Differentiation of Human Mesenchymal Stem Cells on Biodegradable Polyurethane Membranes for Tissue Engineering

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Introduction: Autogenous bone chips are often used to promote healing of critical-size segmental defects. When placed in a bone defect, the chips tend to migrate away from their initial location. Hence, it would be beneficial if they were kept in place by using a resorbable membrane surrounding the chips. Such a membrane can be produced from polyhydroxyacids and/or from biodegradable segmented polyurethanes. The biological activity of such membranes can be promoted by seeding osteogenic cells.

The aim of this study was to determine whether membranes made from biodegradable polyurethane promote the growth of human mesenchymal stem cells and their differentiation into osteoblasts.

Materials and Methods: Porous polyurethane (PU) membranes (Fig. 1a) with a thickness of 200 μm were cut into discs with a diameter of 8 mm and fixed between two polytetrafluoroethylene (PTFE) rings, to prevent the membrane from floating in the culture medium. The rings with membranes were packed in double pouches, sterilized using a cold-cycle ethylene oxide (ETO) process and subsequently evacuated at 50ºC and $3x10^{-1}$ mbar for 10 hours. Human mesenchymal stem cells (HMSC) were seeded onto the surface of the membranes and onto the surface of the cell culture polystyrene dish (monolayer) which served as a control. Cells were seeded at a density of 50000 cells/well (in a 24-well plate) and were cultured in an incubator for 1, 14, 21 and 28 days in a complete culture medium (αMEM supplemented with 10% FCS, 1% pentomycin-streptomycin, non-essential amino acids, and 100 μM L-ascorbic acid 2-phosphate). After 2 days, 10mM β-glycerophosphate and 10nM dexamethasone were added. The medium was changed three times per week. Cell morphology was observed with SEM. Calcein/EthD1 staining was used to evaluate cell viability. Cell growth and differentiation were assessed by measuring DNA, alkaline phosphatase (ALP) activity and mRNA expression levels. Labeling with radioactive Ca^{45} was used to determine mineral deposition.

Results and Discussion: The cells adhered and spread on the PU membranes (Fig. 1b). Calcein/EthD1 staining proved the high viability of the cells cultured on the PU membranes (data not shown). The amount of DNA and ALP activity as well as ALP mRNA expression increased with time of culture (Fig. 2). The cells underwent calcification (data not shown). The results of the study demonstrate that porous polyurethane membranes promote growth, spreading and differentiation of HMSC into osteoblasts. Such membranes can potentially be used in the clinic to stabilize bone chips or synthetic granular biomaterials at the defect site. Moreover, when seeded with HMSC they may additionally promote healing of bone defects.

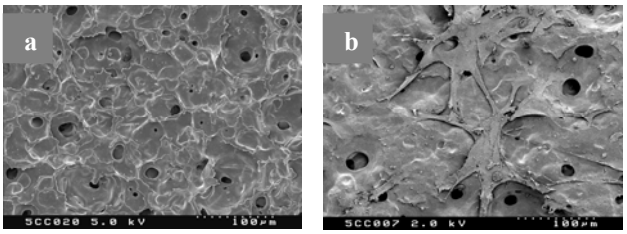

Fig. 1. Original surface of the polyurethane membrane (a); HMSC on the surface of the PU membrane after 48 h of culture (b).

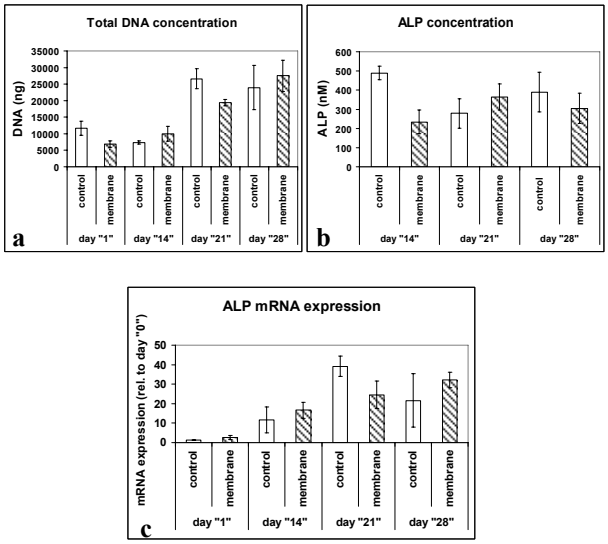

Fig. 2. DNA (a), ALP activity (b) and ALP mRNA expression (c) on the polyurethane membranes and monolayer culture (control) seeded with HMSC at various times of cell culture.

Conclusions: Microporous membranes made from biodegradable polyurethane promote attachment, differentiation and proliferation of human mesenchymal stem cells. The membranes might potentially be used as protective covers to keep autogenous bone chips at the required location. Seeding of the membranes with osteogenic cells might additionally promote bone healing.