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Melt electrospinning writing of defined scaffolds using polylactide-poly (ethylene glycol) blends with 45S5 bioactive glass particles

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ABSTRACT

Advances in Tissue Engineering (TE) demand strategies to apply new biomaterials and processing technologies. In this context, especially computer aided additive manufacturing (AM) has turned out as a promising tool to tailor scaffold architectures with high precision. However, there is currently a lack of appropriate AM methods, since the majority face serious drawbacks regarding printing accuracy and speed, as well as limitations regarding the range of applicable biomaterials. Here, we present the simple AM processing of poly(lactide-*block*-ethylene glycol-*block*-lactide) (PLA-PEG-PLA) triblock copolymers via melt electrospinning writing (MEW). Furthermore, we demonstrate the accurate deposition of fibers (fØ = 31 ± 2 μm) made of copolymer blends with low-molecular PLA incorporating solid 45S5 bioactive glass (BG) particles. Thus, we introduce MEW for the printing of highly porous, particle loaded scaffolds with micron-sized composite fibers with potential application in bone TE.

1. Introduction

MEW has been introduced as high resolution AM method for printing of highly porous fiber scaffolds composed of synthetic biodegradable polymers [1,2]. Using this process, polymer melts can be written via a high voltage field to highly defined scaffolds with submicron-sized fibers [3]. While many different materials have been used for electrospinning in general [4,5], only a few are yet reported for MEW. Amongst these are poly(ε-caprolactone) (PCL), as low melting standard material [4], as well as the photo-cross-linkable copolymer poly(L-lactide-co-ε-caprolactone-co-acryloyl carbonate) [6] and poly(2-ethyl-2-oxazoline) [7] as water-soluble polymer.

To broaden the range of available biomaterials for MEW, PLA-PEG-PLA copolymers were investigated in this study. As previously described, PLA-PEG-PLA meshes can be easily processed by solution electrospinning and such membranes exhibit an increased hydrolysis rate due to an enhanced water uptake [8]. Since BGs are known for their ability to stimulate bone tissue formation and regeneration via ion release [9,10], additional blends with 45S5 BG particles were prepared for MEW printing. Indeed, biopolymer-BG composites have been intensively investigated and also presented as melt electrospun non-woven scaffolds [11], however, the applicability via MEW for the development of highly defined AM printed bone scaffolds has not been reported yet.

PLA-PEG-PLA triblock copolymers were synthesized via ring-opening polymerization and characterized by Nuclear Magnetic Resonance (NMR) spectroscopy, Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). To enhance the MEW processing performance, two blends with low molecular weight PLA (10 and 20 wt%) were prepared and investigated regarding the minimum necessary printing temperature T_{min} for homogenous fiber deposition. Finally, we also prepared a composite including 5 wt% 45S5 BG particles that also could be printed to regular scaffold patterns for potential use in TE.

2. Materials and methods

The synthesis was conducted using PEG with M_n = 10 kg·mol^{-1} (Fluka, Buchs, Switzerland) as initiator, 100% racemic D,L-lactide (Sigma-Aldrich, Steinheim, Germany) as monomer, tin(II) 2-ethylhexanoate (Sigma-Aldrich, Steinheim, Germany) as catalyst and toluene (Alfa Aesar, Karlsruhe, Germany) as solvent. A detailed description can be found elsewhere [8,12]. NMR (recorded at 300 MHz, Bruker Fourier 300, Bruker Corporation, Billerica, USA) showed the successful synthesis of PLA-PEG-PLA: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.57 (3H, PLA), 3.65 (4H, PEG), 4.20 and

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4.40 ppm (1H, PLA's methine protons of the last repetition unit; 4H, PEG's methylene protons connecting PEG and PLA), 5.15 ppm (1H, PLA). Molecular weight and dispersity was analyzed by GPC (Shimadzu, Duisburg, Germany) with a SCL-10A VP system controller, a SIL-10AD VP auto injector, a LC-10AT VP pump, a FCV-10AL VP degasser, a RID-10A refractive index detector run at 40 °C, and a CTP-10AC VP column oven run at 40 °C, equipped with a Phenogel™ 5 μm linear (2) LC column (300 × 7.8 mm, 100–10,000 kg·mol^{-1} (Phenomenex, Aschaffenburg, Germany); M_n = 30 kg·mol^{-1}, M_w = 72 kg·mol^{-1}, Đ = 2.4).

