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Unleashing the potential of supercritical fluids for polymer processing in tissue engineering and regenerative medicine

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ABSTRACT

One of the major scientific challenges that tissue engineering and regenerative medicine (TERM) faces to move from benchtop to bedside regards biomaterials development, despite the latest advances in polymer processing technologies.

A variety of scaffolds processing techniques have been developed and include solvent casting and particles leaching, compression molding and particle leaching, thermally induced phase separation, rapid prototyping, among others. Supercritical fluids appear as an interesting alternative to the conventional methods for processing biopolymers as they do not require the use of large amounts of organic solvents and the processes can be conducted at mild temperatures. However, this processing technique has only recently started to receive more attention from researchers. Different processing methods based on the use of supercritical carbon dioxide have been proposed for the creation of novel architectures based on natural and synthetic polymers and these will be unleashed in this paper.

1. Scaffolds for tissue engineering and regenerative medicine

The concept of tissue engineering and regenerative medicine (TERM) has been proposed in the early 90s by Langer and Vacanti [1]. TERM is defined as an interdisciplinary field of research to provide solutions for tissue regeneration and repair, based on the use of scaffolds, cells, and bioactive molecules, and combinations of two or more of these elements. The scaffold should not act merely as a support for cell growth but it should be able to deliver active compounds and to incorporate cell signaling molecules that promote cell attachment, growth, and proliferation, enhancing the regeneration process. Nonetheless, 20 years later scientists are still facing major challenges to obtain such multifunctional constructs and to address specific critical issues, such as the vascularization and innervation. Scaffolds development for tissue engineering and regenerative medicine should comply with a series of different requirements which are summarized in Table 1. Ideal scaffolds should be biocompatible, biodegradable and promote cellular interactions and tissue development and exhibit mechanical and physical properties compatible with the requirements of the tissue to be regenerated. The preparation of 3D matrices must result, hereafter in structures with adequate porosity, interconnectivity, pore size distribution and mechanical properties which make then suitable for the tissue to be engineered.

Advances in the state of the art in the field of biomaterials involve the proposition of both new materials and the design of new processing technologies. In recent years, biodegradable polymers made from renewable resources constitute an important material innovation as they decrease dependence on fossil fuel resources and reduce the amount of waste material. Natural origin polymers have been extensively used for scaffolds preparation, mostly due to their versatility and biodegradability [2,3]. Natural origin biomaterials can be divided into two large groups, proteins and polysaccharides. Different review papers give detailed insights on the characteristics of particular proteins and polysaccharides used for tissue engineering and regenerative medicine [4–6]. Proteins can be defined, in general terms as polymer structures composed by distinct amino-acids linked by peptide bonds. Twenty amino-acids

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Table 1
Summary of the ideal properties of a 3D scaffold for tissue engineering and regenerative medicine.

	Properties
Biocompatibility	Elicit an adequate response in the host patient
Degradability	Degradation rate compatible with the growth rate of neotissue
Mechanical properties	Sufficient mechanical strength to withstand the biological forces and maintain cell physical integrity
Porosity and pore size	Open pore, interconnected and adequate pore size for cell growth and vascularization
Sterilization	Materials should be easily sterilized without compromising their structure and bioactivity
Surface properties	Adequate surface properties, both chemically and topographically, in order to promote cell adhesion and proliferation

constitute the building blocks of these polymers. Their molecular structure can, hence, mimic the extracellular matrix and direct growth, migration and orientation of cells during the regeneration processes. Among these materials, collagen, gelatin, silk fibroin, elastin and fibrin are the most widely reported [7]. On the other hand, polysaccharides are polymers composed of one or two different sugar monomers (monosaccharides) linked by glycosidic bonds. Differences in monosaccharides, molecular weight and chain conformation confer the intrinsic characteristics of the different polymers. Polysaccharides have demonstrated good hemocompatibility properties. Chitin, chitosan, alginate, carrageenan, starch, hyaluronic acid and chondroitin sulfate are examples of well documented polysaccharides in TERM applications [8–11]. These polymers, both proteins and polysaccharides exhibit interesting properties that can make them highly suitable for a number of biomedical applications. The disadvantages of natural polymers come from the variability from batch to batch production, limited processability and poor mechanical properties. Nonetheless their advantages surpass the identified drawbacks. Biopolymers have sequences that resemble the extracellular matrix, facilitating cell growth and attachment and promoting cellular interaction. Furthermore, the biocompatibility, degradation rate, non-cytotoxicity, availability and low cost make them an interesting alternative to synthetic polymers [4,5].

