Available online at www.sciencedirect.com

ScienceDirect

Procedia CIRP 49 (2016) 72 – 75

www.elsevier.com/locate/procedia

The Second CIRP Conference on Biomanufacturing

From 3D hierarchical scaffolds for tissue engineering to advanced hydrogel-based and complex devices for *in situ* cell or drug release

Antonio Gloria[a,*,+], Teresa Russo[a,+], Diogo F.Lopes Rodrigues[a], Ugo D'Amora[a], Francesco Colella[b], Giovanni Improta[c], Maria Triassi[c], Roberto De Santis[a], Luigi Ambrosio[a]

[a] *Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, V.le J.F. Kennedy 54 - Pad. 20, Mostra d'Oltremare, 80125 Naples, Italy*
[b] *Department of Chemical, Materials and Industrial Production Engineering - University of Naples "Federico II", P.le V. Tecchio 80, 80125, Naples, Italy.*
[c] *Department of Public Health, University of Naples "Federico II", via Pansini 5, 80131 Naples, Italy*

* Corresponding author. Tel.: +390812425942; *E-mail address:* angloria@unina.it

\+ Both first authors

Abstract

In the past few years, researchers have focused on the development of three-dimensional (3D) advanced scaffolds and multifunctional hydrogel-based materials.
As reported in literature, 3D polymer-based composite scaffolds for tissue engineering have been manufactured through conventional and advanced manufacturing techniques, and different injectable materials and hydrogel-based systems have been proposed and studied.
The aim of the current research was to define an approach in the development of multifunctional tools spanning from 3D hierarchical scaffolds for soft tissue engineering to advanced hydrogel-based devices for *in situ* cell or drug release. The mechanical/rheological behaviour as well as the structural/functional features of the designed devices were discussed and analyzed.

Peer-review under responsibility of the scientific committee of The Second CIRP Conference on Biomanufacturing

Keywords: Injectable Materials; Multilayer Hydrogels; Mechanical/Rheological Properties; Additive Manufacturing; Hierarchical Scaffolds

1. INTRODUCTION

Over the past years, in the field of tissue repair and regeneration, researchers' attention has been focused on natural and synthetic polymers. In particular, polymer-based composite materials may be designed to possess enhanced mechanical and functional properties, however showing adequate flexibility, strength and structural integrity at the same time [1, 2].

With regard to the tissue engineering approach, porous scaffolds with improved bioactivity and tailored properties may be developed by processing polymer-based materials. In this context, advanced devices may be designed in the form of "solids" or injectable formulations, taking into account the specific application [3-25]. Hydrogel-based composites and semi-interpenetrating polymer networks (semi-IPNs) have been widely developed and analysed as patches to promote the regeneration of hard and soft tissues or injectable devices for *in situ* drug or cell release, reducing surgical invasiveness [5, 7, 8, 17-19, 25]. As an example, in the nucleus pulposus regeneration, as well as in the treatment of central nervous system neurodegenerative disorders (i.e., Alzheimer's and Parkinson's diseases), or cardiovascular disease, advanced hydrogel constructs may play a crucial role as reservoir systems to deliver specific biomolecules to targeted sites.

Peer-review under responsibility of the scientific committee of The Second CIRP Conference on Biomanufacturing
doi:10.1016/j.procir.2015.07.036

Even though polymeric devices have been mainly developed for cartilage and intervertebral disc regeneration, in the field of bone tissue engineering both injectable materials and 3D “solid” scaffolds have been properly reinforced with inorganic micro/nanoparticles to design high functional devices [11, 12, 20-22, 25].

Anyway, the strategy relies on the combination of cells with appropriate scaffolds and biomolecules. A scaffold should typically possess an interconnected pore network able to support cell adhesion, proliferation and differentiation, also promoting the extracellular matrix deposition, which is crucial for the regeneration process.

The possibility to design multifunctional and complex structures has been provided by the advances in 3D scaffold fabrication techniques achieved through the additive manufacturing approach [12, 14-16, 20-25].

