Macromolecular Symposia

www.ms-journal.de

Integrated Design Strategy for Additively Manufactured Scaffolds in Tissue Engineering

Pierpaolo Fucile, Teresa Russo, Roberto De Santis, Massimo Martorelli,* Michelina Catauro, and Antonio Gloria**

Additive manufacturing technologies allow for the direct fabrication of 3D scaffolds with improved properties for tissue regeneration. In this scenario, design strategies and 3D fiber deposition technique are considered to develop advanced scaffolds with different lay-down patterns, tailored mechanical and biological properties. 3D poly(ϵ-caprolactone) scaffolds are manufactured and surface-modified (i.e., aminolysis). The effect of surface modification on the mechanical and biological performances of the designed 3D scaffolds is assessed.

1. Introduction

The need for functional devices which are able to replace or repair tissues or organs is overwhelming.[1–5] Over the past few years, synthetic polymers such as poly(ϵ-caprolactone) (PCL) have become popular in many applications and new advances are being made for the design of novel scaffolds for tissue engineering.[6–11]

In this scenario, additive manufacturing allows for the design of advanced scaffolds with tailored architectures, mechanical and functional properties. Conventional fabrication methods are not able to precisely control the pore size and geometry, as well as the spatial distribution of pores.[4,5,9–11]

Design represents the main creative activity of engineering. Usually, the engineers benefit from a series of steps to create functional products and processes. Even though different simplified and generalized models have been reported in the literature, the common stages of the engineering design process generally include research, design requirements, feasibility, concept generation, preliminary design, detailed design, design for manufacturability, and production planning.[12]

Conceptual design is considered the first phase of design. It provides a description of the proposed system in terms of a set of integrated ideas and concepts with a special focus on its function, structure and behavior.

Additively manufactured PCL scaffolds with controlled structural and surface properties were already developed.[12,13] The efficacy of a two-step procedure for peptide grafting was already demonstrated. The analytical quantification of functional groups and/or peptides at the interface was also performed.[13]

Thus, the combination of design methods with additive manufacturing and functionalization/bioactivation strategies can lead to the development of advanced scaffolds for tissue regeneration.

Accordingly, the current research aimed at providing a further insight into the design and analysis of 3D additively manufactured and surface-modified PCL scaffolds.

2. Results and Discussion

Integrated design strategies together with the structure-property relationship play an important role in the development of advanced materials for specific applications.[7,8]

The potential of reverse engineering, computer-aided design and theoretical analysis has been widely reported in the literature. Accordingly, over the past years the advances in methodologies [14–17] and design strategies [17–19] have pushed the research towards the development of innovative devices in different fields. Many efforts have been made in engineering biomedical devices, especially focusing on materials,[4,10,20–22] as well as on experimental [8–10,16] and theoretical [17–19] studies.

It is frequently stressed that the functional behavior of 3D additively manufactured scaffolds is dependent on the pore spatial distribution as well as on the geometrical and architectural features.[9,13]

For this reason, an optimization strategy was considered to develop the 3D scaffolds, also focusing on the technological features related to the fabrication process.

In particular, 3D PCL scaffolds with required functional and mass transport properties were manufactured using predefined or optimized lay-down patterns and unit-cell architectures. The pore geometry and porosity as well as the mechanical and mass

P. Fucile
Department of Advanced Biomedical Sciences
University of Naples Federico II
Naples 80131, Italy

T. Russo, R. De Santis, A. Gloria
Institute of Polymers
Composites and Biomaterials – National Research Council of Italy
V.le J.F. Kennedy 54 – Mostra d'Oltremare Pad. 20, Naples 80125, Italy
E-mail: teresa.russo@cnr.it; antonio.gloria@cnr.it

M. Martorelli
Department of Industrial Engineering
Fraunhofer JL IDEAS – University of Naples Federico II
P.le Tecchio 80, Naples 80125, Italy
E-mail: massimo.martorelli@unina.it

M. Catauro
Department of Engineering
University of Campania "Luigi Vanvitelli"
via Roma 29, Aversa I-813031, Italy

DOI: 10.1002/masy.202000263

Table 1. Typical results from compression tests performed on neat and surface-modified 3D scaffolds (fiber diameter of 400 μm, strand distance of 600 μm, layer thickness of 300 μm): modulus (E) and maximum stress (σ_{max}).