Scaffolds processing has long been studied, and a variety of processing techniques have been reported in the literature. Conventionally, 3D structures or particulate systems can be obtained by processes such as solvent casting–particle leaching, freeze–drying–particle leaching, thermally induced phase separation, compression molding, injection molding, extrusion, foaming, wet spinning and electrospinning, as well as others [12]. The advantages of these processes have, however, to be weighed against the fact that these normally involve the use of large amounts of organic solvents, and further purification and drying steps are often needed. Additionally, some of these techniques are performed at high temperatures, which may degrade thermo-labile components. In order to overcome these drawbacks and to comply with the principles of sustainable chemistry, there is a need to develop new polymer processing technologies. Following the green chemistry principles, the use of natural renewable raw materials coupled with environmentally friendly technologies has been the focus of the research carried out and the different methodologies proposed for biomaterials development will be explored in this paper. Supercritical fluid technology is considered an alternative green technology as it does not promote the emission of greenhouse gases and conventionally uses substances that can be recycled and reused. Carbon dioxide is the most commonly used supercritical fluid as it has low critical parameters (T_c 304 K and P_c 7.4 MPa), it is non-toxic, non-flammable and ready available, and it has been considered as a GRAS (Generally Regarded as Safe) solvent. There are a number of different techniques that may take advantage of supercritical fluids. In this paper we review some of the techniques which have been explored for the development of biomaterials, specially focused on tissue engineering applications [13–18].

2. Supercritical fluid foaming

The gas foaming technique has been explored by several authors for the preparation of polymeric porous structures mainly from poly-DL-lactic acid (PDLLA), polyglycolic acid (PGA), and blends of these two polymers (PLGA), as well as from poly-ε-caprolactone (PCL), especially due to their thermal properties [19–21]. In this technique, the polymer is exposed to carbon dioxide at the saturation pressure and temperature, which plasticizes the polymer and reduces the apparent glass transition temperature or melting point. On venting the CO_2 by depressurization, thermodynamic instability causes supersaturation of the carbon dioxide dissolved in the polymer matrix and hence, nucleation of cells occurs. The success of gas foaming technique relies on the extent of carbon dioxide solubility in the polymer and the ability, hereafter to decrease the glass transition temperature of the material. The reduction of glass transition temperature is a thermodynamic effect due to intermolecular interactions between carbon dioxide and the polymer. For these reasons the application of gas foaming technique is limited to amorphous polymers or semi-crystalline polymers with low T_g.

After the pioneer work of Goel and Beckam who demonstrated the feasibility of preparation of microcellular foams using carbon dioxide as foaming agent [22,23] and the pivotal work of Mooney [24], who described for the first time the preparation of porous scaffolds for tissue engineering using this technology, several reports have been described in the literature. The attractive processing conditions (pressure and temperature) are particularly suited for the preparation of controlled delivery systems with thermolabile molecules, as is the case of proteins or growth factors [25–33].

PDLLA scaffolds loaded with nanoparticles containing platelet lysates (PL NP's) were prepared using this technology [31]. PL are a high concentration of platelets in a small volume of plasma that, when activated, release several growth factors that can act toward the healing process and the formation of a mesh of micro/nanofibers. PL are prepared from the patient's own blood, thus eliminating immunogenic and disease transmission concerns due to its autologous origin. PL can be used as either a growth factor-releasing agent alone or a loaded biopolymer scaffold for simultaneous cell delivery [34].

Fig. 1 presents the release profile of proteins from the nanoparticles alone and from the nanoparticles incorporated in the scaffold. The release profile experiments were carried out in phosphate buffer solution with a pH of 7.4, at 37 °C and 60 rpm. Protein release profile was quantified by micro-BCA analysis which demonstrated that a controlled release of the proteins from the 3D construct was successfully achieved.

Additionally, as can be observed from Fig. 1, the release rate can be controlled by the incorporation of the NP's within the 3D matrix, which avoids the initial burst release observed for the NP's alone. *In vitro* biological experiments demonstrate that protein activity was not compromised and it could induce the osteogenic differentiation of human adipose stem cells [31].

