In Vitro Growth and Viability of Cells Encapsulated within Hyaluronic Acid-based Hydrogels

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Statement of Purpose: The goal of this study was to test the hypothesis that encapsulation within Hyaluronic Acid (HA)-based hydrogels can support the viability and growth of human cells. Growth factor-containing hydrogels composed of covalently crosslinked, chemically modified HA, gelatin (Gtn), and heparin (Hp) have been shown to promote neovascularization, and therefore have potential to provide a scaffold for therapeutically implanted cells. However, survival of encapsulated cells *in vivo* remains problematic, possibly because of toxicity of the hydrogel components, or because diffusional limitations due to a lack of initial vasculature in and around the gel implant lead to cell starvation. Accordingly, these experiments investigated the growth characteristics and maximum achievable density of the human cell line HEK293 when seeded into HA-based hydrogels *in vitro*.

Methods: HEK293 cells were transfected with the plasmid pDsRed1-Cl to constitutively produce red fluorescent protein. Thiol-modified HA, Gtn and Hp (HA-DTPH, Gtn-DTPH and Hp-DTPH) were prepared as previously described[1,2]. HA-DTPH and Gtn-DTPH solutions were mixed in ratios of 100:0, 90:10, 80:20, and 50:50 (w/w), with 0.3% (w/w) Hp-DTPH when desired. 10.7 µl (for 25 µl gels) or 42.6 µl (for 100 µl gels) of this solutions were aliquotted into 96 well plates, and HEK293 cells added at a series of initial seedings from 5000 – 800,000. For growth factor experiments, 25 ng (for 25 µl gels) or 100 ng (for 100 µl gels) of vascular endothelial growth factor (VEGF) and/or keratinocyte growth factor (KGF) was added to each well. Gels were formed around the cells by crosslinking with sufficient PEGDA to achieve a 1:2 ratio of acrylate to thiol functionalities and a final gel concentration to 1% (w/v). The solutions were mixed by gentle pipetting and allowed to gel for 30 minutes at room temperature.

Cell concentration within the hydrogels was measured every 24 hours. Using a fluorescent plate reader, cells were excited at 536 nm and their emission measured at 595 nm. This reading was compared to a standard to determine the number of cells in each well. Statistical significance between samples was determined with two-way ANOVA and Fisher's post-hoc PLSD analysis, with significance taken at $p < 0.05$.

Results/Discussion: HEK293 cells could be successfully transfected (figure 1), and stably viable cell lines established. Subsequently, recombinant cells survived the gelation process and could be viably encapsulated within HA:Gtn hydrogels containing Hp, VEGF, and KGF, as well as all combinations of the three additives. In all cases, the cell concentration remained higher than that of unencapsulated control cells ($p < 0.05$). Cell viability was not affected by gel thickness, as growth characteristics remained similar for cells encapsulated in 100 µl hydrogels compared to those encapsulated in 25 µl hydrogels. In addition, growth characteristics were similar for different initial seed densities, regardless of gel thicknesses ($p > 0.1$).

The HA:Gtn ratio of the hydrogel significantly affected cell viability ($p < 0.05$), with highest cell densities achieved in the 50:50 HA:Gtn hydrogel. Importantly, HEK293 cells achieved the greatest cell density, 2.4×10^7 cells/ml, when encapsulated with both VEGF and KGF ($p < 0.05$). The HA only hydrogel was the only non-growth factor containing case that maintained a cell count significantly above zero for the entire 3 week growth curve ($p < 0.05$).

Conclusions: (1) The gel components HA-DTPH, Gtn-DTPH, Hp-DTPH, and PEGDA, as well as the growth factors VEGF and KGF are not inhibitory to cell survival. (2) Diffusion of nutrients through the hydrogel matrix is not a limiting factor for cell viability in hydrogels 70µm in diameter. (3)Increasing the concentration of Gtn-DTPH to 50% in the gel allows the cells to have faster growth rates and grow to higher maximum densities.

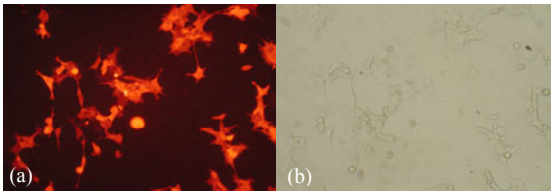
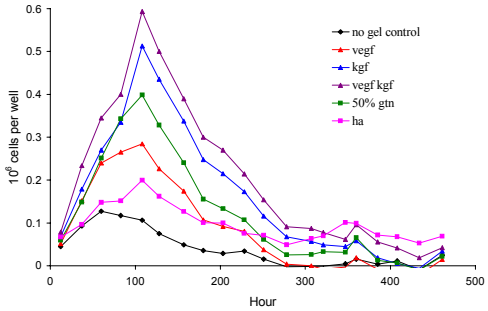


Figure 1. HEK293 cells 3 weeks post transfection, 40×, (a) fluorescence and (b) phase contrast microscopy.

Figure 2. Cell growth curves. Recombinant HEK293 cells encapsulated with both VEGF and KGF achieved the highest maximum cell density of all cases.

References:

1. Shu XZ. Biomacromolecules 2002;3(6):1304-1311.



2. Shu XZ. Biomaterials 2004;25:1339–1348