

New strategies in polymeric biomaterials functionalisation

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INTRODUCTION: The development of biodegradable polymers is suitable for a variety of biomedical applications. Polyurethanes (PUs) have been widely employed as elastomeric biomaterials due to their excellent mechanical properties and relatively good biocompatibility¹. A series of polyester-urethanes were synthesised. Furthermore both surface and bulk modification strategies were performed in order to obtain bioactive materials able to elicit specific cellular responses and directing new tissue formation.

METHODS: PUs were synthesised using a two step procedure, as previously described². The building block were: poly(ϵ -caprolactone) diol ($M_n=1250$ or 2000 , Sigma-Aldrich); L-lysine ethylester-diisocyanate (Kyowa Hakko Kogyo Co.) or 1,6-diisocyanatohexane (Sigma-Aldrich); the chain extender was 1,4-cyclohexane dimethanol (Aldrich). In the first bulk modification approaches, N-Boc Serinol (1,1-Dimethylethyl [2-hydroxy-1 (hydroxymethyl)ethyl] carbamate Sigma-Aldrich), has been selected as chain extender to introduce amino groups in the PU side chains. The Boc group was then removed after polymerization by treating a polymer solution with trifluoroacetic acid. In order to study the reactivity of functionalised PU versus carboxyl groups of biomolecules, a model reaction with N-Boc phenylalanine was carried out using the carbodiimide chemistry. Another bulk modification involved the employ of a synthetic chain extender, a diammine containing the RGD sequence, synthesised using a phase-solid synthetic approach. The surface modification was carried out by a two-stage method. PU films were first treated with argon RF plasma reactor (Plasma System Junior SN 001/072, Europlasma). After exposure to air and the subsequent deposition of acrylic acid (AA) plasma copolymerization was carried out. Gelatine (Gel, Type A, Sigma) and poly(L-lysine) (PolyLys, Sigma-Aldrich) were finally immobilized by carbodiimide coupling. The PUs characterization was carried out by Differential Scanning Calorimetry, Size Exclusion Chromatography and several spectroscopy analyses. Preliminary *in vitro* tests were performed using NIH-3T3, Human Mesenchymal and Fibroblast cells to evaluate the PUs biocompatibility.

RESULTS: Concerning the surface modification, the analysis related to the immobilization of Gel and PolyLys confirm the successful grafting. ATR studies shown the presence of amide signals and XPS analysis revealed changing in chemical composition and the presence of amide groups, according to protein bonds. Regarding the bulk modification, spectroscopic studies confirmed the successful functionalisation of PUs with peptides or aminoacids. Cell test demonstrated that PUs and functionalised PUs can be used as valid substrate for tissue engineering. Cell test shown that surface grafting of PolyLys is preferable instead of Gel for the activation of cellular processes (figure 1). Test performed with Human Mesenchymal and Fibroblast cells, elucidate that cell adhesion is better on the films functionalised with RGD.

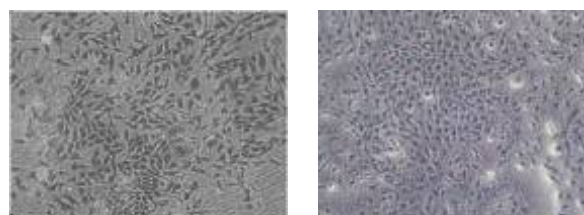


Fig. 1: Optical microscopy pictures of NIH-3T3 mouse fibroblasts cell cultures grown on PU grafted with polylysine (left) and gelatine (right).

DISCUSSION & CONCLUSIONS: A series of PUs and functionalised PUs were synthesised. The results suggest that plasma treatment with AA is an attractive way to introduce carboxylic groups on PU surfaces and immobilise biomolecules. Moreover the introduction of amino groups or peptides in the PU side chains are promising approaches to insert bioactive molecules. This PUs result good candidates in biomedical application.

REFERENCES: ¹N.M.K. Lamba, K.A. Woodhouse, S.L. Cooper (1998) *Polyurethanes in biomedical Application* CRC Press LLC. ² Ciardelli G, Rechichi A, Cerrai P, Tricoli M, Barbani N, Giusti P. (2004) *Macromol Symp* **218**:261-71.

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