One of the ideas of tissue engineering is to couple materials and cells from the patient which will be implanted on the defect site for tissue regeneration. This approach involves the

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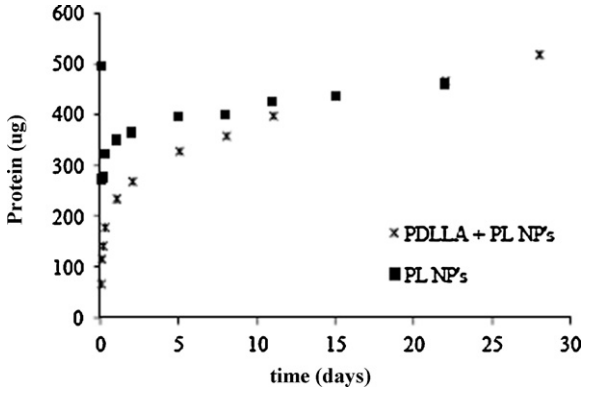

Fig. 1. Protein release profile from PDDLA scaffolds loaded with PL NP's.

collection and expansion of a large number of cells. Cells are cultured in 2D surfaces and recovered by mechanical or enzymatic methods that may compromise their functionality. The development of appropriate cell culture substrates which can reduce the large volume/surface ratio of the 2D culture plates and the existing methods for cell recovery was the rational for the development of a 3D themosensitive matrix [35]. PDLLA structures impregnated with a thermosensitive polymer, poly(N-isopropylacrylamide), PNIPAAm, that was *per se* polymerized under supercritical conditions were prepared by supercritical fluid foaming. The lower critical solution temperature (LCST) of this PNIPAAm was determined by dynamic light scattering and it was found to be ~32 °C, which is close to biological temperature and it is one of the features that makes this polymer interesting for biomedical applications. The hydrophobic nature of the materials at 37 °C allows cell attachment and growth (Fig. 2a) while when lowering the temperature under 32 °C the surfaces become hydrophilic and cells can be detached from the surface (Fig. 2b). Fig. 3 is a scanning electron micrograph of the cells attached to the thermosensitive PDLLA constructs. Our studies, performed with a fibroblast cell line (L929) demonstrate that detached cells maintain their viability and could be further used in TERM applications.

3. Supercritical fluid foaming of hydrogels

The supercritical foaming technique had until recently been restricted to amorphous or semi-crystalline polymers, with low glass transition temperatures. Foaming of hydrogels involves the dissolution of carbon dioxide in the water phase allowing a similar process to conventional gas foaming. Tsioptsias and coworkers critically reviewed and discussed the mechanism of hydrogel foaming [36]. Hydrophilic crystalline polymers can be foamed in a dense carbon dioxide atmosphere in the presence of water. This technology has been described for the preparation of different porous scaffolds. Annabi and coworkers reviewed and discussed different emerging technologies designed for the preparation of porous hydrogels with controlled microarchitecture [37]. Table 2 presents the polymeric materials prepared, cross-linking agents, operating conditions and reported applications.

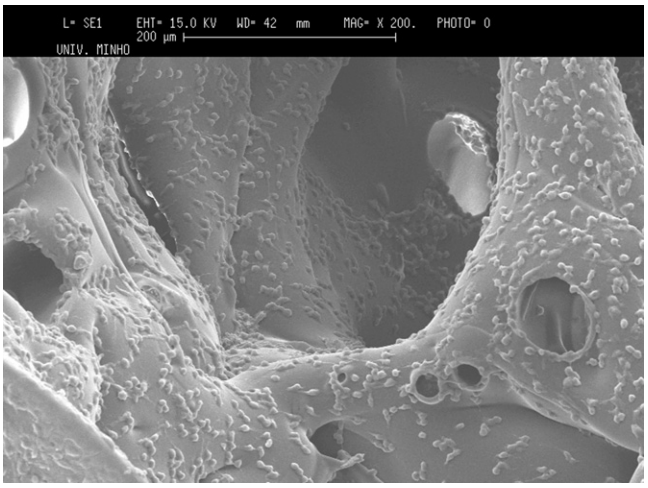

Fig. 3. SEM image of PDLLA construct seeded with L929 cells after 7 days culture.

The work developed on the production of hydrogel foaming revealed a new field of application in which supercritical fluid technology demonstrates once more its high versatility. The production of homogenous matrices is dependent on many operating conditions and thus it is not easy to control the final morphology of the structures. Ji et al. have suggested the addition of a surfactant to the process in order to stabilize the structure which is formed upon depressurization of the system [42]. In their work they explore the feasibility of using acacia gum, a naturally derived surfactant. The results presented show that the addition of the surfactant decreased the interfacial tension between CO_2–water, which results in a significant increase in the hydrogel porosity.

