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Procedia Engineering 59 (2013) 233 – 239

Procedia Engineering

www.elsevier.com/locate/procedia

3rd International Conference on Tissue Engineering, ICTE2013

Systematic analysis of injectable materials and 3D rapid prototyped magnetic scaffolds: from CNS applications to soft and hard tissue repair/regeneration

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Abstract

Over the past years, polymer-based materials have attracted research interest in the field of tissue repair and regeneration. As reported in literature, different injectable systems have been proposed, trying to reduce surgical invasiveness. In a first step of the current research, the rheological and functional features of injecatble hydrogel-based materials for central nervous system applications or soft tissue regeneration (collagen/PEG semi-IPNs) as well as for hard tissue engineering (alginate/iron-doped hydroxyapatite) were evaluated. Then, the study was also devoted to the development of 3D nanocomposite poly(ε-caprolactone)/iron-doped hydroxyapatite scaffolds for bone tissue engineering, providing a preliminary approach to assess magnetic attraction forces.

Selection and peer-review under responsibility of the Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Centro Empresarial da Marinha Grande.

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Selection and peer-review under responsibility of the Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Centro Empresarial da Marinha Grande
doi:10.1016/j.proeng.2013.05.116

Keywords: Injectable Materials; Rheology; Nanocomposites; Rapid Prototyping; Magnetic Scaffolds.

1. Introduction

Over the past few years, polymer-based materials have attracted research interest in the field of tissue repair and regeneration. Polymer-based composite materials should present improved mechanical properties, better flexibility and structural integrity. This means that porous scaffolds with enhanced bioactivity and controlled properties can be obtained by processing polymer-based materials.

Polymer-based materials may be developed in the form of "solids" or injectatble formulations, considering their specific applications [7-20].

In particular, most of the research attention has been widely devoted to the design and applications of injectable systems and hydrogel-based composites [5-7-8] in order to promote the regeneration of soft and hard tissues, trying to reduce surgical invasiveness. They should promote efficient biomolecular interactions with cells, also regulating their basic functions, guiding the spatially and temporally complex multi-cellular processes of tissue formation, thus facilitating the restoration of structure and function of damaged tissues [9]. Furthermore, in order to repair bone, cartilage, intervertebral disc, adipose tissue, neural, and cardiac tissue, hydrogel-based materials have been widely analysed as cell delivery systems providing a controlled release of drugs, proteins, cells, gene and other immobilized biomolecules [9].

As an example, with regard to central nervous system (CNS) neurodegenerative disorders, the development of collagen/polyethylene glycol (PEG) hydrogels for *in situ* cell or drug release should represent a great challenge as well as a minimally invasive solution which should play a crucial role to successfully optimize surgical approaches [7-8]. PEG hydrogel cultures with soluble factors were investigated by Mooney et al. (2011), evidencing how the hydrogel environment may influence neural cell composition and also describing soluble factors that may be useful in generating neuronal-enriched populations within the specific hydrogel environment [10].

In the field of bone tissue repair and regeneration, hydrogels suitably combined with solid micro/nanoparticles can provide high-performance and functional systems [9].

Taking into account the load-bearing function, the reconstruction of a hard tissue such as bone introduces a crucial feature that has to be considered in order to regenerate a highly organized and specialized tissue. The bone tissue cells are highly susceptible to mechanical stimulation and, therefore, the ideal bone scaffold should allow immediately to withstand external forces.

In designing injectable hydrogel-based composites with magnetic properties and 3D "solid" magnetic scaffolds for bone tissue repair and regeneration, the rationale should be summarized in the possibility to obtain structures that can be manipulated *in situ* by applying external magnetic fields, also able to control specific processes at cell level by releasing biomolecules, in turn linked to magnetic nanocarriers [19].

A 3D rapid prototyped magnetic scaffold should be considered as a valuable candidate for bone tissue engineering. An ideal scaffold should possess a morphologically controlled and tailored structure with an interconnected pore network. Furthermore, a superparamagnetic scaffold [17-19] should provide the fascinating possibility to magnetically "switch-on/switch-off" it in order to deliver biofactors and stem cells, or to stimulate cell adhesion, proliferation and differentiation.

