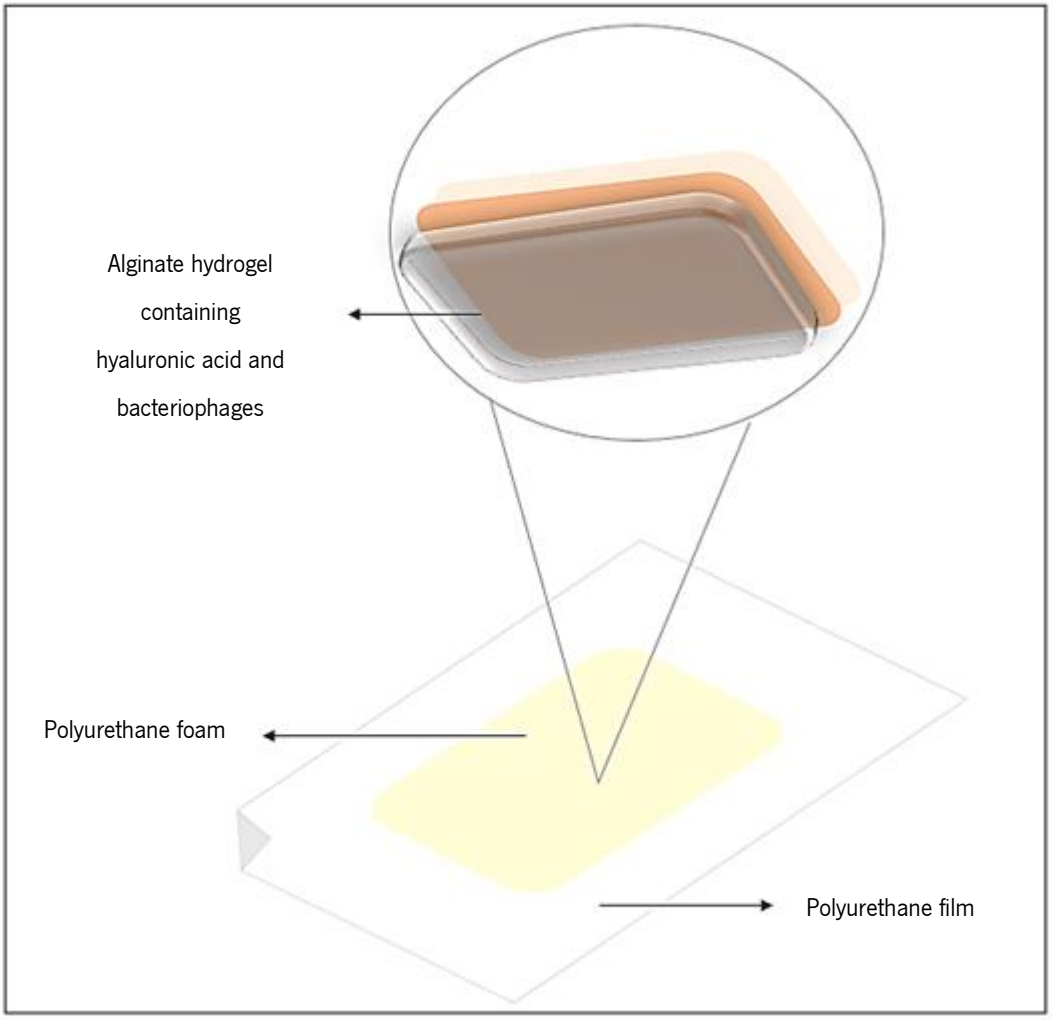


Figure 4.3. Schematic representation of the new multifunctional bioactive dressing.

4.3.2. Materials list and functional specifications

The *BioMultiDress* is a wound care dressing made up of five main materials – polyurethane film, polyurethane foam, alginate hydrogel, hyaluronic acid and bacteriophages - which in combination will provide a dynamic response with release of active agents in the injured site. Each one of these materials have its own function in the overall intended purpose of the device, as it can be seen in table 4.3.

Table 4.3. *BioMultiDress* material composition.

Material / Component	Function	Wound Contact
Polyurethane film	Secure the dressing to skin	No
Polyurethane foam	Absorption of the exudate	Yes
Alginate hydrogel	Hydrating action	Yes
Hyaluronic acid	Regeneration of the dermis and epidermis	Yes
Bacteriophages	Reduction / elimination of the microbial load	Yes

Overall, the major function of each one of the dressing materials are shown in the table above, although their role is more embracing than the described. More specifically, the polyurethane film should be adhesive to intact skin (non-adherent to the wound), hypoallergenic, transparent, impermeable to fluids and secretions, permeable to oxygen / water vapor and impenetrable by external microorganisms [80]. Also, the polyurethane foam consists of two or three layers, including a hydrophilic wound contact surface, making them highly absorbent and a hydrophobic backing, preventing exterior leakage. That material should be non-adherent to the wound area, hypoallergenic, skin-friendly and should ensure optimum conditions at the wound injured site, controlling the local humidity, allowing the thermal insulation and the absorption of the exudate, preventing maceration of the surrounding skin tissue [81]. Moreover, and as previously mentioned, the alginate hydrogel will be incorporated into the polyurethane foam with hyaluronic acid and bacteriophages.

Therefore, the therapeutic mechanism associated to this dressing relies in the central absorbent region with the combination of two therapeutic agents, hyaluronic acid and bacteriophages, through a hydrogel with alginate. Thereby, the hydrating action of the alginate hydrogel will promote tissue hydration, favoring autolytic debridement and will contribute to angiogenesis / granulation. Additionally, the therapeutic agents, hyaluronic acid and bacteriophages, will act on the regeneration of the dermis and

epidermis and on the reduction / elimination of the microbial load, respectively. The hyaluronic acid will contribute to the treatment of the injury, since it is a polysaccharide molecule present in the extracellular matrix of the skin, being that its hygroscopic properties will play essential functions like: 1) expanding the extracellular space for the formation of a new matrix with stability and elasticity; 2) allowing an increase in cell migration, namely fibroblasts; 3) modulate the inflammatory response and facilitate the reorganization and contraction of collagen during repair, increasing the speed of healing [82, 83]. On the other hand, the bacteriophages, being natural predators of bacteria, act as natural antibacterial agents and consequently, they will be the agents responsible for the reduction / elimination of microbiological contamination in the wound [84].

4.3.3. Product intended use and mode of action

The *BioMultiDress* is indicated for the covering of chronic, non-healing wounds, providing the treatment and alleviation/comfort on the injured site. This dressing is intended to be used in wounds with delayed healing process due to the presence of bacteria, like for example: venous leg ulcers, arterial leg ulcers, diabetic foot ulcers or pressure ulcers.

Hereupon, to comprehend how this therapeutic feature will be achieved, it is important to explain how the device acts upon the wound as well as to understand the mechanism behind the healing process. Therefore, the *BioMultiDress* mode of action can be divided into three main processes which will dynamically interact with each other: 1) drainage of the exudate and promotion of a controlled local moisture and thermal isolation, 2) controlled release of phages and hyaluronic acid and 3) the decrease of bacteria load and promotion of tissue regeneration (Figure 4.4).

The wound coverage given by the dressing will, primary, protect and comfort the damaged skin surface. Regarding the first process, the contact between the wound fluid and the dressing, more specifically the polyurethane foam, will maintain a moist and warm environment at the surface of the wound which will contribute for the formation of granulation tissue and epithelialization.

Additionally, and attending to the second process, the alginate hydrogel is essential at this stage of releasing the two therapeutic agents (bacteriophages and hyaluronic acid). As a crosslinked polymer, it will allow the controlled release of the therapeutic substances through a controlled diffusion mechanism, plus its properties contributes to tissue hydration and autolytic debridement, favoring the angiogenesis process.

Finally, regarding the third process, it is expected at that stage that the effect of the therapeutic agents, previously released throughout the alginate hydrogel diffusion power, were noticed so that the

skin repair process can occur. Therefore, the therapeutic mechanism associated to this device passes through the action of two substances, which will act on essential phases for the healing of an infected wound. First the decreasing of the bacteria load provided by the antibacterial effect of phages when infect bacteria, and second, the regeneration of the dermis and epidermis of the injured skin, promoted by the hyaluronic acid.

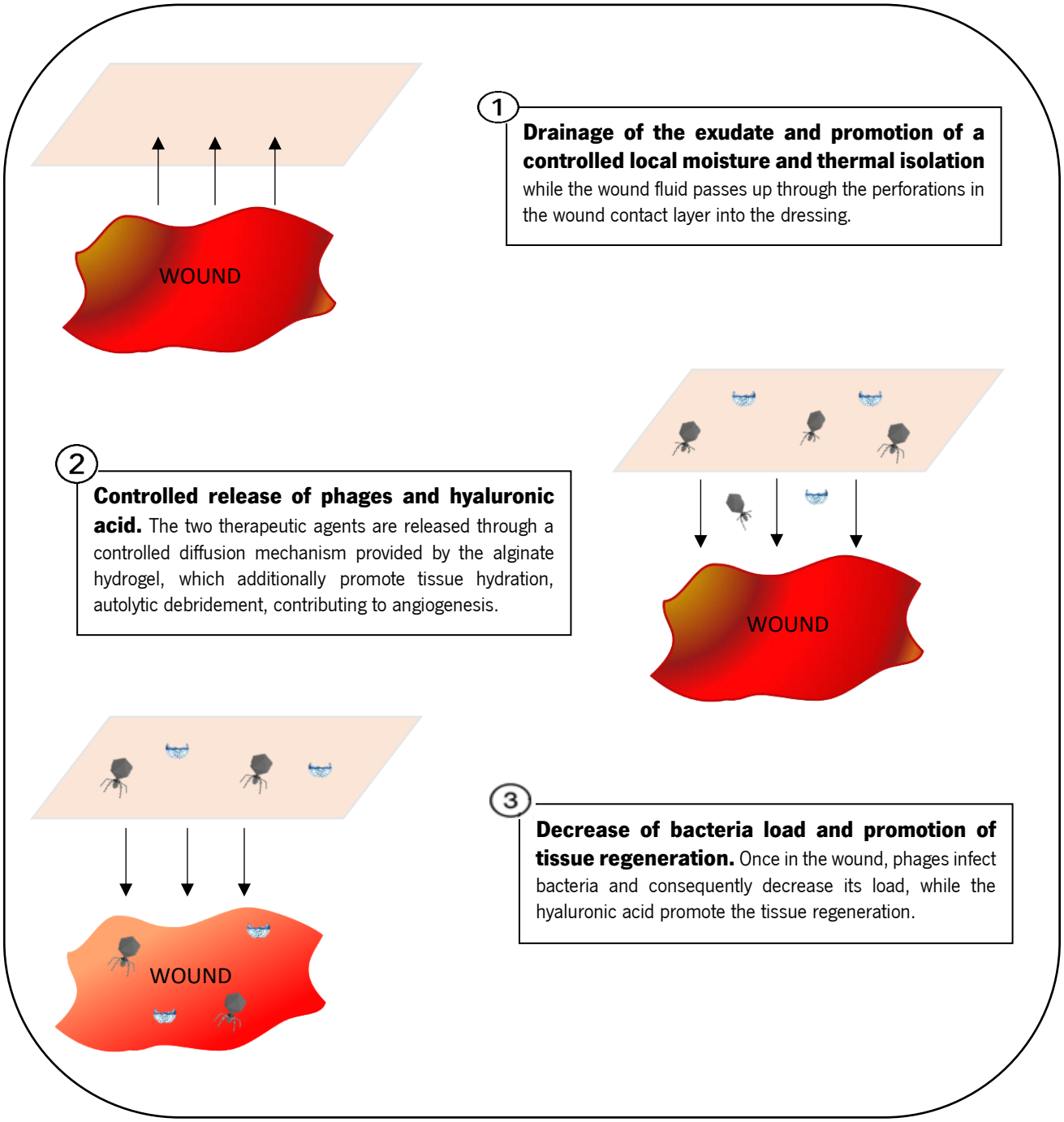


Figure 4.4. Representative scheme of the *BioMultiDress* mode of action upon the wound.

Overall, the *BioMultiDress* appears as a response to the global problem that chronic wounds represent, affecting mobility and patient quality of life, in some cases leading to death, also representing medical costs for both patients and health systems. The novelty of this dressing relies in the singularity of the characteristics presented in only one product, namely: 1) the combination of several materials in a single device, allowing different functions, 2) the bioactivity of the materials, allowing controlled release of incorporated therapeutics, 3) the use of two therapeutic agents, which will allow a conjugated treatment, simultaneously promoting the regeneration of the tissues and the reduction / elimination of the microbiological contamination and 4) the use of innovative antimicrobial agents, bacteriophages, which fight against antibiotic resistant bacteria.

4.3.4. Incorporation of a medicinal substance

The *BioMultiDress* is indicated for the treatment and alleviation of chronic wounds, allowing the protection and comfort of the injury site by maintaining a moist and warm environment, absorbing the exudate and enhancing the formation of granulation tissue and epithelialization. Allied to that principal intended action, this medical device incorporates, as an integral part, ancillary substances- bacteriophages - which will act upon the wound, minimizing the presence of one of the most prevalence factor that impairs the healing process - bacteria.

Medical devices incorporating, as an integral part, a medicinal substance which, if used separately, may be considered to be a medicinal product as defined in Medicinal Products Directive (MPD) 2001/83/EC and which is liable to act upon the body with action that is ancillary to that of the device, required supplementary information regarding the quality, safety and usefulness of the intended substance according to the methods specified in Annex I to Directive 2001/83/EC.

Foremost, there are three conditions that the intended substances incorporated in the device, which in this specific study case of the *BioMultiDress* are bacteriophages, must meet: 1) if used separately, may be considered to be a medicinal product, 2) be liable to act upon the human body and 3) the action be ancillary to that of the device [85]. Thus, as it has been showed, bacteriophages incorporating the *BioMultiDress* accomplish the three conditions because they are considered biological medicinal products by EMA (section 4.2.2.), plus, as also already mentioned, phages are capable of act on human body and its intended action will complement the primary purpose of the device.

Therefore, adding an ancillary medicinal substance to a device make the regulatory process more demanding, then the role of a Notified Body, in this class of medical devices, is particularly significant

to attest the compliance of the devices with this characteristic. According to the MEDDEV 2. 1/3 rev 3 guidelines, the intervention of the notified body is required to access the need of the substance as integral part of the dressing regarding its usefulness and the purpose of the dressing. The notified body is also responsible for the scientific consultation next to the competent authorities designated by the Member States (in case of Portugal- INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde I.P.) or the European Medicines Agency (EMEA) on the quality and safety of the substance. In case that some changes were made to the ancillary substance, mainly in the manufacturing process, the notified body should be recall and once again consult the competent authorities to approve the introduced changes and assure that the quality and safety are maintained.

Hereupon, being a dressing that incorporate bacteriophages as integral part, the *BioMultiDress* documentation should include some data concerning the quality and safety of this medicinal product and Figure 4.5 compiles the relevant information that should be submitted in the certification procedure.

Figure 4.5. Compilation of the data required in the Certification Procedure for the *BioMultiDress*, as a device incorporating, as an integral part, a biological medicinal product.