The possibility to carry out at the same time chemical cross-linking of the structures presents several advantages, in particular the fact that it exhibits faster reaction rates than the processes

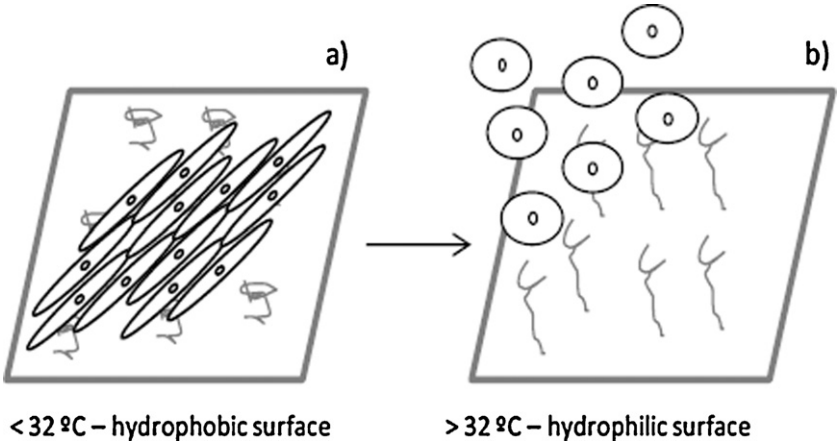

Fig. 2. Schematic representation of the thermosensitive mechanism for (a) temperatures < LCST, the surface is hydrophobic and cells attach and (b) temperatures > LCST, surface is hydrophilic and cells detach.

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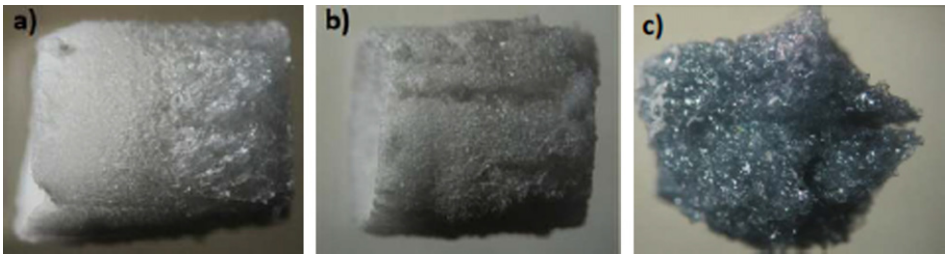

Fig. 4. (a) Collagen scaffold freeze dried; (b) collagen cross-linked with genipin (atmospheric conditions, 7 days); and (c) collagen cross-linked with genipin under high-pressure carbon dioxide (overnight).

carried out at atmospheric conditions. One of the examples is the cross-linking reaction of marine-origin collagen with genipin. The success of the cross-linking reaction is easily observed by the characteristic blue color of cross-linked genipin. Fig. 4 illustrates the differences between the reaction under atmospheric conditions and under 5.5 MPa.

After 7 days of cross-linking reaction under atmospheric conditions, the collagen scaffold does not present the same intensity in blue color as the scaffold cross-linked overnight under dense carbon dioxide atmosphere, which is a clear indication of the advantages of high pressure processes. Furthermore, it is possible to promote a double foaming stage of the hydrogel, upon depressurization of the system, which can enhance the morphological properties of the construct to be used in TERM applications, particularly increasing the porosity and interconnectivity of the samples [43].

Although there are some concerns regarding the cytotoxicity of different crosslinking agents available, in particular genipin, the *in vitro* biological tests performed to the scaffolds produced do not indicate any toxic effects on the chondrocytes seeded on the materials [43]. The results obtained suggest that marine origin collagen crosslinked with genipin is able to sustain cell adhesion and proliferation, therefore these matrices could be used in cartilage tissue regeneration strategies.

A different strategy for the development of porous hydrogel scaffolds was described by Partap and coworkers [44]. In this approach a reactive emulsion templating in which carbon dioxide acts both as reagent and template takes place. 3D calcium alginate hydrogels were produced by combining an emulsion templating technique and a gelation reaction. The materials prepared have interesting properties which make them suitable for potential application in soft tissue engineering regeneration. A similar approach has been described for the preparation of porous biomaterials from dextran and gelatin, two other natural polymers [45,46]. These works report the production of a highly porous and interconnected structure by a supercritical CO_2-in-water emulsion templating method. Although this method has the great advantage of avoiding the use of organic solvents both in the processing and purifications steps, it does not meet the requirements needed in terms of final morphology of the structures for some cell types [46].

