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Biodegradable gelatin microcarriers in tissue engineering

In vitro studies on cartilage and bone

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ABSTRACT

Tissue engineering is a multidisciplinary field that combines cells, biomaterial scaffolds and environmental factors to achieve functional tissue repair. This thesis focuses on the use of macroporous gelatin microcarriers as scaffolds in tissue engineering applications, with a special focus on cartilage and bone formation by human adult cells *in vitro*.

In our first study, human articular chondrocytes were seeded on macroporous gelatin microcarriers. The microcarriers were subsequently encapsulated in coagulated blood-derived biological glues and cultured under free-swelling conditions for up to 17 weeks. Even in the absence of recombinant chondrogenic growth factors, the chondrocytes remained viable and metabolically active for the duration of the culture period, as indicated by an increased amount of cell nuclei and extracellular matrix (ECM). The ECM showed several cartilage characteristics, but lacked the cartilage specific collagen type II. Furthermore, ECM formation was seen primarily in a capsule surrounding the tissue-engineered constructs, leading to the conclusion that the used *in vitro* models were unable to support true cartilage formation.

The capacity of human dermal fibroblasts to produce cartilage- and bone-like tissue in the previously mentioned model was also investigated. Under the influence of chondrogenic induction factors, including TGF-β_1 and insulin, the fibroblasts produced cartilage specific molecules, as confirmed by indirect immunohistochemistry, however not collagen type II. Under osteogenic induction, by dexamethasone, ascorbate-2-phosphate and β–glycerophosphate, the fibroblasts formed a calcified matrix with bone specific markers, and an alkaline phosphatase assay corroborated a shift towards an osteoblast like phenotype. The osteogenic induction was enhanced by flow-induced shear stress in a spinner flask system.

In addition, four different types of gelatin microcarriers, differing by their internal pore diameter and their degree of gelatin cross-linking, were evaluated for their ability to support chondrocyte expansion. Chondrocyte densities on the microcarriers were monitored every other day over a two-week period, and chondrocyte growth was analyzed by piecewise linear regression and analysis of variance (ANOVA). No differences were seen between the different microcarriers during the first week. However, during the second week of culture both microcarrier pore diameter and gelatin cross-linking had significant impacts on chondrocyte density.

Lastly, a dynamic centrifugation regime (f=12.5 mHz for 16 minutes every other day) was administered to chondrocyte-seeded microcarriers, with or without encapsulation in platelet rich plasma (PRP), to study the possible effect of dynamic stimuli on cartilage formation. Presence of PRP enhanced the structural stability of the tissue-engineered constructs, but we were not able to confirm any dose-response pattern between ECM formation and the applied forces. After 12 weeks, distinct gelatin degradation had occurred independent of both dynamic stimuli and presence of PRP.

In summary, this thesis supports a plausible use for gelatin microcarriers in tissue engineering of cartilage and bone. Microcarrier characteristics, specifically gelatin cross-linking and pore diameter, have been shown to affect chondrocyte expansion. In addition, the use of human dermal fibroblasts as an alternative cell source for cartilage and bone formation *in vitro* was addressed.