495g Bioreactor Optimization of Scaffold Seeding for Cartilage Tissue Engineering

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Cartilage tissue engineering is a promising therapeutic alternative to current treatments for arthritis and osteoarthritis, and also offers a non invasive method for testing new drugs and therapies. Clinically relevant tissues can be developed through controlled in vitro cultivation of chondrocytes seeded on biodegradable scaffolds in bioreactors. The fluid environment within the bioreactor affects the quality of cell-scaffold constructs, and thus their potential use as implants. The current study focuses on the effects of the fluid environment on cell seeding using the wavy-walled bioreactor (WWB), which provides a wide range of hydrodynamic forces. Its unique design enhances mixing at low fluid shear, and is characterized by fluid recirculation and higher axial mixing than that in a conventional spinner flask (SF). These differences result in increased rates of formation and size of chondrocyte aggregates in suspension cultures in the WWB.

The seeding of chondrocytes onto scaffolds is a crucial step in cartilage construct cultivation that requires efficient cell attachment to scaffolds, uniform spatial distribution of cells, and fast kinetics. All of these requirements are affected by the hydrodynamic environment; mixing increases seeding efficiency, turbulent flow provides more uniform cell distribution in scaffolds, and chondrocyte aggregation increases cell seeding kinetics. We have characterized the hydrodynamic environment within the bioreactors using computational fluid dynamics (CFD) modeling by simulating the 3-D velocity fields around the scaffolds and evaluating the shear-stress applied on scaffolds for the bioprocessing conditions. The kinetics of chondrocyte attachment to polyglycolic acid (PGA) scaffolds was investigated at three seeding densities (2.5, 5 and 10×10^6 cells/construct) for scaffolds positioned in three hydrodynamic zones inside the bioreactors: (i) in the center of WWB, (ii) in the lobes of WWB, and (iii) in the center of SF. Three independent experiments, each with 3-day parallel cultures encompassing all 9 combinations of bioreactor geometry/seeding density were carried out. Seeded cell-scaffold constructs were sampled at 1, 2 and 3 days for biochemical and histological analyses. The average, maximum, and minimum levels of shear stress applied on the construct were correlated to the chondrocyte seeding data obtained from experiments. We investigated the following construct properties, which are likely to be modulated by the hydrodynamic environment during cell seeding: (a) construct cell density (DNA), (b) cell spatial distribution, (c) GAG deposition.

In order to optimize cell seeding, a model-driven bioreactor optimization method was utilized for seeding chondrocytes on scaffolds. This method consists of a two layer modeling framework, the first one being a hydrodynamic model based on CFD simulations, and the second an Artificial Neural Network (ANN) model for correlating cell seeding data with hydrodynamic parameters. The hydrodynamic parameters and the experimental data obtained from our seeding studies were used for the generation of the ANN model. This model was used in a constrained nonlinear programming approach to optimize the operating conditions and tailor the cultivation of cartilage constructs. The optimum operation conditions suggested by this model were experimentally validated. The experimental results demonstrate that this method improves the success of cell seeding, and suggests that further optimization can be achieved with more sophisticated models that include the prediction of oxygen and nutrient distribution in the bioreactor.

The ANN model allows for fast and cost-efficient optimization of tissue engineering bioreactors, significantly reducing the number of necessary experiments for tissue cultivation. This study represents the first step in the development of a tool capable of predicting the optimal bioprocessing conditions for a given set of desired tissue properties.