In a first step of the research, the aim was to design injecatble hydrogel-based materials for CNS applications or soft tissue regeneration (collagen/PEG semi-IPNs) as well as for hard tissue engineering (alginate/iron-doped hydroxyapatite).

Accordingly, rheological and injectability tests were performed in order to obtain important information on the functional properties of the injectable systems in terms of viscoelasticity and flow behavior.

In a second step, the goal of the current study was to design 3D nanocomposite magnetic scaffolds characterized by a morphologically controlled structure, also providing a preliminary approach to assess magnetic attraction forces.

2. Materials and Methods

Ultra pure type I collagen from bovine skin (3 mg/ml), PEG (M_w 1900-2200 g/mol), sodium hydroxide, Dulbecco′s Modified Eagle′s Medium (DMEM), Poly(ε-caprolactone) (PCL M_w 65,000 Da) and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis-MO, USA). Sodium alginate copolymer was supplied by Fluka whilst bioresorbable iron-doped hydroxyapatite (FeHA) nanoparticles were prepared according to the method previously described [21].

2.1. Injectable Materials

Collagen-PEG semi-IPNs were prepared by promoting collagen fibrillogenesis in the presence of PEG.

PEG powder dissolved in DMEM was suitably mixed with the collagen solution, in order to obtain different final PEG (up to 1.2 mg/ml) and collagen concentrations (up to 1.8 mg/ml). The solutions were incubated at 37°C for 1h in order to allow collagen fibrillogenesis. After the incubation, the collagen was completely fibrillated and collagen-PEG semi-IPNs were obtained.

Alginate/FeHA materials were prepared by embedding FeHA nanoparticles (1 wt%) in sodium alginate aqueous solution (80 mg/ml).

2.2. Nanocomposite Magnetic Scaffolds

PCL pellets were dissolved in THF with stirring at room temperature. FeHA nanoparticles and then ethanol were added to the PCL/THF solution during stirring. A PCL/ FeHA nanoparticles weight ratio (wt/wt) of 80/20 was used. An ultrasonic bath (Branson 1510 MT, Danbury, CT) was also used to optimize the FeHA nanoparticle dispersion in the polymer solution. Accordingly, a homogeneous paste was obtained, and then, the solvent was totally removed. Successively, PCL/FeHA (80/20 wt/wt) pellets were made.

3D nanocomposite magnetic scaffolds with a $0_2°/90_2°$ pattern were preliminary manufactured by the processing of PCL/FeHA pellets through a 3D fiber deposition technique.

In particular, nanocomposite scaffolds were built by extruding and alternatively depositing the fibers along the 0° direction and the 90° direction between two successive layers. PCL/FeHA (80/20 wt/wt) pellets were initially placed in a stainless steel syringe and then heated at a temperature of 130–140°C with a heated cartridge unit placed on the mobile arm of a bioplotter dispensing machine (Envisiontec GmbH, Gladbeck, Germany). Successively, a nitrogen pressure of 8.5–8.9 bar was applied to the syringe through a cap. The stainless steel nozzle used to extrude the PCL/FeHA fibers was characterized by an inner diameter of about 600 μm.

The designed nanocomposite scaffolds were characterized not only by the fiber diameter (depending on the needle diameter and/or the deposition speed) but also by the fiber spacing (strand distance, that is, center-to-center distance) and layer thickness, which influenced the overall pore size. A deposition speed of 30 mm/min was used.

2.3. Rheological Analysis and Injectability Measurements

Rheological analysis was carried out on all the different formulations of the proposed injectable materials.

A rheometer (Bohlin Gemini, Malvern Instruments, Malvern, UK) equipped with a parallel plate geometry was used.

Preliminary tests were performed at a fixed oscillation frequency to determine the linear viscoelastic region, then small amplitude oscillatory shear tests were carried out at 37 °C with frequency ranging from 0.01 Hz to 10 Hz. The storage modulus (*G'*) and loss modulus (*G"*) were evaluated as follows:

$$G' = \frac{\tau_0}{\gamma_0}\cos\delta \tag{1}$$

$$G'' = \frac{\tau_0}{\gamma_0} \sin \delta \tag{2}$$

where δ is the phase shift between the input and output signals (the stress, τ, and the strain, γ, respectively), while τ_0 and γ_0 represent stress and strain amplitudes, respectively.

