Bone Tissue Engineering:

3D PCL-based nanocomposite scaffolds with tailored properties

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Abstract

In the field of reconstructive surgery, a great challenge is represented by bone injuries beyond the self-repair threshold. Autologous bone grafts may be considered the gold standard. Anyway, such approach is limited by the amount of tissue required for grafting and by donor site morbidity. To overcome these drawbacks, bone tissue engineering represents a promising solution. 3D fully biodegradable and nanocomposite scaffolds for bone tissue regeneration, consisting of poly(ε-caprolactone) (PCL) reinforced with hydroxyapatite (HA) nanoparticles, were developed using an additive manufacturing process. The effect of nanoparticles and architecture (i.e., lay-down pattern) on the mechanical/functional and biological properties was discussed.

Keywords: Bone; Tissue Regeneration; Nanocomposite Scaffolds; Additive Manufacturing

1. Introduction

The need for substitutes to repair or replace tissues or organs due to trauma, disease, or congenital problems is overwhelming. In the field of reconstructive surgery, bone injuries beyond the self-repair threshold represent a great challenge.

Many efforts have been made in the development of advanced materials and fabrication techniques to design devices in the field of prosthetic and tissue engineering [1-25].

However, in terms of organ transplantation, many problems are basically related to the shortage of donors as well as the side effects of immunosuppressive agents.

Even though many surgical procedures may be adopted and different options are available for bone regeneration, autologous bone grafts harvested from healthy bone can be considered the gold standard. Anyway, donor site morbidity, low mechanical performances, long recovery periods, and complex graft shaping process are crucial features [22, 23].

In this context, bone tissue engineering may be seen as a promising solution [22, 23]. This approach clearly involves the synergistic combination of cells with appropriate scaffolds and/or specific biomolecules.

The most promising approach in tissue engineering involves the seeding of porous biocompatible and biodegradable scaffolds with cells to promote tissue regeneration [22].

Taking into account the regeneration strategies, the load-bearing function of bone plays a crucial role and introduces additional considerations. If a highly specialized and
organized tissue such as cortical bone is considered, during walking the regenerated tissue must withstand forces which are greater than the body weight [23].

For this reason, interconnectivity, pore shape and size are essential characteristics for the biological and mechanical performances of the scaffolds, which should be immediately capable of withstanding the forces acting on the bone segment after the implantation. Furthermore, to reproduce a physiological tissue regeneration process, the mechanical and biological features should be properly combined [23].

With regard to scaffold manufacturing techniques, conventional processes (i.e., solvent casting/particulate leaching, freeze drying, gas foaming, solution casting, phase separation, etc.) do not allow to control the spatial distribution of pores, pore size and geometry, and the micro-architecture of the scaffolds. To overcome the drawbacks related to conventional techniques, further fabrication methods have been introduced as a new group of non-conventional techniques in the biomedical field. The introduction of additive manufacturing methods has allowed to develop customized scaffolds with a reproducible internal morphology and high architectural control, complex 3D shapes, and tailored mechanical and mass transport properties. Specifically, the expression "additive manufacturing" refers to a group of technologies able to fabricate objects in a layer-by-layer fashion, starting from a 3D computer design of a specific object [22, 23].

Bone is a natural composite which possesses unique remodelling properties to adapt its microstructure to external mechanical loads [26]. Structure and properties of bone (i.e., cortical and trabecular bone) significantly vary as a function of the anatomical location. It can be considered as a nanostructured composite consisting of organic and inorganic phases [27, 28]. The extracellular matrix is mainly composed of hydroxyapatite (HA) nanocrystals as mineral phase, type I collagen (90% of the organic phase) and water [29, 30].

Taking into account the nanotechnology approach, as nanocomposites show properties which are better than their microcomposite counterparts, advanced polymer-based materials able to mimic the natural nanostructure of the human tissue (i.e., bone) have been developed. As a consequence, nanocomposite scaffolds should represent the natural choice for developing structures for bone tissue engineering. Accordingly, the current research focused on the strategies in designing 3D nanocomposite PCL/HA scaffolds for bone regeneration using an additive manufacturing method (i.e., 3D fibre deposition). The effect of nanoparticles and architecture (i.e., lay-down pattern) on the mechanical/functional and biological properties was discussed.

2. Materials and Methods

Poly(ε-caprolactone)/hydroxyapatite (PCL/HA) nanocomposite pellets were first prepared and then properly processed to develop scaffolds using an additive manufacturing method. PCL pellets (Mw = 65,000, Sigma-Aldrich, St. Louis, MO) were dissolved in tetrahydrofuran (THF, Sigma-Aldrich, St. Louis, MO). During stirring HA nanoparticles and, then, ethanol were added to the PCL/THF solution. A PCL/filler weight ratio (w/w) of 90/10 was used. To optimize the dispersion of nanoparticles in the solution, an ultrasonic bath (Branson 1510 MT, Danbury, CT) was also employed.

