



Scaffolds with tubular/isotropic Bi-modal pore structures by gas foaming and fiber templating

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ABSTRACT

The aim of this study was to engineer a scaffold for tissue engineering with double shape pore architecture, with tubular and isotropic pores. A novel method is herein proposed, based on the combination of gas foaming and fiber templating. In particular, the tubular pores have been obtained by leaching of melt-spun saccharide fibers (cotton candy) within CO₂-foamed poly(ϵ -caprolactone). The optimization of the scaffold has been conducted via the selection of the proper saccharide, its thermal treatment to stabilize the spun fiber (crystallization), the formation of the polymer–fiber composite, the selection of the proper gas-foaming processing parameter and the final leaching in water.

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1. Introduction

In regenerative medicine, the ability to design scaffolds characterized by a tubular porosity for vascularization or nerve growth remains, to date, a critical limiting step [1–3].

In order to form tubular porosities in 3-D structures, few methods have been proposed in the literature. These include the following: (i) thermally-induced phase separation with controlled heat transfer direction, to promote unidirectional crystal growth [4,5]; (ii) centrifugal casting technique, in which a suspension is spun into a cylindrical, rapidly freezing mold to achieve a single porous tube [3]; (iii) rolling and sealing porous mesh of foam sheets [6,7]; (iv) extrusion of tubular constructs [8,9]; (v) electrospinning of small diameters fibers around a mandrel [10] and (vi) fiber templating [1,2,11,12]. Of the cited method, only the latter allows to fine tune the architecture of multi-pore tubular structures, since methods (ii)–(v) allow to produce of single-tube structures and method (i) provides a limited control over the pore size, position and interconnections.

Saccharose (β -D-fructofuranosyl- α -D-glucopyranoside), commonly known as sucrose or table sugar, is the most abundant carbohydrate and is extracted from sugar cane and sugar beet [13]. As a carbohydrate, it is a natural polymer and exerts, like synthetic polymers, viscoelasticity, semicrystallinity, glass transition and melting. When spun to fiber, after melting in the spinning head of a cotton candy

machine, it solidifies in an amorphous state. The glass transition temperature of dried saccharose is around 65 °C, but it steeply decreases with humidity content (down to room temperature at 5.5wt%), significantly limiting the handling of the fibers [14]. One way to circumvent this problem is to thermal treat the fiber, in order to induce re-crystallization [15] or to use different saccharides characterized by lower hygroscopicity, in order to preserve the glassy state of the biopolymer at room temperature. Stabilized saccharide fibers, hence, may be used as templates to leave, when removed with water, channel-like pores suitable for guiding growth of vascular or nerve cells. However, to allow the nutrients to reach the growing tissue, an additional isotropic, open porosity has to be formed among the fibers.

Recent investigations reported that poly(ϵ -caprolactone) (PCL) scaffolds with 60% overall porosity and characterized by a macroporosity of the order of hundreds of microns interconnected with a microporosity of tens of microns may improve cell colonization and proliferation in three dimensions [16].

The aim of this work was (1) to produce thermally stable saccharide fibers by melt spinning and (2) to fabricate poly(ϵ -caprolactone) (PCL) scaffold with bi-modal (oriented/isotropic) pore architecture, for vascular and nerve tissue engineering, by combining gas foaming and fiber templating techniques.

2. Materials

Materials: Isomalt (Squires Kitchen Ltd., Farnham, UK), sacrose (Eridania S.p.A., Bologna, Italy) and PCL (CAPA 6'800,

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Solvay Interlox Ltd., Southampton, UK) were vacuum dried overnight at room temperature under reduced atmosphere before use. Commercial purity grade CO₂ (SOL S.p.A., Monza, Italy) was used as foaming agent. Dichloromethane (DCM), a good solvent for PCL and not for saccharides, has been purchased from Sigma-Aldrich S.r.l., Milan, Italy.

3. Methods

Scaffold preparation: The procedure utilized for preparing the scaffold is summarized in the scheme reported in Fig. 1:

- 10 g of saccharide in its original powder form was delivered to and melted in the rotating heated head of a commercial cotton candy machine (Zuccherosa mod. 5XHZF100, Optima Electra S.p.A., Modena, Italy). The spun saccharide fibers were collected on a rectangular (10 × 5 cm² L H) plate to preserve orientation.
- PCL-saccharide fiber composites were produced by solution casting and by compression molding. In the first method, PCL was dissolved in DCM (1/5 g/mL) at 40 °C under magnetic stirring to obtain a viscous, concentrated solution, which is then poured on to the saccharide fibers in a Petri dish. DCM is finally allowed to evaporate at 0 °C under reduced atmosphere. In the second method, alternating PCL and saccharide fiber layers were compression molded in a P300P hot press (Dr. Collin GmbH, Ebersberg, Germany) at 100 °C and 2 MPa

for 2 min and then were allowed to cool to room temperature under pressure.