On the other hand the potential to develop advanced hydrogel-based systems with optimized properties clearly benefits from accurate mechanical/rheological and functional analyses.

Accordingly, the aim of the present research was to define an approach in the development of multifunctional tools spanning from 3D hierarchical scaffolds for soft tissue engineering to advanced hydrogel-based devices for *in situ* cell or drug release. The mechanical/rheological behaviour as well as the structural/functional features of the designed devices were analysed.

2. MATERIALS AND METHODS

Several injectable collagen-based materials were prepared by promoting collagen fibrillogenesis at 37°C for 1h. Furthermore, multilayer systems consisting of three collagen-based layers, with different compositions, were also fabricated combining a conventional method with an electrospray-based technique.

The viscosity as a function of the shear rate was assessed through steady state shear measurements at a temperature of 37°C in a wide range of shear rate, and the viscoelastic properties (storage modulus and loss modulus) were studied through small amplitude oscillatory shear tests. All the tests were carried at a temperature of 37°C, using a rheometer (Gemini, Bohlin Instruments). The injectability properties were also investigated simulating the clinical practice, and scanning electron microscopy (SEM) were carried out to assess the morphological features of the injectable materials and of the multilayer systems.

On the other hand, 3D poly(ε-caprolactone) scaffolds with different lay-down patterns were fabricated through an additive manufacturing process (i.e., 3D fiber deposition technique).

Poly(ε-caprolactone) (PCL, M_W = 65 000, Sigma-Aldrich, St. Louis, MO) pellets were placed in a syringe and then heated at a temperature of 100–120°C using the cartridge unit placed on the mobile arm of a 3D plotter (Envisiontec GmbH, Gladbeck, Germany).

In particular, 3D PCL scaffolds were built by injecting/extruding and depositing the fibers along well defined directions between two successive layers according to the selected lay-down patterns (i.e., from 0°/90° to 0°/45°/90°/135° or specific radial and circumferential layers).

A nitrogen pressure of 8.5 bar was then applied to the syringe through a cap. The stainless steel nozzle used to extrude the PCL fibers possessed an inner diameter of about 500 μm.

Different customized 3D PCL scaffolds were fabricated according to the specific application. They were characterized by the fiber diameter (depending on the deposition speed and/or the needle inner diameter), the fiber spacing (center-to-center distance) and layer thickness, which influenced the pore size. A deposition speed of 45 mm/min was employed.

3D complex hierarchical structures were also obtained by properly loading the collagen-based gels into the interconnected pore network of the fiber deposited scaffolds. Both acellular or cell-loaded micro/nanocomposite gels were considered.

Compression tests were carried out on the 3D scaffolds as well as on the gel-loaded hierarchical structures at 1 mm/min using an Instron 5566 testing machine.

3. RESULTS AND DISCUSSION

Results from small amplitude shear tests on the injectable and multilayer systems evidenced that G’ was always higher than G’’ in the frequency range analysed, and the values of both dynamic moduli increased with frequency (Fig. 1).

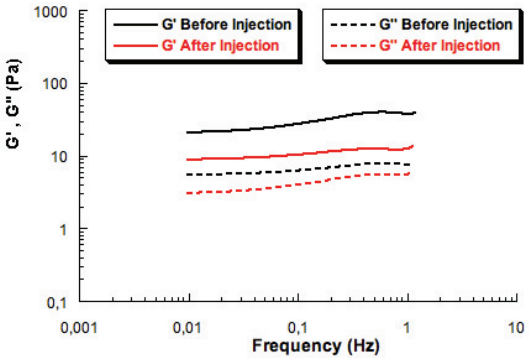

Figure 1. Results from small amplitude oscillatory tests on a collagen-based composite: storage modulus (G’) and loss modulus (G’’) as function of frequency. A typical effect of the injection through clinical needles.

The viscosity decreased with increasing the shear rate (shear thinning behaviour) (Fig. 2), and the composition (i.e., collagen concentration and amount of gelatin particles) influenced the viscoelastic properties as well as the flow behaviour and injectability.