The DSC analysis (204 F1 Phoenix, Netzsch, Selb, Germany) was conducted with temperature ramps of 10 K min^{-1} and −10 K min^{-1}. For comparison of material characteristics, the second heating cycle was used and resulted in a glass transition temperature $T_g \approx 21$ °C for pure PLA-PEG-PLA. For sample imaging a stereomicroscope (SM; Discovery V20, Carl Zeiss Microscopy GmbH, Göttingen, Germany) and two scanning electron microscopes (SEM; Supra 25 and Discovery V20, Carl Zeiss Microscopy GmbH, Göttingen, Germany) were used. Energy dispersive X-ray spectroscopy (EDX) was conducted at 10 kV and 5 mm using a silicon drift detector (INCA Energy 350 AzTec Advanced system,

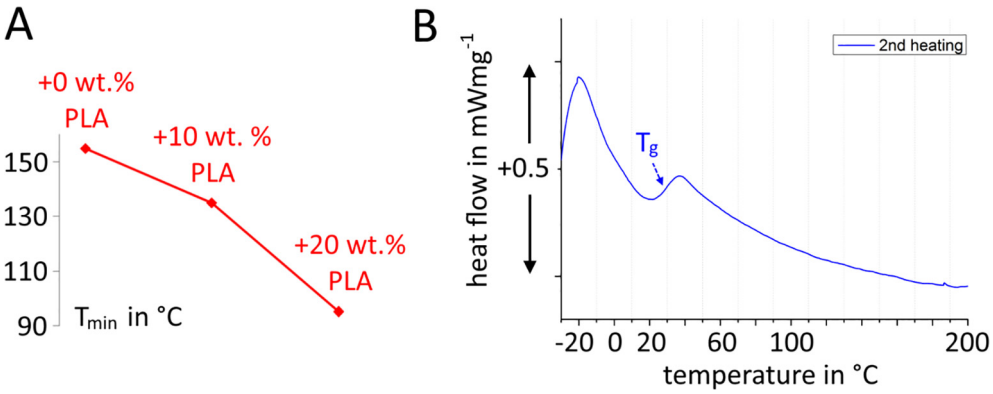

Fig. 1. Thermal characteristics. A) Minimum printing temperature to obtain continuous fibers. Via addition of PLA, T_{min} decreased. B) DSC plot of the PLA-PEG-PLA/PLA (10 wt %) blend including 5 wt% 45S5 Bioglass®. A T_g slightly above room temperature could be observed.

