Reverse Engineering and Additive Manufacturing towards the design of 3D advanced scaffolds for hard tissue regeneration

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Abstract – 3D Printing and Additive Manufacturing technologies represent powerful tools for the direct fabrication of lightweight structures with improved and tunable properties. In current research, Fused Deposition Modeling (FDM)/3D fiber deposition technique was considered to design 3D multifunctional scaffolds with complex morphology, tailored biological, mechanical and mass transport properties. Polymeric and nanocomposite materials were used for scaffold design and optimization, with a particular focus on bone tissue engineering. As an example, poly(ε-caprolactone) (PCL), and PCL-based nanocomposite scaffolds were fabricated and analyzed. The effects of structural and morphological features (i.e., sequence of stacking, fiber spacing, pore size and geometry) as well as of nanoparticle inclusion on the mechanical performances were reported. Furthermore, the possibility to design 3D customized scaffolds for mandibular defect regeneration (i.e., symphysis and ramus) was also considered.

Keywords—Additive manufacturing, reverse engineering, nanocomposites, scaffold design.

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I.ⁿ INTRODUCTION

Design for Additive Manufacturing (DfAM) method holds different aspects, ranging from conceptual design, process selection, design steps, and design for manufacturing [1-3]. Reverse Engineering (RE), Additive Manufacturing (AM), computer-aided design (CAD) and theoretical/experimental analyses have been already reported in the literature for their key role in the development of a wide range of polymeric and composite devices in the biomedical field [4-9].

Polymeric and polymer-based composite materials have been designed and analyzed as interesting alternatives to metals.

In this context, Additive Manufacturing (AM) technologies offer the possibility to build an object in a layer-by layer fashion, taking into account the exact specifications related to the CAD model.

By using the mathematical method known as topology optimization, the selection of the optimum material layout for a specific design will be properly performed [10,11].

Using a finite element mesh, the structure can be discretized, whereas the material points and the density become the elements and the design variables, respectively [10]. Topological optimization represents a key point in the field of tissue engineering, since the design of 3D biodegradable porous structures (i.e., scaffolds) involves different aspects: mechanical performances and mass transport properties (i.e., permeability, diffusion), and appropriate morphology and geometry are required to reproduce the complex 3D anatomic defects [11,12].

Cell metabolic activity, biocompatibility, degradation, bioresorbability should be also taken into account. Hierarchical porous structures can be properly designed starting from volume fraction and shape of a "unit cell".

Pore size and shape, interconnectivity of 3D scaffolds and, consequently, mechanical behavior and cell-material interaction are strictly connected to the adopted scaffold fabrication method (i.e., "conventional" and "advanced" techniques).

As the precise control on pore shape and interconnectivity is not simply tunable through conventional methodologies [13-17], different innovative techniques have been optimized and proposed as "advanced" methods in the biomedical field.

In this scenario, AM has demonstrated the potential to design and optimize 3D porous scaffolds with complex shapes, reproducible internal morphology and architectural features, tunable mechanical and mass transport properties [17-21].

Furthermore, over the past years the advances in design strategies and methodologies and CAD-FE modeling have pushed the research towards the development of innovative structures for different applications [22-30].

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Accordingly, the current research was focused on the design and analysis of 3D advanced and multifunctional scaffolds for hard tissue regeneration. Examples of design strategies for the development of customized scaffolds were also reported.

II. Materials and Methods

3D polymeric (poly(ε-caprolactone), PCL) and PCL-based nanocomposite scaffolds with different lay-down patterns were fabricated by FDM/3D fiber deposition technique.

Pellets consisting of poly(ε-caprolactone) (PCL) and PCL-based nanocomposites (PCL loaded with inorganic nanoparticles) were heated using the cartridge unit on the mobile arm of a 3D plotter.

A controlled injection/extrusion process through a specific needle was performed for the development of the proposed 3D scaffolds.

Fibers were deposited along specific directions between two successive layers, according to the specific lay-down pattern. A nitrogen pressure of about 8.0-8.5 bar was applied to the cartridge.