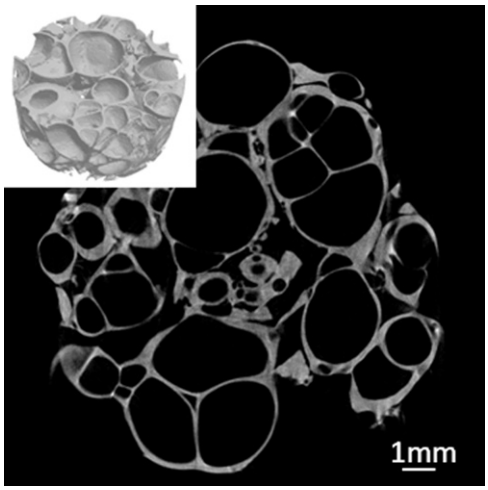

Fig. 5. Micro-CT 2D image and 3D reconstruction of a PDLLA scaffold loaded with ulvan beads.

4. Subcritical sintering

Subcritical fluid sintering is a process that takes place at conditions close to the supercritical region of the solvent used. The process relies on the slight plasticization of the polymeric particles which are fused together, creating a 3D environment. Few papers report the use of this technique for the preparation of scaffolds for TERM applications, despite this technique uses lower operating conditions than supercritical fluid foaming, therefore being interesting for the processing of thermosensitive molecules such as proteins, growth factors or even cells. The possibility to encapsulate cells in hydrogel beads and design a structure which is able to support their growth and proliferation is an interesting approach for the preparation of 3D systems for regenerative medicine [47,48]. This was the driving force for the development of PDLLA matrices loaded with a natural polymer extracted from green algae, ulvan [49,50]. As an example, Fig. 5 represents a 2D image of a PDLLA scaffold sintered in the presence of ulvan beads obtained by micro-computed tomography together with a 3D reconstruction of the structure [51].

Table 2
Overview of hydrogels processed by supercritical fluid foaming.

Polymer	Cross-linking agent	Operating conditions		Reported applications	Reference
		Pressure (MPa)	Temperature (K)		
α-Elastin	Glutaraldeyde	3.0–15.0	310	Tissue engineering	[38]
Tropoelastin/α-elastin	Glutaraldeyde	6.0	310	Tissue engineering	[39]
α-Elastin	Hexamethylene diisocyanate	6.0	298	Tissue engineering	[40]
Chitosan	Glutaraldeyde genipin	6.0–16.0	277–310	Skin/cartilage regeneration	[41]
Chitosan/gelatin	Glutaraldeyde	5.5–11.9	297–323	–	[36]
Chitosan + acacia gum	Glutaraldeyde	6.0–15.0	277	Tissue engineering	[42]

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Fig. 6. SPLA-based matrices prepared by supercritical assisted phase inversion.

5. Supercritical assisted phase inversion

Supercritical fluid phase inversion process offers an attractive and alternative process to obtain solvent free structures. This technique involves casting of a polymer solution onto an inert support followed by immersion of the support with the cast film into a bath filled with a non-solvent for the polymer. The contact between the solvent and the non-solvent causes the solution to be phase-separated. When the non-solvent used is carbon dioxide a solid dry product is obtained, and there is no need for further processing steps. Several studies report different polymers processed using this technique including PLLA [52], poly(methyl methacrylate) [53], polysulfone/polycaprolactone [54], poly(vinylidene fluoride) [55], and poly(vinyl-alcohol) [56]. This technology has also given proofs of the successful preparation of natural-based porous materials. This is a versatile method which allows the production of porous 3D matrices and at the same time prepare impregnated 3D constructs with bioactive compounds, which can be inorganic or pharmaceutical agents [57–59]. Fig. 6 shows scanning electron microscope images of natural-based polymers prepared using this technique. A commercial blend of starch-poly lactic acid (SPLA) alone or combined with bioglass® after 7 days of immersion in simulated body fluid or dexamethasone is shown.

The morphology of the structures prepared with and without active agents is characterized by a rough surface with micro- and macro-porosity which may enhance cell adhesion and proliferation. The presence of active agents is particularly important in order to promote an effective bone tissue regeneration. While bioactive glass may induce the mineralization of the tissues and the formation of a hydroxyapatite layer which mimics the bone calcium phosphates, dexamethasone may be used as an inducer of osteogenic differentiation of stem cells. The presence of dexamethasone, which was used as a model drug, further demonstrates the possibility to prepare a controlled drug delivery system able to sustain drug release up to 21 days.

