Multilayered Chitosan and Hyaluronan Hydrogels

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Statement of Purpose: Currently hydrogel beads can be made using several methods, including solvent exchange, agglutination, fluctuation, and pH shifts[1,2]. However, all of these methods yield beads consisting of a single, homogeneous form. The goal of this work was to develop and assess multilayered chitosan/hyaluronan beads that have the potential to act as delivery vehicles for cells, vaccines, and/or chemotherapeutic agents.

Materials and Methods: Multilayered hydrogels were made using a Var V1 electrostatic bead generator (Nisco Engineering, Zürich, CH) capable of producing beads as small as 150 µm. The multilayered beads were created by first dissolving 3 wt% chitosan (Acros Organics, Geel, BE) in diH_20 and 1% acetic acid (Fisher, Hampton, NH, US). In a separate container, 0.03 wt% hyaluronan (Fluka, St. Louis, MO, US) was dissolved in diH_20 with 2% acetic acid. After the two solutions were dissolved via 30°C heat and stirring, the two solutions were mixed together at room tempeature at a ratio of 1ml of 0.03% hyaluronan solution to 10ml of 3% chitosan solution. This new mixture, with both hyaluronan and chitosan, was then immediately placed in a syringe and was dripped into the electrostatic bead generator using a syringe pump. The syringe pump was set to 5 ml/hr while the electrostatic bead generator was set to 7 KV and 10mm separation. As the beads dripped from an 18-gauge needle, they were collected in a 0.5 N NaOH (Fisher) stirred bath, where the beads gelled and retained their droplet shape. After the beads formed in the NaOH bath, they were removed and washed with phosphate buffered saline (PBS) (Cellgro, Manassas, VA, US). Once the pH of the solution was below 8, lactic acid (Sigma, St. Louis, MO, US) was added drop-wise into the solution with the beads, while stirring, until the pH was around 6.5. The solutions were then left for 10 hours. Afterwards the solution was exchanged with PBS several more times until the pH reached 7.4. These beads were tested with D1 (ATCC CRL-12424) murine multipotent bone marrow stromal cells. The cells were seeded at 2000 per hydrogel bead in a low attachment 6-well plate (Corning, Manassas, VA, US) and placed on an orbital shaker. Cells from the same cell line were also tested with control hydrogels fashioned without the multilayered technique. After 8 days of culture, the deoxyribonucleic acid (DNA) concentration was measured using a Quant-iT Picogreen assay (Invitrogen, Carlsbad, CA, US).

Results: The acid bath treatment removed the chitosan material from the outer layer of the hydrogels. When compared to a single layered bead, a distinct difference can be seen (Figure 1A).

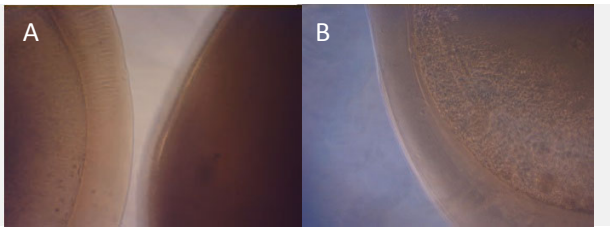

Figure 1 (10X objective): A) Image of multilayered hydrogel on left, control hydrogel on right B) Image of D1 cells growing on a multilayered hydrogel

The multilayered hydrogel scaffolding allows the cells to grow between the two layers (Figure 1B). Cells were also grown on control beads and the DNA concentrations of each hydrogel type were compared. With triplicate samples, and a two-tailed Student's t-test, the results were found to be significantly different, with a P-value < 0.01. Results for these data are shown in Figure 2.

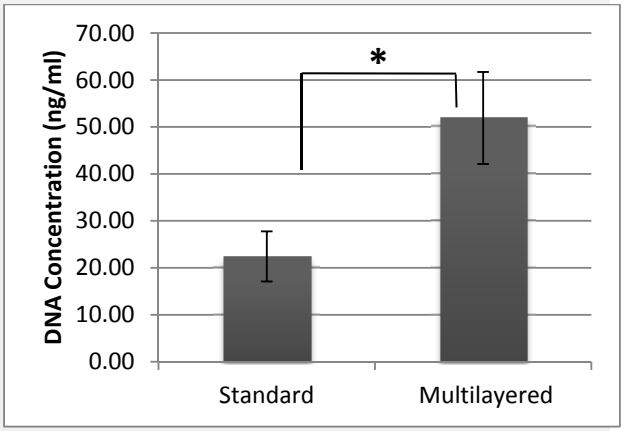

Figure 2: Picogreen results suggest a significantly higher number of D1 cells growing on the multilayered hydrogels

Conclusions: The results indicate cells attach and grow between the two layers of the multilayered hydrogel beads. This feature can be useful for cells that are sensitive to shear stress forces or for encapsulation techniques. The two layers consist of different concentrations of components. The inner core consists of chitosan and hyaluronan while the outer layer consists of mostly hyaluronan. This characteristic gives each layer unique solubility and hydrophilic properties, allowing the potential to load and retain therapeutic agents, growth factors, or other additives.

References:

1:Brannon-Peppas L, Peppas N. Equilibrium swelling behavior of pH-sensitive hydrogels. Chemical Engineering Science. 1991;46(3):715-22.
2: Suh F, Matthew H. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. Biomaterials. 2000;21(24):2589-98.

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