

Surface Modification of a Polyether-urethane with RGD-containing Peptides for Enhanced Cell Attachment and Signalling

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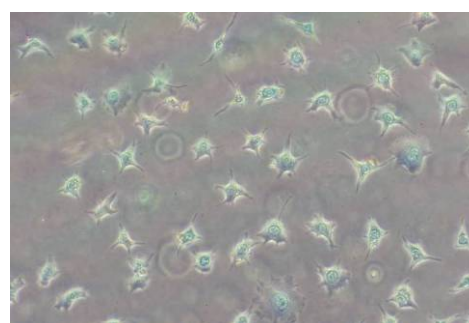
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INTRODUCTION: The chemical modification of polyurethane with RGD-containing peptides offers a means of encouraging the adhesion, spreading and proliferation of cells cultured on its surface. This study assesses the efficacy of a modification procedure using surface analysis techniques and preliminary cell culture studies.

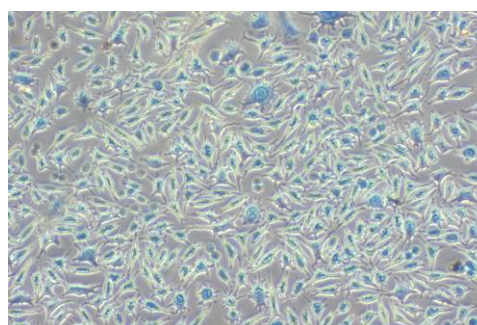
METHODS: A commercially available polyurethane was dissolved in dimethylacetamide and spin-coated on to glass discs. Amine groups were introduced to the surface using reversible swelling to sterically trap polyethyleneimine within the polymer network. Reductive amination was used to couple partially oxidised dextran to the amine groups. The Gly-Arg-Gly-Asp-Ser-Pro-Lys (GRGDSPK) domain of fibronectin or a recombinant fragment of fibrillin-1 was then covalently bound to the dextran layer again using reductive amination. The surfaces were characterised by FTIR and fluorescence analysis using fluorescamine. L929 murine fibroblasts were cultured on the surfaces for up to 4 days. Image analysis was used to assess the cell number and an Alamar Blue redox assay was used to quantify the metabolic activity of the cells.

RESULTS & DISCUSSION: Fluorescence analysis and FTIR spectra verified the amination and dextran attachment steps of the modification. The immobilisation of the peptides was verified with FTIR. The cells cultured on the GRGDSPK-modified surface proliferated extensively. The cell counts show that after 4 days there were more cells on this surface than on the positive glass control. The results of the Alamar Blue assay show an increase in the metabolic activity of the cells on this surface over the 4-day time period. The fibrillin-modified surface also supported cell proliferation to a greater extent than on the plain polyurethane (Fig 1). Cell number increased steadily over the 4 days with the quantity after 2.5 days similar to that on the glass control. The results of the Alamar Blue assay show an increase over

the 4 days. As the dextran coating appeared to limit cell attachment it is clear that it is the peptides that have caused the high level of proliferation.



A



B

Fig. 1: L929 fibroblasts cultured for 4 days on A) plain polyurethane and B) fibrillin-modified polyurethane.

CONCLUSIONS: Both the GRGDSPK and the fibrillin-1 fragment caused an increase in cell adhesion and proliferation compared to the plain polyurethane. Further work will seek to analyse further the changes in cell behaviour including the cell cycle and extracellular matrix production.

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