The obtained nanocomposite pellets consisting of PCL loaded with HA nanoparticles were processed using an additive manufacturing technique (i.e., 3D fibre deposition).

Specifically, 3D block-shaped scaffolds (5.0 mm in length - l, 5.0 mm in width - w and 8.0 mm in height - h) were built layer-by-layer, depositing the fibres along specific directions according to a specific lay-down pattern. The PCL/HA pellets were placed in a stainless steel syringe and heated to a temperature of 130 °C using a heated cartridge unit placed on the mobile arm of a 3D plotter dispensing machine (Envisiontec GmbH, Germany). A nitrogen pressure of 8.0 bar was properly applied to the process the material. The material was extruded through a nozzle with an inner diameter of 400 μm, and the fibre was deposited at a speed of 30 mm/min.

Three different lay-down patterns were adopted (0/90°, 0/60/120° and 0/45/90/135°) for polymeric (PCL) and nanocomposite scaffolds (PCL/HA) maintaining a fibre spacing (i.e., centre-to-centre distance between two fibres) of 800 μm.

Both polymeric and nanocomposite scaffolds were characterized by a fibre diameter of 400 μm, a layer thickness of 320 μm and a strand distance (i.e., centre-to-centre fibre distance) of 800μm.

Morphological analysis was carried out on the 3D scaffolds using a scanning electron microscope (FEI Quanta FEG 200 apparatus, The Netherlands). The in vitro biological behaviour of human mesenchymal stem cells was studied and cell constructs were also analysed by confocal laser scanning microscopy (CLSM, Zeiss LSM 510/Confocor 2) at different times after cell seeding (7, 14 and 21 days). Compression tests were carried out at a rate of 1 mm/min using an INSTRON 5566 testing machine. The tests were performed in physiological conditions. In particular, a strain limit of 0.4 mm/mm (40%) was considered.

3. Results and Discussion

The mechanical behaviour of the 3D PCL and PCL/HA scaffolds is similar to that already reported for rapid prototyped structures [22, 23]. Specifically, with regard to the stress-strain curves, an initial linear region followed by a region with lower stiffness was observed. A stiff region of the stress-strain curve was finally evidenced.
The compressive modulus (E) was evaluated from the slope of the initial linear region of the stress-strain curve. To analyse the effect of nanoparticles and architecture, compressive modulus and maximum stress are reported as mean value ± standard deviation (Tab. 1).

<table>
<thead>
<tr>
<th>Lay-down pattern</th>
<th>PCL</th>
<th>PCL/HA 90/10</th>
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<tbody>
<tr>
<td></td>
<td>E</td>
<td>σ\text{max}</td>
</tr>
<tr>
<td>0°/90°</td>
<td>60.1 ± 6.1</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td>0°/60°/120°</td>
<td>41.1 ± 3.7</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>0°/45°/90°/135°</td>
<td>30.4 ± 4.0</td>
<td>4.7 ± 0.5</td>
</tr>
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Table 1. Results from compression tests performed on 3D scaffolds up to a strain limit of 0.4 mm/mm: modulus and maximum stress reported as mean value ± standard deviation.

As expected, both the architecture and the inclusion of HA affected the mechanical performances of the developed scaffolds. The lay-down pattern influenced the mechanical behaviour of the scaffolds. Scaffolds with a 0°/90° pattern showed a compressive modulus and maximum stress which are greater than those obtained for 0°/60°/120° and 0°/45°/90°/135° patterns. These results are consistent with those reported in the literature [22]. As already demonstrated [22], a decrease in the amplitude of the deposition angle (from 0°/90° to 0°/45°/90°/135°) corresponds to a larger contact area (i.e., fused area), thus leading to a decrease of the stress locally experienced by the 3D scaffold [22]. The fibres deposited with a smaller lay-down pattern (0°/45°/90°/135°) showed a decreased stiffness [22].

Furthermore, SEM images showed a fully interconnected pore network, and the obtained pore geometry and size were consistent with the theoretical values defined during the manufacturing process.

The differences founded in terms of cell viability according to pore size and shape were already discussed for PCL scaffolds in terms of angle amplitudes. On the other hand, SEM and CLSM analyses allowed to analyse cell adhesion and spreading on the nanocomposite fibres of the scaffolds. In particular, if compared to PCL scaffolds, at 7 days after seeding, better cell adhesion and spreading were observed in the case of nanocomposite structures. To this aim, an example, figure 2 reports results from CLSM analyses performed on both polymeric and nanocomposite scaffolds at 7 days after seeding, showing cell adhesion to the nanocomposite fibres of the scaffold.

4. Conclusion

The introduction of HA strongly improved the compressive mechanical properties as well as the biological performance of scaffolds in terms of cell adhesion and spreading. As evidenced by CLSM analysis, if compared to the neat PCL scaffolds, the improvement of cell adhesion may be ascribed to a synergistic effect of surface chemistry and topography.

References


