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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