The morphology of the scaffolds was characterized by Scanning Electron Microscopy (SEM) at different steps of the design process, focusing on the fiber diameter, strand distance (centre-to-centre distance) and layer thickness. The mechanical behavior of the 3D scaffolds was properly analyzed.

Compression tests on 3D scaffolds and nanoindentation analyses on polymeric and nanocomposite fibers were carried out to assess the effect of the inclusion of inorganic nanoparticles (i.e., hydroxyapatite – HA nanoparticles) on the mechanical behavior and local surface properties.

In this context, an INSTRON 5566 testing machine and a Nanotest Platform (Micromaterials, U.K.) were used. Compression tests were carried out at 1 mm/min up to a strain of 0.4 mm/mm, whereas nanoindentation analyses were performed in a specific load range (1-5 mN), employing a diamond pyramid-shaped Berkovich-type indenter tip.

With regard to nanoindentation tests, trapezoidal load functions were considered using specific values for loading-unloading rates (i.e., 300 μN/s) and load hold periods (i.e., 20 s). Load-depth curves were reported and hardness values were evaluated using the Oliver and Pharr method.

Hardness (H) was evaluated as follows:

$$H = \frac{P_{max}}{A_c} \qquad (1)$$

where P_{max} and A_c represent the applied peak load and the projected contact area at the specified load, respectively.

The biological performances of the developed scaffolds were also evaluated to assess the effect of nanoparticle inclusion.

In brief, 3D scaffolds were prepared for cell seeding according to a reported protocol [20]. They were seeded with bone marrow-derived human mesenchymal stem cells (hMSCs) (1x104 cells/sample).

Cell viability was assessed using the Alamar Blue assay (AbD Serotec Ltd,UK).

Confocal laser scanning microscopy (CLSM) and rhodamine phalloidin staining were employed to study cell adhesion and spreading at different days after seeding.

CLSM images of cell-scaffold constructs were analyzed using Image J software and a shape factor [20].

The shape factor was calculated as follows:

$$\Phi = \frac{4\pi A}{P^2} \qquad (2)$$

where P and A are the perimeter and area of a cell, respectively.

Taking into consideration that the greatest area-to-perimeter ratio is achieved for circular objects, a shape factor of 1 indicates a perfect circle.

On the other hand, a thin thread-like object has the lowest shape factor approaching zero [20].

Finally, 3D customized nanocomposite scaffolds for mandibular defect regeneration (i.e., symphysis and ramus) were designed and fabricated by integrating different techniques: 3D scanning, 3D modeling and additive manufacturing.

A scheme of the production process of such scaffolds may be summarized in the following Figure 1.

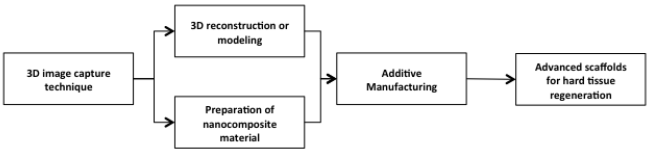

Fig. 1: Customized nanocomposite scaffolds - production process scheme.

CT scans were performed to capture the image and, hence, shape and size of a human mandible.

The obtained point clouds were processed through specific software, and, successively, the 3D model of the human mandible was reconstructed using Rapidform software and Materialise Magics.

III. Results

3D polymeric and nanocomposite scaffolds with desired shapes and required functional and mass transport properties were developed.

Results from compression tests showed stress-strain curves (Figure 2) characterized by an initial linear region, followed by a region with a lower slope. Then, the slope of the curve increased (i.e., stiff region) up to the strain limit of 0.4 mm/mm.