Viscosity as a function of shear rate was also recorded by steady shear measurements at 37 °C.

With regard to the injectability measurements, a syringe equipped with a typical clinical catheter was mounted on an INSTRON 5566 testing machine (Bucks, UK) and loaded with 0.5 ml of material. The piston was driven at a speed of 40 mm/min and the applied load for injecting the materials into and through the needle was measured. In order to estimate the friction between the piston and the syringe walls, an empty syringe was also tested.

2.4. Magnetic Attraction Force

The attraction force was evaluated at different values of distance (i.e. displacement) from the upper surface of the nanocomposite PCL/FeHA scaffolds by using a neodymium magnet. The experimental setup is reported in figure 1.

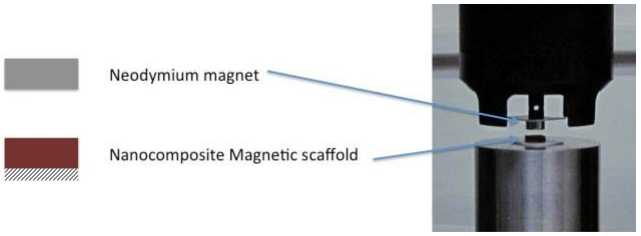

Fig.1. Experimental setup used to evaluate the magnetic attraction force at different values of distance from the upper surface of the PCL/FeHA scaffold.

Briefly, an INSTRON 5566 testing machine was used and the attraction force of the neodymium magnet, which moved away from the nanocomposite scaffold at a constant (crosshead) speed of 10 mm/min, was evaluated while increasing the distance from the upper surface of the structure.

3. Results

3.1. Rheological Analysis and Injectability Measurements

Small amplitude oscillatory shear tests have indicated that collagen/PEG semi-IPNs show a gel-like behavior, with the storage modulus *G'* always being higher than the loss modulus *G"* in the investigated frequency range (Fig. 2, left). Both dynamic moduli depend on frequency and collagen/PEG composition, rising with frequency and final collagen concentration.

Steady shear tests have shown that viscosity decreases as the shear rate increases (shear thinning behavior), suggesting the injectability of the proposed collagen-PEG semi-IPNs (Fig. 2, right). Anyway, viscosity values depend on collagen/PEG composition, generally increasing with collagen concentration.

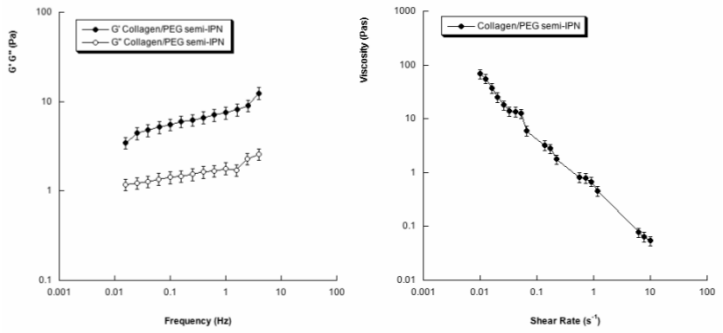

Fig. 2. Results from rheological tests on a specific collagen/PEG semi-IPN: storage modulus (G') and loss modulus (G'') as function of frequency (left); viscosity as function of shear rate (right).

Sodium alginate solutions and alginate/FeHA materials have shown mechanical spectra characterized by a crossover frequency, f_c (Fig. 3, left). In particular, at low frequencies ($f<f_c$) these materials show a predominant viscous character (G''>G'), however, as the frequency increases the storage modulus approaches the viscous one and at high frequency ($f>f_c$) G' exceeds G'', thus suggesting a predominant elastic character.

The inclusion of FeHA nanoparticles does not dramatically alter the viscoelastic moduli of the proposed materials, but crossover frequency, f_c, is slightly shifted to higher values.

On the other hand, steady state shear measurements have highlighted a shear thinning behaviour for the materials, thus suggesting their injectability (Fig. 3, right).

