Porous Polyurethane Composites as Scaffolds for Bone Regeneration: Comparison between Micro- and Nano- Sized Hydroxyapatite Fillers

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Statement of Purpose: Composite materials can be used for scaffolds production, thus merging the appealing properties of polymers, in terms of chemico-physical properties and ease of fabrication into 3D porous structures, with mechanical strength and osteoconductive properties of phosphate ceramics [1]. Among polymer matrices, polyurethanes (PU) combine versatility in reagents chemistry, mechanical properties and biological performance with easy processability into porous structures. We have developed PU foams via a gas foaming procedure by addition of water as expanding agent during synthesis, and experimented their use as scaffold able to support cell adhesion [2], and to be integrated in the subcutaneous tissue in the rat model [3]. This work was aimed at investigating the development of PU-based composites by use of micro- and nano- sized hydroxyapatite (HA) fillers, and their potentiality to be used as scaffold for bone regeneration.

Methods: PU-based composites were synthesized with a co-expansion process in the presence of 25% w/w_{polyol} micro-sized HA (A6021, Plasma Technik) [PU-Cmicro] or ad hoc sintered nano-sized HA [PU-Cnano], using MDI prepolymer (Bayer, Germany), a polyether-polyol mixture, FeAA as catalyst and water (2% w/w_{polyol}) as expanding agent, as previously described [2]. A PU-foamed matrix [PU-foam] was produced and used as control. Crystallinity of micro- and nano-sized HA powders was investigated by X-ray diffractometry (XRD, Philips PW 1710) and granulometry by SEM (Stereoscan Cambridge 360). PU-foam and PU-Composites were characterized for morphology at SEM, density, average pore size and open pore percentage. Mechanical compressive properties were investigated under dry and wet (in distilled water, at 37±1°C) conditions. *In vitro* direct cytocompatibility tests were performed with the human osteosarcoma cells line MG63 (ECACC No. 86051601) up to 7 days. Each sample (Ø = 6 mm, h = 2 mm) was seeded with a cell density of 10^4 cells/ml. Cell viability was evaluated by MTT colorimetric assay (M5655, Sigma), and cell morphology was observed at SEM.

Results: As observed by XRD, micro-sized HA showed higher cristallinity when compared to the nano-sized one, whose diffraction pattern showed wider peaks, typical of a nano-sized powder. Physico-morphological properties of the obtained PU-foam and PU-Composites are shown in Table 1.

Table 1: Properties of PU-foam and PU-Composites

	Density [g/cm^3]	Average pore size [µm]	Open porosity [%]
PU-foam	0.081±0.004	255	69±4
PU-Cmicro	0.143±0.007	289	69±4
PU-Cnano	0.127±0.006	271	91±3

The addition of both micro and nano HA increased the material density with respect to the PU matrix, without affecting the expansion process. This was confirmed by the satisfactory values of open porosity and average pore size of PU-Composites.

The presence of micro-HA improved the compression stiffness and strength both in dry and wet conditions ($p > 0.05$). In contrast, nano-sized HA, seemed to weaken the resulting material, if compared to PU-foam and PU-Cmicro ($p < 0.05$). This can be attributed to the high quantity of HA (25% w/w_{polyol}) that, when micro-sized, was adequate for the preparation of the composite, but too high, due to the increased surface area, when nano-sized.

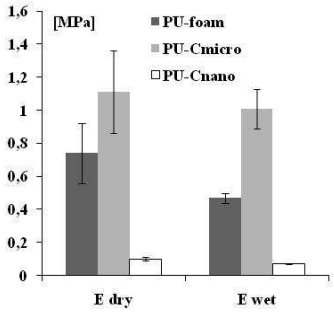

Figure 1: Values of compressive elastic modulus of PU-foam, PU-Cmicro and PU-Cnano in dry and wet conditions

A good *in vitro* cytocompatibility was shown by both PU-Composites, up to 7 days of culture (Figure 2). The MTT assay confirmed a good cell viability onto all the PU scaffolds at every time point.

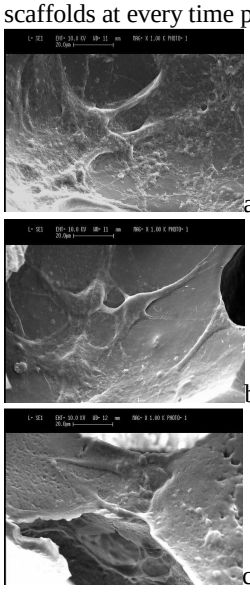

Figure 2: MG63 cell morphology at SEM after 7 days on (a) PU-foam, (b) PU-Cmicro and (c) PU-C nano

Conclusions: With the proposed co-expansion method good results have been obtained in the case of micro HA powder. However, biomimetic composites can be prepared with nano-sized HA fillers. Although our PU-Cnano have shown cytocompatibility, mechanical performance has to be improved. Future developments are focused on the production of PU-Composites with a reduced amount of nano-sized HA, also varying its cristallinity (Progentix Orthobiology BV).

References: [1] Hutmacher DW. J Tissue Eng Regen Med. 2007;1:245-260.
[2] Zanetta M. Acta Biomater. 2009;5:1126-1136.
[3] Farè S. 2007 SFB Annual Meeting, p.190.

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