Considering the data provided on Figure 4.5, it is perceptible that the information is divided into five groups, including the one referring to the substance itself, the one related to the substance when incorporating the device, the pre-clinical studies information, as well as the data concerning the clinical evaluation and the labelling.

The dossier containing information relative to the bacteriophages quality procedures should be prepared as described by the internationally agreed format for the preparation of applications to be submitted to regulatory authorities in Europe, USA or Japan, named "Common Technical Document (CTD)", especially regarding the Module 3 "Quality", concerning chemical pharmaceutical and biological documentation for chemical active substance(s) and biological medicinal products [86]. Moreover, qualitative and quantitative data should be also addressed when phages incorporated in the wound dressing in order to access if some changes have occurred during its incorporation process. Then, the stability of the product must be verified to confirm if the desired function of the dressing is maintained throughout its shelf-life.

Furthermore, documentation on the pre-clinical studies should comprise information on pharmacodynamics of the substance, for example, its availability after incorporation, and information on pharmacokinetics, like for example concerning the phages release process from the dressing. The resulting data from the assessment on toxicity and biocompatibility of the dressing should also be provided.

Considering that *BioMultiDress* are in class III (see section- 4.3.5. Classification), like others which incorporates, as an integral part, a medicinal product, the clinical evaluation is required (according to the Annex X of the Directive 93/42/ EEC). Likewise, the information regarding the safety of the product, including the dressing and the bacteriophages, must be provided and for that, there are two standards that described the methodology to procedure for the clinical investigations, namely ISO 14155-1:2010 and ISO 14155-2:2010.

Lastly, the details provided by the manufacturer should comprise information on safety and usefulness of, not only the intended device, but also concerning the ancillary medicinal substance incorporated [85].

4.3.5. Classification

Determine a medical device classification is the basic procedure to structure all the following steps. The *BioMultiDress* is a wound care dressing that, in regulatory terms, falls within the definition of a medical device established in the Medical Devices Directive (MDD) 93/42 / CEE. Regarding the duration of use, this dressing is not intended to act on human body more than 30 days, thus it is classified as short-term device.

Additionally, this product incorporates bacteriophages as antimicrobial agents, which are consider medicinal products as previously discussed in section 4.2.2. Consequently, their antimicrobial effect is primarily intended to be related to the human body, being the interpretation of rule 13, Annex IX of the MDD 93/42/EC amended by Directive 2007/47/EC, applicable and consequently the device is classified as a class III.

Moreover, such class III devices are also described in *MEDDEV guideline 2.1/3 Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Rev. 3, 5.2015*. According to this guideline, the *BioMultiDress* is consider a medical device incorporating as an integral part, an ancillary medicinal substance. The following example are listed in the referenced guideline, in section B.4.1 *Examples of medical devices incorporating, as an integral part, an ancillary medicinal substance*:

"– Wound dressings, surgical or barrier drapes (including tulle dressings) with antimicrobial agent."

Overall, the *BioMultiDress* is classified as a *short-term class III medical device incorporating, as an integral part, an ancillary medicinal substance* and on table 4.4 are represented the different classification criteria, as well as the its definitions given by the respective legal documents.

Finally, attending the risk classification, the CE-certification process requires the involvement of a Notified Body to verify the documentation and clinical evaluation.

Table 4.4. *BioMultiDress* Classification given by the different classification criteria present on Annex IX of MDD.

***BioMultiDress* Classification**		
Classification Criteria	Legal Documentation	Definition
Medical Device	Medical Device Directive (MDD) 93/42 / CEE, Article 1- Definitions, scope	"Any instrument, appliance, apparatus, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: - diagnosis, prevention, monitoring, **treatment or alleviation of disease;** - diagnosis, monitoring, **alleviation of or compensation for an injury** or handicap; - investigation, **replacement** or modification **of the anatomy** or of physiological process; - control of conception; and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but that may be assisted in its function by such means"
Short term device	MDD 93/42/EC amended by Directive 2007/47/EC, Annex IX- Classification criteria, 1.1. Duration	"Normally intended for continuous use for not more than 30 days."
Class III	MDD 93/42/EC amended by Directive 2007/47/EC, Annex IX- Classification criteria, 4.1. Rule 13	"All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product, as defined in Article 1 of Directive ►**M5** 2001/83/EC ◄, and which is liable to act on the human body with action ancillary to that of the devices, are in Class III."
Medical device incorporating as an integral part, an ancillary medicinal substance	MEDDEV guideline 2.1/3 Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Rev. 3, 5.2015	"– Wound dressings, surgical or barrier drapes (including tulle dressings) with antimicrobial agent**."**

4.4. *BioMultiDress* Technical Requirements

4.4.1. Essential Requirements

The compliance of the medical device *BioMultiDress* with the provisions of Council Directive MDD 93/42/EEC amended by Directive 2007/47/EC will be demonstrated by suitable product tests. These will be performed during development and production according to harmonized standards and other valid national and international standards in order to fulfil the essential requirements of the intended directive.

The essential requirements checklist is a determinant part of the technical documentation of a medical device, as already explained in Chapter 3. In this very preliminary phase of the product development, it is obviously no possible to do a proper ER checklist because the product is still an idea in progress. Nevertheless, the technical file is a dynamic document that can be updated at any time, so, although it is not possible at this stage to describe the manufacturing methods used to prove certain conformity of a requirement, one can rather complete the ER checklist with information regarding the applicability of each requirement to the product in question, mentioning to the relevant supportive documents like standards and guidelines. The following table 4.5. shows an excerpt of the *BioMultiDress* Essential Requirements checklist, however the full table could be consulted in Annex I on the section *Annexes*.

Table 4.5. Excerpt of the full BioMultiDress Essential Requirements Checklist.

Essential Requirements	**Applicable (yes/no)**	**Standards/ Guidelines/ Requirements applied by the manufacturer**
I.GENERAL REQUIREMENTS 1- The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons provided that any risks which may be associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety. – reducing, as far as possible, the risk of use error due to the ergonomic features of the device and the environment in which the device is intended to be used (design for patient safety), and – consideration of the technical knowledge, experience, education and training and where applicable the medical and physical conditions of intended users (design for lay, professional, disabled or other users).	Yes	EN 980:2008 EN 1041:2008 EN ISO 10993 EN ISO 13485:2012 EN ISO 14971: 2012 ISO 14971:2007 Directive 93/42/EEC

4.4.2. Relevant Standards Applied

In order to accomplish compliance with the Essential Requirements, it is foreseeable the use of some standards during the development process of the intended device- *BioMultiDress*. Logically, at this initial study phase of the device, the following list of standards, guidelines and other relevant documents was done based on predictions, after a research on similar products and guideline documents given by regulatory agencies. Despite it is almost certainty the use of each one of them, others may be added to the technical file of the product, if justified during the manufacturing process. Thereby, table 4.6 contemplates the standards/guidelines that should be consider to achieve a proper compliance with Medical Devices Directive essential requirements with the view to CE marking for the device.

Table 4.6. List of standards/guidelines applicable to *BioMultiDress*.

Standard/Guideline	Description
EN 556-1	Sterilization of medical devices – Requirements for terminally sterilized medical devices to be labelled "Sterile" - Part 1: Requirements for terminally sterilized medical devices, 2001 [H]
EN 980	Graphical symbols for use in the labelling of medical devices, 08.2008 [H]
EN 1041	Information supplied by the manufacturer of medical devices, 2008
EN 1939	Self-adhesive tapes - Determination of peel adhesion properties, 2003 (AFERA 4001)
EN ISO 10993-1	Biological evaluation of medical devices - Part 1: Evaluation and testing, 2009
EN ISO 10993-5	Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity, 2009
EN ISO 10993-7	Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals, 2008
BS EN ISO 10993-10	Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity, 2013
EN ISO 10993-12	Biological evaluation of medical devices – Part 12: Sample preparation and reference materials, 2012
EN ISO 11135-1	Sterilization of health care products - Ethylene oxide - Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices, 2007 [H]
EN ISO 11607-1	Packaging for terminally sterilized medical devices - Part 1: Requirements for materials, sterile barrier systems and packaging systems, 2009 [H]
EN ISO 11607-2	Packaging for terminally sterilized medical devices - Part 2: Validation requirements for forming, sealing and assembly processes, 2009 [H]
EN ISO 11737-1	Sterilization of medical devices - Microbiological methods - Part 1: Determination of a population of microorganisms on products, 2006 [H]
DIN 13019	Adhesives for first aid – Dimensions, 2016

Standard/Guideline	Description
EN ISO 13485	Quality Management Systems - Requirements for regulatory purposes, 07.2012
EN 13726-2	Test methods for primary wound dressings - Part 2: Moisture vapour transmission rate of permeable film dressings, 2002 [H]
BS EN ISO 14644-1	Cleanroom and associated controlled environments - Part 1: Classification of air cleanliness, 2015);
BS EN ISO 14644-2	Cleanroom and associated controlled environments – Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644, 2015
BS EN ISO 14644-3	Cleanroom and associated controlled environments – Part 3: Test methods, 2005
BS EN ISO 14644-4	Cleanroom and associated controlled environments – Part 4: Design, construction and start up, 2001
BS EN ISO 14644-5	Cleanroom and associated controlled environments – Part 5: Operations, 2004
BS EN ISO 14644-6	Cleanroom and associated controlled environments Part 6: Vocabulary 2007
EN ISO 14971	Medical devices – Application of risk management to medical devices, 2012
EN ISO 15225	Nomenclature – Specification for a nomenclature system for medical devices for the purpose of regulatory data exchange, 2000 [H]
MEDDEV 2.1/3	Guidance document - Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Rev. 3. 05.2015
MEDDEV 2.4/1	Guidelines for the Classification of Medical Devices, Rev. 9, 06.2010
MEDDEV 2.7.1	Evaluation of Clinical Data: A Guide for Manufacturers and Notified Bodies. 06.2016
CPMP/ICH/2736/99 ICH Topic Q1A	Stability Testing of new Drug Substances and Products, Note for Guidance on Stability Testing: Stability Testing of New Drug Substances and Products (CPMP/ICH/2736/99) EMEA 2003
ASTM F 88 Rev. A	Standard Test Method for Seal Strength of Flexible Barrier Materials, 2007
ASTM F 1929	Standard Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration, 1998
ASTM D 3079-94	Standard Test Method for Water Vapor Transmission of Flexible Heat-Sealed Packages for Dry Products, 2003

5. Biological Evaluation of Medical Devices

5.1. General considerations

The appropriate assessment of device safety represents a central question in the medical devices industry, since the ensuring of patient safety is priority. As previously discussed, compliance with standards is fundamental to guarantee product's conformity with the legal requirements and for that, worldwide federations, like ISO, plays an essential role in the preparation of such documents which are recognized in most regulatory systems.

The International Standard ISO 10993 concerning *Biological Evaluation of Medical Devices* serves as a framework which addresses the determination of potential risks arising from the use of medical devices. This standard, composed of parts 1 to 20, compiles numerous International and National Standards and Guidelines regarding the biological evaluation of medical devices and mostly aims to assess the effect of the device on tissues. Thus, the device classification attending the nature and duration of the interaction with human tissues is imperative to properly delineate a complete and fully suitable biological evaluation, which may subsequently be an integral part of the entire risk management process.

Wherefore, material characterization, described on ISO 10993-18 and ISO/TS 10993-19, represents the starting point for the device biological evaluation. The flowchart represented in Figure 5.1, shows the different steps in the chemical characterization process, determinants in the definition of the pathways to the overall biological evaluation.

Once this assessment is done and the device biological evaluation route is defined, it is necessary to develop a biological evaluation plane according to the nature and duration of the contact of the intended device with the organism, consequently, the categorization of the device is the next step. Considering the type of contact, three sub- categories emerged: *Surface- contacting devices*, *External communicating devices and Implant Devices* (Figure 5.2). Surface- contacting devices are those which contact with intact skin (e.g. external prostheses) or mucosal membranes (e.g. urinary catheters) or even breached/ compromised body surfaces (e.g. dressings for ulcers), while the External communicating devices shall be categorized according to the contact site into blood path, if the device contact serve to conduct into the vascular system (e.g. solution administration sets) or, circulating blood, if the device contacts

circulating blood (e.g. dialysers); tissue, bone or dentin contact devices (e.g. arthroscopes) also belong this class. The last sub-categorization devices attending contact nature are the Implant Devices and include devices which contact with tissue/bone (e.g. replacement joints) or blood (e.g. heart valves).

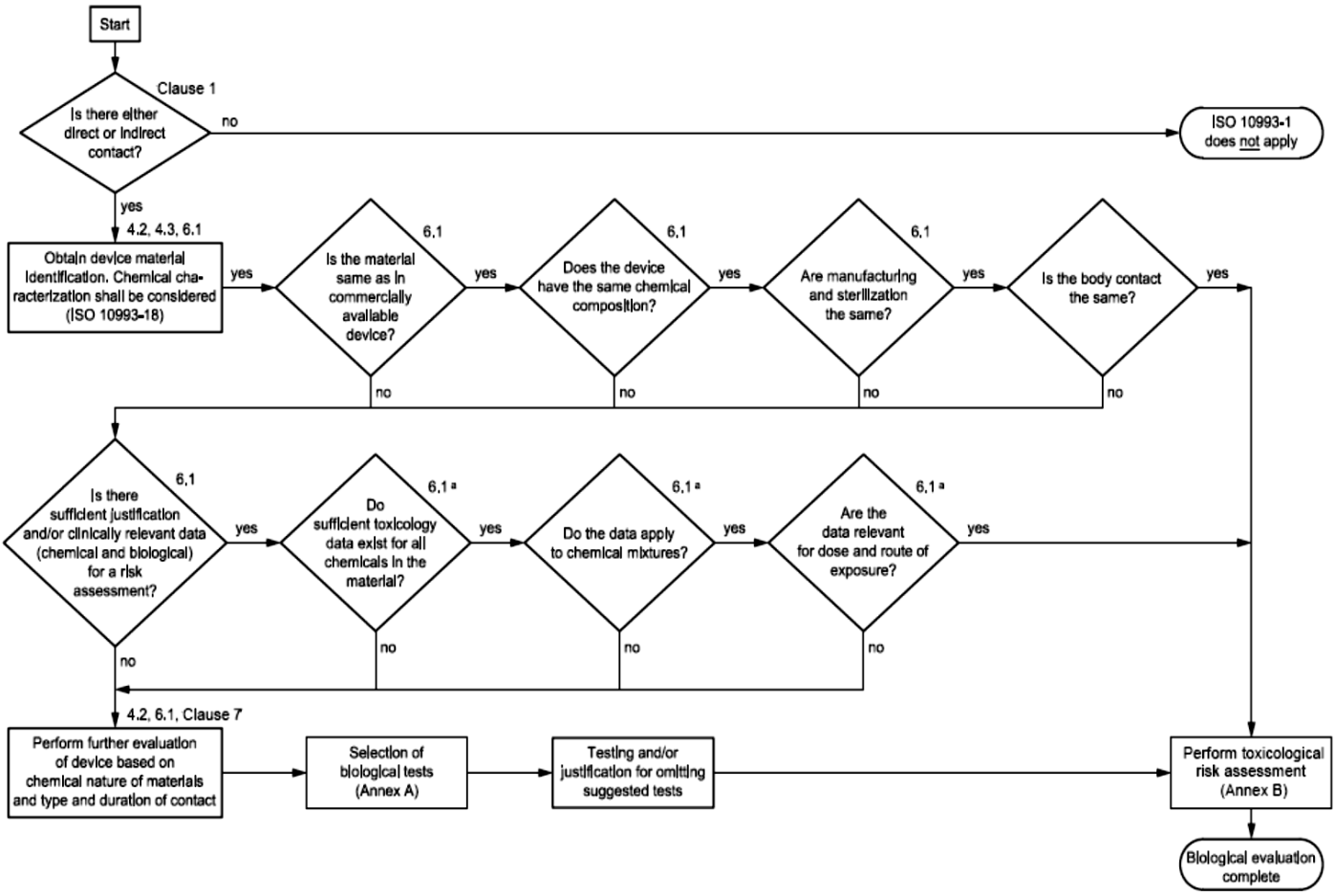


Figure 5.1. Summary of the systematic approach to a biological evaluation of medical devices within risk management process.

