SYNOVIAL FIBROBLASTS, BONE MARROW MSC OR CHONDROCYTES COMBINED WITH CHONDROGIDE FOR MENISCAL REPAIR

**Abstract**

The long term repair of meniscal lesions is still unsatisfactory. Successful hyaline cartilage regeneration using the ACI technique in combination with ChondroGide (CGide) membrane prompted us to examine whether CGide could be developed for fibrocartilage tissue-guided regeneration in surgical treatment of meniscal defects. CGide is a collagen/III membrane produced by Geistlich Pharma. We have initiated an in vitro study to identify the most clinically relevant cell source for meniscal repair. Several cell candidates, including primary chondrocytes, synovial membrane fibroblasts (SMF) and bone marrow derived mesenchymal stem cells (MSC) may contribute towards fibrocartilage formation in combination with CGide. We have established cell cultures allowing adhesion, proliferation and differentiation of all three cell types on CGide. Gene expression and matrix synthesis were comparatively analysed by real-time RT-PCR and histology from cells cultured on CGide under cartilage differentiating and control conditions.

**Methods**

Human cartilage and synovial membrane were collected at autopsy in accordance with ethical regulations of Canton Bern, Switzerland. Bone marrow was obtained at the Inselspital Bern, Switzerland with the appropriate approval. Cartilage and synovial membranes were enzymatically digested to liberate cells, and MSC were isolated via Ficoll gradient centrifugation followed by attachment to plastic. All cell types were cultured in monolayer, in pellet cultures and on CGide either in media containing 10% FCS or in serum-free media supplemented with ITS+, 100 nM dexamethasone, 50 µg/ml ascorbic acid and 10 ng/ml TGFβ1. TaqMan real-time PCR was performed and monitored using the ABI Prism 7700 Sequence Detection System. Analyzed genes included TGFβ1, COL1A2, COL3A1, SOX9 and COMP. Comparative Ct method was used for analysis and 18S RNA served as internal control for cDNA input. Proteoglycan synthesis was evaluated via Alcian blue and Safranin O staining, and collagen deposition via Masson Trichrome staining.

**Results Section**

To establish cell culture on CGide membranes, cells were seeded on the rough side of the membrane and allowed to adhere and subsequently proliferate for three weeks. Subsequently chondrogenesis was induced in CGide cultures, and monolayer and pellet cultures were prepared in parallel. Fibrocartilage formation was analyzed after three weeks. All cell types were able to attach, proliferate and form cell layers to surround the CGide. The newly formed cartilaginous tissue had fibrous-like appearance. Synovial fibroblasts most closely penetrated the membrane and re-arranged its architecture. Alcian blue staining and Masson’s Trichrome staining indicated the presence of highly sulphated glycosaminoglycans and collagens in all samples, albeit only weakly in samples cultured in 10% FCS (Figure 2). Pellet cultures from all cell types stained positive for Alcian blue, Safranin O and Masson Trichrome indicating cells’ chondrogenic capacity. In contrast, no positive Safranin O staining was observed in CGide samples suggesting the production of less mature proteoglycans.

**Discussion**

The aim of this study was to evaluate three cell candidates which could be combined with CGide membrane to produce fibrocartilage for meniscal repair. We could demonstrate that SMF, MSC and chondrocytes adhered, proliferated and differentiated on CGide thereby confirming that CGide represents a suitable scaffold for cell growth and differentiation. The interaction between cells and scaffolds is an interactive process and it was reflected in different gene expression profiles of three cell types analyzed. Our preliminary data indicated that SMF have most potential for cartilage formation when grown on CGide in the presence of TGFβ1. To further investigate whether SMF are the most suitable cells for fibrocartilage tissue formation required for meniscal repair, our ongoing efforts comprise studies of growth factors and cytokines, including matrix degradation enzymes, produced by three cell types grown on CGide.

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