Study of the *in vivo* degradation of polyurethanes 3D foams in the rat animal model

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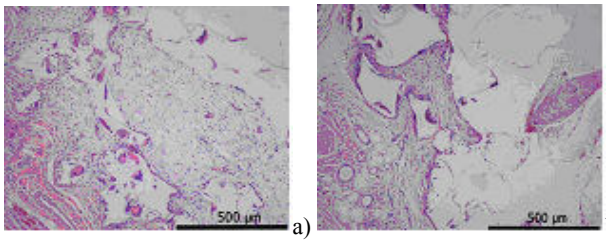
Statement of Purpose: Biodegradable polymer foams are receiving increasing attention as matrices for Tissue Engineering. We have recently set up a process to obtain biocompatible crosslinked PU foams with a controlled range of pore size and open porosity (Tanzi MC. J Appl Biomat Biomech. 2003;1:58) and slow degradation rate. The main advantage expected by the use of polymer materials with slow degradation rate, compared with traditionally used biodegradable polymers, is the slow release of non toxic degradation products which do not evoke aspecific inflammatory response. Two types of PUFs were developed from two different polyether-polyol mixtures (EC and EF, with water uptake, $W_{eq\%}$, respectively = 110 and 600). EF-based foams proved to be more flexible than the EC-based ones, both foam types showing higher compressive properties in the dry state than in the wet state (Farè S. CCT 2003, ISTEC-CNR Eds, 365). *In vitro* cytocompatibility tests with different cell lines (i.e. Saos-2, primary human hosteoblasts and human bone marrow stromal cells) already provided evidence of an adequate PUFs cytocompatibility.
In this work we investigated the *in vivo* behavior of both foams types in the rat model to evaluate the extent of inflammatory reaction and the biodegradation rate.

Methods: Two EC (EC1 and EC2) and one EF (EF1) foams were synthesized as previously described (Tanzi MC. J Appl Biomat Biomech. 2003;1:58).
These PUFs were characterized for density, pore size and open porosity. The foams were sterilized by gas plasma (Sterrad® 100S Sterilization System, Ethicon). To evaluate the possible release of low MW cytotoxic products due to sterilization, the extracts in PBS were analyzed by HPLC and those in culture medium (at 2 and 7 days of incubation) by *in vitro* indirect cytotoxicity assays. These tests were performed using the L929 cell line (density=$5*10^3$ cells/well), and cell vitality was evaluated at 24h by MTT biochemical assay (Sigma, 5655).
For the *in vivo* tests, sterilized discs (Ø=10 mm; h=2 mm) of the PU foams were implanted in the dorsal subcutaneous tissue of CD male rats. According to the ISO 10993-6 standard practice, the implanted samples and surrounding tissues were histologically inspected by hematoxilin and eosin staining at 1, 4, and 12 weeks after the operation.

Table 1: Foams properties.

Foam	Density [g/cm^3]	Ø [µm]	Open porosity [%]
EC1	0,20 ± 0,02	691	35 ± 7
EC2	0,20 ± 0,01	955	74 ± 11
EF1	0,12 ± 0,02	395	95 ± 3

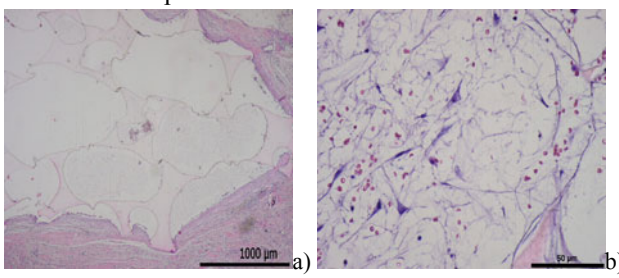
Results/Discussion: The three foams showed different properties (Table 1), EF1 having the lowest density and the highest open porosity, EC2 the highest average pore size.
Gas plasma sterilization increased the open porosity of the foams with a low percentage of open pores (EC1 and EC2). It did not cause degradation of all the foams as no cytotoxic effects of the extracts were observed
The inflammatory response evoked by PU foams after 1, 4 and 12 weeks of implantation was considered physiological at all time points. The presence of several FBGC at the material/tissue interface (Fig. 1a), tissue neovascularization and new peripheral nerves formation (Fig. 1b) were noticed, particularly in the case of EF1 foam.



a) b)

Figure 1. Representative histological images of the implants after 4 weeks: a) EF1; b) EC1.

At both 4 and 12 weeks the EC2 foam seemed to be less filled by connettive tissue (Fig. 2a) than EC1 and EF1, probably due to the high pore size that is a hindrance to tissue colonization. Probably due to its higher hydrophilicity, the EF1 foam was highly colonized by connettive tissue, and also seemed to promote the formation of mesenchimal tissue (Fig. 2b). Histological analyses after 12 weeks of implantation showed that biodegradation, with material fragmentation, begun only for EF1 foam. The next step of the investigation will consider the explants at 26 weeks.



a) b)

Figure 2. Representative histological images of the implants after 12 week: a) EC2; b) EF1.

Conclusions: The described PU foams are demonstrating a good *in vivo* behavior and therefore they represent a promising choice for future application in Tissue Engineering. Particularly, the EF-type foams seem to promote the development of a mesenchimal tissue that, under particular stimuli, could produce a specific tissue, e.g. cartilage and derma or, in association with calcium phosphates, bone tissue.

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