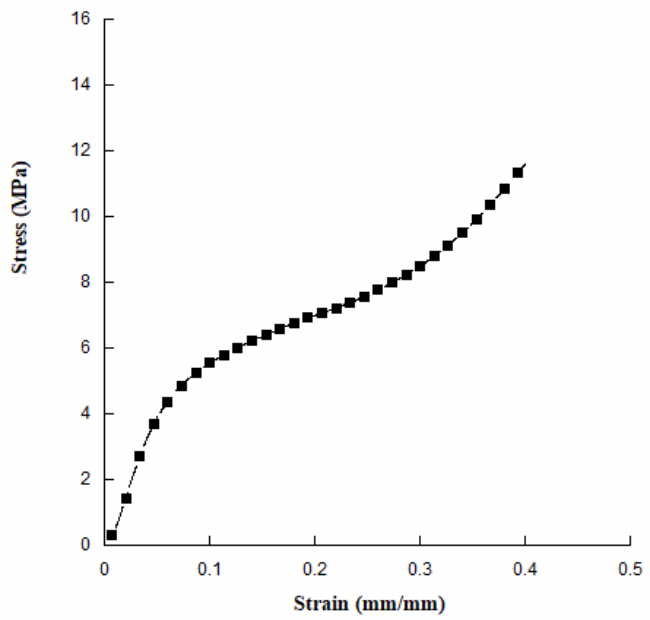

Fig. 2: Typical results from compression tests. Stress-strain curves for 3D PCL scaffolds with specific lay-down pattern and geometric features, tested up to a strain of 0.4 mm/mm.

PCL scaffolds with different architectural features showed a wide range of values for modulus (i.e., from 23.0 to 90.1 MPa) and maximum stress (from 4.1 to 15.7 MPa), according to the adopted lay-down pattern (i.e., 0/90°, 0/45/90/135°, 0/60/120°), fiber diameter, fiber distance and layer thickness.

Concerning nanoindentation measurements, in the investigated load range, tests on PCL fibers provided values of hardness ranging from 0.46 ± 0.03 GPa to 0.28 ± 0.02 GPa (Figure 3).

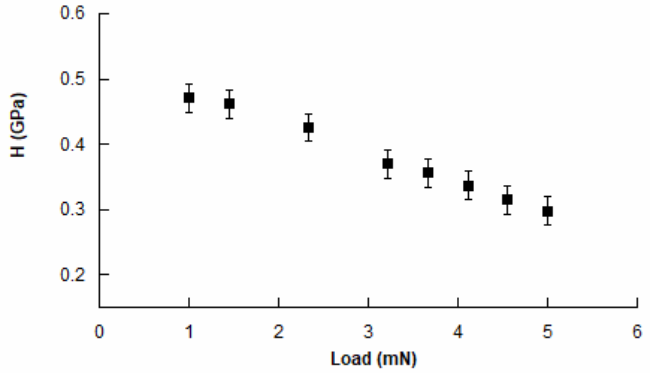

Fig. 3: Results obtained from nanoindentation tests on PCL fibers. Hardness as a function of the applied load. Data are reported as mean value and error bar represents the standard deviation.

In comparison to the neat PCL structures, the inclusion of inorganic (i.e., HA) nanoparticles generally led to an increase in compressive modulus and fiber hardness.

In vitro biological tests were performed to evaluate the influence of the inorganic nanoparticles on the biological behavior of hMSCs.

Typical results obtained from the Alamar Blue assay are reported in Figure 4.

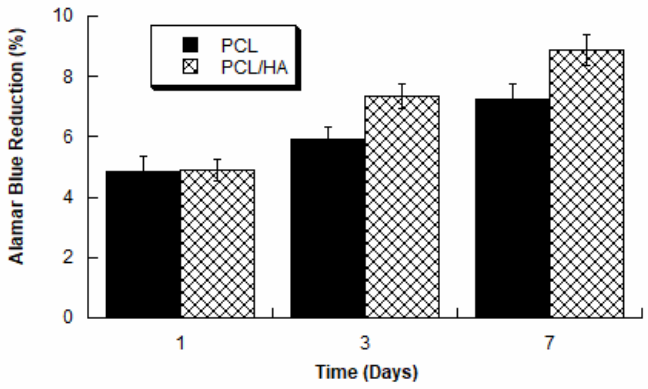

Fig. 4: Percentage of Alamar Blue reduction as a function of time for PCL and PCL/HA nanocomposite scaffolds. Data are reported as mean value and error bar represents the standard deviation.

