In Vitro Response of Human Osteoprogenitor Cells to Cross-Linked Poly(Lactide-co-Caprolactone) Dimethacrylate for Bone Repair

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INTRODUCTION

Bone defects above a critical size do not heal without intervention. Implantation of resorbable bone substitute materials can switch the mechanism of healing from formation of a collagen callus across the defect followed by mineralisation to creeping substitution of implant material with new bone at the resorbing interfaces, making use of the ability of bone to remodel its structure over time.

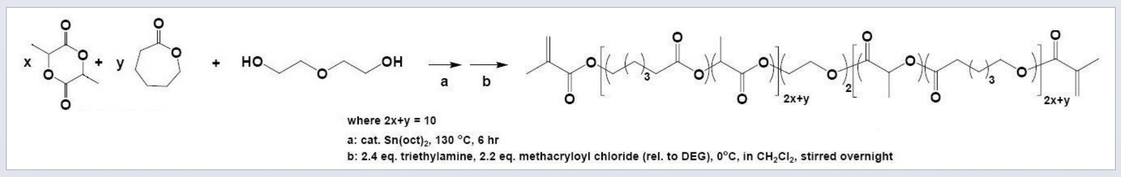
Resorbable α-hydroxyacid polymers are an attractive choice for controlled implant degradation. The rate of degradation in the body may be tuned by varying the monomer molecular weight or composition in copolymers. An amorphous microstructure gives a desirable linear degradation profile, but increased resorption rate, in comparison to a semicrystalline polymer.

A crosslinked copolymer of D,L-lactide (LA) and ε-caprolactone (CL) was used in this study. Similar materials were first produced and characterised by Davis et al. [1]. Here, we study *in vitro* biocompatibility and specifically osteogenic potential over a longer timescale of 14 days.

Osteoprogenitor cells were cultured on copolymer samples with two different compositions. We compared two types of macroscopic structure; a dense disc, and a scaffold produced by two photon polymerisation. Metabolic activity and expression of alkaline phosphatase (ALP), a marker of differentiation to osteoblast phenotype, were measured to determine osteogenic potential.

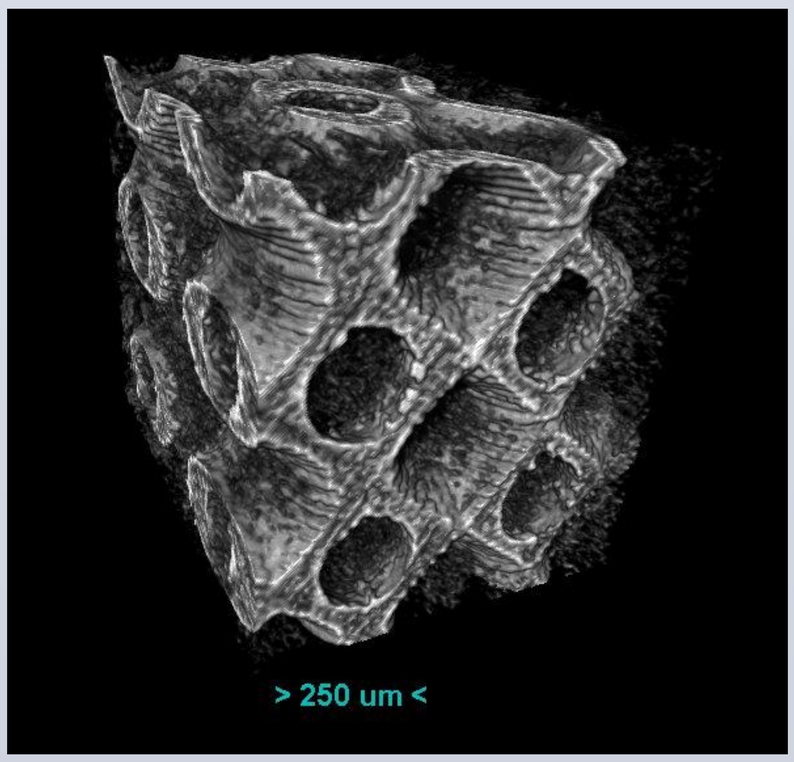
MATERIALS AND METHODS

D,L-lactide and ε-caprolactone monomers were polymerised according to the protocol shown below.