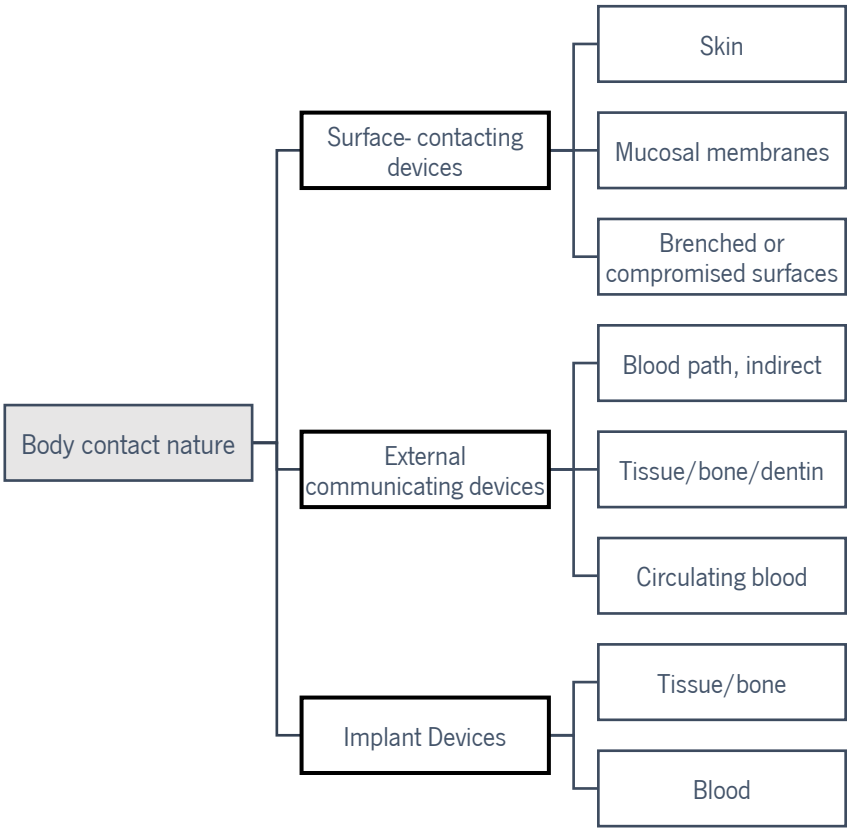


Figure 5.2. Categorization of medical devices by nature of body contact.

Beside the categorization by the device- body interaction, duration of contact is the other key criterion that supports the determination of the applicable biological evaluation tests. Thus, the anticipated duration of contact could be sub-divided into three types: *Limited exposure* (A), *Prolonged exposure* (B) and *Permanent contact* (C). The first one is referred to the devices whose use/contact, being single, multiple or repeated, does not exceed 24 hours while in the prolonged exposure devices this is likely to exceed 24 hours but not 30 days. Finally, the long-lasted devices classified as "Permanent contact devices" are those which the use exceed 30 days.

Biological evaluation tests comprise Cytotoxicity, Delayed-type hypersensitivity, Irritation (including intracutaneous reactivity), Systemic toxicity (acute), Subacute and subchronic toxicity, Genotoxicity, Implantation, Haemocompatibility, Chronic toxicity, Carcinogenicity, Reproductive and developmental toxicity, Biodegradation, Toxicokinetic studies and Immunotoxicology. The applicable tests for a particular medical device will depend of the device specific characteristics, including the nature of body contact and the duration of use, therefore the ISO 10993 comprise a framework which aims to

guide the development of a biological evaluation program, setting out different tests according to medical device categorization (Figure 5.3).

Medical device categorization by			Biological effect							
nature of body contact (see 5.2)		contact duration (see 5.3) A – limited (⩽ 24 h) B – prolonged (> 24 h to 30 d) C – permanent (> 30 d)	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface device	Skin	A	X [a]	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External communicating device	Blood path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X	X		X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

[a] The crosses indicate data endpoints that can be necessary for a biological safety evaluation, based on a risk analysis. Where existing data are adequate, additional testing is not required.

Figure 5.3. Biological evaluation tests. *From [47].*

Besides the performance of biological tests, the medical device safety can be also assured by the scientific literature reviewing.

5.2. *Biomultidress* Biological Evaluation Tests

As described above, *Biomultidress* is constituted by 5 materials: polyurethane film, polyurethane foam, hydrogel, hyaluronic acid and phages. In this first phase of investigation, the biological evaluation tests will be conducted for the phages.

Regarding all the categories shown on Figure 5.2, the *Biomultidress* could be categorized as a *Surface- contacting device* for *branched or compromised surfaces,* since the device is a dressing which will be used on branched skin characteristic of chronic wounds. Additionally, as regards to the anticipated duration of contact, this device is classified as *Prolonged exposure*, although its use does not exceed a

few days. Thus, following the guide previously shown in Figure 5.3, the applicable tests that need to be performed aiming the development of a biological evaluation of the *Biomultidress* are cytotoxicity, sensitization and irritation or intracutaneous reactivity of the device Figure (5.4).

Medical device categorization by			Biological effect							
nature of body contact (see 5.2)		contact duration (see 5.3) A – limited (⩽ 24 h) B – prolonged (> 24 h to 30 d) C – permanent (> 30 d)	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface device	Skin	A	X [a]	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		

Figure 5.4. Representation of the *BioMultidress* applicable biological tests according to its categorization.

5.2.1. Tests for *in vitro* cytotoxicity

The *in vitro* medical devices cytotoxicity is described by tests methods presented on ISO 10993-5 concerning Tests for *in vitro* cytotoxicity. These tests consist on the evaluation of biological parameters, such as cell death, the inhibition of cell growth, colony formation, and other effects resulted of the interaction of mammalian cells with the device and/or extracts of a device.

The purpose of this standard is to give a wide range of procedures so the choice of one or more protocols depends on the characteristics of the sample to be evaluated. Therefore, there are three different categories of tests listed on this document, namely extract test, direct contact test and indirect contact test. Part 5 of ISO 10993 also compiles annexes with examples of quantitative test protocols which can be performed to support the cytotoxicity evaluation of the intended device, specifically the Neutral red (NR) uptake cytotoxicity test, the Colony formation cytotoxicity test, the MTT cytotoxicity test and the XTT cytotoxicity test. For the evaluation of the *BioMultiDress* cytotoxicity, the NR uptake test was performed.

The neutral red (3-amino-m-dimethylamino-2-methlphenazine hydrochloride) is a weak cationic dye used to identify viable cells in culture. The measurement of neutral red uptake is based on a protocol

first described by Borenfreund and Puerner [87]. This assay consists on the uptake of NR into lysosomes and its subsequent accumulation on living cells, indicating quantitative viability. If cells were damaged or dead, NR is no longer retained within the vacuoles cells, so it quantifies the number of viable, uninjured cells after their exposure with test agents, through reduction in the absorbance.

Phages have been used for therapeutic purposes in Georgia, Russia and Poland and no adverse effects during human and animal experimentation have been reported, so they have been assumed as "clinically safe" [88]. However, there is not much scientific evidence concerning the cytotoxic potential of bacteriophages or their bacterial lysis products. Although, since bacterial cell products can have cytotoxic effects on cells through, for example, the release of bacterial toxins [89] and given the foreseeable clinical application of phages in *BioMultiDress*, it is necessary to test and guarantee that phage preparations are not harmful. In this study, the cytotoxic effect of the addition of three different therapeutic phages was evaluated, in mouse fibroblast 3T3 cells, using neutral red uptake test.

The phages used in this assay, named *Enterococcus faecalis* phage 09-2, *Enterococcus faecalis* phage 80-2 and *Enterococcus faecium* phage C410 (the names attached to the strains are temporary), were previously isolated and generously provided by a colleague of the Bacteriophage Biotechnology Group of the Centre of Biological Engineering. The selection of these phages was based on the good lytic spectrum and in vitro efficacy presented for bacteria frequently isolated from chronic wounds, *Enterococcus spp.*

The following procedures were based on the test protocol described in Annex A of the ISO 10993-5:2009 - Neutral Red Uptake cytotoxicity test (Annex II).

5.2.2. Materials and Methods

Briefly, BALB/c 3T3 cells, purchase from ATCC, were seeded into three 96-well plates, in a cell suspension of 1×10^5 cells/ml of DMEM culture medium (89% Dulbecco's Modified Eagle's Medium (DMEM) (Biochrom), 10% of Fetal Bovine Serum (FBS) (ThermoFisher) and 1% of Penicillin Streptomycin (Pen/Strep) (ThermoFisher)) per well and then the cells were maintained for 24h at 37 °C, 5 % of CO_2 and > 90% humidity in a cell incubator (Heracell 150, Heraeus). Additionally, it was prepared a positive control and a negative control, submitted to the same conditions. In the second day, after the 24h incubation period, the culture medium was removed and 100 µl of treatment medium containing the appropriate concentrations of phages (10^8 PFU/ml and 10^7 PFU/ml) was added. Then, the negative control was prepared with DMEM culture medium and for the positive control it was used different concentrations of sodium dodecyl sulfate (SDS) (ThermoFisher) (0,20 mg/ml, 0,15 mg/ml, 0,10 mg/ml

and 0,05 mg/ml). The blank was only prepared with culture medium, without 3T3 cells. The 96 well plate containing these preparations, whose schematic representation is illustrated in Figure 5.5, was incubated for 24h at 37 °C, 5 % of CO_2 and > 90% humidity. In the third day, after the 24h treatment period, the culture medium was removed and the cells were washed with 150 µl pre- warmed phosphate-buffered saline (PBS). The stock solution of Neutral Red was supplied by ThermoFisher and prepared using 0,4% of NR in 100 mL of sterile water, which was then mixed (1,25 %) with DMEM culture medium. The next step was to add 100 µl of pre- filtered and pre- warmed of the previously prepared solution of Neutral Red (1,25 %) to each well and incubate at 37 °C in a humidified atmosphere of 5 % CO_2 for 3h. After this time, the solution was removed and cells were washed with 150 µl of pre-warmed PBS. Subsequently, it was added 150 µl of NR desorb (ETOH/ acetic acid) solution to all wells and then the plate was shaken 10 min in an agitator (ES-20/60, Orbital Shaker-Incubator – BIOSAN) until NR was extracted from the cells and forms a homogeneous solution. The last procedure was to detect NR absorption at 540 nm in a spectrophotometer (Synergy HT – Bio-Tek) for further analysis of the cells viability (%).

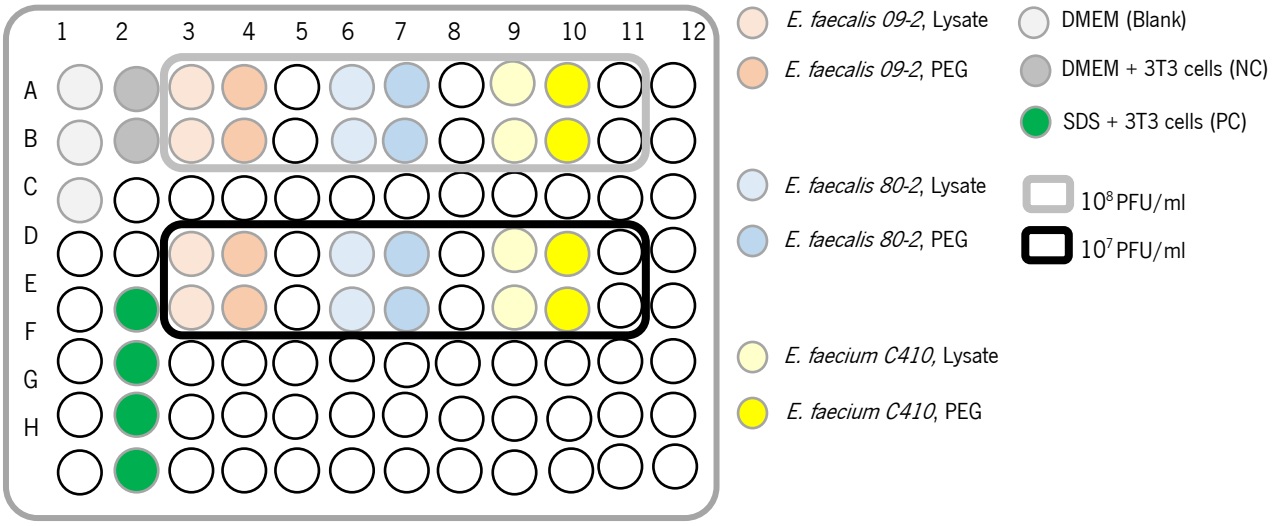


Figure 5.5. Schematic representation of the 96 well plate used for the preparation of the cytotoxicity assay.

5.2.3. Results and Discussion

Figure 5.6. shows the results of viability assays using three different phages, at two different concentrations (10^8 PFU/ml and 10^7 PFU/ml) and two different conditions represented on graph A, Phage lysate, and graph B, Phage purified using polyethylene glycol (PEG) precipitation. The purpose of purified preparations is to minimize the bacterial debris due to the foreseeable clinical application of these phages as antimicrobial agents in the *BioMultiDress*.

In this study, the viability of the 3T3 cells was assessed comparing with the percentage of negative control (untreated cells), which is 100 %.

Generally, the 24 h treatment with *E. faecalis* phage 09-2 and *E. faecalis* phage 80-2 was not toxic to any 3T3 cells conditions tested. The same was verified for the *E. faecium* phage C410 when its concentration was 10^7 PFU/ml, in all conditions tested. However, the lysate of phage C410 at a concentration of 10^8 PFU/ml showed some toxicity, causing a cell loss of about 30 % of cells.