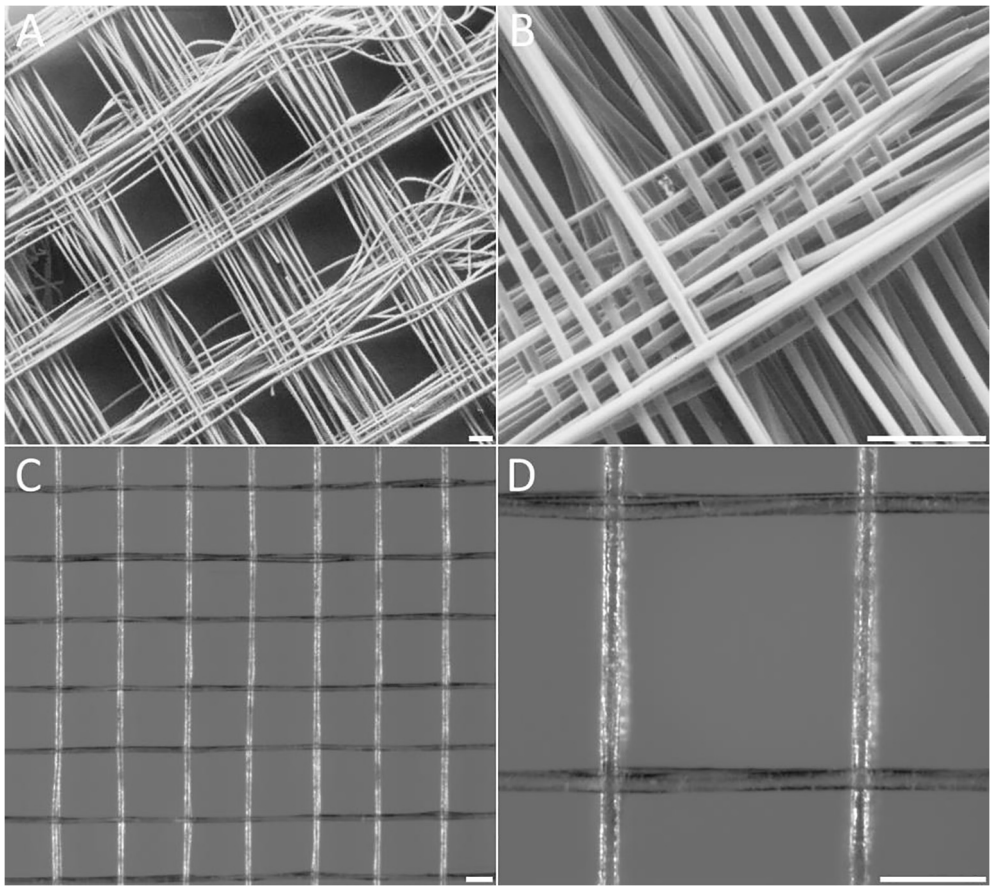

Fig. 2. Images of printed scaffolds. A–B) SEM images of pure PLA-PEG-PLA fibers exhibited printing instabilities. A) Overview of the defective structure. B) Close-up view with non-regular fiber diameters. C–D) SM images of PLA-PEG-PLA/PLA (10 wt%) fibers with 5 wt% 45S5 Bioglass®. C) Overview indicating a well-structured scaffold with 10 layers (5 layers in 0° and 90° direction). D) Close-up view with homogeneous composite fibers with a diameter of 31 ± 2 μm. Scale bars = 200 μm.

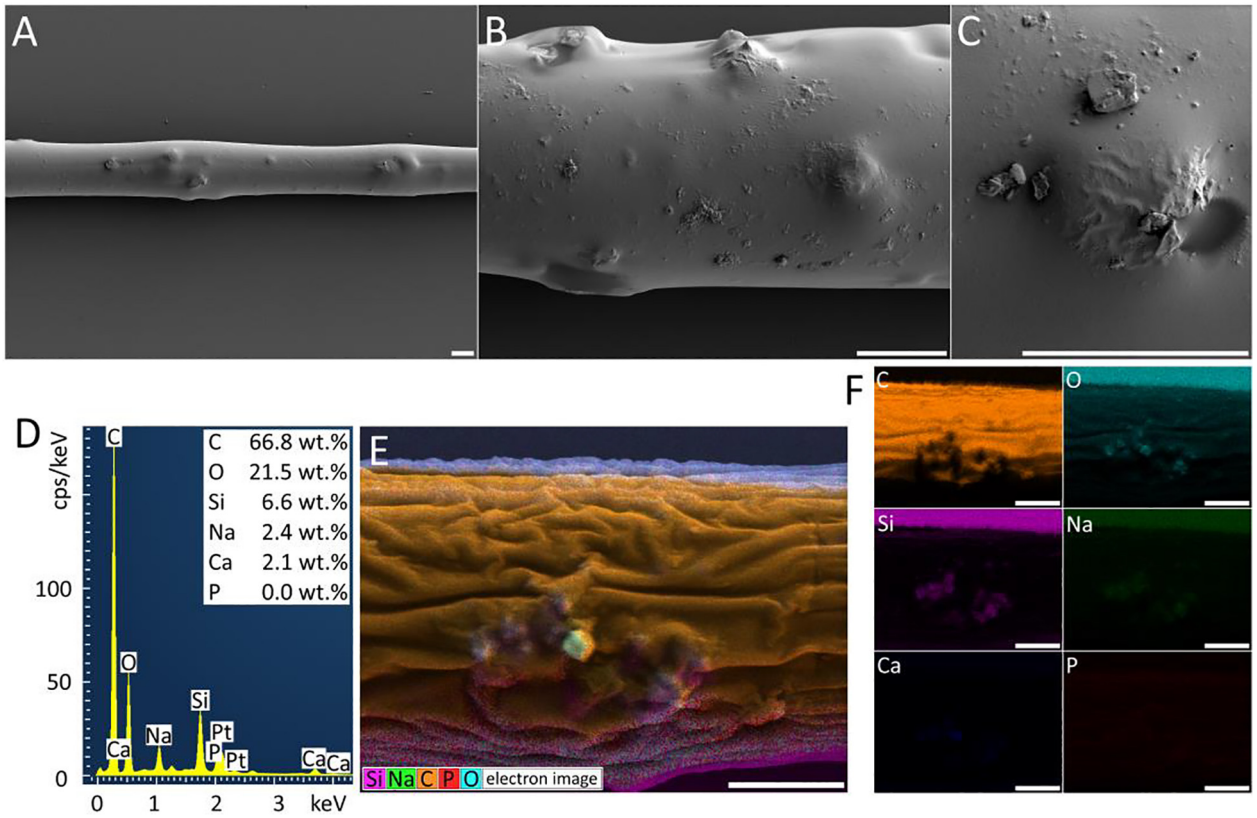

Fig. 3. Platinum coated single composite fibers on a glass slide. A–C) SEM images. A) Overview image of a fiber. B) Close-up view of the fiber surface. C) Particles partially beneath and on top of the fiber. D–F) EDX measurement. D) Element analysis indicating the presence of 45S5 Bioglass®. E–F) Element images. Scale bars = 10 μm.