The discussion between the advantages and benefits of natural over synthetic polymers has long passionate scientists, particularly in applications where synthetic polymers have long been established. A straightforward comparison between these polymers is very difficult to demonstrate. Several parameters affect the biological performance of the materials and the *in vitro* behavior. Morphological, topological, chemical and mechanical properties will surely have an influence on the cellular response to a particular material and they cannot be dissociated, most of the times. Natural polymers, as already mentioned present intrinsic properties which make them very attractive for biomedical applications. The biological performance of scaffolds based on poly(L-lactic acid) (PLLA) and the blend SPLA processed by the same methodology was assessed using a fibroblast cell line. In this example we show that the same processing methodology can be applied for the preparation of a synthetic and a natural-based scaffold. After seven days of culture the SPLA scaffold presented a good interaction with cells in which a layer of fibroblasts could be detected. In this matrix it was also noticeable that cells started to migrate into the pores of the scaffold (Fig. 7).

The evaluation of the cell viability and DNA quantification assay allows the conclusion that while in early contact times SPLA appears to induce better cellular response than PLLA, these differences are attenuated for longer periods of time.

The possibility to prepare polymeric blends using this technique was evaluated in another work [60]. The preparation of polymeric blends of chitosan and PLLA has long been a challenging process due to the fact that chitosan has limited solubility in most organic solvents and it undergoes degradation before melting. At the same time the solvents able to dissolve PLLA act as an anti-solvent for chitosan solutions leading to polymer precipitation. For these reasons it is difficult to encounter a suitable process for the preparation of polymeric blends of these two materials. We have successfully achieved this by the supercritical assisted phase inversion method. Fig. 8 represents a photo and SEM images of the blends prepared. The homogeneity of the structures prepared is confirmed by the chemical mapping of the scaffolds obtained by FTIR analysis.

This methodology was also proposed for the development of an autologous based scaffold from platelet lysates. Novel PL-based scaffolds were produced by supercritical fluid technology and different structures were prepared using genipin as a cross-linking agent. The morphological characteristics of the scaffolds produced, such as porosity, interconnectivity and mean pores size were assessed by scanning electron microscopy and micro-computed tomography. Fig. 9 presents the summary of the *in vitro* cell seeding studies performed using a chondrocyte cell line (ATDC5) for the different culture periods (1, 3, 7 and 14 days) [30].

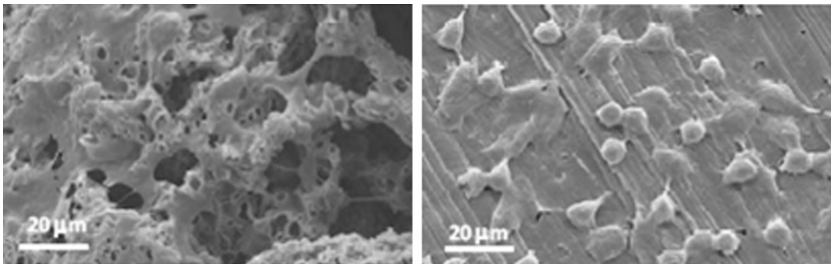

Fig. 7. Fibroblast like cells (cell line L929) cultured on SPLA (left) and PLLA (right) scaffolds after seven days.

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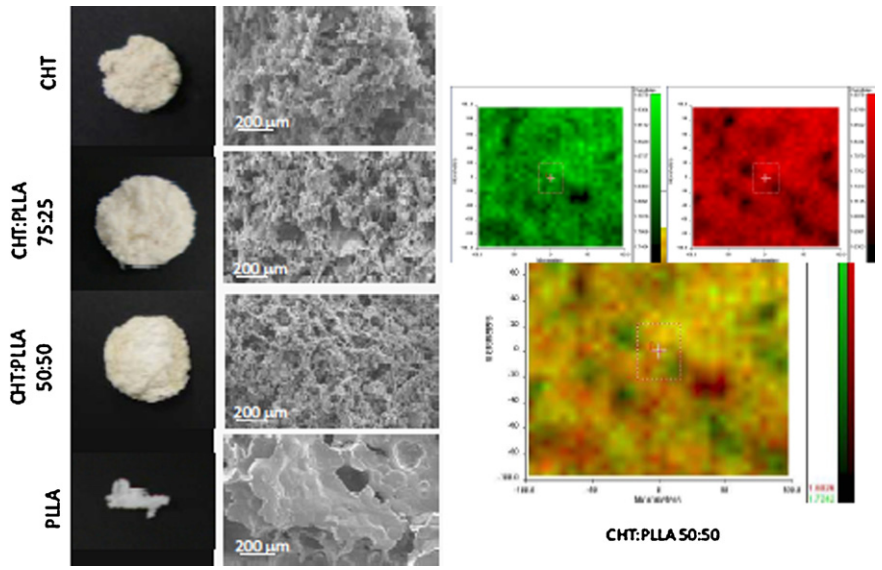