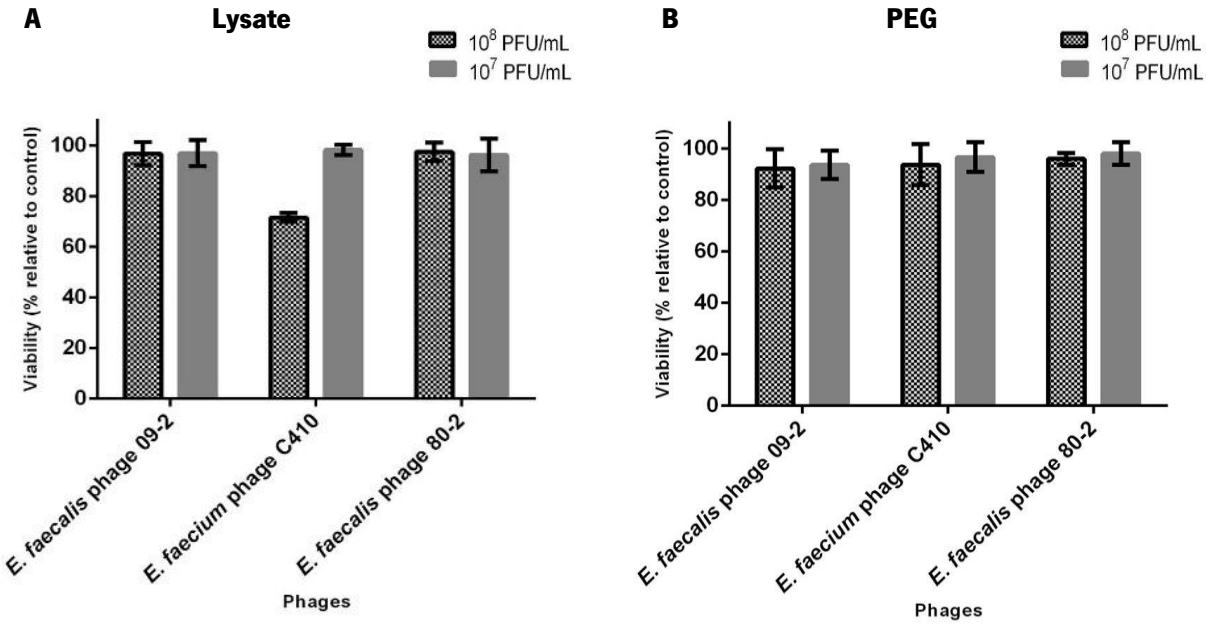


Figure 5.6. 3T3 mouse fibroblast cell viability (%) following 24 h exposure to *Enterococcus faecalis 09-2* phage*, Enterococcus faecalis 80-2* phage *and Enterococcus faecium C410* phage. The cells were exposed to the three phages at two different concentration (10^8 PFU/ml and 10^7 PFU/ml) and two different conditions **(A)** Phage lysate, **(B)** Phage purified with PEG. Results were calculated as percentage of negative control (cells without phage treatment), considered 100%. Error bars represent standard deviations from three independent experiments performed in duplicate.

Considering the presented results and the requirements of the Annex A - Neutral Red Uptake cytotoxicity test – of the ISO 10993- 5:2009, the three studied phages were non-cytotoxic since the cell viability was superior to 70 % of the control for all the conditions and concentrations tested.

Overall, despite it was observed some cell death after a 24 h treatment with *E. faecium* phage C410 at the concentration of 10^8 PFU/ml, according to the limits established in ISO 10993-5, the three phages shall be considered non-cytotoxic.

Furthermore, these results allied to some other studies [90, 91], showing that phages have no potential cytotoxic effect on cell viability, encourage new investigations towards the therapeutic application of phages in the treatment of topical infections, for example.

6. Conclusions and Final Considerations

6.1. Conclusions

Chronic wounds are a serious health condition that affects more than 50 million people worldwide. Bacterial infection is the major cause why these wounds do not heal, so it is urgent to find new therapeutic solutions that improve the healthcare associated to infected chronic wounds.

BioMultiDress emerges in response of this demand, so this research work intended to guide the development of a novel dressing to treat infected chronic wounds, focusing on the regulatory issues attached to the approval process of this type of medical device.

To accomplish this, first it was carried out a comprehensive analysis of the medical device sector. Despite the existence of some differences between the regulatory guidelines for medical devices of EMA and FDA, it is clear that for both regulatory agencies the risk classification is a key factor for the definition of the pathway towards the regulatory approval of medical devices.

Based on Medical Devices Directive 93/42/EEC, it was proposed that the *BioMultiDress* should be classified as a short-term class III medical device incorporating, as an integral part, an ancillary medicinal substance. As demonstrated, it is crucial to have a technical documentation showing how the device conforms to the essential requirements in order to place a CE marking on the device, so in the case of a class III medical device, as it is *BioMultiDress*, this documentation is a *Design Dossier*. Some of the elements of this type of documentation, like the essential requirements checklist and the standards applied, were prepared to compile in a draft version of the *BioMultiDress Design Dossier.*

Additionally, it was provided an exhaustive collection of the documentation that needs to be consider concerning the quality and safety of bacteriophages, since they are the biological medicinal products incorporated in the *BioMultiDress.* This documentation includes all the data that should be collected and submitted for the Certification Procedure of the product.

Moreover, as discussed, the determination of the potential risks arising from the use of medical devices is of extremely importance, since the ensuring of safety is priority. Part of the entire risk management process is in the ISO 10993-5 concerning Biological Evaluation of Medical Devices, which evaluate the effect of the device on tissues. In this work, it was performed part of the biological evaluation of *BioMultiDress* by assess the cytotoxic effect of the bacteriophages. Comparing the results with the limit established by the ISO 10993-5, it was verified that bacteriophages are not consider cytotoxic to the cells, which encourage the incorporation of this type of medicinal products as antimicrobial agents in the dressing.

Overall, this research provides an insight of what are the major regulatory questions related to the development of novel medical device, particularly a class III medical device with a medicinal product

in its composition. The findings of this research provide a consistent investigation that could support and guide the medical devices manufacturers and regulatory authorities towards the development of a product like *BioMultiDress*.

6.2. Final Considerations

BioMultiDress could be a valuable solution to treat infected chronic wounds, a disease that brings so much individual and economic burdens worldwide. Although this research may be a starting point for the development of such product, several paths can be taken in order to continue the findings herein achieved.

For example, further studies on sensitization and irritation could be performed in order to complete the biological evaluation of bacteriophages and so assess its *in vitro* safety as a component of the final product.

Another improvement that could be explored in *BioMultiDress* is the capacity to monitor the treatment progress by an external dressing color change. This could be achieved by the addition of an halochromic indicator incorporated into the polyurethane foam which is sensitive to changes in pH of the surrounding medium.

Finally, a well-defined regulatory framework regarding the clinical trials for the application of bacteriophages may be the opportunity to explore the potential of phage therapy and consequently increase interest and facilitate the development of products such as *BioMultiDress.*

References

References

1. Guest, J.F., et al., *Health economic burden that wounds impose on the National Health Service in the UK.* BMJ Open, 2015. **5**(12): p. e009283.
2. Hjort, A. and F. Gottrup, *Cost of wound treatment to increase significantly in Denmark over the next decade.* J Wound Care, 2010. **19**(5): p. 173-4, 176, 178, 180, 182, 184.
3. Purwins, S., et al., *Cost-of-illness of chronic leg ulcers in Germany.* Int Wound J, 2010. **7**(2): p. 97-102.
4. Posnett, J., et al., *The resource impact of wounds on health-care providers in Europe.* J Wound Care, 2009. **18**(4): p. 154-161.
5. *Using Nanova Therapy System in Practice: a round table discussion. London: Wounds UK. 2015;11(4):Supplement.*
6. Gad, S.C. and M.G. McCord, *Materials in Medical Device Design*, in *Safety Evaluation in the Development of Medical Devices and Combination Products*, I. Healthcare, Editor. 2008. p. 49–60.
7. Lucintel, *Growth Opportunities in the Global Medical Device Market*, in *Trends, opportunities and forecast in this market to 2021 by application type (surgical & infection control, general medical devices, cardiovascular, home healthcare, other device roof), and region (North America, Europe, Asia Pacific and Rest of the World)*. 2016.
8. WHO. *Medical Devices*. 2017 [cited 2017 19 Jan]; Available from: http://www.who.int/medical_devices/definitions/en/.
9. EMA. *European Medicines Agency, About us*. 2016 Nov 8]; Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/document_listing/document_listing_000426.jsp&mid=.
10. FDA. *U. S. FOOD & DRUG ADMINISTRATION, FDA Mission*. 2016 [cited 2017 Nov 8]; Available from: http://www.fda.gov/AboutFDA/WhatWeDo/default.htm.
11. Supra, J., *New European Medical Device Regulations Will Become Effective on May 25, 2017*. 2017.
12. IMDRF. *International Medical Device Regulators Forum*. 2017 [cited 2017 Feb 7]; Available from: http://www.imdrf.org/index.asp.
13. Institute of Medicine (US) Committee on the Public Health Effectiveness of the FDA 510(k) Clearance Process; Wizemann T, e.P.H.E.o.t.F.k.
14. James B. Shore, J.A.F., *GHTF SG3 Document and Flowchart*, in *Proactive Supplier Management in the Medical Device Industry*, Q. Press, Editor. 2016, Seiche Sanders.
15. FDA. *U. S. FOOD & DRUG ADMINISTRATION, Recent Final Medical Device Guidance Documents. Medical Device Accessories – Describing Accessories and Classification Pathway for New Accessory Types*. 2017 [cited 2017 Feb 9]; Available from: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm418448.htm.
16. PinsentMasons. *Case Update: Brain Products GmbH v BioSemi*. 2012 [cited 2012 Feb 9]; Available from: http://www.pinsentmasons.com/en/media/legal-updates/case-update-brain-products-gmbh-v-biosemi/.
17. *Manual on borderline and classification in the community Regulatory framework for medical devices Version 1.17 (09-2015); . p. 6-7.*
18. *U. S. Government Publishing Office, Electronic Code of Federal Regulations, Title 21: Food and Drugs, PART 3—PRODUCT JURISDICTION, 3.2. (e)Definitions. [cited 2017 Feb 20]; Available*

from: http://www.ecfr.gov/cgi-bin/text-idx?SID=ff8b6b795927bf2afddb0200997e3d37&mc=true&node=se21.1.3_12&rgn=div8.
19. Jeary, T., *A European perspective and guide to key regulatory considerations for combination products.* Regulatory Rapporteur, Topra, 2015. **12**.
20. Santos, I.C.T., *Product development methodologies: the case of medical devices*. 2013, Faculdade de Engenharia da Universidade do Porto.
21. Speer, J., *A Step-by-Step Guide to Determine How Your Medical Device will be Classified*. 2015.
22. Commission, E., *MEDDEV 2. 4/1 Rev. 9, MEDICAL DEVICES: Guidance document-Classification of medical devices*. 2010.
23. French-Mowat, E. and J. Burnett, *How are medical devices regulated in the European Union?* J R Soc Med, 2012. **105 Suppl 1**: p. S22-8.
24. FDA. *U. S. FOOD & DRUG ADMINISTRATION, Product Classification*. 2017 [cited 2017 Feb 23]; Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPCD/classification.cfm.
25. WHO, *Medical devices: managing the mismatch: an outcome of the priority medical devices project.* World Health Organization, 2010.
26. *GDMN Agency, Global Medical Device Nomenclature, GMDN*. [cited 2017 7 Jul]; Available from: https://www.gmdnagency.org/.
27. *FDA. U. S. FOOD & DRUG ADMINISTRATION, History*. [cited 2017 Mar 8]; Available from: https://www.fda.gov/AboutFDA/WhatWeDo/History/default.htm.
28. Kramer, D.B., et al., *Ensuring medical device effectiveness and safety: a cross–national comparison of approaches to regulation.* Food Drug Law J, 2014. **69**(1): p. 1-23, i.
29. *FDA. U. S. FOOD & DRUG ADMINISTRATION, FDA's Role in Regulating Medical Devices*. [cited 2017 Mar 8]; Available from: https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/HomeHealthandConsumer/HomeUseDevices/ucm204884.htm.
30. *ISO. International Organization for Standardization. All about ISO.* . [cited 2017 Mar 14]; Available from: https://www.iso.org/about-us.html.
31. *EMA. European Medicines Agency. History of EMA.* [cited 2017 Mar 1]; Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/general/general_content_000628.jsp.
32. Dr. Simone Wurster, D.T.P., Dr. Patrick Murphy (TU Berlin), Dr. Florian Fritz, Roger von Laufenberg (VICESSE), Jolien van Zetten (NEN), Cristina Pauner, Artemi Rallo (UJI) and Rosario García Mahamut (UJI), Rosamunde van Brakel, Alessia Tanas (VUB), *D2.1: Report on security standards and certification in Europe - A historical/evolutionary perspective*. 2017. p. 31-39.
33. *European Commission. Medical Devices Regulatory framework. Current legislation.* 2017 [cited 2017 Mar 1]; Available from: https://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework_pt.
34. Sorenson, C. and M. Drummond, *Improving medical device regulation: the United States and Europe in perspective.* Milbank Q, 2014. **92**(1): p. 114-50.
35. *Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. Official Journal. L 169/21, 12.7.1993*.
36. *European Commission. Notified bodies.* [cited 2017 Mar 2]; Available from: https://ec.europa.eu/growth/single-market/goods/building-blocks/notified-bodies_pt.
37. Infarmed. Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. *Autoridade de designação*. 2016 [cited 2017 Mar 7]; Available from: http://www.infarmed.pt/web/infarmed/entidades/dispositivos-medicos/autoridade-designacao.
38. Emergo, *Europe. The regulatory process for medical devices.* 2017.

