A Systematic Method to Quantify Cell Viability in the Bio-Manufacturing Process

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Introduction: Bio-manufacturing is a method to incorporate living cells in the fabrication of bio products, such as the cell-encapsulated scaffolds used in tissue engineering. Incorporation of cells is limited by harsh conditions present during the manufacturing process. Specifically, cells are subjected to sustained mechanical forces such as hydrostatic pressure and shear stress. Within a physiological range, these forces elicit both acute and chronic adaptive responses. When the forces exceed certain thresholds, cell damage results. Cell viability varies with the bio-manufacturing process, and depends upon cell manipulation techniques, cells types, and process parameters. Although cell viability can be measured and investigated experimentally, a systematic method to relate cell viability to bio-manufacturing process parameters has not yet been well documented in the literature. Such a method is essential for reproducible control over the distribution of living cells within the bio-products, for example, for tissue scaffolds to mimic the highly organized cells and cell aggregates in native tissues.

Methods: Our method consists of three steps. The first step is to monitor the response of cells to given hydrostatic pressures and shear stresses and, on the basis, to develop experimental models for cell damage. Specifically, pressurized air with a controllable magnitude is applied to the cell culture stored in a container; and in the application of shear stress, the cell culture is sheared under torsion flow within the gap between a cone and a plate of a rheometer. Cell viability after varying levels and durations of applied force are measured and, based on the results obtained, the relationship between the percent cell damage and mechanical forces can be established. The second step is to represent the forces and their duration experienced by cells during the bio-manufacturing process, based on the fundamentals of the fluid flow involved in this process. The outcome of this step allows for the representation of the process-induced forces and their duration in every cell path in the process. Thirdly, based on the results from the above two steps, the percent cell viability in every cell path can be determined, and then the cell viability in the process is obtained by integration.

Experiments and Results: Sodium alginate (Sigma, St. Louis, MO) was dissolved in water to form a 6% (w/v) solution. Schwann cells and 3T3 fibroblasts (ATCC, Manassas, VA) were grown in DMEM supplemented with 5% fetal bovine serum and 1% antibiotic. The cells were then incubated at 37°C in a humidified atmosphere of 95% air and 5% CO2. Immediately prior to the use in experiments, cell cultures were blended with alginate solution to form 50% (v/v) cell suspensions. The cell suspensions were applied by hydrostatic pressures and shear stresses for varying time periods; and the percents of cell damage were measured for establishing the relationship between the percent cell damage and the applied forces. Next, 2 mL of cell suspension was loaded into the syringe of our bio-manufacturing system [1] for dispensing. In the experiments, the process parameters (i.e., air pressure to the cell suspension) and the structure parameters (i.e., the size and geometry of the needles for dispensing) were altered; and the percent cell damage was measured for each of the parameter settings. Meanwhile, the percent cell damage was predicted by using the systematic method outlined previously. The agreement between the experimental results and predicted values shows the effectiveness of our method. Part of the results obtained has been reported in our recent publication [2]. This abstract reports another promising result (Figure 1), showing the influence of needle geometry on cell damage in the bio-manufacturing process. In the experiments, two types of needle geometry, i.e., cylindrical and tapered, were used for dispensing cell suspensions at a same flow rate. The results shown in Figure 1 illustrated that tapered needle lead to less cell damage. This suggests that, in order to preserve cell viability in the bio-manufacturing process, the use of the tapered nozzles should be preferable than the use of cylindrical ones. Figure 1 also shows the agreement between the experimental results and the predicted values by means of our method, regardless of the variation of needle geometry.

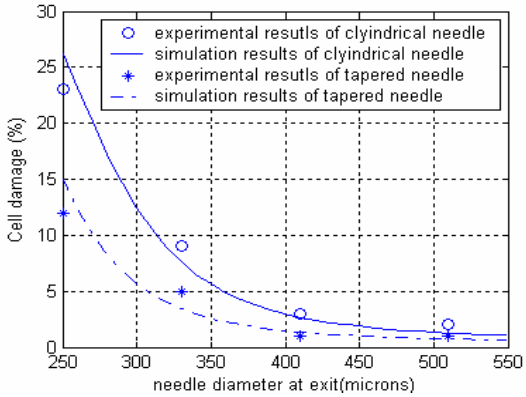

Figure 1. Schwann Cells damage in the bio-manufacturing process

Conclusions: Cell damage is one key issue in the bio-manufacturing process. The percent cell damage can be well represented by using the systematic method outlined in this abstract. The experimental results show that the needle geometry has a significant influence on the cell damage rate. Tapered nozzles cause less cell damage than cylindrical ones at the same flow rate, suggesting that tapered needle is preferable in the dispensing-based bio-manufacturing process in order to preserve cell viability.

References:

[1] Chen XB, *ASME J. of Manu. Sci. and Eng.*, 2008, 130(2): 21003-1-7.
[2] Li MG, *Tissue Eng.* 2009 (in press).