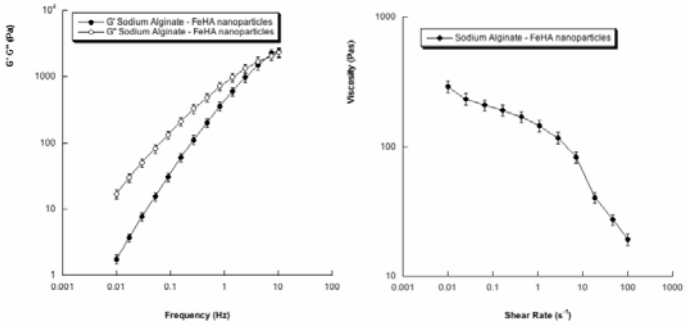

Fig. 3. Results from rheological tests on alginate/FeHA materials: storage modulus (G') and loss modulus (G'') as function of frequency (left); viscosity as function of shear rate (right).

Injectability measurements have provided interesting information about the injection of all the proposed materials through clinical catheters. A typical load-displacement curve obtained from injectability measurements is reported in figure 4.

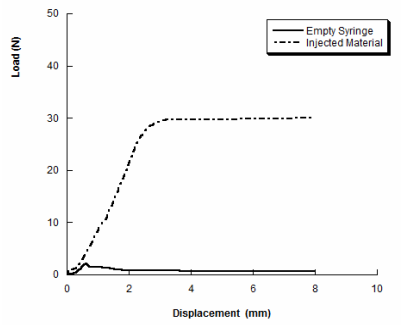

Fig. 4. Typical load-displacement curve obtained from injectability measurements on alginate/FeHA materials.

Load-displacement curves obtained from the injectability tests have basically evidenced an initial linear region. After a maximum load occurred, load values generally dropped, then reaching a plateau. At the end of the plateau-like region all the materials were completely injected.

However, collagen/PEG semi-IPNs have provided values of maximum load lower than those obtained from alginate/FeHA materials.

All the results obtained from the injectability measurements could be properly used to assess some functional features and rheological information benefiting from the basic principles of capillary extrusion rheometry. Clearly, it is worth noting that several assumptions should be taken into account: the pressure drop along the length of the needle is considered to be equivalent to that measured above the piston; the entrance pressure, shear flow pressure drop in the syringe body and friction between the piston and syringe wall will be ignored. The obtained results should be suitably compared and integrated with those provided by steady state shear measurements.

3.2. Magnetic Attraction Force

The performed measurements have highlighted the possibility to magnetically attract the designed nanocomposite PCL/FeHA scaffold by using a neodymium magnet. Specifically, the attraction force has been numerically evaluated and a typical load-displacement curve is reported in figure 5.

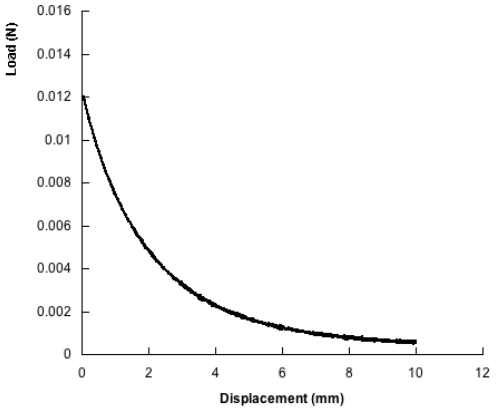

Fig. 5. Magnetic attraction force as function of the distance (i.e. displacement) from the upper surface of the nanocomposite PCL/FeHA scaffold.

It is worth noting that the attraction force attains a maximum value when the neodymium magnet is in close contact with the upper surface of the scaffold.

The values of the attraction force clearly decrease by increasing the distance of the neodymium magnet from the upper surface of the scaffold.

4. Conclusion

Collagen/PEG semi-IPNs and alginate/FeHA materials were designed and analyzed as injectable systems for biomedical applications spanning from CNS to soft and hard tissue repair/regeneration. With regard to bone tissue engineering, 3D nanocomposite PCL/FeHA scaffolds with a tailored architecture were also developed, and a preliminary approach to assess the magnetic attraction force was carried out.

Acknowledgements

The authors would like to thank Fondazione Cariplo (NANOBRAIN project, Ref. no: 2011-0335), MAGISTER project (NMP3-LA-2008-214685) and FIRB MERIT project (GAE: P0000570), for providing financial support to this research.

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