3D Cell Culture Microenvironment: Rationale For Material Selection and Method of Fabrication
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Introduction
Presented here is a rationale for creation of a three-dimensional, biomimetic cell culture material based on principles of cell biology and cellular turgor.¹ The biomimetic hydrogel described is intended for use as an in vitro research tool as well as an in vivo cell and / or drug delivery platform. Constituent materials for this construct were chosen for their structural attributes as well as their inherent biologic properties supporting the chondrocyte phenotype.² ³ Fluorescence microscopy and RT-PCR are presented as methods for in situ study of cells embraced by the 3-D culture system.

Materials and Methods
Acid form hyaluronan (HY⁺) (Mw=633,000) and chitosan (CT-NH₃⁺) (Mw=430,000) were filter sterilized, lyophilized, blended as dry particles at mass ratio of HY⁺=1: CT-NH₃⁺=1.44 and hydrated with dextran at 15µL / 1.0mg. 500µL dextran, containing 25x10⁶ C8161 malignant melanoma cells, hydrated 33 mg HY⁺-CT-NH₃⁺ dry blend to create a malleable, cylindrical matrix within 2.5 minutes of hydration (Fig. 1). Insoluble polyelectrolytic complex (PEC) fibers of HY⁺ and CT-NH₃⁺ formed immediately after hydration (Fig. 2).⁴

Results
Fluorescence microscopy and RT-PCR were investigated as means for studying cell behaviors in situ. Fig. 3a is a differential interference contrast (CID) image of C8161 cells attached to HY-CT-PEC fibers (60x objective). Fig. 3b is a frozen section image of C8161 cells treated with calcein-AM immediately after HCP-h matrix formation.

Discussion and Conclusions
This 3D cell culture matrix, known by the acronym HCP-h, is an irreversible hydrocolloid that relies on self-assembled, insoluble PEC fibers, the dispersion phase, to provide structural and mechanical properties for the matrix. Hydrocolloid formation is simple, rapid and accomplished without use of potentially toxic crosslinking agents.

Constituent materials (unreacted HY⁺ and CT-NH₃⁺) and mechanical properties of the matrix initiate biochemical cues supporting the chondrocyte phenotype and can act in co-operation with soluble signaling factors to effect differentiation of pluripotent cells toward a specific phenotypic destination. HY and CT are US FDA approved at the Class III level (HY) and the Class II level (HY and CT), making both attractive candidates for human and veterinary therapeutic applications.

References

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