Results in Engineering 12 (2021) 100282

Contents lists available at ScienceDirect

Results in Engineering

journal homepage: www.sciencedirect.com/journal/results-in-engineering

A possible method to avoid skin effect in polymeric scaffold produced through thermally induced phase separation

Marco Friuli [*], Paola Nitti, Marta Madaghiele, Christian Demitri

Department of Engineering for Innovation, University of Salento, Lecce, 73100, Italy

ARTICLE INFO

Keywords:
Scaffolds
Tissue engineering
Skin effect

ABSTRACT

Scaffold's morphology and in particular pore architecture is a key parameter for cell viability and tissue regeneration. Usually, morphology is managed through Thermally Induced Phase Separation (TIPS) consisting of controlled quenching and freeze-drying to remove ice crystals to create porosity. Nevertheless, the so-called skin effect, a less-/non-porous layer, usually occurring at scaffold's air–liquid or material-mold interface. Skin effect reduces scaffold's performance then the layer have to cut out with consequent loss of material and damage risks. Here, it is presented a possible method to avoid skin effect at mold-polymer interface in biopolymer-based scaffolds. It is based on producing scaffolds not directly in a mold but on a previously frozen distilled water surface. SEM analysis showed the absence of skinned surfaces and a uniform pore pattern in shape and size.

1. Introduction

Scaffolds are crucial devices in tissue engineering. Numerous production techniques have been developed to obtain specifically engineered devices to reproduce natural tissue behavior in order to improve cell-material interaction to promote and guide the neo-tissue genesis. In particular, pore size and generally device's morphology resulted among the more influencing features. Consequently, numerous techniques have been studied to finely control every influencing parameter. Among them, thermally induced phase separation (TIPS) based methods are widely used for the fabrication of controlled morphology scaffolds [1,2]. Generally, TIPS are based on separation of a polymer solution into polymer-rich and polymer-lean phases by quenching to a temperature lower than either the cloud point of the solution followed by a freeze-drying to remove the polymer-lean phase and yields the porous polymer scaffold [3]. Pore architecture depends on thermal cycle (cooling rate, thermal gradient etc.) applied to induce phase separation. Nevertheless, although TIPS guarantees to control porosity within the whole volume, formation of a less-/non-porous layer (so-called skin effect [4]) usually occurs at the scaffold's outer surface at the air–liquid or at material-mold interface. Skin effect restricts cells adhesion to material and it jeopardizes the effort to produce a specifically architecture scaffold limiting all those processes involving in tissue regeneration [5–8]. For example, skin effect reduces cells infiltration inside the scaffold limiting nutrients and waste substances exchange compromising cell's vitality [9]. Consequently, skinned surfaces presence obliges to cut scaffolds partially with consequent shape, material and features loss in particular for thin scaffolds [9–13]. In the present work, we tested an easy TIPS-based methodology to reduce skin effect. The method called TIPS/ice is based on producing scaffolds not directly in a mold but pouring the initial polymeric solution on a previously frozen distilled water surface [12]. Actually, it has been tested in sodium carboxymethyl cellulose (CMC) and sodium alginate (SA) slurries but it could be potentially extended to other polymeric solutions.

2. Material and methods

Sodium Carboxymethyl Cellulose (CMC) and Sodium Alginate (SA) (Medium molecular Weight polymer powder, Sigma Aldrich) were used to prepare three different polymer solutions: CMC, SA and CMC+SA (200 ml at 1%wt of polymer, 0.5%wt of CMC and 0.5%wt of SA for CMC+SA). Each solution was prepared in a beaker at room temperature and neutral pH by adding the polymer powder to 200 ml of distilled water and then mixing at room temperature until clear solution then stored at 4 °C to avoid water evaporation. Distilled water dishes were prepared by pouring distilled water into a Petri dish placed on a planar surface to form a 0.5 cm layer and then by freezing it at −10 °C. From each solution were prepared samples through TIPS/ice and traditional TIPS protocol and compared through morphological analysis.

* Corresponding author.
E-mail address: marco.friuli@unisalento.it (M. Friuli).

https://doi.org/10.1016/j.rineng.2021.100282
Received 29 July 2021; Received in revised form 3 September 2021; Accepted 9 September 2021
Available online 13 September 2021