Lay-down pattern	Neat scaffolds		Surface-modified scaffolds	
	E [MPa]	σ_{max} [MPa]	*E* [MPa]	σ_{max} [MPa]
0°/60°/120°	72.6 6.0	13.2 0.8	70.1 6.3	13.0 0.9
0°/90°	96.1 7.0	14.6 0.9	93.5 8.0	14.2 0.8

The results are reported as mean value ± standard deviation.

transport properties of the 3D PCL scaffolds were tailored by varying the lay-down pattern (i.e., sequence of stacking) and further parameters.

The stress-strain curves obtained from compression tests were consistent with those reported in the literature.[9,13] The compressive modulus was evaluated from the slope of the linear region of the stress-strain curve. **Table 1** reports typical values of compressive modulus and maximum stress obtained for neat and surface-modified PCL scaffolds.

Results from compression tests (Table 1) evidenced that the surface modification process did not significantly alter the compressive properties (i.e., modulus and maximum stress) of the manufactured scaffolds.

Furthermore, nanoindentation was considered to assess the effect of the treatment on the surface properties of the scaffolds. This technique provides the possibility to map the surface mechanical properties. In the investigated load range, nanoindentation measurements on the surface-modified PCL fibers provided hardness values ranging from 0.12 to 0.05 GPa, which were lower than those found for the neat PCL fibers (from 0.40 to 0.21 GPa). The observed differences were statistically significant ($p < 0.05$).

Although the surface modification reduced the hardness, the treatment did not negatively affect the compressive mechanical performance of the additively manufactured scaffolds, also confirming the previously obtained results.[13]

On the other hand, in vitro biological tests were performed to evaluate the behavior of hMSCs. **Figure 1** reports the results as percentage of Alamar Blue reduction over time.

For the different kinds of constructs, the values of Alamar Blue reduction significantly increased ($p < 0.05$) over the investigated time period; thus, indicating cells survival and proliferation. Even though at day 1 no significant differences ($p > 0.05$) were observed among the different groups, the effect of the surface modification was well evident at 3 and 7 days after cell seeding. Specifically, for each lay-down pattern, if compared to the neat structures, higher values of Alamar Blue reduction were found for the surface-modified scaffolds, suggesting that the surface modification significantly improved cell viability/proliferation ($p < 0.05$).

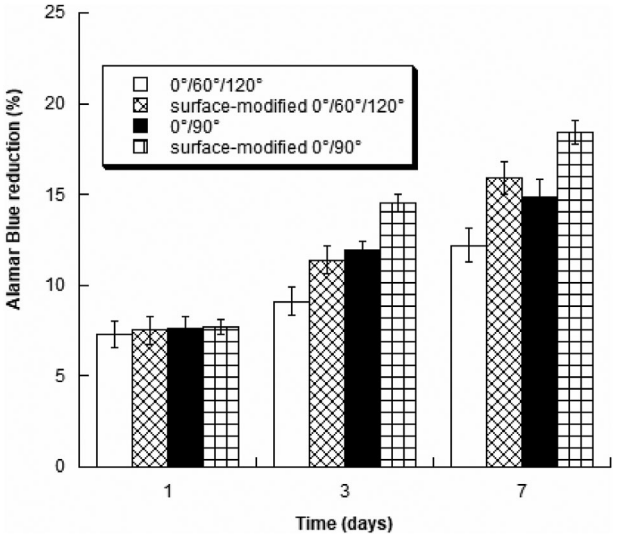

Figure 1. Typical results from in vitro biological tests: percentage of Alamar Blue reduction evaluated for the cell-laden scaffolds. Data are reported as mean value and error bar represents the standard deviation.

