

THE USE OF BIODEGRADABLE POLYURETHANE SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING: A PRELIMINARY STUDY

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INTRODUCTION: To improve treatment options for articular cartilage injuries, current research has increasingly focused on the *in vitro* generation of cell-polymer constructs for implantation. Biomaterial scaffolds should provide a three-dimensional structure, support growth and activity of the cells and allow for the maintenance of their differentiated phenotype. Various biocompatible materials have been tested for their potential as suitable matrices for generating cartilaginous tissue from seeded cells. The use of synthetic resorbable polymers offers interesting possibilities, because their biochemical and biomechanical properties can be varied and they can be manufactured with a high degree of reproducibility. Stable polyurethanes have widely been used in implantable devices for various biomedical applications. Since recently, experimental biodegradable polyurethanes have gained an increasing interest. The aim of the present study was to evaluate the capability of novel biodegradable polyurethane scaffolds to support attachment, growth and phenotype of chondrocytes *in vitro*.

METHODS: Three-dimensional polyurethane scaffolds with an average pore size of 150 μm and a pore-to-volume ratio of 85 % were cut into discs of 8 mm in diameter and 4 mm in thickness. After sterilization with ethylene oxide, discs were seeded with bovine articular chondrocytes by active cell seeding in spinner flasks. 8×10^6 cells were seeded per scaffold, and cell-polymer constructs were cultured in tissue culture plates for up to 6 weeks. After 2, 14, 28, and 42 days, they were analysed biochemically, biomechanically and histologically. Cell viability was assessed by fluorescence staining. DNA contents of the constructs were measured, and amounts of glycosaminoglycans and total collagen were determined in constructs and in the culture media. Collagen type I and type II were specifically identified by western blot analysis. Gene expression of aggrecan, procollagen type I and type II was quantified by real-time RT-PCR using TaqMan PCR analysis. Compressive stiffness of the constructs was assessed by stress-strain behaviour. For histological evaluation constructs were embedded in methyl methacrylate and sections were stained with toluidine blue.

RESULTS: Fluorescence staining showed a high degree of viability and a uniform distribution of the cells throughout the scaffold two days after seeding. After an initial decrease, the DNA content remained nearly stationary during the culture period. Amounts of glycosaminoglycans and total collagen steadily increased with time in culture, indicating the formation of a new extracellular matrix within the construct. However, up to 80% of the totally synthesized matrix molecules were found in the culture medium. On the transcriptional level, a decrease in aggrecan and procollagen II mRNA expression was noticeable, whereas procollagen I expression was increased, indicating dedifferentiation of the seeded chondrocytes. Western blot analysis also demonstrated that, in addition to the cartilage specific type II collagen, type I collagen was present in the constructs and in the culture media. Toluidine blue staining confirmed the homogeneous distribution of the cells in the scaffolds two days after seeding and the formation of a rich proteoglycan containing extracellular matrix by day 42 of culture. An increase in compressive stiffness was also observed, although the compressive modulus was still ten times lower than that of native articular cartilage.

DISCUSSION & CONCLUSIONS: These preliminary experiments demonstrate that polyurethane based biodegradable scaffolds are able to support attachment and extracellular matrix production of bovine articular chondrocytes. The limitations of the system are the diffusion of large amounts of matrix molecules into the culture medium and the accumulation of type I collagen. Possible strategies to improve and maintain the chondrocytic phenotype of the cells include the modification of the polymer scaffold (pore size, active groups) and the modulation of the chondrocytic activity by specific growth factors. In addition, due to the favourable mechanical properties of the polyurethane matrix, stimulation of chondrocytes by mechanical loading can be considered in order to improve the formation of a functional cartilage-like extracellular matrix.