39. *Guidance Technical Documentation and Design Dossiers for Non Active Medical Devices.TÜV SÜD Product Service GmbH.* 2015; Available from: http://www.tuev-sued.de/uploads/images/1446043428118222590797/guidance-design-dossiers.pdf.
40. *Infarmed. Avaliação da Conformidade. Dispositivois Médicos.* [cited 2017 Jun]; Available from: http://www.infarmed.pt/web/infarmed/entidades/dispositivos-medicos/avaliacao-da-conformidade.
41. NBOG. *Notified Body Operations Group* [cited 2017 Jun 21]; Available from: http://www.nbog.eu/.
42. *U.S. Government Publishing Office, Electronic code of federal regulations, Title 21, Chapter I, Subchapter H.* [cited 2017 Jun 21]; Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=033c04bff3b0464c6359c0d1e1a630a9&mc=true&node=pt21.8.820&rgn=div5.
43. Solutions, M. *The 3 D's of Medical Device Documentation*. 2014 [cited 2017 Jun 19]; Available from: http://www.meritsolutions.com/life-sciences/the-3-ds-of-medical-device-documentation/.
44. *Recomendation NB-MED/2.5.1/Rec5 Technical Documentation, revision 4, 03.02.2000.* . Available from: http://www.team-nb.org/?q=node/64.
45. *GHTF/SG1/N011:2008 - Summary Technical Documentation for Demonstrating Conformity to the Essential Principles of Safety and Performance of Medical Devices (STED), February 21st, 2008.*; Available from: http://www.imdrf.org/documents/doc-ghtf-sg1.asp.
46. *Guidance MEDDEV 2.12/1 rev.8 – Medical Devices Vigilance System. January 2013.*; Available from: http://ec.europa.eu/DocsRoom/documents/15506.
47. *ISO International Organization for Standardization (2009) ISO 10993-1:2009 - Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process. 2009.*
48. Boateng, J.S., et al., *Wound healing dressings and drug delivery systems: a review.* J Pharm Sci, 2008. **97**(8): p. 2892-923.
49. Sen, C.K., et al., *Human skin wounds: a major and snowballing threat to public health and the economy.* Wound Repair Regen, 2009. **17**(6): p. 763-71.
50. *WHASA- Wound Healing Association of Southern Africa. [cited 2016 25/06]; Available from:* http://www.whasa.org/A_Aboutus.html.
51. *Gordon J. Shumate, DPM. [cited 2016 25/06]; Available from:* http://www.reddingpodiatrist.com/foot-problems/wounds/.
52. Situm, M. and M. Kolic, *[Atypical wounds: definition and classification].* Acta Med Croatica, 2012. **66 Suppl 1**: p. 5-11.
53. Mustoe, T.A., K. O'Shaughnessy, and O. Kloeters, *Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis.* Plast Reconstr Surg, 2006. **117**(7 Suppl): p. 35S-41S.
54. Gosain, A. and L.A. DiPietro, *Aging and wound healing.* World J Surg, 2004. **28**(3): p. 321-6.
55. Guo, S. and L.A. Dipietro, *Factors affecting wound healing.* J Dent Res, 2010. **89**(3): p. 219-29.
56. Bumpus, K. and M.A. Maier, *The ABC's of wound care.* Curr Cardiol Rep, 2013. **15**(4): p. 346.
57. Powers, J.G., L.M. Morton, and T.J. Phillips, *Dressings for chronic wounds.* Dermatol Ther, 2013. **26**(3): p. 197-206.
58. Morin, R.J. and N.L. Tomaselli, *Interactive dressings and topical agents.* Clin Plast Surg, 2007. **34**(4): p. 643-58.
59. Sarabahi, S., *Recent advances in topical wound care.* Indian J Plast Surg, 2012. **45**(2): p. 379-87.
60. Smith N, O.J., Greenwood JE, *Local management of deep cavity wounds – current and emerging therapies.* Browse Journals, 2015.

61. Goldenheim, P.D., *An appraisal of povidone-iodine and wound healing.* Postgrad Med J, 1993. **69 Suppl 3**: p. S97-105.
62. *Wound infection in clinical practice. An international consensus.* Int Wound J, 2008. **5 Suppl 3**: p. iii-11.
63. Hiro, M.E., et al., *Comparative evaluation of silver-containing antimicrobial dressings on in vitro and in vivo processes of wound healing.* Eplasty, 2012. **12**: p. e48.
64. Lipsky, B.A. and C. Hoey, *Topical antimicrobial therapy for treating chronic wounds.* Clin Infect Dis, 2009. **49**(10): p. 1541-9.
65. Tascini, C., et al., *Microbiology at first visit of moderate-to-severe diabetic foot infection with antimicrobial activity and a survey of quinolone monotherapy.* Diabetes Res Clin Pract, 2011. **94**(1): p. 133-9.
66. Jun, J.W., et al., *Bacteriophage application to control the contaminated water with Shigella.* Sci Rep, 2016. **6**: p. 22636.
67. Cowley, L.A., et al., *Analysis of whole genome sequencing for the Escherichia coli O157:H7 typing phages.* BMC Genomics, 2015. **16**: p. 271.
68. Thiel, K., *Old dogma, new tricks–21st Century phage therapy.* Nat Biotechnol, 2004. **22**(1): p. 31-6.
69. Kutter, E., et al., *Phage therapy in clinical practice: treatment of human infections.* Curr Pharm Biotechnol, 2010. **11**(1): p. 69-86.
70. Housby, J.N. and N.H. Mann, *Phage therapy.* Drug Discov Today, 2009. **14**(11-12): p. 536-40.
71. Kingwell, K., *Bacteriophage therapies re-enter clinical trials.* Nat Rev Drug Discov, 2015. **14**(8): p. 515-6.
72. Rhoads, D.D., et al., *Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial.* J Wound Care, 2009. **18**(6): p. 237-8, 240-3.
73. Mendes, J.J., et al., *Wound healing potential of topical bacteriophage therapy on diabetic cutaneous wounds.* Wound Repair Regen, 2013. **21**(4): p. 595-603.
74. Parracho, H.M., et al., *The role of regulated clinical trials in the development of bacteriophage therapeutics.* J Mol Genet Med, 2012. **6**: p. 279-86.
75. *Directive 2001/83/EC of the European Parliament and of the council of 6 November 2001 on the community code relating to medicinal products for human use (consolidated version: 16/11/2012). In: EudraLex—The Rules Governing Medicinal Products in the European Union, Volume 1: Pharmaceutical Legislation: Medicinal Products For Human Use. European Commission.*http://ec.europa.eu/health/files/eudralex/vol1/dir_2001_83_consol_2012/dir_2001_83_cons_2012_en.pdf*.*
76. Verbeken, G., et al., *European regulatory conundrum of phage therapy.* Future Microbiol, 2007. **2**(5): p. 485-91.
77. Monk, A.B., et al., *Bacteriophage applications: where are we now?* Lett Appl Microbiol, 2010. **51**(4): p. 363-9.
78. *United States Environmental Protection Agency. 2005. Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato specific bacteriophages: exemption from the requirement of a tolerance. Federal Register 70: 76700-76704. Available from:* http://www.epa.gov/EPA-PEST/2005/December/Day-28/ *p24540.pdf. Accessed October 31, 2011. Verbeken G, De Vos D, Vaneechoutte M, Merab.*
79. *Bren L. 2007. Bacteria-eating virus approved as food additive.FDA Consum, 41, 20-22.*
80. Dutra, R.A., et al., *Using transparent polyurethane film and hydrocolloid dressings to prevent pressure ulcers.* J Wound Care, 2015. **24**(6): p. 268, 270-1, 273-5.
81. Jones, V., J.E. Grey, and K.G. Harding, *Wound dressings.* BMJ, 2006. **332**(7544): p. 777-80.

82. Nyman, E., et al., *Hyaluronic acid, an important factor in the wound healing properties of amniotic fluid: in vitro studies of re-epithelialisation in human skin wounds.* J Plast Surg Hand Surg, 2013. **47**(2): p. 89-92.
83. Voigt, J. and V.R. Driver, *Hyaluronic acid derivatives and their healing effect on burns, epithelial surgical wounds, and chronic wounds: a systematic review and meta-analysis of randomized controlled trials.* Wound Repair Regen, 2012. **20**(3): p. 317-31.
84. Azeredo, J. and I.W. Sutherland, *The use of phages for the removal of infectious biofilms.* Curr Pharm Biotechnol, 2008. **9**(4): p. 261-6.
85. *MEDDEV 2.1/3 rev 3, MEDICAL DEVICES: Guidance document - Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Guidelines relating to the application of: the Council Directive 90/385/EEC on active implantable medical devices and the Council Directive 93/42/EEC on medical devices, May 2015,* http://ec.europa.eu/consumers/sectors/medical-devices/files/meddev/2_1_3_rev_3-12_2009_en.pdf.
86. *Common Technical Document for the Registration of Pharmaceuticals for Human Use.-Quality Overall Summary of Module 2 and Module 3: Quality, July 2003.* http://ec.europa.eu/health//sites/health/files/files/eudralex/vol-2/b/update_200805/ctd_05-2008_en.pdf.
87. Borenfreund, E. and J.A. Puerner, *Toxicity determined in vitro by morphological alterations and neutral red absorption.* Toxicol Lett, 1985. **24**(2-3): p. 119-24.
88. Henein, A., *What are the limitations on the wider therapeutic use of phage?* Bacteriophage, 2013. **3**(2): p. e24872.
89. Los, F.C., et al., *Role of pore-forming toxins in bacterial infectious diseases.* Microbiol Mol Biol Rev, 2013. **77**(2): p. 173-207.
90. Henein, A. E., Hanlon, G. W., Cooper, *A Partially Purified Acinetobacter baumannii Phage Preparation Exhibits no Cytotoxicity in 3T3 Mouse Fibroblast Cells*. *Front. Microbiol.* 2016. **7** (1).
91. Merabishvili, M. *et al. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials*. PLoS One, 2009. **4,** e4944.

Annexes

Annex I: Draft version of the Design Dossier of *BioMultiDress*

Design Dossier

BIOMULTIDRESS

Innovative bioactive dressing for the treatment of chronic wounds

Draft Version

October 2017

Contents

1. Product description

1.1. General Description

The *BioMultiDress* is a novel multifunctional dressing with antibacterial and regenerative properties for the treatment of chronic wounds, comprised by two major regions: a surface adhesive region (polyurethane film) and a central absorbent region (polyurethane foam). In the central absorbent region, it will be incorporated the alginate hydrogel with two therapeutic agents, hyaluronic acid and bacteriophages.

The product will be supplied as sterile single-use device.

The composition of the *BioMultiDress* is shown on Figure 1.

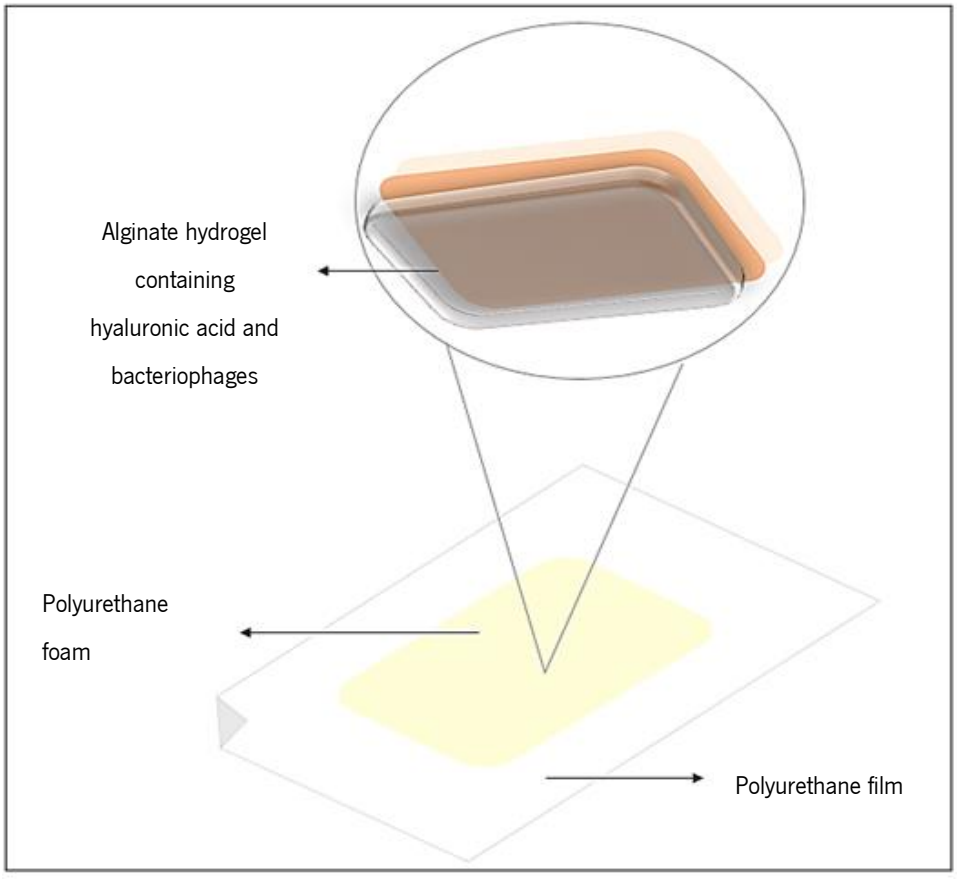


Figure 1- Schematic representation of the new multifunctional bioactive dressing.

1.2. Materials list

The *BioMultiDress* will be composed of the following materials shown in table 1.

Table 1- *BioMultiDress* material composition.

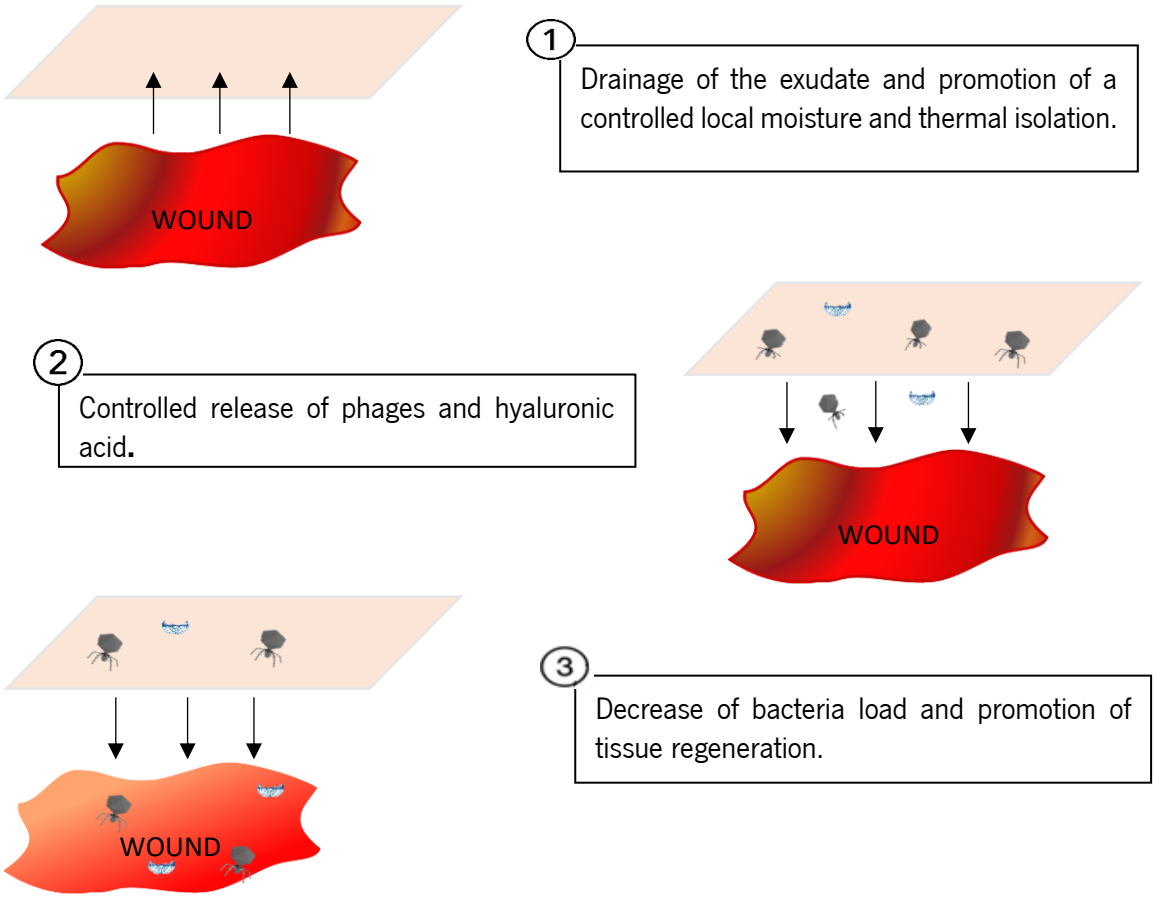
Material / Component	**Function**	**Wound contact**
Polyurethane film	Secure the dressing to skin	No
Polyurethane foam	Absorption of the exudate	Yes
Alginate hydrogel	Hydrating action	Yes
Hyaluronic acid	Regeneration of the dermis and epidermis	Yes
Bacteriophages	Reduction / elimination of the microbial load	Yes

1.3. Description of the intended use and operation of the device

The *BioMultiDress* is indicated for the covering of chronic, non-healing wounds, providing the treatment and alleviation/comfort on the injured site.

