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In vivo biocompatibility and vascularization of biodegradable porous polyurethane scaffolds for tissue engineering

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Abstract

Scaffolds for tissue engineering should be biocompatible and stimulate rapid blood vessel ingrowth. Herein, we analyzed in vivo the biocompatibility and vascularization of three novel types of biodegradable porous polyurethane scaffolds. The polyurethane scaffolds, i.e., PU-S, PU-M and PU-F, were implanted into dorsal skinfold chambers of BALB/c mice. Using intravital fluorescence microscopy we analyzed vascularization of the implants and venular leukocyte–endothelial cell interaction in the surrounding host tissue over a 14 day period. Incorporation of the scaffolds was analyzed by histology, and a WST-1 assay was performed to evaluate their cell biocompatibility in vitro. Our results indicate that none of the polyurethane scaffolds was cytotoxic. Accordingly, rolling and adherent leukocytes in venules of the dorsal skinfold chamber were found in a physiological range after scaffold implantation and did not significantly differ between the groups, indicating a good in vivo biocompatibility. However, the three scaffolds induced a weak angiogenic response with a microvessel density of only ~47–60 and ~3–10 cm/cm^2 in the border and centre zones of the scaffolds at day 14 after implantation. Histology demonstrated that the scaffolds were incorporated in a granulation tissue, which exhibited only a few blood vessels and inflammatory cells. In conclusion, PU-S, PU-M and PU-F scaffolds may be used to generate tissue constructs which do not induce a strong inflammatory reaction after implantation into patients. However, the scaffolds should be further modified or conditioned in order to accelerate and improve the process of vascularization.

Keywords: Scaffold; Polyurethane; Vascularization; Biocompatibility; Dorsal skinfold chamber

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1. Introduction

The primary aim of tissue engineering is the development of biological tissue substitutes that can restore, maintain or improve tissue function [1]. For this purpose, isolated cells may be seeded onto a scaffold, which serves as a three-dimensional matrix for the cells to attach, to proliferate and to finally form a functional tissue construct that can be implanted into the patient. Accordingly, scaffolds for tissue engineering should be biocompatible without inducing a strong inflammatory reaction after implantation into the host [2]. Moreover, they should exhibit a porous structure with interconnecting pores of adequate size to allow a rapid and sufficient vascularization. This is thought to be a major prerequisite for the long-term survival of the cells within the tissue construct [3]. In order

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to fulfil these criteria, the development of novel scaffold types differing in material composition and architecture has become an expanding field in biomaterials research during the last years.

Synthetic scaffolds that have often been used in tissue engineering consist of ceramics such as hydroxyapatite and β-tricalcium phosphate [4–6] or are composed of bioresorbable and biodegradable polymers, in particular poly(α-hydroxyacids) [7,8]. At present, both types of materials are also combined in composite scaffolds [9,10]. However, implanted ceramics have the disadvantage that they are brittle and occupy the space that would otherwise be filled up with viable tissue. On the other hand, scaffolds created from poly(α-hydroxyacids) may undergo plastic deformation under loading, which can be accompanied by a significant size reduction of the scaffold pores. Alternatively, polyurethanes may be used as biomaterials for scaffold fabrication [11]. By modification of their chemical structure, these polymers can be made biostable or biodegradable as well as rigid or flexible. Accordingly, scaffolds based on polyurethanes have already been used for the generation of various tissue constructs such as nerve conduits [12], vascular grafts [13], cartilage [14–16] and cancellous bone graft substitutes [17,18].

The aim of the present study was to analyze in vivo the angiogenic and inflammatory host tissue reaction to three novel types of porous polyurethane scaffolds, i.e., PU-S, PU-M and PU-F. These scaffolds have been developed for tissue engineering by introducing labile units into the stable polyurethane chains, ensuring an almost frictionless integration into the host tissue due to the flexible material properties of these segmented polyurethanes [19]. The polyurethanes were prepared by introducing molecules that influence the surface topochemistry, degradation and mechanical properties of the scaffolds, which in turn could potentially modulate the cell–material interactions. The PU-S scaffold has been used extensively for cartilage tissue engineering. It has unconfined compressive stiffness of ~22–145 kPa depending on its structure and has been shown to be particularly well suited for biomechanical stimulation studies [14]. PU-M is a new polyurethane with mechanical properties close to those of PU-S, and has been synthesized with the objective of developing a faster degrading polyurethane, because of the presence of thiourea chemical functions more sensitive to hydrolysis than the urethane functions [20]. Finally, PU-F was synthesized following a previous study showing that isoprenoid molecules could positively influence cell viability and morphology. PU-F scaffolds have an unconfined compressive of stiffness of ~10–57 kPa [21]. For our experiments, we implanted the scaffolds into the dorsal skinfold chamber, which is a well-established model for the in vivo analysis of biocompatibility, vascularization and tissue incorporation of biomaterials by means of intravital fluorescence microscopy [2,22–24].

2. Materials and methods

2.1. Animals

For our experiments we used 12- to 16-week-old BALB/c mice with a body weight of 22–25 g. The mice were housed one per cage and had free access to tap water and standard pellet food (Altromin, Lage, Germany). All experiments were approved by the local governmental animal care committee and were conducted in accordance with the German legislation on protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85–23 Rev. 1985).

2.2. Scaffold production

Linear segmented polyurethanes were prepared as described previously [19]. Briefly, 1,6-hexamethylene-diisocyanate was reacted with a polyol (poly(ε-caprolactone) diol; 530 g mol^{-1}) and chain extenders in the presence of a catalyst (dibutyl tin dilaurate, 100 ppm) at 80 °C in *N*,*N*-dimethylformamide. 1,4,3,6-dianhydro-D-sorbitol was the chain extender used in the synthesis of the polyurethane PU-S, bis(2-mercaptoethyl) ether in the synthesis of the polyurethane PU-M, and 1,4,3,6-dianhydro-D-sorbitol with 3,7,11-trimethyl-2,6,10-dodecatrien-1-diaminobutane amide were the chain extenders in the polyurethane PU-F synthesis. The reactants were left to react for 1–2 days, depending on the reactivity of the chain extenders and the mixture viscosity. The three different polyurethanes were then precipitated in a 2:1 mixture of water:ethanol and washed extensively in deionized water before drying at constant weight under vacuum.

Scaffolds of the three different polyurethanes were produced by combining phase-inversion and sodium phosphate dibasic salt leaching techniques as previously reported [19]. Briefly, 12 g of polyurethane were added in 85 ml of *N*,*N*-dimethylformamide. After complete polyurethane dissolution, 40 ml of acetone and 5 ml of water were slowly added to avoid precipitation. Subsequently, sodium phosphate dibasic heptahydrate with a particle size range of 200–600 μm (sieved) was added (weight equivalent of polyurethane solution), mixed manually until a homogeneous paste was obtained and poured rapidly in a mould. The mixture was left to set and dry. The salt was leached out by washing the obtained block extensively in warm water. Subsequently, the polyurethane sponge was washed in ethanol and dried under vacuum. In order to produce scaffolds of comparable size, the polyurethane sponge with an initial size of 2 × 10 cm was sliced into 1 mm thick sheets using a water-jet cutting tool. Then, 3 × 3 mm scaffolds were cut into the polyurethane sheets using a razor blade. The scaffolds were washed in ethanol, dried under vacuum and sterilized in a cold-cycle (37 °C) ethylene oxide process and subsequently evacuated at 45 °C and 150 mbar for 5 days.

Structural characterizations of the polyurethane scaffolds were carried out using scanning electron microscopy

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