Oxford Instruments PLC, UK) on 4 nm platinum sputter-coated composite fibers.

The 45S5 BG used [9], was a melt-derived powder exhibiting 45 wt% SiO_2, 24.5 wt% Na_2O, 24.5 wt% CaO and 6 wt% P_2O_5 with a volumetric particle size of D_{50} = 4.3 μm and D_{90} = 7.7 μm (LA-300, Horiba Ltd., Kyōto, Japan). Three blends based on PLA-PEG-PLA were prepared; first: 10 wt% PLA (Resomer 203, M_w = 18–28 kg·mol^{-1}, Boehringer-Ingelheim AG & Co. KG, Ingelheim am Rhein, Germany), second: 20 wt% PLA and third: 10 wt% PLA and 5 wt% BG 45S5. The polymer blends were obtained via dissolution in chloroform with 30 min ultrasonication (Sonorex RK 100 H, Bandelin electronic GmbH & Co. KG, Berlin, Germany). Afterwards, the solvent was removed using a rotary evaporator. For MEW processing a custom-built device was utilized as described elsewhere [7].

3. Results

When printing degradable polymers, T_{min} of the reservoir is an important parameter to determine the long term polymer stability and thus, small temperatures are preferred. Accordingly, the impact of the low molecular weight PLA on T_{min} was studied to obtain suitable blends, as presented in Fig. 1A. The amount of 20 wt% PLA in PLA-PEG-PLA ultimately led to a decrease of 60 °C. However, the also investigated fiber shape fidelity was best for blends with only 10 wt% PLA, which led to the most constant fiber diameter. Thus, only the thermal characteristics of the three component blend with 10 wt% PLA and 5 wt% BG 45S5 is shown in Fig. 1B, where a T_g between room temperature and the physiological body temperature was observed. In contrast, PCL with 5 wt% BG 45S5 could not be used for stable printing. Pre-studies further revealed no MEW processability for the triblock copolymer PCL-PEG-PCL, with a similar molecular weight to PLA-PEG-PLA.

As indicated by Fig. 2A and B, MEW processing of the pure PLA-PEG-PLA triblock copolymer was feasible, however, obtained fibers were inhomogeneous in diameter, accompanied by an insufficient deposition performance. Most regular scaffold architectures were obtained by printing the PLA-PEG-PLA/PLA(10 wt%) blend with 5 wt% 45S5 BG at optimized instrumental parameters (acceleration voltage 4.0 kV, nozzle-collector distance 3.5 mm, pressure 2.0 bar, temperature 142 °C at 5000 mm·min^{-1} collector speed using a 23 G nozzle). Resulting homogeneous composite fibers with diameters of 31 ± 2 μm are shown in Fig. 2C and D. Here, the coefficient of variance was below 10% and, moreover, no fiber diameter instabilities like fiber pulsing [13] were observed. 45S5 BG particles incorporated in the fibers are presented in Fig. 3.

4. Discussion

From the investigated polymer blends, PLA-PEG-PLA including 10 wt% PLA exhibited the most promising printing performance, which can be explained by beneficial flow characteristics induced by the appropriate amount of the low molecular weight PLA additive. Blends with 20 wt% PLA already exhibited an insufficient jet constancy indicating an inhomogeneity of the melt. In contrast, an addition of 5 wt% solid 45S5 BG particles did not influence the MEW processing adversely. Furthermore, we found suitable conditions for a steady melt jet, which is important for accurate direct writing as changes in printing direction can only be realized with a predictable deposition performance.

5. Conclusion

In the present study, the applicability of particle composites based on PLA-PEG-PLA/PLA blends for MEW could be successfully

proven. Since the synthesis and printing is neither particularly expensive, nor time consuming, the novel 45S5 BG-polymer composite represents a promising novel approach for bone TE and cell culture research. Using the MEW process to print the presented composites enables production of differently designed scaffolds, by changing mesh widths and shapes, fiber diameters as well as blend compositions. Future work should investigate the biodegradability and bioactivity in comparison with scaffolds fabricated by standard methods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.matlet.2017.06.096.

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