

GRAFTING COLLAGEN ON POLY(L-LACTIDE) BY GAMMA IRRADIATION

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INTRODUCTION: A key element of tissue engineering is controlling the growth, differentiation and behaviour of cells on biodegradable scaffolds, facilitating their organisation into functional tissue. The initial attachment of a cell to a substrate is mediated by cell-surface adhesion factors. Cell-extracellular matrix (ECM) interactions participating directly in promoting cell adhesion, migration, growth and differentiation are well documented. In this study results of grafting ECM molecules onto poly(l-lactide) by gamma irradiation is reported. The aim of the project is to immobilize protein onto polymers covalently and increase stability of proteins on polymers. The effect of irradiation on the grafting efficiency, surface morphology and the ECM molecule activity were tested.

METHODS: Poly(l-lactide) (PLLA) films of about 500 μm thickness with a molecular weight of 360,000 were used. Gamma irradiation was carried out using a Cesium137 source (IBL 337 from Cis Bio International) and a dose-rate of 3 kGy h⁻¹. Reaction mixtures containing PLLA, acrylic acid (AA), collagen type I were prepared in Schlenk tubes and were deoxygenated by three freeze-thaw cycles in vacuum. The mixtures were then exposed to gamma radiation at room temperature for different time periods ranging from 1 - 7 hours. After irradiation, the PLLA films were washed with distilled water for 30 hours with stirring, to remove un-reacted monomers, non-grafted products and other compounds from the reaction mixture, and were then dried in a vacuum desiccator. The grafted PLLA were characterised by grafting yield, XPS, immunostain (DAKO-HRP collagen ELISA kit).

RESULTS: The grafting yield (i.e. weight change after irradiation) *versus* irradiation dose is plotted in Figure 1. With the exception of the lowest irradiation dose of 3 kGy (1 hour irradiation), the grafting yield increased with increasing irradiation dose. Table 1 shows the XPS results of the collagen type I-grafted PLLA films. Nitrogen was introduced onto the film as a result of the gamma irradiation. With increasing irradiation time (and therefore dose), the percentage of nitrogen increased, indicating that more proteins had grafted onto the PLLA film. The collagen-grafted PLLA films

stained positive (brown) for collagen, as shown in Figure 2.

DISCUSSION & CONCLUSIONS: In our results, XPS confirmed the presence of proteins on the grafted PLLA. Localisation of collagen using immunostaining showed high levels of collagen on

the grafted PLLA, confirming that the grafted collagen in the PLLA was still biologically active. The presence of collagen epitopes on the surface, i.e. having reactivity of the antibody against collagen on the surface of the grafted PLLA, demonstrated that irradiation may not alter

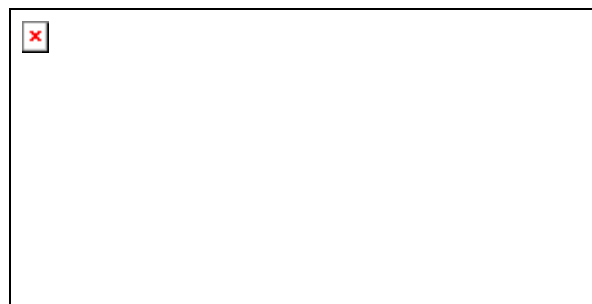


Figure 1 Effect of irradiation dose on the grafting Yield.

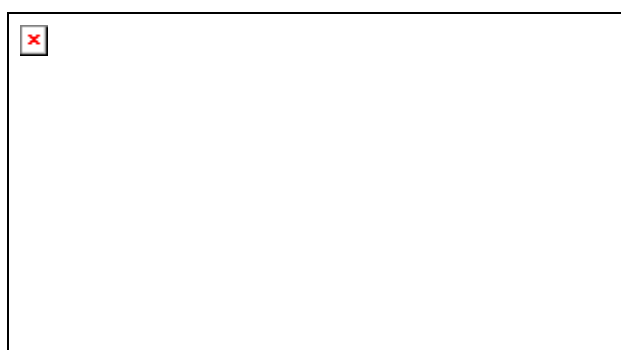


Figure 2 Immunostain of grafted PLLA films

Dose	C	O	N	Si
0kGy	74.3	21.3	0	4.4
3kGy	62.8	36.4	0.43	0.27
15kGy	61.8	34.1	1.7	2.3

Table 1 Elemental analysis of the PLLA films

availability of active binding sites. These results indicate that the single-step procedure of grafting by gamma irradiation could provide a simple but efficient technique to modify the biocompatibility alongside sterilisation of a scaffold for use in tissue engineering.

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