Biodegradable polyurethane scaffolds for tissue engineering

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Introduction: There is an increasing interest in the use of scaffolds for the repair and regeneration of tissues and internal organs. Depending on the tissue to be repaired, the mechanical properties of such scaffolds may vary, ranging from rigid to elastomeric. With this in mind, a new aliphatic biodegradable segmented polyurethane elastomer was recently developed, whereby its interaction with cells was enhanced by incorporation of a biologically active moiety into the backbone chain. This polymer was further processed into porous scaffolds with potential applications as cancellous bone graft substitutes and for articular cartilage repair.

This paper presents the data on the characterization of the scaffolds produced from this new polyurethane.

Materials and Methods

Polyurethane. The polymer used in the study was synthesized from hexamethylene diisocyanate, poly(ε-caprolactone) diol (MW=530 dalton) and 1,4:3,6-dianhydro-furo(3,2-b)furan-3,6-diol chain extender.

Scaffolds. Scaffolds were produced using a combined phase-inversion-salt-leaching process. The solvents used were dimethylformamide, dimethylsulfoxide, methyl-2-pyrrolidone, acetone, ethanol, isopropanol, tetrahydrofuran and water, and the solid porogene was sodium phosphate heptahydrate with defined crystal sizes in the range 90-140 μm. *Characterization:* The molecular weight and polydispersity index of the polyurethane were determined by size exclusion chromatography; thermal characteristics were assessed by differential scanning calorimetry; porosity was determined from the ratio of the nonsolid volume (pores) to the total volume of material including the solid and nonsolid parts; porous structure was observed with a Hitachi model S-4100 field emission scanning electron microscope operated at 2.0 kV. The scaffolds' water permeability was measured using the Merck permeation apparatus. Compressive strengths at 25% of deformation and Young's moduli were determined on both the dry and wet samples using an Instron tester. For *cytotoxicity* study peripheral venous blood was taken from a group of 18-45 years old healthy volunteers. Leukocytes were isolated by gradient centrifugation in Gradisol G with a density of 1.115g/ml. Five ml of blood were layered on three ml of Gradisol and centrifuged for 25 min at 400xg. The leukocytes from the interface were collected, washed twice with DMEM supplemented with 2% calf serum, and suspended in the medium at a density of $2x10^6$ cells/ml. For cell culture experiments the human lung adenocarcinoma cell line A549 (ATCC CCL 165) and the mouse fibroblast-like cell line L929 (ATCC CCL-1) were used. The cells A549 were maintained in Dulbecco`s modified Eagle's minimum essential medium (DMEM), the L929 cell line in an Eagle's culture supplemented with 10% c.s, 2mM L-glutamine, antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). **Cytotoxicity assay:** MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide colorimetric analysis was used to estimate the cytotoxicity of the polyurethane scaffolds. Cell-culture quality polystyrene foil (Hereaus, Germany) was used as a control.

Results and discussion

The polyurethane scaffolds had an interconnected porous structure with an average pore size in the range of 30 to 100 μm and a pore-to-volume ratio of 85%. The compressive strength was 0.65±0.03 MPa, compressive modulus 2.5±0.15 MPa, permeability 0.05 ± 0.0210^{-11} m^2, density 138±5 kg/m^2 and pore-to-volume ratio 87±1%. The molecular weight of the scaffolds decreased with time they were rinsed in water to remove the solid porogene, yet this did not substantially affect their mechanical properties.

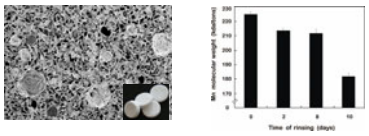

Fig. 1. SEM image of the polyurethane scaffolds used in the study. A macroscopic view is shown in the inset.

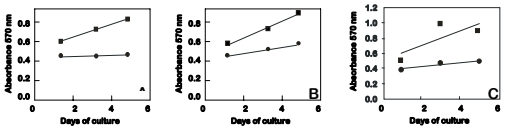

Fig. 2. A. Cytotoxicity of the polyurethane scaffolds determined by MTT assay on: **A.** human peripheral blood leukocytes; **B.** human A549 cells; **C.** mouse L929 cells. ■ - polyurethane; ● - polystyrene.

The potential cytotoxicity of a biomaterial under hypoxic conditions is determined by exposing cell cultures to this material in a low oxygen atmosphere. Subsequent cell survival is determined by the MTT and methylene blue colorimetric assays. The higher the number of surviving cells, the less cytotoxic the material proves to be. In the present study the polyurethane scaffolds turned out to be as good as or better than the control cell culture polystyrene material which is known to be noncytotoxic. These results are in accordance with the data of the parallel *in vivo* study where bone substitutes from the same polyurethane scaffolds were seeded with osteoblasts and tested in the muscle of nude mice. The polyurethane material proved to be biocompatible and promoted new bone formation.

Conclusions: Combined phase-inverse - salt leaching technique allows for the preparation of 3-D microporous biodegradable polyurethane scaffolds with controlled pore sizes and pore-to-volume ratios. The scaffolds have good mechanical properties and undergo elastic recovery under loading. MTT cytotoxicity tests have shown that these scaffolds are noncytotoxic and may be promising candidates for tissue repair and regeneration.