This dressing is intended to be used in wounds with delayed healing process due to the presence of bacteria, like:

- venous leg ulcers
- arterial leg ulcers
- diabetic foot ulcers
- pressure ulcers



1.4. Incorporation of a medical substance

BioMultiDress incorporates, as an integral part, bacteriophages, which are considered to be biological medicinal products, as defined in Medicinal Products Directive (MPD) 2001/83/EC and will act upon the wound as antibacterial agents for the reduction / elimination of microbiological contamination.

The bacteriophages are contained within the alginate hydrogel which allows its controlled release throughout a diffusion mechanism.

1.5. Classification of the device

The *BioMultiDress*, a dressing intended to treat chronic wounds, incorporating bacteriophages as antibacterial agents, is classified as short-term (continuous use for not more than 30 days) device of class III, according to rule no.13.

Rule 13

*"All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product, as defined in Article 1 of Directive ►**M5** 2001/83/EC ◄, and which is liable to act on the human body with action ancillary to that of the devices, are in Class III.*
All devices incorporating, as an integral part, a human blood derivative are in Class III."

The classification of such class III devices is also described in MEDDEV guideline 2.1/3 *Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Rev. 3, 5.2015.* According to this guideline, *BioMultiDress* is consider a *medical device incorporating as an integral part, an ancillary medicinal substance*. The following example is listed in the referenced guideline, in section *B.4.1 Examples of medical devices incorporating, as an integral part, an ancillary medicinal substance:*

"– Wound dressings, surgical or barrier drapes (including tulle dressings) with antimicrobial agent**."**

The CE-certification process requires the involvement of a Notified Body.

2. Technical Requirements

2.1. Identification of technical requirements

The compliance of the medical device *BioMultiDress* with the provisions of Council Directive MDD 93/42/EEC amended by Directive 2007/47/EC is demonstrated by suitable product tests. These will be performed during development and production according to harmonized standards and Pharmacopoeia Monographs and other valid national and international standards, if applicable.

Essential Requirements	**Applicable (yes/no)**	**Standards/ Guidelines/ Requirements applied by the manufacturer**
I.GENERAL REQUIREMENTS 1- The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons provided that any risks which may be associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety. – reducing, as far as possible, the risk of use error due to the ergonomic features of the device and the environment in which the device is intended to be used (design for patient safety), and – consideration of the technical knowledge, experience, education and training and where applicable the medical and physical conditions of intended users (design for lay, professional, disabled or other users).	Yes	EN 980:2008 EN 1041:2008 EN ISO 10993 EN ISO 13485:2012 EN ISO 14971: 2012 ISO 14971:2007 Directive 93/42/EEC
2- The solutions adopted by manufacturer for the design and construction of the devices must conform to safety principles, taking account of the generally acknowledged state of the art. In selecting the most appropriate solutions, the manufacturer must apply the following principles in the following order: — eliminate or reduce risks as far as possible (inherently safe design and construction),	Yes	EN 1041:2008 EN 1041:2008 EN ISO 10993-1:2009 EN ISO 10993-5:2009 EN ISO 10993-7:2008 EN ISO 10993-10:2010 EN ISO 14971:2012
— where appropriate take adequate protection measures including alarms if necessary, in relation to risks that cannot be eliminated,	No. No need since product is medical.	

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
— inform users of the residual risks due to any shortcomings of the protection measures adopted.		EN ISO 980:2008 EN 1041:2008 EN ISO 13485:2012
3. The devices must achieve the performances intended by the manufacturer and be designed, manufactured and packaged in such a way that they are suitable for one or more of the functions referred to in Article 1 (2) (a), as specified by the manufacturer.	Yes	EN ISO 13485:2012 EN ISO 14971:2012 EN ISO 10993 EN 11607:2006
4. The characteristics and performances referred to in Sections 1, 2 and 3 must not be adversely affected to such a degree that the clinical conditions and safety of the patients and, where applicable, of other persons are compromised during the lifetime of the device as indicated by the manufacturer, when the device is subjected to the stresses which can occur during normal conditions of use.	Yes	EN ISO 14971:2012 EN ISO 10993
5. The devices must be designed, manufactured and packed in such a way that their characteristics and performances during their intended use will not be adversely affected during transport and storage taking account of the instructions and information provided by the manufacturer.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 11607:2009 EN 980:2008 EN 1041:2008
6. Any undesirable side-effect must constitute an acceptable risk when weighed against the performances intended.	Yes	EN ISO 14971:2012
6a. Demonstration of conformity with the essential requirements must include a clinical evaluation in accordance with Annex X.	Yes	EN ISO 14971:2012
(II)REQUIREMENTS REGARDING DESIGN AND CONSTRUCTION **7- Chemical, physical and biological properties**		
7.1. The devices must be designed and manufactured in such a way as to guarantee the characteristics and performances referred to in Section I on the 'General requirements'. Particular attention must be paid to: — the choice of materials used, particularly as regards toxicity and, where appropriate, flammability, — the compatibility between the materials used and biological tissues, cells and body fluids, taking account of the intended purpose of the device — where appropriate, the results of biophysical or modelling research whose validity has been demonstrated beforehand.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 10993-5: 2009 EN 556 -1:2001 EN 556-2:2015
7.2. The devices must be designed, manufactured and packed in such a way as to minimize the risk posed by contaminants and residues to the persons involved in the transport, storage and use of the devices and to the patients, taking account of the intended purpose of the product. Particular attention must be paid to the tissues exposed and to the duration and frequency of exposure.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 10993 EN 11737:2009

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
7-3 The devices must be designed and manufactured in such a way that they can be used safely with the materials, substances and gases with which enter into contact during their normal use or during routine procedures; if the devices are intended to administer medicinal products they must be designed to be compatible with the medicinal products concerned according to the provisions and restrictions governing these products and that their performance is maintained in accordance with the intended use	Yes	
7.4. Where a device incorporates, as an integral part, a substance which, if used separately, may be considered to be a medicinal product as defined in Article 1 of Directive 2001/83/EC and which is liable to act upon the body with action ancillary to that of the device, the quality, safety and usefulness of the substance must be verified by analogy with the methods specified in Annex I to Directive 2001/83/EC. For the substances referred to in the first paragraph, the notified body shall, having verified the usefulness of the substance as part of the medical device and taking account of the intended purpose of the device, seek a scientific opinion from one of the competent authorities designated by the Member States or the European Medicines Agency (EMEA) acting particularly through its committee in accordance with Regulation (EC) No 726/2004 ([30]) on the quality and safety of the substance including the clinical benefit/risk profile of the incorporation of the substance into the device. When issuing its opinion, the competent authority or the EMEA shall take into account the manufacturing process and the data related to the usefulness of incorporation of the substance into the device as determined by the notified body. Where a device incorporates, as an integral part, a human blood derivative, the notified body shall, having verified the usefulness of the substance as part of the medical device and taking into account the intended purpose of the device, seek a scientific opinion from the EMEA, acting particularly through its committee, on the quality and safety of the substance including the clinical benefit/risk profile of the incorporation of the human blood derivative into the device. When issuing its opinion, the EMEA shall take into account the manufacturing process and the data related to the usefulness of incorporation of the substance into the device as determined by the notified body. Where changes are made to an ancillary substance incorporated in a device, in particular related to its manufacturing process, the notified body shall be informed of the changes and shall consult the relevant medicines competent authority (i.e. the one involved in the initial consultation), in order to confirm that the quality	Yes	EN 13726:2002

and safety of the ancillary substance are maintained. The competent authority shall take into account the data related to the usefulness of incorporation of the substance into the device as determined by the notified body, in order to ensure that the changes have no negative impact on the established benefit/risk profile of the addition of the substance in the medical device. When the relevant medicines competent authority (i.e. the one involved in the initial consultation) has obtained information on the ancillary substance, which could have an impact on the established benefit/risk profile of the addition of the substance in the medical device, it shall provide the notified body with advice, whether this information has an impact on the established benefit/risk profile of the addition of the substance in the medical device or not. The notified body shall take the updated scientific opinion into account in reconsidering its assessment of the conformity assessment procedure.		
7.5. The devices must be designed and manufactured in such a way as to reduce to a minimum the risks posed by substances leaking from the device. Special attention shall be given to substances which are carcinogenic, mutagenic or toxic to reproduction, in accordance with Annex I to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances ([31]). If parts of a device (or a device itself) intended to administer and/or remove medicines, body liquids or other substances to or from the body, or devices intended for transport and storage of such body fluids or substances, contain phthalates which are classified as carcinogenic, mutagenic or toxic to reproduction, of category 1 or 2, in accordance with Annex I to Directive 67/548/EEC, these devices must be labelled on the device itself and/or on the packaging for each unit or, where appropriate, on the sales packaging as a device containing phthalates. If the intended use of such devices includes treatment of children or treatment of pregnant or nursing women, the manufacturer must provide a specific justification for the use of these substances with regard to compliance with the essential requirements, in particular of this paragraph, within the technical documentation and, within the instructions for use, information on residual risks for these patient groups and, if applicable, on appropriate precautionary measures	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 10993
7.6. Devices must be designed and manufactured in such a way as to reduce, as much as possible, risks posed by the unintentional ingress of substances into the device taking into account the device and the nature of the environment in which it is intended to be used.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 10993

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
8. Infection and microbial contamination		
8.1. The devices and manufacturing processes must be designed in such a way as to eliminate or reduce as far as possible the risk of infection to the patient, user and third parties. The design must allow easy handling and, where necessary, minimize contamination of the device by the patient or vice versa during use.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 10993 BS EN ISO 14644:2015
8.2. Tissues of animal origin must originate from animals that have been subjected to veterinary controls and surveillance adapted to the intended use of the tissues. Notified bodies shall retain information on the geographical origin of the animals. Processing, preservation, testing and handling of tissues, cells and substances of animal origin must be carried out so as to provide optimal security. In particular safety with regard to viruses and other ►M5 transmissible ◄ agents must be addressed by implementation of validated methods of elimination or viral inactivation in the course of the manufacturing process.	No. Device does not contain any tissues of animal origin.	
8.3. Devices delivered in a sterile state must be designed, manufactured and packed in a non-reusable pack and/or according to appropriate procedures to ensure that they are sterile when placed on the market and remain sterile, under the storage and transport conditions laid down, until the protective packaging is damaged or opened.	Yes	EN ISO 14971:2012 EN ISO 13485:2012 EN ISO 11135:2007 EN ISO 11737:2006 EN ISO 11737-2:2009 EN 980:2008 EN 1041:2008
8.4. Devices delivered in a sterile state must have been manufactured and sterilized by an appropriate, validated method.	Yes	EN ISO 11135:2007 EN ISO 11737-1:2006 EN ISO 11737-2:2009
8.5. Devices intended to be sterilized must be manufactured in appropriately controlled (e. g. environmental) conditions.	Yes	EN ISO 14971:2012 BS EN ISO 14644:2015 EN ISO 13485:2012 EN ISO 11737-1:2006 EN ISO 11737-2:2009 EN ISO 11135:2007 EN ISO 10993 EN 980:2008
8.6. Packaging systems for non-sterile devices must keep the product without deterioration at the level of cleanliness stipulated and, if the devices are to be sterilized prior to use, minimize the risk of microbial contamination; the packaging system must be suitable taking account of the method of sterilization indicated by the manufacturer.	No. Device is delivered sterile.	BS EN ISO 14644:2015 EN ISO 11737:2006
8.7. The packaging and/or label of the device must distinguish between identical or similar products sold in both sterile and non-sterile condition.	No. Device is delivered sterile.	EN ISO 11135:2007 EN 980:2008

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
9. Construction and environmental properties		
9.1. If the device is intended for use in combination with other devices or equipment, the whole combination, including the connection system must be safe and must not impair the specified performances of the devices. Any restrictions on use must be indicated on the label or in the instructions for use.	No. No combination with other devices or equipment.	
9.2. Devices must be designed and manufactured in such a way as to remove or minimize as far as is possible: the risk of injury, in connection with their physical features, including the volume/pressure ratio, dimensional and where appropriate ergonomic features, risks connected with reasonably foreseeable environmental conditions, such as magnetic fields, external electrical influences, electrostatic discharge, pressure, temperature or variations in pressure and acceleration, the risks of reciprocal interference with other devices normally used in the investigations or for the treatment given, — risks arising where maintenance or calibration are not possible (as with implants), from ageing of materials used or loss of accuracy of any measuring or control mechanism.	No. Device does not cause injury - no reactive with surround environmental.	
9.3. Devices must be designed and manufactured in such a way as to minimize the risks of fire or explosion during normal use and in single fault condition. Particular attention must be paid to devices whose intended use includes exposure to flammable substances or to substances which could cause combustion.	No. No risk of fire or explosion.	
10. Devices with a measuring function		
10.1. Devices with a measuring function must be designed and manufactured in such a way as to provide sufficient accuracy and stability within appropriate limits of accuracy and taking account of the intended purpose of the device. The limits of accuracy must be indicated by the manufacturer.	No. No measuring function.	
10.2. The measurement, monitoring and display scale must be designed in line with ergonomic principles, taking account of the intended purpose of the device.	No. No measuring function.	
10.3. The measurements made by devices with a measuring function must be expressed in legal units conforming to the provisions of Council Directive 80/181/EEC.	No. No measuring function.	
11. Protection against radiation		
11.1. General		
11.1.1. Devices shall be designed and manufactured in such a way that exposure of patients, users and other persons to radiation shall be reduced as far as possible compatible with the intended purpose, whilst not restricting the application of appropriate specified levels for therapeutic and diagnostic purposes.	No. Device does not contain any substance that emits any level of radiation.	

