

Development of Bioreactor Technology for Tissue Engineering of Human Cartilage

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The aim of cartilage tissue engineering is to generate functional, three-dimensional cartilage constructs *in vitro* using cell culture techniques. Typically, chondrocytes attached to polymer scaffolds are cultivated under conditions that promote cell differentiation and the synthesis of extracellular matrix (ECM). The polymer mesh degrades during cell culture to leave a cartilaginous tissue construct.

A long-term objective of cartilage tissue engineering is to produce transplantable tissues for treatment of injured or diseased joints and for use in plastic and reconstructive surgery. However, engineered cartilage has additional applications in a range of areas, including as a substitute for animals in toxicity trials, in animal-free medical research, and as a production system for therapeutic compounds and growth factors synthesised exclusively in differentiated tissues. The goal of most tissue engineering studies is to develop three-dimensional tissue constructs *in vitro* with the same biochemical, structural and mechanical characteristics as the natural tissue or organ. So far, this goal has not been realised for cartilage. Accordingly, new approaches to improving the properties of tissue-engineered cartilage are required.

Being an avascular tissue, adult cartilage does not have ready access to the nutrients, growth factors, oxygen supply and waste removal functions of the blood stream. Cartilage therefore has very limited capacity for self-repair within the body. A key factor affecting the success of cartilage tissue engineering is whether culture conditions appropriate for cell differentiation and rapid ECM synthesis can be provided *in vitro*. Several aspects of the physical culture environment, such as mixing, hydrodynamic regime, mechanical pressure and oxygen transfer, are known to play important roles in cartilage development. Because these conditions are relatively easily controlled and monitored in bioreactors, significant improvements in the quality of tissue-engineered cartilage may be obtained using bioreactor technology.

In this work, chondrocytes isolated from human fetal tissue were expanded in monolayer culture, then seeded under dynamic conditions on to 15-mm-diameter polyglycolic acid (PGA) mesh discs. The cells were cultivated in two sets of triplicate recirculation column bioreactors that allowed perfusion of medium through the tissues during cartilage development. Each growth chamber was constructed from a short length of glass tube of about 1.5 cm i.d. with a flange opening mid-way along the tube. The PGA disc seeded with chondrocytes was fitted into the flange opening and the tube sections clamped together. Medium from six separate stirred reservoirs was recirculated through the chambers using peristaltic pumps and silicone tubing. In some cultures, a dissolved oxygen electrode was fitted into the recirculation line to measure the properties of the medium leaving the constructs. The bioreactors were placed in an incubator operated at 37°C with a gas atmosphere of 5% CO₂ in air. After 5 weeks of culture, the constructs were harvested and the cell, glycosaminoglycan (GAG) and collagen contents of the tissues were measured and their histological properties examined. In selected experiments, properties of the culture medium, such as the concentrations of lactic acid, sugar and GAG, were also measured.

Cartilage constructs produced in the bioreactors were creamy-white, glossy, and firm to the touch due to the production of cartilage ECM. The principal ECM components, GAG and collagen type II, were present in the tissues and the cells were located in lacuna. A wide range of culture conditions, including the cell seeding density, the thickness and orientation of the PGA

mesh, and the medium flow rate, flow direction and withdrawal-and-replacement program, was found to influence the quality of bioreactor-produced cartilage. Non-uniform distribution of chondrocytes in the scaffold after cell seeding was identified as an important problem resulting in the heterogeneous distribution of extracellular matrix within the tissues. To address this issue, cultures were carried out using separately-seeded PGA scaffolds sutured together in several orientations and combinations. In addition, to enhance oxygen and nutrient transport to the cells and to distribute the hydrodynamic pressure and shear effects more uniformly, the direction of medium flow in the bioreactors was periodically reversed. These strategies resulted in the production of constructs that were cartilaginous throughout the entire tissue cross-section. To date, tissue-engineered cartilage containing up to 1.25% GAG, 0.75% collagen and 90% water has been produced. Further work is currently in progress.