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Geddes, L., Quinn, J., Themistou, E., Buchanan, F., & Carson, L. (2020). *In vitro methodology to predict a delayed inflammatory response towards bioresorbable polymers*. Poster session presented at World Biomaterials Congress 2020: Virtual Meeting.  
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Publisher's PDF, also known as Version of record

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# In vitro methodology to predict a delayed inflammatory response towards bioresorbable polymers

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## INTRODUCTION

Bioresorbable polymers composed of poly(lactide) (PLA), poly(glycolide) (PGA) and their related copolymers are commonly used in orthopaedic applications to help regenerate and repair injured bone tissue. These polymers are widely considered biocompatible, however, throughout the literature there is evidence of a delayed inflammatory reaction occurring months or years after implantation. These reactions generally present as a non-specific FBR and can range in severity<sup>1-6</sup>.

Reports of a delayed inflammatory response occurring in the late stages of degradation has limited wide scale use<sup>1,5</sup>. Very few *in vitro* studies assess long-term biocompatibility of these polymers. Initial testing may yield positive results in pre-clinical studies<sup>7</sup>, however this may not reflect clinical use, as illustrated by a high number of clinical adverse reactions occurring 2-36 weeks after implantation of the CALAXO screw<sup>2</sup>.

*In vitro* biocompatibility screening of these polymers is challenging, due to limitations of methodology, prolonged degradation profiles, the physical and chemical nature of degradation products.

## AIM

Our aim is to develop *in vitro* methodology as a tool to predict the *in vivo* and clinical performance of these bioresorbable polymers.

## METHOD

PDLLGA 85:15 and PLLGA 85:15 (Corbion) were processed by compression moulding, and annealing (PLLGA only) for 4h at 100°C, laser cut into 8mm diameter discs and electron beam sterilised. Samples were degraded in sterile PBS at 47°C and retrieved at predetermined time intervals.

The *in vitro* characterisation of samples was carried out following both standard ISO 10993-5 guidelines, and using a modified direct-contact cytotoxicity assay in 24-well plates, where permeable trans-well supports facilitated the introduction of degraded samples into the wells. After 24 h incubation with fibroblast (L929) and macrophage (RAW264.7) cell lines, an MTT assay was used to determine cytotoxicity.

A flow culture model was investigated as an improved approach to *in vitro* testing. The Quasi Vivo® QV500 system (Kirkstall) is a flow system that can be used to culture cells in a way that better reflects the dynamic nature of the physiological environment. This approach utilises interconnected chambers with cell culture media pumped through the system, allowing cells to be cultured under flow conditions. The components of the QV500 system were connected under sterile conditions in a Class II microbiological safety cabinet. A peristaltic pump (SP-minipump compact, Shenchen Baoding) at a flowrate of 75 µL/min was used to achieve flow conditions.

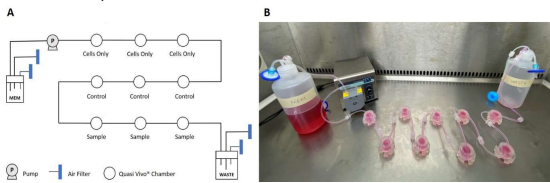


Figure 1. Schematic representation of QV500 system (A) and lab set-up of QV500 system (B)

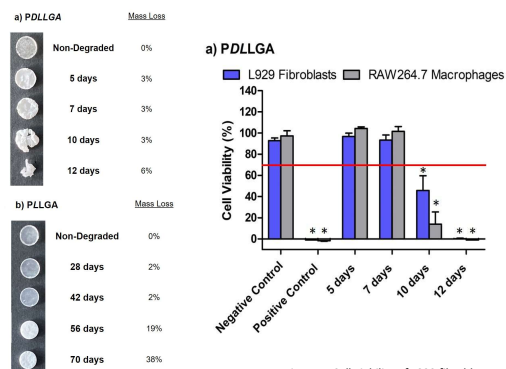


Figure 3. Samples appearance and mass loss of a) PDLLGA and b) PLLGA after degradation at 47°C and drying.

## RESULTS

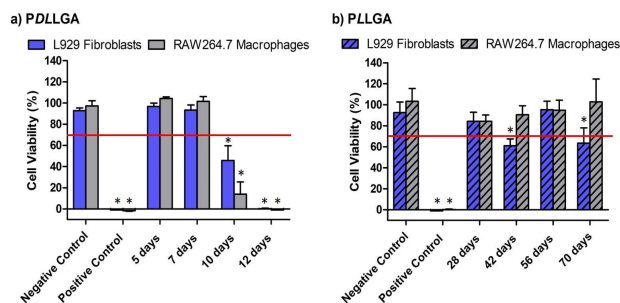


Figure 4. Cell viability of L929 Fibroblasts and RAW264.7 macrophages versus degradation time of a) PDLLGA and b) PLLGA at 47°C. (\* indicates significant difference to the negative control, red line represents cytotoxicity threshold, at 70% viability relative to blank.

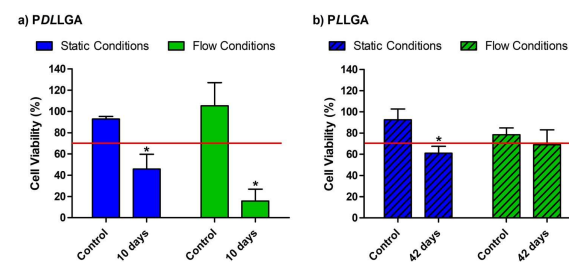


Figure 5. Comparison of the cell viability of L929 fibroblasts using static conditions compared to Quasi Vivo flow conditions when in contact with a) PDLLGA degraded for 10 days and b) PLLGA degraded for 42 days. \*Indicates significant difference relative to blank control. Red line represents cytotoxicity threshold, at 70% viability relative to blank.

## CONCLUSIONS

It can be difficult to extrapolate *in vitro* results to *in vivo* applications as the conditions are very different. However, the use of the increased temperature, 47°C, to accelerate the degradation of PDLLGA and PLLGA has demonstrated great potential for the assessment of bioresorbable polymers with long degradation times as the results extrapolated to 37°C<sup>8</sup> corroborate with the clinical data of adverse reactions occurring, *in vivo*, in the literature.

Efforts were also made in this work to improve upon the static conditions generally used for *in vitro* research. The results validate the feasibility of using the QV500 system as a method to better represent the *in vivo* flow environment. This study has shown differences in the cytotoxicity of PDLLGA and PLLGA. It is recommended that future research into orthopaedic devices focuses on PLLGA.

## ACKNOWLEDGEMENTS

Corbion Biomaterials, The Netherlands, is acknowledged for the kind gift of poly(D, L-lactide-co-glycolide) and poly(L-lactide-co-glycolide). This work has been funded by the Department for the Economy, Northern Ireland (DFE).

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