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
11.2. Intended radiation		
11.2.1. Where devices are designed to emit hazardous levels of radiation necessary for a specific medical purpose the benefit of which is considered to outweigh the risks inherent in the emission, it must be possible for the user to control the emissions. Such devices shall be designed and manufactured to ensure reproducibility and tolerance of relevant variable parameters.	No. Device does not contain any substance that emits any level of radiation.	
11.2.2. Where devices are intended to emit potentially hazardous, visible and/or invisible radiation, they must be fitted, where practicable, with visual displays and/or audible warnings of such emissions.	No. Device does not contain any substance that emits any level of radiation.	
11.3. Unintended radiation		
11.3.1. Devices shall be designed and manufactured in such a way that exposure of patients, users and other persons to the emission of unintended, stray or scattered radiation is reduced as far as possible.	No. Device does not contain any substance that emits any level of radiation.	
11.4. Instructions		
11.4.1. The operating instructions for devices emitting radiation must give detailed information as to the nature of the emitted radiation, means of protecting the patient and the user and on ways of avoiding misuse and of eliminating the risks inherent in installation.	No. Device does not contain any substance that emits any level of radiation.	
11.5. Ionizing radiation		
11.5.1. Devices intended to emit ionizing radiation must be designed and manufactured in such a way as to ensure that, where practicable, the quantity, geometry and quality of radiation emitted can be varied and controlled taking into account the intended use.	No. Device does not contain any substance that emits any level of radiation.	
11.5.2. Devices emitting ionizing radiation intended for diagnostic radiology shall be designed and manufactured in such a way as to achieve appropriate image and/or output quality for the intended medical purpose whilst minimizing radiation exposure of the patient and user.	No. Device does not contain any substance that emits any level of radiation.	
11.5.3. Devices emitting ionizing radiation, intended for therapeutic radiology shall be designed and manufactured in such a way as to enable reliable monitoring and control of the delivered dose, the beam type and energy and where appropriate the quality of radiation.	No. Device does not contain any substance that emits any level of radiation.	
12. Requirements for medical devices connected to or equipped with an energy source		
12.1Devices incorporating electronic programmable systems must be designed to ensure the repeatability, reliability and performance of these systems according to the intended use. In the event of a single fault condition (in the system) appropriate means should be adopted to eliminate or reduce as far as possible consequent risks.	No. Device does not contain any electronic programable system.	

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
12.1a. For devices which incorporate software or which are medical software in themselves, the software must be validated according to the state of the art taking into account the principles of development lifecycle, risk management, validation and verification.	No. Device does not contain software.	
12.2. Devices where the safety of the patients depends on an internal power supply must be equipped with a means of determining the state of the power supply.	No. Device does not contain electronic parts.	
12.3. Devices where the safety of the patients depends on an external power supply must include an alarm system to signal any power failure.	No. Device does not contain electronic parts.	
12.4. Devices intended to monitor one or more clinical parameters of a patient must be equipped with appropriate alarm systems to alert the user of situations which could lead to death or severe deterioration of the patient's state of health.	No. Device does not monitor clinical parameters.	
12.5. Devices must be designed and manufactured in such a way as to minimize the risks of creating electromagnetic fields which could impair the operation of other devices or equipment in the usual environment.	No. Device does not contain any electric parts, which may create any electromagnetic fields.	
12.6. Protection against electrical risks Devices must be designed and manufactured in such a way as to avoid, as far as possible, the risk of accidental electric shocks during normal use and in single fault condition, provided the devices are installed correctly.	No. Device does not contain any electric parts, is not equipped with an energy source.	
12.7. Protection against mechanical and thermal risks		
12.7.1. Devices must be designed and manufactured in such a way as to protect the patient and user against mechanical risks connected with, for example, resistance, stability and moving parts.	No. Device does not contain mechanical parts.	
12.7.2. Devices must be designed and manufactured in such a way as to reduce to the lowest possible level the risks arising from vibration generated by the devices, taking account of technical progress and of the means available for limiting vibrations, particularly at source, unless the vibrations are part of the specified performance.	No. Device does not generate any vibration.	
12.7.3. Devices must be designed and manufactured in such a way as to reduce to the lowest possible level the risks arising from the noise emitted, taking account of technical progress and of the means available to reduce noise, particularly at source, unless the noise emitted is part of the specified performance.	No. Device does not generate any noise.	

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
12.7.4. Terminals and connectors to the electricity, gas or hydraulic and pneumatic energy supplies which the user has to handle must be designed and constructed in such a way as to minimize all possible risks.	No. Device does not contain any terminals and connectors to electricity, gas or hydraulic and pneumatic energy supplies.	
12.7.5. Accessible parts of the devices (excluding the parts or areas intended to supply heat or reach given temperatures) and their surroundings must not attain potentially dangerous temperatures under normal use.	No. Device does not supply heat.	
12.8. Protection against the risks posed to the patient by energy supplies or substances	No. No energy supplies	
12.8.1. Devices for supplying the patient with energy or substances must be designed and constructed in such a way that the flow-rate can be set and maintained accurately enough to guarantee the safety of the patient and of the user.	Yes	
12.8.2. Devices must be fitted with the means of preventing and/or indicating any inadequacies in the flow-rate which could pose a danger. Devices must incorporate suitable means to prevent, as far as possible, the accidental release of dangerous levels of energy from an energy and/or substance source.	No. Device is not intended to monitor any flow rate.	
12.9. The function of the controls and indicators must be clearly specified on the devices. Where a device bears instruction required for its operation or indicates operating or adjustment parameters by means of a visual system, such information must be understandable to the user and, as appropriate, the patient.	No. Device does not contain any controls and indicators.	
13. Information supplied by the manufacturer		
13.1. Each device must be accompanied by the information needed to use it safely and properly, taking account of the training and knowledge of the potential users, and to identify the manufacturer. This information comprises the details on the label and the data in the instructions for use. As far as practicable and appropriate, the information needed to use the device safely must be set out on the device itself and/or on the packaging for each unit or, where appropriate, on the sales packaging. If individual packaging of each unit is not practicable, the information must be set out in the leaflet supplied with one or more devices. Instructions for use must be included in the packaging for every device. By way of exception, no such instructions for use are needed for devices in Class I or IIa if they can be used safely without any such instructions.	Yes	EN ISO 14971:2012 EN 1041.2008 EN 980:2008

Essential Requirements	Applicable (yes/no)	Standards/ Guidelines/ Requirements applied by the manufacturer
13.2. Where appropriate, this information should take the form of symbols. Any symbol or identification colour used must conform to the harmonized standards. In areas for which no standards exist, the symbols and colours must be described in the documentation supplied with the device.	Yes	EN ISO 14971:2012 EN 1041:2008 EN 980:2008
13.3. The label must bear the following particulars: a) the name or trade name and address of the manufacturer. For devices imported into the Community, in view of their distribution in the Community, the label, or the outer packaging, or instructions for use, shall contain in addition the name and address of the authorised representative where the manufacturer does not have a registered place of business in the Community;	Yes	EN 1041:2008 EN 980:2008
b) the details strictly necessary to identify the device and the contents of the packaging especially for the users;	Yes	EN 1041:2008 EN 980:2008
c) where appropriate, the word 'STERILE';	Yes	EN 1041:2008 EN 980:2008
d) where appropriate, the batch code, preceded by the word 'LOT', or the serial number;	Yes	EN 1041:2008 EN 980:2008
e) where appropriate, an indication of the date by which the device should be used, in safety, expressed as the year and month;	Yes	EN 980:2008
f) where appropriate, an indication that the device is for single use. A manufacturer's indication of single use must be consistent across the Community;	Yes	EN 980:2008
g) if the device is costum-made, the words 'custom-made device';	No. Device is not custom-made.	
h) if the device is intended for clinical investigations, the words 'exclusively for clinical investigations';	No. Device is not for clinical investigations.	
i) any special storage and/or handling conditions;	Yes	EN 980:2008
j) any special operating instructions;	Yes	EN 980:2008
k) any warnings and/or precautions to take;	Yes	EN 980:2008
l) year of manufacture for active devices other than those covered by (e). This indication may be included in the batch or serial number;	Yes	
m) where applicable, method of sterilization;	Yes	EN 11135:2007
n) in the case of a device within the meaning of Article 1(4a), an indication that the device contains a human blood derivative.	No. Device does not contain any human blood derivative.	
13.4. If the intended purpose of the device is not obvious to the user, the manufacturer must clearly state it on the label and in the instructions for use.	Yes	EN 1041:2008 EN 980:2008
13.5. Wherever reasonable and practicable, the devices and detachable components must be identified, where appropriate in terms of batches, to allow all appropriate action to detect any potential risk posed by the devices and detachable components.	No. No detachable componentes.	
13.6. Where appropriate, the instructions for use must contain the following particulars:		

Essential Requirements	**Applicable (yes/no)**	**Standards/ Guidelines/ Requirements applied by the manufacturer**
(a) The details referred to in section 13.3 with the exception of (d) and (e)	Yes	
(b) The performances referred to in section 3 and any undesirable side effects.		
(c) If the device must be installed with or connected to other medical devices or equipment in order to operate as required for its intended purpose sufficient details of its characteristics to identify the correct devices or equipment to use in order to obtain a safe combination.	No. Device not intended to be used with any other device.	
(d) All the information needed to verify whether the device is properly installed and can operate correctly and safely plus details of the nature and frequency of the maintenance and calibration needed to ensure that the device operate properly and safely at all times.	No. Medical Device does not contain any part that needs to be maintained or calibrated	
(e) Where appropriate information to avoid certain risks in connections with implantation of the device.	No. Device is not intended to be implanted.	
(f) Information regarding the risks of reciprocal interference posed by the presence of the device during specific investigations or treatment.	Not predictable yet.	
(g) The necessary instructions in the event of damage to the sterile packaging and where appropriate details of appropriate methods of re-sterilization.	Yes	EN ISO 11135:2007 EN 980:2008
(h) If the device is reusable information on the appropriate processes to allow reuse including cleaning, disinfection packaging and where appropriate the method of sterilization of the device to be desterilized and any restriction on the number of reuses. Where devices are supplied with the intention that they be sterilized before use the instruction for cleaning and sterilization must be such that if correctly followed the device will still comply with the requirements in section 1. If the device bears an indication that the device is for single use, information on known characteristics and technical factors known to the manufacturer that could pose a risk if the device would be reused. If in accordance with Section 13.1 no instructions for use are needed, the information must be made available to the user upon request	No. Device is a sterile single use only device.	
(i) Details of any further treatment or handling needed before the device can be used (for example sterilization final assembly etc.).	No. Product is sterile and ready to use.	
(j) In The case of devices emitting radiation for medical purposes details of the nature type intensity and distribution of this radiation.	No. device does not contain any substance that emits radiation.	
(K)Precautions to be taken in the event of changes in the performance of the device.	No	

Essential Requirements	**Applicable (yes/no)**	**Standards/ Guidelines/ Requirements applied by the manufacturer**
(L)Precautions to be taken as regards exposure in reasonably foreseeable environmental conditions to magnetic fields external electrical influences electrostatic discharge pressure or variations in pressure or variations in pressure acceleration thermal ignition sources etc.	No	
(M)Adequate information regarding the medicinal product or products which the device in question is designed to administer including any limitations in the choice of substances to be delivered.	Yes	
(N)Precautions to be taken against any special unusual risks related to the disposal of the device.	Yes	
((o) medicinal substances, or human blood derivatives incorporated into the device as an integral part in accordance with Sections 7.4	Yes	
(P)Degree of accuracy claimed for devices with a measuring function.	No. Device has no measuring function.	
(q) date of issue or the latest revision of the instruction for use.	Yes	EN 980:2008

2.2. Standards applied

The following list of standards includes all standards that are foreseeable to be used during development and in order to demonstrate compliance with Essential Requirements. The list contains Harmonized Standards (H) as well as non-harmonized standards.

Standard/Guideline	Description
EN 556-1	Sterilization of medical devices – Requirements for terminally sterilized medical devices to be labelled "Sterile" - Part 1: Requirements for terminally sterilized medical devices, 2001 [H]
EN 980	Graphical symbols for use in the labelling of medical devices, 08.2008 [H]
EN 1041	Information supplied by the manufacturer of medical devices, 2008
EN 1939	Self-adhesive tapes - Determination of peel adhesion properties, 2003 (AFERA 4001)
EN ISO 10993-1	Biological evaluation of medical devices - Part 1: Evaluation and testing, 2009
EN ISO 10993-5	Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity, 2009
EN ISO 10993-7	Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals, 2008
BS EN ISO 10993-10	Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity, 2013
EN ISO 10993-12	Biological evaluation of medical devices – Part 12: Sample preparation and reference materials, 2012
EN ISO 11135-1	Sterilization of health care products - Ethylene oxide - Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices, 2007 [H]
EN ISO 11607-1	Packaging for terminally sterilized medical devices - Part 1: Requirements for materials, sterile barrier systems and packaging systems, 2009 [H]
EN ISO 11607-2	Packaging for terminally sterilized medical devices - Part 2: Validation requirements for forming, sealing and assembly processes, 2009 [H]
EN ISO 11737-1	Sterilization of medical devices - Microbiological methods - Part 1: Determination of a population of microorganisms on products, 2006 [H]
DIN 13019	Adhesives for first aid – Dimensions, 2016

Standard/Guideline	Description
EN ISO 13485	Quality Management Systems - Requirements for regulatory purposes, 07.2012
EN 13726-2	Test methods for primary wound dressings - Part 2: Moisture vapour transmission rate of permeable film dressings, 2002 [H]
BS EN ISO 14644-1	Cleanroom and associated controlled environments - Part 1: Classification of air cleanliness, 2015);
BS EN ISO 14644-2	Cleanroom and associated controlled environments – Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644, 2015
BS EN ISO 14644-3	Cleanroom and associated controlled environments – Part 3: Test methods, 2005
BS EN ISO 14644-4	Cleanroom and associated controlled environments – Part 4: Design, construction and start up, 2001
BS EN ISO 14644-5	Cleanroom and associated controlled environments – Part 5: Operations, 2004
BS EN ISO 14644-6	Cleanroom and associated controlled environments Part 6: Vocabulary 2007
EN ISO 14971	Medical devices – Application of risk management to medical devices, 2012
EN ISO 15225	Nomenclature – Specification for a nomenclature system for medical devices for the purpose of regulatory data exchange, 2000 [H]
MEDDEV 2.1/3	Guidance document - Borderline products, drug-delivery products and medical devices incorporating, as an integral part, an ancillary medicinal substance or an ancillary human blood derivative, Rev. 3. 05.2015
MEDDEV 2.4/1	Guidelines for the Classification of Medical Devices, Rev. 9, 06.2010
MEDDEV 2.7.1	Evaluation of Clinical Data: A Guide for Manufacturers and Notified Bodies. 06.2016
CPMP/ICH/2736/99 ICH Topic Q1A	Stability Testing of new Drug Substances and Products, Note for Guidance on Stability Testing: Stability Testing of New Drug Substances and Products (CPMP/ICH/2736/99) EMEA 2003
ASTM F 88 Rev. A	Standard Test Method for Seal Strength of Flexible Barrier Materials, 2007
ASTM F 1929	Standard Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration, 1998
ASTM D 3079-94	Standard Test Method for Water Vapor Transmission of Flexible Heat-Sealed Packages for Dry Products, 2003

3. Design

3.1. Biocompatibility tests

The following section will contain information on the preclinical evaluation of the different raw materials and a biocompatibility evaluation of the finished product *BioMultiDress* wound dressing.
The following materials have contact with the skin:

- Polyurethane film
- Polyurethane foam
- Alginate hydrogel
- Hyaluronic acid
- Bacteriophages

The other materials used do not contact with the patient´s skin, so no biocompatibility evaluation of the single raw materials is performed. Any potential leaching of these materials into the skin contacting surfaces will be considered in the biocompatibility studies performed with the finished product.
To date, biocompatibility data is only available for the cytotoxicity test of one of the raw materials- the bacteriophages.

a) Documentation on raw materials

Toxicological tests were performed on bacteriophages in order to demonstrate the biocompatibility of *BioMultiDress*. The selection of biocompatibility tests was based on the provisions of ISO 10993, "Biological evaluation of medical devices – part 1: Guidance on selection of tests". According to this standard, the *BioMultiDress* is categorized as

Surface-contacting device, in contact with breached or compromised surfaces, prolonged exposure (single use or contact likely to exceed 24 h but not 30 days).