Fig. 8. Images of the different chitosan:PLLA scaffolds prepared (left); Chemical mapping of the scaffolds obtained by FTIR analysis: green mapping depicts the chitosan characteristic band at 1650 cm^{-1} and red mapping is assigned to PLLA characteristic band at 1750 cm^{-1} (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

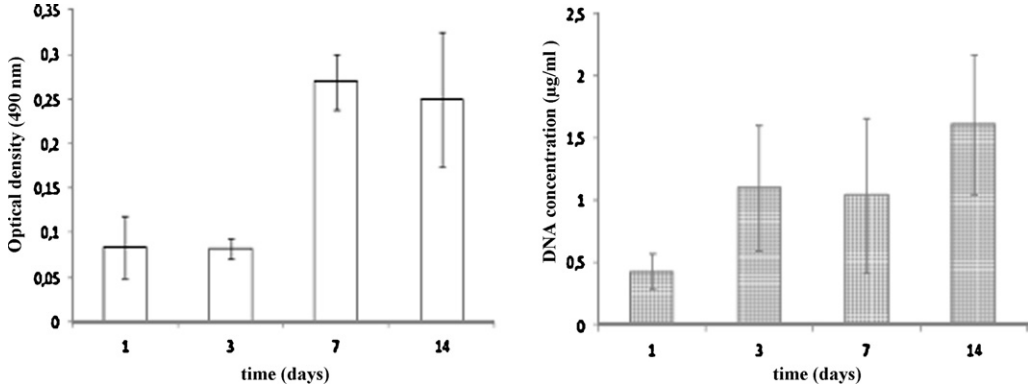

Fig. 9. Cell viability and proliferation on PL scaffolds crosslinked with 0.25 wt% genipin. MTS assay (left) and DNA quantification (right).

The morphological analysis of the systems indicate that these may be suitable for tissue engineering purposes and are able to sustain growth factors release *in vitro* up to 14 days. The 3D constructs prepared show a good biological response, which was demonstrated in the viability and proliferation assays performed using the standard MTS protocol and DNA quantification by PicoGreen method. These structures are particularly appealing for tissue engineering applications since supercritical assisted phase inversion explored the dual role of PL both as structural matrix and as growth factor-releasing system to promote cell proliferation and differentiation.

6. Supercritical fluid drying

The drying process is a crucial process in the preparation of 3D architectures and particles. Solvent removal by commonly used drying processes involves phase transitions which can compromise the integrity of the structures. It is important to notice that supercritical fluid drying does not involve any phase transition as the process occurs in the supercritical region where there are no phase boundaries, therefore this may present several advantages over the conventional methods (Fig. 10).

The main drawback of this process is that CO_2-phylic solvents have to be used and the matrices prepared from aqueous solutions need to go through an additional solvent-exchange step. The most commonly used solvents in this procedure are acetone, methanol or ethanol. It has been reported, for example, that the use of ethanol leads to higher shrinkages than acetone in the case of resorcinol-formaldehyde organic and carbon aerogels [61]. Rinki discusses in their work [62] a comparison between freeze-dried chitosan scaffolds and scaffolds dried by supercritical carbon dioxide. They

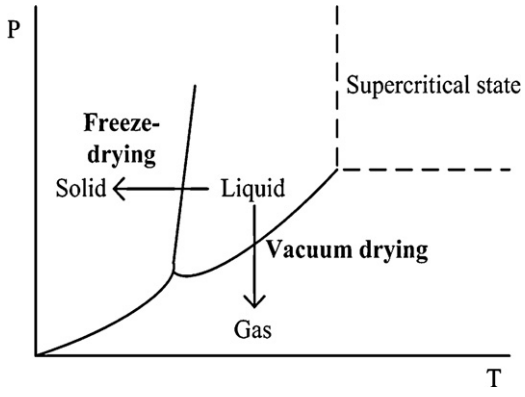

Fig. 10. Phase transitions involved in different drying processes.