We choose a low ratio of initiator to monomer of 1:10 to give an appropriate viscosity for further two-photon polymerisation (2PP), and to give an amorphous final structure. 2PP is an additive manufacturing process which allows complex microstructures to be defined using CAD software, with a voxel size of 5x1x1μm. This is ideal for producing complex scaffold architectures which maximise permeability [2] (in this case a Schwartz P triply periodic structure) at an optimal scale for cell migration (500 μm unit cell). An X-ray tomography image of the resulting scaffold is shown below.

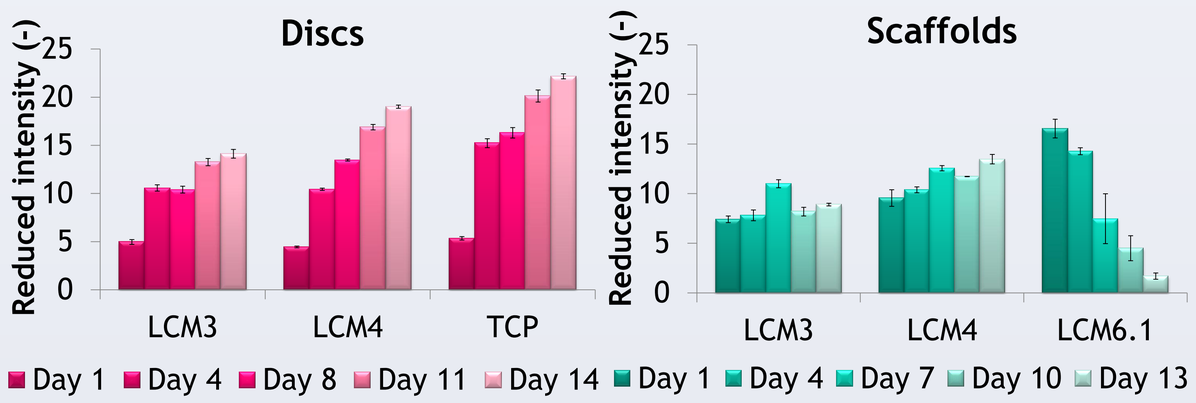
LCM is a tapered copolymer consisting of crosslinked oligomers of 20 monomer units. LCM3 has a nominal lactide content of 70wt% and LCM4 contains 80wt% lactide. These compositions provide the superior mechanical properties of poly(lactide) while retaining the lower degradation rate of poly(caprolactone).



RESULTS

Cell culture experiments used primary human osteoblasts isolated from the bones of patients undergoing elective knee-replacement surgery. Cells at passage 4 were seeded onto disc and scaffold samples of LCM materials and suitable control materials and cultured for 28 days. Different materials were used as positive controls to match the sample morphology; tissue culture plastic (TCP) was chosen as a control for the disc samples, and Vitoss FOAM (a commercial scaffold made of ß-TCP with 20wt% collagen) was used for the scaffold study.

