Combinatorial Screening of Chondrocyte Response to Tissue Engineering Hydrogels

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Introduction: Tissue engineering strategies for cartilage repair rely on the use of chondrocytes, growth factors, and an engineered extracellular matrix. We have applied combinatorial methods to investigate the interplay between these variables in determining the proliferation and differentiation of fetal bovine chondrocytes in an *in vitro*, three-dimensional cell culture model. Cell culture can be carried out using defined media containing insulin-like growth factor-I (IGF), basic fibroblast growth factor (b-FGF), and transforming growth factor-beta (TGF-β), which are all known to be crucial for promoting chondocyte proliferation and differentiation.[1] The matrix used also can play an important role, [2] and in our experiments we have screened collagen type I and alginate as potential hydrogels. These variables will be screened using design-of-experiment methods to characterize the response surface of this system.

Experimental: We have used design-of-experiment methods in order to understand the interplay between the variables of cell density, growth factor concentrations, and extracellular matrix. Fetal bovine chondrocytes were cultured for three days in defined media conditions containing Dulbecco's modified Eagle's medium (DMEM, BioWhittaker), IGF-I (Sigma), b-FGF (Sigma), and TGF-β (Sigma), and 4 mM $CaCl_2$. Mixtures of the growth factors were added to the well plates prior to the addition of cells or matrix polymers. Cell culture was performed by seeding 10,000 chondrocytes in 96-well plates (Daigger) using 5% alginate (FMC Biopolymers) and 5% collagen type I (Sigma) solutions as three-dimensional matrices. Alginate was dissolved in phosphate buffer solution and mixed with the cell suspension prior to addition to the well plates. Acetic acid solutions of collagen were neutralized in 1 mM NaOH solution and allowed to gel at 37 ºC for 5 min prior to addition to the well plates. Total volume was 200 μL.

All permutations of the following variables were analyzed for both hydrogels and equimolar mixtures of the two:

Variable	High value	Low value
Cell number	10,000	1,000
IGF (ng/mL)	100	1
TGF-b (ng/mL)	1	0.01
b-FGF (ng/mL)	10	0.1

The DNA content of each well was measured to assess chondrocytes proliferation using the CyQuant Cell Proliferation Assay Kit (Molecular Probes). Production of extracellular matrix components such as collagen type II and aggrecan was measured using real-time polymerase chain reaction (RT-PCR) to assess differentiation. RNA was extracted for RT-PCR analysis using 800 μL Trizol reagent (Invitrogen) and the concentrations analysed with the Bio-Rad iCycler (Bio-Rad).

Results: The proliferation data were analyzed using standard methods of statistical analysis. A full-factorial design was used, which allows complete characterization of all single variables as well as interactions between multiple variables. Influence of single variables and coupling between variables is measured by averaging the difference in responses over all conditions. As an example, the influence of cell concentration on proliferation is measured by taking the average of all cell proliferation across all the other variables at high cell concentration. From this, the average of the cell proliferation across all the other variables at low cell concentration is subtracted. In a similar manner, the influence of coupling between two variables (e.g. IGF-I concentration and cell concentration) can be estimated. Shown in Figure 1 is a table of differential response for chondrocyte proliferation in alginate hydrogels.

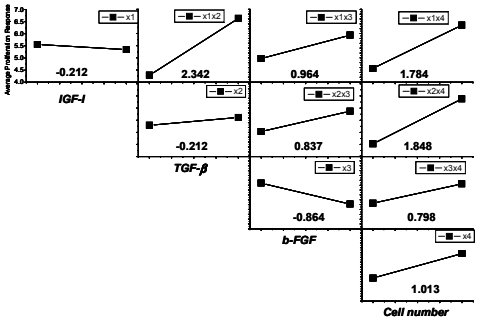

Figure 1: Proliferation response in alginate hydrogels.

Conclusions: The strongest variable is cell number but growth factor contributions are significant and there appear to be strong synergistic effects between all of them. The relative influence of the growth factors changes in the various hydrogels, suggesting specific interactions between the matrix and cells or growth factors is capable of enhancing the performance of tissue engineered medical products.

References:

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