3. Conclusion

Within the limitations of the current research, the following conclusions were reached:

1) An integrated approach was reported to develop advanced scaffolds for tissue regeneration. Design strategies were combined with additive manufacturing (i.e., 3D fiber deposition technique) and functionalization routes.
2) The strategy confirmed the potential of tailoring the performances of the additively manufactured scaffolds to guide cell behavior.

4. Experimental Section

Design problem, additive manufacturing and theoretical/experimental analyses were the basic steps of the current research.

3D poly(ϵ-caprolactone) scaffolds with two different lay-down patterns (0°/90° and 0°/60°/120°) were fabricated by an additive manufacturing technique based on injection/extrusion methods (i.e., 3D fiber deposition/fused deposition modeling). In brief, pellets consisting of poly(ϵ-caprolactone) (PCL) were heated using the cartridge unit on the mobile arm of a 3D plotter (Envisiontec GmbH, Germany). 3D PCL scaffolds were fabricated by injecting/extruding the material through a needle. The fibers were deposited along specific directions between two successive layers according to the selected lay-down pattern. A nitrogen pressure of 7.0 bar and deposition speed of 40 mm min^{-1} were employed. The additively manufactured scaffolds were characterized by the fiber diameter, fiber spacing (i.e., strand distance, center-to-center distance) and layer thickness. The fabricated PCL scaffolds were surface-modified (i.e., aminolysis).[13]

The effect of surface modification was evaluated through mechanical and biological tests.

Mechanical compression tests were performed on the 3D scaffolds at 1 mm min^{-1} up to a strain of 0.5 mm min^{-1}, using an INSTRON 5566 testing machine. The "apparent" stress (σ) and strain (ϵ) were calculated as reported in previous works.[9,13]

Nanoindentation analyses were carried out on neat and surface-modified PCL fibers using Nanotest Platform (Micromaterials, U.K.) in a well-defined load range (1–5 mN). A diamond pyramid-shaped Berkovich-type indenter tip was employed. Trapezoidal load functions characterized by specific values for load hold periods (i.e., 20 s) and loading-unloading rates (i.e., 300 μN s^{-1}) were considered. Hardness values were evaluated from the load-depth curves, according to the Oliver and Pharr method.

Cell viability and proliferation were analyzed at different time points using the Alamar Blue assay (AbD Serotec Ltd, UK) and the results were reported as a percentage of Alamar Blue reduction. Neat and

surface-modified scaffolds were prepared for cell seeding following a reported protocol.[8,9] They were seeded with bone marrow-derived human mesenchymal stem cells (hMSCs) using 1×10^4 cells/sample. The experiments were done at least three times in triplicate.

The data were analyzed by ANOVA followed by Bonferroni post hoc test. A value of $p < 0.05$ was considered statistically significant.

Acknowledgements

The authors gratefully acknowledge Rodolfo Morra (Institute of Polymers, Composites and Biomaterials – National Research Council of Italy) for providing information on the mechanical test methods.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

computer-aided design, design for additive manufacturing, mechanical analysis, scaffold design