The Alamar Blue assay is based on a redox reaction occurring in the mitochondria of the cells. The colored product is transported out of the cell and can be measured through a spectrophotometer.

The percentage of Alamar Blue reduction is related to the number of viable cells. Thus, a significant increase of Alamar Blue reduction suggests that hMSCs can survive and proliferate throughout the scaffolds.

Consequently, a higher reduction rate would indicate a higher number of viable cells.

The obtained results suggested that, even though there were no differences between PCL scaffolds and PCL/HA nanocomposite structures at day 1, the presence of HA significantly improved cell viability/proliferation at 3 and 7 days (Figure 4).

Cell adhesion and spreading were further investigated through the evaluation of the shape factor using CLSM images.

Typical values of the shape factor were evaluated at 1, 3 and 7 days after cell seeding (Figure 5).

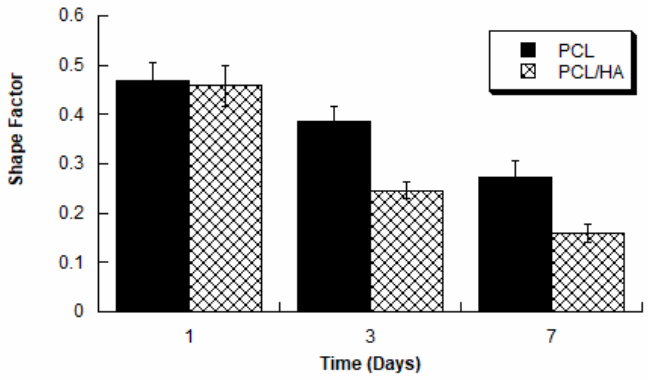

Fig. 5: Values of shape factor obtained from CLSM images of hMSCs on PCL and PCL/HA nanocomposite scaffolds. Data are reported as mean value and error bar represents the standard deviation.

It is worth noting that the cell shape factor significantly decreased over time for the two kinds of cell-scaffold constructs reported in Figure 5.

Even though at day 1 similar values were found for the two kinds of cell-scaffold constructs, at 3 and 7 days a lower shape factor was achieved for PCL/HA nanocomposite scaffolds. Anyway, a reduction of the shape factor should indicate better cell adhesion and spreading, since the more elongated the cell, the lower the shape factor [20].

The obtained findings confirmed the effect of the HA inclusion in improving cell adhesion.

Finally, taking into account the obtained results in terms of mechanical and biological performances, the reverse engineering approach was also adopted to develop functional nanocomposite structures for mandibular defect regeneration (i.e., symphysis and ramus) (Figure 6).

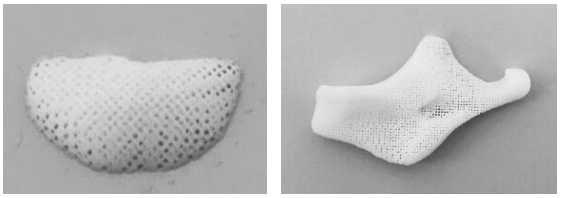

Fig. 6: Customized nanocomposite scaffolds for mandibular defect regeneration (i.e., symphysis and ramus).

IV. Conclusions

A systematic study on the effect of structural and morphological features (i.e., lay-down pattern, filament distance, pore size and geometry) as well as of the inclusion of inorganic nanoparticles was performed, the aim being to define a design strategy for the development of customized scaffolds for hard tissue regeneration.

Starting from neat PCL scaffolds, the results were briefly summarized. The possibility to modulate the performances of 3D additively manufactured structures through an appropriate material-design combination was stressed.

Although similar profile of stress-strain curves were obtained for different kinds of polymeric and nanocomposite scaffolds, differences were found in terms mechanical properties. These findings were also consistent with those already reported in the literature [15-21]. The inclusion of inorganic nanoparticles would improve both the mechanical and biological performances of PCL scaffolds.

Benefiting from the obtained experimental results, as well as from the reverse engineering approach and additive manufacturing, the feasibility to develop customized scaffolds for mandibular defect regeneration (i.e., symphysis and ramus) was also demonstrated.

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