In Vitro Migration and Proliferation of Human Osteoblasts in Injectable In Situ Crosslinkable Poly(caprolactone fumarate) Scaffolds

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Introduction

Many clinical situations, such as spinal arthodesis, total joint arthroplasty, osteoprotic insufficiency fractures, and bone loss after skeletal trauma might be amenable to treatment options which include biodegradable scaffolds that can be injected and crosslinked in-situ to fill a defect. For minimally invasive applications, injectable systems that can be crosslinked in-situ by chemical redox initiation and can promote migration and proliferation of osteoblasts from the surrounding tissues would be attractive [1]. We recently developed a poly(caprolactone-fumarate) (PCLF) macromer that self-crosslinks in the absence of a crosslinking agent. Above its melting point of 45-55C, the copolymer is a viscous liquid and it can be used as an injectable matrix to fill irregularly shaped defects [2]. At physiological temperature, the macromer self-crosslinks and hardens in-situ physically by crystallization and chemically by radical polymerization. The objective of this work was to study migration and proliferation of osteoblasts in porous PCLF scaffolds in vitro.

Methods

PCLF was synthesized by condensation polymerization of PCL with fumaryl chloride in methylene chloride with triethylamine as the catalyst [2]. Three PCLs with number average molecular weight (Mn) of 340, 760, and 1200 Daltons were used. The polydispersity indices of the PCLs were 1.7, 1.8, and 1.8, respectively, as determined by gel permeation chromatography (GPC). The PCLs were dried under vacuum of less than 5 mm Hg at 60C for at least 12 h before the reaction. The molar ratio of fumaryl chloride to PCL was 0.9. A typical reaction for PCL with Mn of 760 was as follows. In a three-neck reaction flask, 40 mmol of PCL was dissolved in 300 ml of methylene chloride under nitrogen atmosphere. Thirty-six mmol of fumaryl chloride and 72 mmol of triethylamine dissolved in 25 ml of methylene chloride were added dropwise to the reaction with stirring. The reaction vessel was placed in an ice bath to limit the temperature rise of the exothermic reaction. After the addition of fumaryl chloride and triethylamine, reaction was continued for 24 h under ambient conditions. After completion of the reaction, solvent was removed by rotovaporation at ambient temperature and reduced pressure, the residue was dissolved in anhydrous ethyl acetate, and the by-product triethylamine hydrochloride salt was removed by filtration. Ethyl acetate was removed by vacuum distillation. The polymer was redissolved in methylene chloride and precipitated twice in ice cold ethyl ether. The polymer was dried in vacuum (less than 5 mmHg) at ambient temperature for at least 12 h and stored at -20C until used.

NMR and FTIR were used to confirm the presence of the fumarate group in the macromer. The presence of peaks at 6.8 ppm in the NMR spectrum attributable to the hydrogen atoms of the unsaturated double bonds of the fumarate group, and the presence of a band due to the ester carbonyl stretching vibration centered at 1725 cm-1 in the FTIR spectra, confirmed the incorporation of fumarate monomers into the PCL macromer. GPC was used to

determine the molecular weight and polydispersity of the PCLF macromer. Monodisperse polystyrene standards with Mn of 0.474, 6.69, 18.6, and 38 kD, and polydispersities of less than 1.1, were used to construct the calibration curve. The PCLF in this study had a number average molecular weight of 3680 daltons and polydispersity index of 2.3.

Scaffolds were prepared by self-crosslinking of the fumarate carbon-carbon double bonds via free radical polymerization with sodium chloride salt particles as the porogen [3]. Benzoyl peroxide and dimethyl toluidine were used as the free radical initiator and accelerator, respectively. A typical procedure for fabrication of scaffolds was as follows. 1 g of PCLF was mixed with 5.0 g of porogen in a scintillation vial, corresponding to 75% by volume porosity. Fifty ul of initiator solution (50 mg of benzoyl peroxide in 250 ul of NVP) and 40 ul of accelerator solution (60 ul of dimethyltoluidine in 940 ul of methylene chloride) were added and mixed thoroughly. NVP, in amount less than 2% by weight of PCLF, was used to dissolve the initiator and not as a crosslinking agent. The polymerizing scaffold was transferred into a mold and pressed manually and crosslinked. After crosslinking, cylindrical specimens with diameter and length of 5 mm by 8 mm were cut. The salt was leached out by placing the scaffolds in distilled water for 3 days, during which time water changes occurred every 12 hours. The scaffolds were dried in a controlled atmosphere at ambient temperature for 1 d and in vacuum of less than 5 mmHg for at least 12 h. The disks were sterilized with ethanol overnight and washed with sterile PBS before adding to the well plate.