- PCL-saccharide fiber composites were gas foamed by a custom-designed equipment [17]. Samples 3 mm thick, with a diameter of 15 mm were saturated with CO₂ at 60 °C and 10 MPa for 2 h in a pressure vessel (mod. BC-1, High Pressure Equipment Company, Erie, PA, USA). The vessel was then cooled to 36 °C and pressure-quenched to ambient pressure at 32 MPa/s [18,19].
- Saccharide fibers were leached by multiple washes of water to achieve the final PCL scaffold with dual scale pore structure.

Scaffold characterization: Thermal properties of the fibers were assessed via differential scanning calorimetry (DSC), by a Q2920 (TA Instruments, New Castle, DE, USA), with scans from room temperature to 200 °C at 10 °C/min under N₂. The scaffold morphology was investigated by scanning electron microscopy (SEM), using an S440 (Leica Microsystems S.r.l., Milan, Italy). Optical microscopy was utilized to assess the stability of the fibers in ambient conditions by a Z16 APO-PLANAPO 2.0 × (Leica Microsystems S.r.l., Milan, Italy).

4. Results and discussion

In order to produce the dual-porosity scaffolds, the first step was to fabricate the saccharide fibers by a cotton candy machine. As briefly described in the introduction, the presence of water is detrimental for the stability of the filamentary structure and, therefore, the commercial cotton candy machine was modified to have fiber spinning in a nitrogen atmosphere. Despite this appliance, fibers from saccharose were very unstable for their high hygroscopicity and proved difficult to handle for further processing. In fact, when the spun saccharose fibers (which are in the amorphous state because of the temperature quench exerted in the spinning process) absorb water, they go through the glassy-to-rubbery transition, thereby softening and gluing together, actually losing their filamentary structure.

To overcome this problem, we investigated the preparation of fibers starting from isomalt, a saccharide characterized by very low hygroscopicity as compared to saccharose and has similar processing conditions [20]. In fact, isomalt fibers proved to be very stable when exposed to ambient conditions; even after 6 h (data not reported) they did not glue together, keeping initial shape and stiffness.

In its “as received” form, isomalt is semi-crystalline, as evidenced in the DSC trace reported in Fig. 2A, where both the water evaporation in the range 80–110 °C and melting in the range 110–150 °C are evident [21]. When it is produced in fibers by melt spinning, it cools down quickly into the amorphous state,

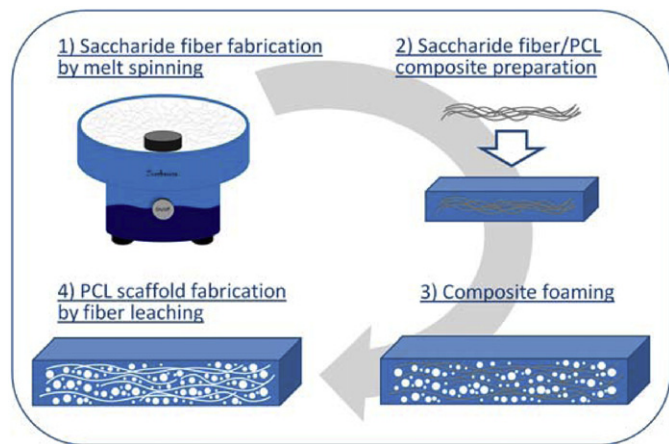


Fig. 1. Scheme of the procedure utilized for preparing the scaffold; saccharide fiber (1); PCL-saccharide fiber composite (2); foamed PCL-saccharide fiber composite (3); PCL scaffold with dual scale porosity (4).

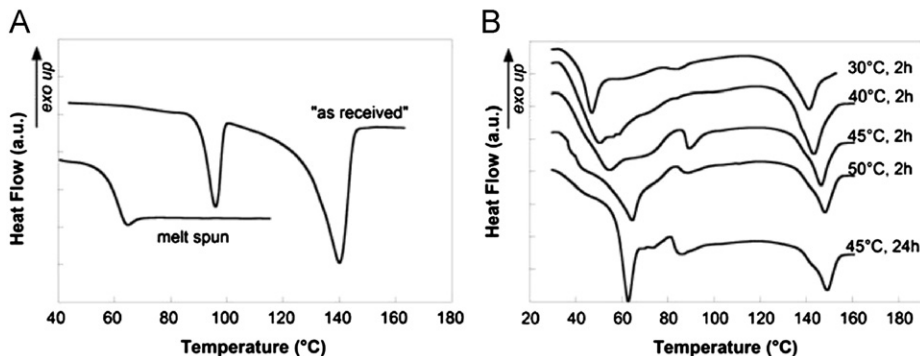


Fig. 2. DSC thermograms of “as received” and spun isomalt (A); thermally treated isomalt fibers (B).

with a glass transition temperature around 60 °C. As observed above, these fibers are rather stable at room conditions. However, they rapidly dissolved in DCM and were also unstable at molding temperature, therefore making the production of the PCL/fiber composite difficult (data not reported). In order to overcome this problem, amorphous fibers were thermally treated under reduced atmosphere at temperatures ranging from 30 to 50 °C, up to 24 h, to allow for the development of crystals. Fig. 2B reports the DSC thermograms of thermally treated fibers, while Table 1 reports the thermal properties and crystallinity of the fibers as a function of the thermal treatment.