[1] P. Lichte, H. C. Pape, T. Pufe, P. Kobbe, H. Fischer, *Injury* **2011**, *42*, 569.
[2] J. Lee, M. M. Farag, E. K. Park, J. Lim, H. Yun, *Mater. Sci. Eng. C* **2014**, *36*, 252.
[3] A. Oryan, S. Alidati, A. Moshiri, N. Maffulli, *Journal of Orthopaedic Surgery and Research* **2014**, *9*, 18.
[4] P. Fucile, I. Papallo, G. Improta, R. De Santis, A. Gloria, I. Onofrio, et al. *Proc. of IEEE* **2019** *II Workshop on Metrology for Industry 4.0 and IoT (MetroInd4.0&IoT)*, Naples, 4–6 June **2019**, 33–37, 8792891.
[5] N. Rocco, M. B. Nava, G. Catanuto, A. Accurso, M. Martorelli, et al. *Proc. of IEEE* **2019** *II Workshop on Metrology for Industry 4.0 and IoT (MetroInd4.0&IoT)*, Naples, 4–6 June **2019**, 39–42, 8792910.
[6] T. Baradaran, S. S. Shafiei, S. Mohammadi, F. Moztarzadeh, *Materials Today Communications* **2020**, *23*, 100913.
[7] A. Shapourzadeh, S. - M. Atyabi, S. Irani, H. Bakhshi, *Int. J. Biol. Macromol.* **2020**, *150*, 152.
[8] T. Russo, V. Peluso, A. Gloria, O. Oliviero, L. Rinaldi, G. Improta, R. De Santis, V. D'Antò, *Nanomaterials* **2020**, *10*, 577.
[9] R. De Santis, A. Gloria, T. Russo, U. D'Amora, S. Zeppetelli, A. Tampieri, T. Herrmannsdörfer, L. Ambrosio, *Virtual Phys. Prototyping* **2011**, *6*, 189.
[10] T. Russo, U. D'Amora, A. Gloria, M. Tunesi, M. Sandri, S. Rodilossi, D. Albani, G. Forloni, C. Giordano, A. Cigada, A. Tampieri, R. De Santis, L. Ambrosio, *Proc. Eng.* **2013**, *59*, 233.
[11] I. Bružauskaitė, D. Bironaitė, E. Bagdonas, E. Bernotienė, *Cytotechnology* **2016**, *68*, 355.
[12] A. Lanzotti, M. Martorelli, T. Russo, A. Gloria, *Mater. Sci. Forum* **2018**, *941*, 2154.
[13] A. Gloria, F. Causa, T. Russo, E. Battista, R. Della Moglie, S. Zeppetelli, R. De Santis, P. A. Netti, L. Ambrosio, *Biomacromolecules* **2012**, *13*, 3510.
[14] V. Pagliarulo, F. Farroni, P. Ferraro, A. Lanzotti, M. Martorelli, P. Memmolo, D. Speranza, F. Timpone, *Optics and Lasers in Engineering* **2018**, *104*, 71.
[15] M. Martorelli, C. Pensa, D. Speranza, *Journal of Maritime Archaeology* **2014**, *9*, 81.
[16] D. Solari, L. M. Cavallo, P. Cappabianca, I. Onofrio, I. Papallo, et al. *Proc. of IEEE* **2019** *II Workshop on Metrology for Industry 4.0 and IoT (MetroInd4.0&IoT)*, Naples, 4–6 September **2019**, 28–32, 8792878.
[17] P. Ausiello, S. Ciaramella, A. Fabianelli, A. Gloria, M. Martorelli, A. Lanzotti, D. C. Watts, *Dent Mater* **2017**, *33*, 690.
[18] P. Ausiello, S. Ciaramella, F. Garcia-Godoy, A. Gloria, A. Lanzotti, S. Maietta, M. Martorelli, *Dent Mater.* **2017**, *33*, e39.
[19] A. Gloria, S. Maietta, M. Martorelli, A. Lanzotti, D. C. Watts, P. Ausiello, *Dent Mater* **2018**, *34*, 1063.
[20] M. Catauro, C. Pagliuca, L. Lisi, G. Ruoppolo, *Thermochim. Acta* **2002**, *381*, 65.
[21] M. Catauro, G. Laudisio, A. Costantini, R. Fresa, F. Branda, *J. Sol-Gel Sci. Technol.* **1997**, *10*, 231.
[22] R. Cioffi, P. Pernice, A. Aronne, M. Catauro, G. Quattroni, *J. Eur. Ceram. Soc.* **1994**, *13*, 143.