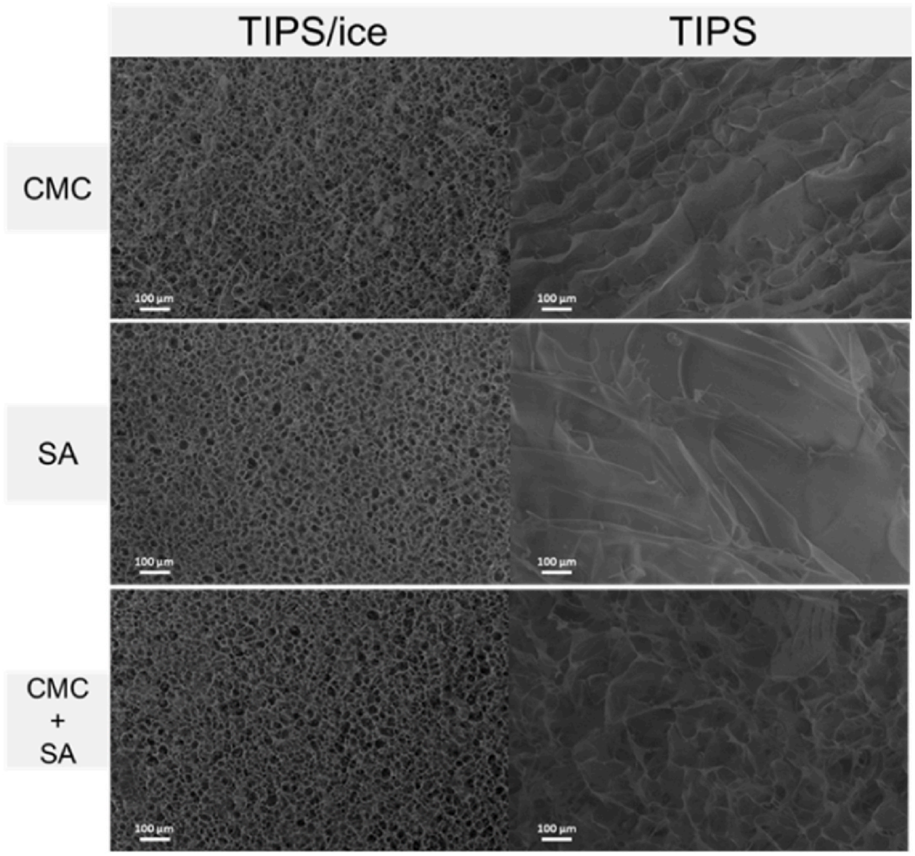

Fig. 1. SEM analysis (Magnitude 200x, scale bar 100 μm). Top view of the Polymer-ice interface in samples produced through TIPS/ice method (left) and Polymer-Petri interface in samples produced through standard TIPS (right).

3. TIPS/ice and TIPS samples preparation

TIPS/ice and TIPS methods differ only in the use of the ice dish. TIPS/ice samples (CMC, SA and CMC+SA TIPS/ice, left side in Fig. 1) were prepared by slowly pouring a 0.5 cm layer of each polymeric solution (at room temperature) directly onto the frozen dish (−10 °C) previously prepared into the Petri dish. The system (Polymer on ice dish) was frozen at −10 °C and then freeze-dried for 24h to produce porosity and, at the same time, remove the entire ice dish at the bottom. TIPS samples (right side in Fig. 1) were prepared by directly pouring the polymeric solution (0.5 cm layer) into a Petri dish then freezing at −10 °C and freeze-drying for 24h.

4. Morphological analysis

Scanning electronic microscope was performed on each sample through SEM EVO® 40, Carl Zeiss AG to compare surface morphology between TIPS/ice and TIPS produced samples. Top view of the Solution-ice and solution-Petri interface were analyzed for each sample and reported in Fig. 1.

5. Experimental results

The following Fig. 1 shows the difference in pores size and pattern between the samples produced through the two different methods. It emerges as using TIPS/ice method a uniform pattern of mainly closed macro-pores was obtained and skin effect was completely absent. Differently, when the sample is directly poured and frozen into a Petri dish as mold, a random morphology was obtained and, in particular in SA, emerged the presence of a skinned layer. Skin effect is also present in the other samples and it could be noticed as lack of clearness in CMC/TIPS and CMC+SA/TIPS samples.

6. Conclusions

SEM analysis showed as the TIPS/ice procedure could avoid skin effect and promote a well patterned surface morphology. Even though a deeper analysis on morphology formation mechanism must be carried out, we hypothesize that when room temperature slurry is poured onto ice, a thin water film is produced and phase separation process is not hindered by Petri bottom. This could lead to a complete rearrangement of polymer-rich phase or, alternatively, to an interpenetrated ice crystal growth at frozen water-scaffold interface. Tests have been carried out using a simple frozen flat surface, but potentially more complex shapes could be realized by pouring polymeric solutions in an “ice mold” produced through CAM-CAD methods to customize scaffold’s shape. Furthermore, it could be possible to produce different morphologies by controlling slurry/ice temperature or composition or to promote cross-linking reaction by adding crosslinking agents to ice molding (e.g. calcium chloride for SA crosslinking).

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was supported in conceptualization and research by University of Salento within the Industrial PhD Program “Dottorati innovativi a caratterizzazione industriale” and by Mr. Donato Cannoletta for SEM analysis.