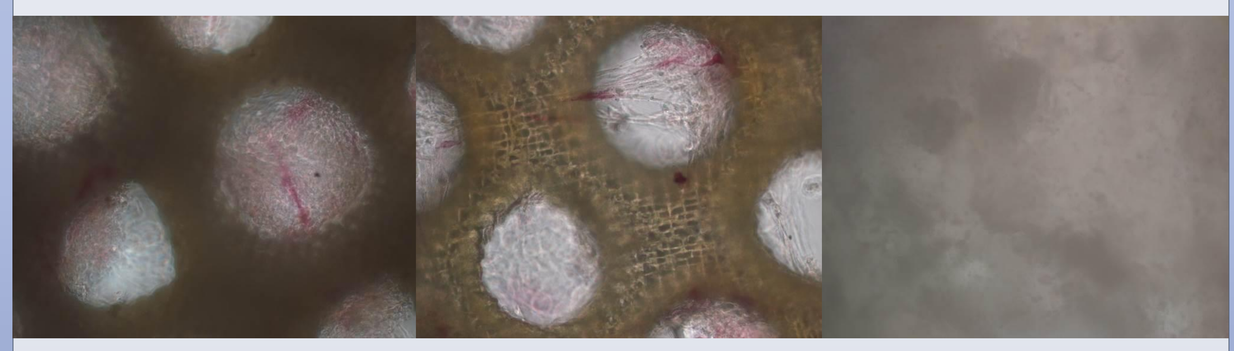
AlamarBlue assay was used to measure cell population metabolic activity on discs (n=5) and scaffolds (n=2).



At Day 7, scaffolds were fixed and stained for ALP and imaged. A positive red stain indicates the presence of ALP.

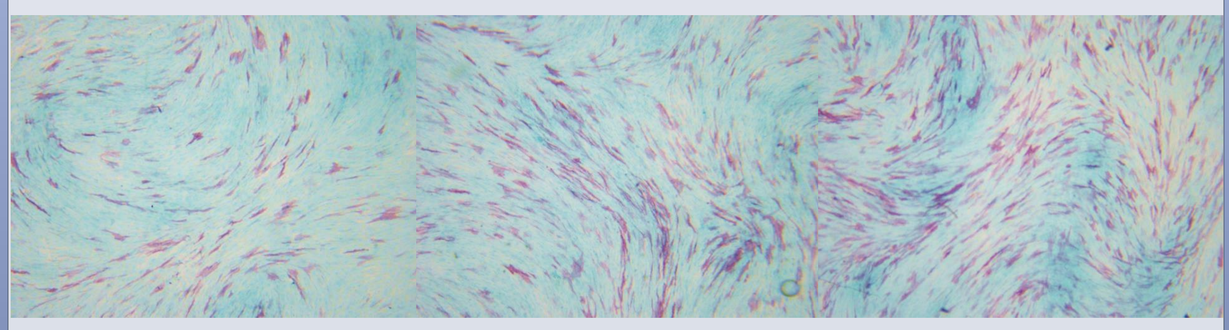
Scaffolds:

LCM3 LCM4 Vitoss



Discs:

LCM3 LCM4 TCP



For scaffold samples, cell adhesion was lower for the LCM materials than the control, but subsequent proliferation was significantly higher than the Vitoss control, despite its status as a clinically approved bone substitute material. There were no significant differences in attachment or proliferation between the three LCM materials tested.

CONCLUSIONS and FURTHER WORK

Short chain co-oligomers of D,L-lactide and ε-caprolactone were synthesised. Oligomers were methacrylated and crosslinked via two methods to produce different morphologies; a dense disc and a CAD-defined scaffold structure.

Scaffold structure was characterised using X-ray tomography.

Osteogenic potential of disc and scaffold samples was tested by cell culture of primary human osteoprogenitor cells over 14 days.

Cell population metabolic activity does not appear to vary significantly with either copolymer composition or oligomer molecular weight over the range studied. AlamarBlue results show very different behaviour in LCM scaffolds in comparison with a commercial control material, Vitoss.

Osteoprogenitor cells showed good attachment and proliferation when cultured on the LCM materials in both disc and scaffold form. This is a promising result for the *in vivo* biocompatibility of the LCM materials.

REFERENCES

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2. Rajagopalan S. *et al*. Med. Imag. Anal., 2006, 10: 693-712

ACKNOWLEDGEMENTS

The authors wish to thank EU Framework Program 7 (Grant 263363 - InnovaBone) for funding this work, the EPSRC for funding for LB, and the NIHR for funding for RB.