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

Laura Brown[1], Jessica Gwynne[1], David Shepherd[1], Leander Poocza[2], Gerhard Hildebrand[2], Klaus Liefeth[2], Roger Brooks[3], Serena Best[2]

[1]CCMM, Department of Materials Science & Metallurgy, University of Cambridge, United Kingdom
[2]Institute for Bioprocessing and Analytical Measurement Techniques e.V., Heilbad Heiligenstadt, Germany
[3]Orthopaedic Research, Addenbrooke's Hospital, University of Cambridge, United Kingdom
lvb26@cam.ac.uk

INTRODUCTION

Autologous bone grafts remain the gold standard for treatment of critical size bone defects[1]. However, this approach has significant drawbacks; limited supply, a second injury site, increased infection risk. Various materials have been investigated as synthetic bone substitutes. Resorbable α-hydroxyacids are an attractive choice for controlled implant degradation. Copolymers of lactide and glycolide have been widely studied for applications such as resorbable sutures. Copolymers containing the longer chain caprolactone monomer have a longer degradation time, which is tunable with composition[2]. Such materials may be a good choice for a bone substitute material, which should ideally resorb at a rate to match the ingrowth of new bone.

EXPERIMENTAL METHODS

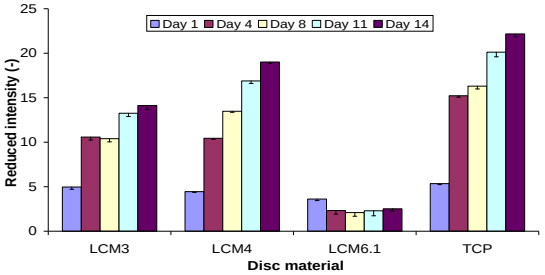
Poly(lactide-co-caprolactone) dimethacrylate (LCM) materials were synthesised by stannous catalyzed ring-opening polymerisation of cyclic caprolactone and the D,L-lactide dimer, using diethylene glycol as the initiator. LCM is a tapered copolymer consisting of crosslinked oligomers of 10 (LCM6.1) or 20 (LCM3,4) monomer units. LCM4 and LCM6.1 materials have a nominal lactide content of 80wt%, LCM3 contains 70wt% lactide. After methacrylation, crosslinking was performed by two methods to give different microstructures. Disc samples were UV-crosslinked. Scaffolds samples were produced by spatially selective two-photon polymerisation, an additive manufacturing technique, to create a well-defined structure from a CAD model. Our chosen structure is a Schwartz P unit cell with cell parameter 500 µm. Pore interconnectivity gives this structure high permeability - the scale chosen reflects optimum pore size for bone cell migration[3].

Primary human osteoprogenitor cells isolated from bone tissue of patients undergoing total knee replacement were cultured for two weeks on gamma-sterilised disc and scaffold samples in 24-well microplates. Cell proliferation was monitored by alamarBlue assay throughout and cell differentiation by ALP staining at Day 7. For disc samples, tissue culture plastic discs of similar size were used as a positive control for good cell response; for scaffolds, a clinically successful bone substitute scaffold, Vitoss, was used as positive control.

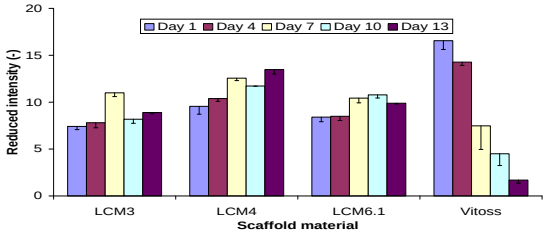
RESULTS AND DISCUSSION

For disc samples (Fig 1a), cell proliferation was comparable to the control material for LCM3 and LCM4 materials, suggesting little effect of copolymer composition over the range studied. Cell proliferation was lower for the LCM6.1 material, perhaps due to lower methacrylation efficiency resulting in an acidic oligomer component. This might be improved by increasing the methacrylate:oligomer ratio.

For scaffold samples (Fig 1b), 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, suggesting two-photon polymerisation improves the gel content of the LCM6.1 material compared with 'bulk' UV crosslinking.



(a)



(b)

Figure 1: alamarBlue intensity, normalised to no cell control, for disc (a) and scaffold (b) samples

Osteoprogenitor cells on all LCM materials showed ALP staining at day 7, indicating support of a more differentiated cell phenotype.

CONCLUSION

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.

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