The categorization determines the tests schedule for general toxicological evaluation. Table 2 lists the type of test conducted on raw material – bacteriophages (the Irritation/Intracutaneous Reactivity and Sensitization tests were not yet performed).

Table 2- Biocompatibility tests applicable to the *BioMultiDress*.

Test	**Report date**	**Standard**	**Result**
Cytotoxicity test (Neutral Red Uptake cytotoxicity test)	2017-07-05	EN ISO 10993-5:2009	Showed no cytotoxicity
Irritation/Intracutaneous Reactivity	Not done yet	BS EN ISO 10993-10:2013	
Sensitization	Not done yet	BS EN ISO 10993-10:2013	

The ***cytotoxicity test*** was performed using semi-confluent monolayer of BALB/c 3T3 cells. The negative control was prepared with DMEM culture medium and in the positive control it was used different concentrations of sodium dodecyl sulfate (SDS) (0,20 mg/mL, 0,15 mg/mL, 0,10 mg/mL and 0,05 mg/mL). The exposure of the cells to the test material extract did not show any sign of toxicological and biological critical cell damage and growth inhibitions. The results of the controls showed that the test was working as expected and was sensitive.

Furthermore, the biocompatibility test performed show that bacteriophages which will come into contact with the patient´s skin are considered as non-cytotoxic and meets the requirements of the ISO 10993-5. Consequently, the bacteriophages can be considered as suitable for the intended use.

Annex II: Neutral Red Uptake cytotoxicity test protocol of the International Standard ISO 10993-5 on Biological Evaluation of Medical Devices

Annex A

(informative)

Neutral red uptake (NRU) cytotoxicity test

A.1 General

The following test protocol is based on, and describes only, those parts of Annex C of Reference [1], which are relevant for this test.

A.2 Experimental procedure

A.2.1 Basic procedure

BALB/c 3T3 cells are seeded into 96-well plates and maintained in culture for 24 h (~ 1 doubling period) to form a semi-confluent monolayer (see Reference [5] for more information on cell maintenance and culture procedures). They are then exposed to the test compound over a range of concentrations. After 24 h exposure, NRU is determined for each treatment concentration and compared to that determined in control cultures. For each treatment (i.e. concentration of the test chemical), the inhibition of growth percentage is calculated, if the extract exhibits a cytotoxic effect on the cells. The $IC50$ (i.e. the concentration producing 50 % reduction of NRU) is calculated from the concentration-response and expressed as a dilution percentage of the extract. The neat extract is designated as 100 % extract.

A.2.2 Material

A.2.2.1 Cell line

BALB/c 3T3 cells, clone 31 (e.g. ECACC86110401, European Collection of Cell Cultures, Salisbury, Wiltshire SP4 0JG, UK; CCL-163, American Type Culture Collection [ATCC], Manassas, VA, USA) and JCRB 9005, prepared from CCL-163[ATCC], Human Science Research Resources Bank, Osaka, Japan.

A.2.2.2 Technical equipment

A.2.2.2.1 Incubator, 37 °C, humidified, 5 % CO2/air [alternatively, in the absence of a suitable buffer in the cell culture medium, 7,5 % CO2/air may be used because cells are very sensitive to pH changes; however 5 % is more commonly used in most laboratories, while HEPES [acid 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid] is added for better buffering.

A.2.2.2.2 Laminar flow cabinet, standard: “biological hazard”.

A.2.2.2.3 Water bath, 37 °C.

A.2.2.2.4 Inverse phase contrast microscope.

A.2.2.2.5 Laboratory burner.

A.2.2.2.6 Centrifuge, optionally equipped with microtitre plate rotor.

A.2.2.2.7 Laboratory balance.

A.2.2.2.8 96-well plate photometer, equipped with 540 nm filter

A.2.2.2.9 Shaker, for microtitre plates.

A.2.2.2.10 Cell counter or **hemacytometer**.

A.2.2.2.11 Pipetting aid.

A.2.2.2.12 Pipettes, 8-channel pipettes, dilution block.

A.2.2.2.13 Cryotubes.

A.2.2.2.14 Tissue culture flasks, 80 cm^2, 25 cm^2.

A.2.2.2.15 96-well tissue culture microtitre plates.

A.2.2.3 Chemicals, media and sera

A.2.2.3.1 Dulbecco’s Modification of Eagle’s Medium (DMEM), without L-glutamine

A.2.2.3.2 L-glutamine, 200 mM, or **glutamax**.

A.2.2.3.3 Newborn calf serum (NBCS).

IMPORTANT — Foetal calf serum (FCS) shall not be used. FCS causes a strongly reduced O.D. due to the formation of vacuoles in the cells.

Due to lot variability of NBCS, first check a lot for growth-stimulating properties with 3T3 cells (20 h to 25 h doubling time) and then reserve a sufficient amount of NBCS.

A.2.2.3.4 Trypsin/EDTA solution.

A.2.2.3.5 Phosphate-buffered saline (PBS), without Ca^{2+} and Mg^{2+} (for trypsinization).

A.2.2.3.6 HEPES (see A.2.2.2.1).

A.2.2.3.7 PBS, with Ca^{2+} and Mg^{2+} (for rinsing).

A.2.2.3.8 Penicillin/streptomycin solution.
A.2.2.3.9 Neutral red (NR).

A.2.2.3.10 Dimethyl sulfoxide (DMSO), analytical grade.

A.2.2.3.11 Ethanol (ETOH), analytical grade.

A.2.2.3.12 Glacial acetic acid, analytical grade.

A.2.2.3.13 Distilled water or **any purified water suitable for cell culture**.

A.2.2.4 Preparations

A.2.2.4.1 General

All solutions (except NR stock solution, NR medium and NR desorb), glassware, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard).

A.2.2.4.2 Media

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

(A) For freezing

- 20 % NBCS

-7 % to 10 % DMSO

(B) For routine culture

- 10 % NBCS
- 4 mM L-glutamine or glutamax
- 100 IU/ml penicillin
- 100 μg/ml streptomycin
- 20 mM HEPES

(C) For treatment with test samples

- 5 % NBCS
- 4 mM glutamine or glutamax
- 100 IU/ml penicillin
- 100 μg/ml streptomycin
- 20 mM HEPES

Complete media should be kept at 4 °C and stored for no longer than two weeks.

The serum concentration of treatment medium is reduced to 5 %, since serum proteins can mask the toxicity of the test substance. Serum cannot be totally excluded because cell growth is markedly reduced in its absence.

A.2.2.4.3 Neutral red (NR) stock solution

- 0,4 g NR dye
- 100 ml H_2O

Make up prior to use and store in the dark at room temperature for up to two months. Commercially prepared neutral red stock solutions may be used up to their expiration dates when stored according to the label.

A.2.2.4.4 Neutral red (NR) medium

- 1 ml NR stock solution
- 79 ml DMEM

The NR medium should be incubated overnight at 37 °C and centrifuged at 600*g* for 10 min (to remove NR crystals) before adding to the cells. Alternative procedures (e.g. millipore filtering) can be used as long as they guarantee that the NR medium is free of crystals. Aliquots of the NR medium should be maintained at 37 °C (e.g. in a water bath) before being added to the cells. They should be used within 30 min of preparation and within 15 min of removing from 37 °C storage.

A.2.2.4.5 Ethanol/acetic acid solution (NRdesorb)

- 1 % glacial acetic acid solution
- 50 % ethanol
- 49 % H2O

Prepare immediately prior to use. Do not store for longer than 1 h.

A.2.2.4.6 Preparation of sample extract

Samples are extracted in accordance with ISO 10993-12.

A.2.3 Methods

A.2.3.1 General

For routine cell culture methods, see Annex C of Reference [1].

A.2.3.2 Quality check of assay (I); positive control (PC)

A positive control shall be included in every cytotoxicity test.

With regard to the many chemicals backed by sufficient history or intra- and interlaboratory repeat tests, sodium lauryl sulfate (SLS, CAS # 151-21-3) is one of the most frequently tested, and is therefore recommended as a PC.

It is recommended that SLS be tested in a four-concentration scale: 0,05 mg/ml; 0,1 mg/ml; 0,15 mg/ml; 0,2 mg/ml.

The historical mean, *IC*50, of SLS (Spielmann et. al., 1991 [10]) is 0,093 mg/ml.

The 95 % CI (confidence interval) is 0,070 mg/ml to 0,116 mg/ml.

A test meets acceptance criteria, if the *IC*50 for SLS is within the 95 % CI.

The use of positive reference materials and negative reference materials is recommended [e.g. ZDEC and ZDBC (see footnote 1 on page 2 and 3.2 and 3.4)].

A.2.3.3 Quality check of assay (II); blank

The absolute value (not relative to the blank) of optical density (*OD*540 of NRU) obtained in the untreated blank indicates whether the 1×104 cells seeded per well have grown exponentially with normal doubling time during the two days of the assay.

A test meets the acceptance criteria if the mean *OD*540 of blanks is W 0,3.

To check for systematic cell seeding errors, blanks are treated under extraction conditions (see A.2.2.4.6) and are placed both at the left side (row 2) and the right side (row 11) of the 96-well plate (row 1 and row 12 shall not be used; for plate layout, see Annex E in Reference [1]).

A test meets acceptance criteria if the left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to ensure that cell quantity is consistent. Microscopic evaluation obviates the need for two rows of blanks.

A.2.3.4 Quality check of concentration-response

The *IC*50 derived from the concentration-response should be supported by at least two, or if possible, three responses between 10 % and 90 % inhibition of NRU. If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor.

A.2.3.5 Concentrations of test sample extracts

A.2.3.5.1 Range finder experiment

Test eight concentrations of the sample extract by diluting the stock solution with a constant factor, covering a large range, e.g. half-log intervals. If the reduction of viability of the cell culture with the highest concentration of the sample extract is 30 % or less, then the material has to be considered non-cytotoxic and no further main experiment is necessary.

A.2.3.5.2 Main experiment

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller (e.g. 6 10 = 1,47). Try to cover the relevant concentration range (between 10 % and 90 % effect) with at least three points of a graded effect, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations.

A.2.3.6 Test procedure

IMPORTANT — After thawing from stock, passage two to three times before using the cells in the test.

Table A.1 shows the work flow of the test procedure.

1st day

- Prepare a cell suspension of 1×10^5 cells/ml in culture medium. Using a multichannel pipette, dispense 100 µl culture medium only into the peripheral wells of a 96-well tissue culture microtitre plate (= blanks, see Appendix E in Reference [1]). In the remaining wells, dispense 100 µl of a cell suspension of 1×10^5 cells/ml (= 1×10^4 cells/well). Prepare one plate per sample extract to be tested, one plate for the PC and one plate for the negative control material if available.
- Incubate cells for 24 h (5 % CO_2, 37 °C, > 90 % humidity) so that cells form a half-confluent monolayer. This incubation period ensures cell recovery, and adherence and progression to exponential growth phase.
- Examine each plate under a phase contrast microscope to ensure that cell growth is relatively even across the microtitre plate. This check is performed to identify experimental errors.

2nd day

– After 24 h incubation, aspirate culture medium from the cells.
– Per well, add 100 µl of treatment medium containing either the appropriate concentration of sample extract, or the negative control, or the PC or nothing but vehicle (blank).
– Incubate cells for 24 h (5 % CO_2, 37 °C, > 90 % humidity).

3rd day

After 24 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record changes in morphology of the cells due to cytotoxic effects of the test sample extract, but do not use these records for the calculation of the highest tolerable dose (HTD) or any other quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells can indicate experimental error and can be cause for rejection of the assay.

The measurement of NRU is based upon that of Ellen Borenfreund (Borenfreund and Puerner[3]). The uptake of NR into the lysosomes/endosomes and vacuoles of living cells is used as a quantitative indication of cell number and viability.

– Wash the cells with 150 µl pre-warmed PBS. Remove the washing solution by gentle tapping. Add 100 µl NR medium and incubate at 37 °C in a humidified atmosphere of 5 % CO_2 for 3 h.

– After incubation, remove the NR medium, and wash cells with 150 µl PBS.

– Decant and blot PBS totally (optionally, centrifuge the reversed plate).

– Add 150 µl NR desorb (ETOH/acetic acid) solution to all wells, including blanks.

– Shake the microtitre plate rapidly on a microtitre plate shaker for 10 min until NR has been extracted from the cells and forms a homogeneous solution.

– Measure the absorption of the resulting coloured solution at 540 nm in a microtitre plate reader, using the blanks as a reference. Save raw data in a file format (e.g. ASCII, TXT, XLS) appropriate for further analysis of the concentration-response and calculation of IC_{50}.

A.2.4 Data analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test sample extract by using the mean NRU of the six replicate values per test concentration. This value is compared with the mean NRU of all blank values (provided blanks have met the blank acceptance criteria).

Table A.1 — 3T3 NRU cytotoxicity test work flow

Time h	Procedure
00:00	Seed 96-well plates: 1×10^4 cells/100 µl DMEM culture medium/well Incubate (37 °C/5 % CO_2/22 h to 24 h) ↓
24:00	Remove culture medium ↓
24:00	Treat with eight concentrations of test sample extract in treatment medium (100 µl) (untreated blank = treatment medium) Incubate (37 °C/5 % CO_2/24 h) ↓
48:00	Microscopic evaluation of morphological alterations Remove treatment medium Wash once with 150 µl PBS Add 100 µl NR medium Incubate (37 °C/5 % CO_2/3 h) ↓
51:00	Discard NR medium Wash once with 150 µl PBS Add 150 µl NR desorbing fixative (ETOH/acetic acid solution) ↓
51:40	Shake plate for 10 min
51:50	Detect NR absorption at 540 nm (i.e. cell viability)

Relative cell viability is then expressed as a percentage of untreated blank. If achievable, the eight concentrations of each compound tested should span the range of no effect up to total inhibition of cell viability. If the relative cell viability for the highest concentration of the sample extract (100 % extract) is less than 70 % of the control group, the concentration of a test chemical reflecting a 50 % inhibition of cell viability (i.e. *IC*50) is determined from the concentration-response. This can be done either by applying

- a manual graphical fitting method,

The use of probability paper with "X = log" and "Y = probit" scales is recommended because in most cases the concentration-response function will become almost linear in the relevant range. Semi-log paper could also be used for this technique.

or

- any appropriate non-linear regression procedure (preferably a Hill function4) or a logistic regression) to the concentration-response data.

Before using the *IC*50 for further calculations, the quality of the fit should be appropriately checked.

If the relative cell viability for the highest concentration of the sample extract (100 % extract) is W 70 % of the control group, then the material shall be considered non-cytotoxic.

4) Hill functions are monotonous and sigmoidal in shape and represent an acceptable model for many dose response curves.