References

[1] C. Liu, Z. Xia, J.T. Czernuszka, Design and development of three-dimensional scaffolds for tissue engineering, Chem. Eng. Res. Des. 85 7 (2007) 1051–1064, https://doi.org/10.1205/cherd06196. https://www.sciencedirect.com/science/article/pii/S0263876207731428.

[2] W. Trisunaryanti, K. Wijaya, T. Triyono, A.R. Adriani, S. Larasati, Green synthesis of hierarchical porous carbon prepared from coconut lumber sawdust as Ni-based catalyst support for hydrotreating Callophyllum inophyllum oil, Results in Engineering 11 (2021) 100258, https://doi.org/10.1016/j.rineng.2021.100258. https://www.sciencedirect.com/science/article/pii/S2590123021000591.

[3] C. Demitri, M.G. Raucci, A. Giuri, V.M. De Benedictis, D. Giugliano, P. Calcagnile, et al., Cellulose-based porous scaffold for bone tissue engineering applications: assessment of hMSC proliferation and differentiation, J. Biomed. Mater. Res. 104 3 (2016) 726–733, https://doi.org/10.1002/jbm.a.35611, 10.1002/jbm.a.35611.

[4] N. Kasoju, D. Kubies, T. Sedlačík, O. Janoušková, J. Koubková, M.M. Kumorek, et al., Polymer scaffolds with no skin-effect for tissue engineering applications fabricated by thermally induced phase separation, Biomed. Mater. 11 (1) (2016), 015002, https://doi.org/10.1088/1748-6041/11/1/015002.

[5] N. Calabriso, E. Stanca, A. Rochira, F. Damiano, L. Giannotti, B. Di Chiara Stanca, et al., Angiogenic properties of concentrated growth factors (CGFs): the role of soluble factors and cellular components, Pharmaceutics 13 5 (2021) 635, https://doi.org/10.3390/pharmaceutics13050635. https://pubmed.ncbi.nlm.nih.gov/33946931. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8146902/.

[6] B. Palazzo, A. Gallo, A. Casillo, P. Nitti, L. Ambrosio, C. Piconi, Fabrication, characterization and cell cultures on a novel composite chitosan-nano-hydroxyapatite scaffold, Int. J. Immunopathol. Pharmacol. 24 (1 Suppl 2) (2011) 73–78, https://doi.org/10.1177/03946320110241s214.

[7] E. Varoni, M. Tschon, B. Palazzo, P. Nitti, L. Martini, L. Rimondini, Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response, Connect. Tissue Res. 53 6 (2012) 548–554, https://doi.org/10.3109/03008207.2012.712583.

[8] P. Nitti, N. Gallo, L. Natta, F. Scalera, B. Palazzo, A. Sannino, et al., Influence of nanofiber orientation on morphological and mechanical properties of electrospun chitosan mats, Journal of Healthcare Engineering 2018 (2018) 3651480, https://doi.org/10.1155/2018/3651480.

[9] M. Friuli, P. Nitti, L. Cafuero, A. Prete, M.S. Zafar, M. Madaghiele, et al., Cellulose acetate and cardanol based seed coating for intraspecific weeding coupled with natural herbicide spraying, J. Polym. Environ. 28 11 (2020) 2893–2904, https://doi.org/10.1007/s10924-020-01821-9.

[10] P. Nitti, N. Gallo, B. Palazzo, A. Sannino, A. Polini, T. Verri, et al., Effect of l-Arginine treatment on the in vitro stability of electrospun aligned chitosan nanofiber mats, Polym. Test. 91 (2020) 106758, https://doi.org/10.1016/j.polymertesting.2020.106758. https://www.sciencedirect.com/science/article/pii/S0142941820315580.

[11] P. Nitti, S. Kunjalukkal Padmanabhan, S. Cortazzi, E. Stanca, L. Siculella, A. Licciulli, et al., Enhancing bioactivity of hydroxyapatite scaffolds using fibrous type I collagen, Front Bioeng Biotechnol 9 (2021) 631177, https://doi.org/10.3389/fbioe.2021.631177. https://pubmed.ncbi.nlm.nih.gov/33614615. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7890361/.

[12] M.G. Raucci, U. D'Amora, A. Ronca, C. Demitri, L. Ambrosio, Bioactivation routes of gelatin-based scaffolds to enhance at nanoscale level bone tissue regeneration, Front Bioeng Biotechnol 7 (2019) 27, https://doi.org/10.3389/fbioe.2019.00027. https://pubmed.ncbi.nlm.nih.gov/30828576. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6384229/.

[13] L. Salvatore, V.E. Carofiglio, P. Stufano, V. Bonfrate, E. Calò, S. Scarlino, et al., Potential of electrospun poly(3-hydroxybutyrate)/collagen blends for tissue engineering applications, Journal of Healthcare Engineering 2018 (2018) 6573947, https://doi.org/10.1155/2018/6573947.