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Table 3
Strengths and weaknesses of the different supercritical fluid technologies used for processing 3D architectures for TERM applications.

Technology	Strengths	Weaknesses
Foaming	Very good control over final morphology of the materials	Limited to amorphous or semi-crystalline polymers with low T_g
	Does not require the use of organic solvents	Not suitable for natural polymers processing
	Incorporation of active agents in a single step operation	
	Proteins can be processed without significant loss of activity	
Foaming of hydrogels	Possibility to process natural-based materials	Limited control over the final morphology of the scaffolds
	Double foaming technique, which enhances morphological properties of the scaffolds	
	Possibility to crosslink the structure in a one step process	
	Subcritical conditions can be used	
Sintering	Does not require the use of organic solvents	Limited to amorphous or semi-crystalline polymers with low T_g
	Low pressure operation ($P < P_c$)	Not suitable for natural polymers processing
	Low time of operation	
Phase inversion	Possibility to process natural-based materials	Use of organic solvent
	Formation of polymeric blends	The use of aqueous solutions is possible but requires the use of ethanol as co-solvent
	Formation of hybrid materials, with an organic and inorganic phase	
	Incorporation of pharmaceutical drugs	
	Possibility to cross-link structures	
Drying	Prevents shrinking of the structures when compared to other drying techniques	Need high pressure equipment
	Impregnation of active compounds during drying step	Use of ethanol, methanol or acetone

conclude that the SCF dried scaffolds presented more suitable properties for tissue engineering, such as higher surface area, pore size and bioactive behavior unlike the freeze dried scaffolds. Supercritical fluid drying has been applied to produce different chitosan scaffolds with or without the addition of a second component [63,64]. Together with chitin these have been the natural polymers most widely studied for the production of 3D architectures [65]. Chitin presents, however, major challenges due to its limited solubility [66,67]. In order to overcome the solubility limitations of chitin in common organic solvents, the possibility of using ionic liquids has been recently explored [65]. In our work the application of green chemistry principles, coupling ionic liquids as solvents for natural polymers together with supercritical fluids has demonstrated the possibility to develop ultralight highly porous chitin structures [68]. Chitin was dissolved in 1-butyl-3-imidazolium acetate at high temperature. The solution gelified at room temperature, and the ionic liquid is exchanged by ethanol. Afterwards, the solvent is removed by supercritical fluid drying. The preparation of chitin matrices following this procedure has shown the prevention of the shrinkage of the matrix of chitin based scaffolds and resulted in a highly porous interconnected structure. Using the same approach particles were produced. Materials in the particulate form can be used *per se* as drug delivery devices, but the interest in their production increases further when a 3D structure can be produced. Particle agglomeration is a method commonly used to produce stable matrices for tissue engineering and regenerative medicine [69–72].

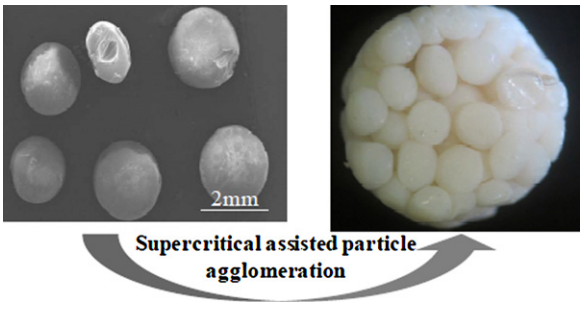

Fig. 11. Chitosan microspheres obtained after supercritical fluid drying.

Supercritical fluid drying has been tested for the preparation of chitosan 3D structures from a novel process so-called supercritical particle agglomeration (Fig. 11) [73].

In this approach the particles together with a gellan gum solution were loaded and pressed in a mold, which was added to promote agglomeration, and were placed in the high pressure vessel for critical point drying. The 3D matrices produced present an interesting structure with adequate porosity and mean pore size for bone tissue engineering applications.

7. Perspectives

Natural polymers present interesting inherent characteristics which make them attractive to be used where synthetic polymers have traditionally been applied. Nonetheless, their processability is often limited. In this paper we presented different methodologies, based on supercritical fluid technology, for materials processing in particular for different TERM applications. Table 3 presents the strengths and weaknesses of each of the technologies described in this paper.

The results documented in the literature suggest that the application of supercritical fluids, especially carbon dioxide, can be a successful alternative overcoming the drawbacks of the conventional processing techniques concerning the biomaterials development of natural based polymers.

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