Table 1
Thermal properties of the isomalt fibers as a function of the thermal treatment.

Thermal treatment	Melting temperature (°C)	Melting enthalpy (J/g)	Degree of crystallinity ^a (dimensionless)
As received	140.05	99.46	0.74
30 °C; 2 h	141.06	9.80	0.07
40 °C; 2 h	143.04	12.40	0.09
45 °C; 2 h	146.30	9.40	0.07
50 °C; 2 h	147.90	7.70	0.06
45 °C; 24 h	148.11	31.96	0.24

^a Melting enthalpy for 100% crystalline isomalt is 135 J/g [18].

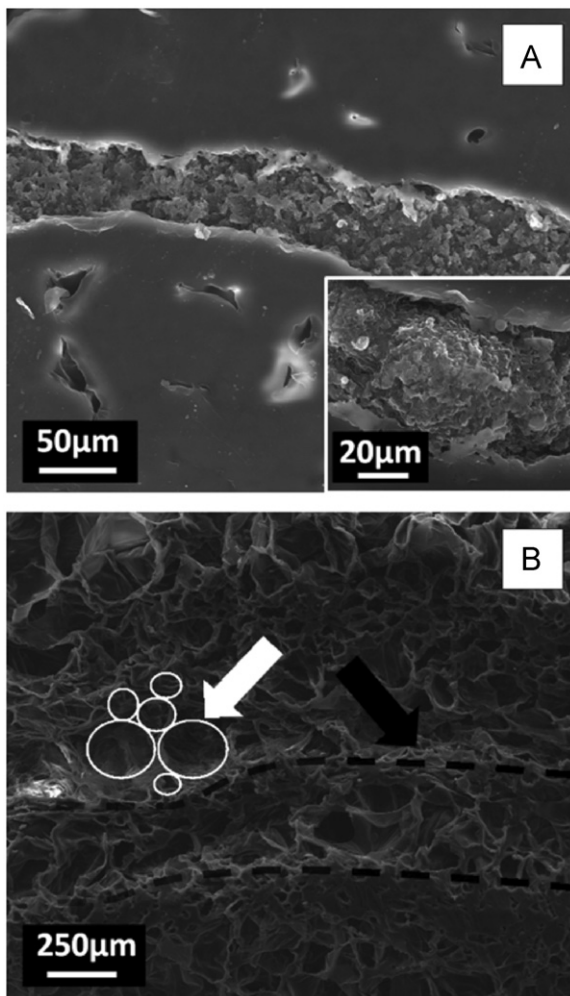


Fig. 3. SEM micrographs of PCL-isomalt composite produced by compression molding (A); PCL scaffold after foaming and isomalt fiber removal with evidenced isotropic porosity (white arrow and lines) and tubular porosity (black arrow and dashed lines) (B); (lines are drawn to guide the eye).

The DSC traces evidence the efficacy of the thermal treatment in inducing the crystallization, as it is proved by the occurrence of the melting peak around 140–150 °C. It is worth of note that the melting peak shifts progressively toward higher temperatures with the increase of the treatment temperature, as a consequence of the increased crystal perfection. Finally, treatment at 45 °C for 24 h was selected, since it gave the maximum degree of crystallinity, as an indication of maximum achievable stability of the fibers.

Treated isomalt fibers have been subsequently utilized to produce the PCL/fiber composite, both by solution casting and compression molding. The results obtained by the solution casting process (not shown) evidenced the good dispersion of the fibers within the polymeric matrix, while macroscopic pores formed as a consequence of DCM evaporation were present in the polymeric phase. Conversely, macroscopic defects were absent in compression molded PCL/fiber composites, in which thermally treated fibers retained their fibrillar form after processing (Fig. 3A).

The composite prepared by compression molding was finally foamed and the isomalt fiber leached out in water, to achieve the final scaffold (see Fig. 3B). Here, it is possible to evidence pores homogeneously distributed within the scaffold and the tubular pore left by the fiber. The complete isomalt fiber removal was assessed by gravimetric measurements and gave PCL scaffold with a final porosity of 70% and tubular network spanning the whole composite, suitable for vascularization or nerve growth.

5. Conclusions

It has been proved how, in a simple and versatile way, it is possible to produce polymeric scaffolds characterized by dual shape (tubular/isotropic) pore architecture. In particular, (i) we produced isomalt fibers by melt spinning, (ii) we thermal treated the fibers for the production of fiber-polymer composites with PCL, by compression molding or solution casting, and (iii) after PCL-foaming and fiber leaching, we produced the final scaffold, suitable for those fields of tissue engineering requiring, for vascularization and/or nerve reconstruction, tubular porosities in addition to random size and position pores.

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