A human osteoblastic cell line hFOB was seeded on the scaffolds at a density of 1x10^6 cells per scaffold in agarose coated 48-well plates with growth media for 24 h [4]. Then the scaffolds were transferred to uncoated tissue culture plates and grown in mineralization media. At 1, 7, 14, 21 d the scaffolds were treated with calcein AM and propidium iodide for live and dead cells, respectively, and imaged by confocal microscopy.

Results

Scaffolds were fabricated from the PCLF copolymer with salt as the porogen, BP as the chemical initiator, and DMT as the accelerator. The SEM of a cross-section of the PCLF scaffold with Mn of 3680 daltons and 75% by volume salt content is shown in Figure 3. The SEM micrograph confirms the formation of a three-dimensional porous and interconnected PCLF scaffold in the absence of a crosslinking agent.

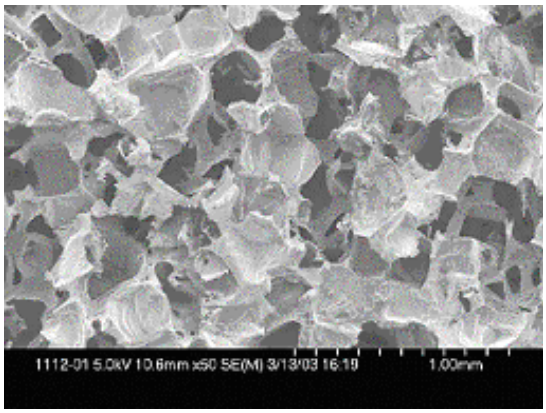

Figure 1

The distribution of live hFOB cells, stained with calcein AM, after 1, 7, and 21 days of culture in osteogenic media is shown in Figure 2a, 2b, and 2c, respectively.

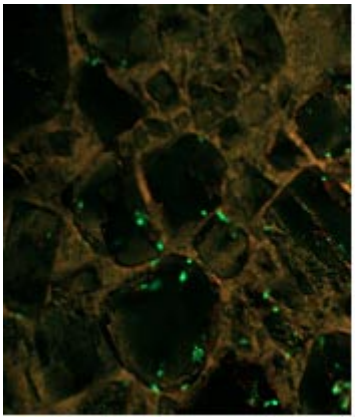
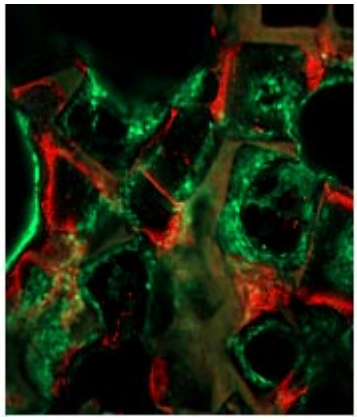
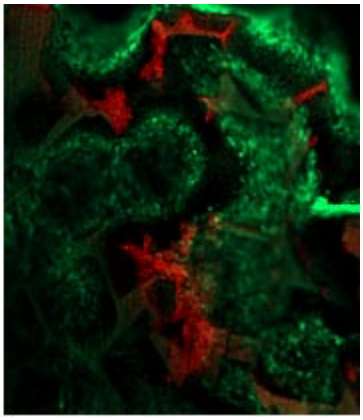

Figure 2a **Figure 2b** **Figure 2c**

Extensive migration and proliferation of hFOB cells were observed after 7 d compared to 1 d of culture. After 21 d, cells and extra-cellular matrix occupied the pore volume of the scaffold. These results demonstrate that PCLF, as an injectable and in-situ crosslinkable scaffold, is able to support osteoblast attachment, migration, and proliferation in vitro.

Conclusions

A novel self-crosslinkable degradable biomaterial was synthesized by copolymerization of fumaryl chloride with poly(caprolactone). Results from cell migration study with confocal microscopy demonstrate that PCLF, as an injectable and in-situ crosslinkable scaffold, is able to support osteoblast attachment, migration, and proliferation in vitro.

Acknowledgements

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