Development of a Novel *in situ* forming Tissue Engineering Scaffold

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Statement of Purpose: Tissue engineering offers the promise of a potentially limitless supply of biological tissue to combat degenerative diseases and trauma. A typical tissue engineering approach requires the combination of cells, a scaffold for cell growth, and biological factors. One of the most promising classes of materials used as tissue engineering scaffolds is hydrogels. These materials are attractive as tissue engineering scaffolds because they are aqueous, they can mimic the extracellular environment, and can be designed to be bioactive [1].

When dealing with the complex geometries of biological defects, it can be beneficial for a material to form *in* situ. Consequently, *in situ* forming hydrogels have emerged as a promising tissue engineering materials. Herein we report the biological testing of a novel family of *in situ* forming hydrogels that contain two components, a biological macromolecule (gelatin) and a reactive polyalkylene oxide cross-linker. These hydrogels allow both for cell proliferation and cell viability and our results indicate that this hydrogel could be an appropriate choice as an *in situ* forming, tissue engineering scaffold.

Methods: Biological testing was conducted by seeding cells on hydrogels and assaying for cell proliferation and viability. L929 fibroblasts were grown in DMEM supplemented with 10% FBS, hereafter referred to as fibroblast growth medium. Bovine skin gelatin was dissolved in a borate buffer (pH= 8.25) to obtain 5, 10 and 15 weight percent solutions. A reactive polyalkylene oxide cross-linker was dissolved in Hank's balanced salt solution to obtain 100 mg/mL and 200 mg/mL solutions.

Using a 1:1 dual barrel static mix system the gelatin and cross-linker solutions were injected into a rectangular mold and allowed to cure for 5 minutes at room temperature. The hydrogels were then removed from the mold and soaked in Dulbecco's Phosphate Buffered Saline overnight. Subsequently, cylindrical hydrogel pieces (6.75 mm, n = 10) were cut and placed into a 96-well plate and pre-conditioned with 200 µL of fibroblast growth medium. Next, the medium was removed, fresh medium (n = 3) or 10^4 fibroblasts were added (n = 7). And the cells were allowed to proliferate for 1 week.

On the seventh day, 1 cell-seeded sample was fluorescently stained using a LIVE/DEAD® kit (Invitrogen: Eugene, OR). The remaining samples were freeze dried and digested in a Proteinase K solution. Next the digested samples were assayed according to the Quanti-It™ PicoGreen® kit (Invitrogen). Data obtained was analyzed using JMP 8. Multivariate analysis techniques were applied to the data set to determine the outliers using Jackknife distances. Student's t-test was used to determine statistically significant differences between sample types. All quantitative data are expressed as mean ± standard deviation.

Results: A fluorescent LIVE/DEAD® assay showed viability of L929 fibroblasts on the gelatin-based hydrogels after one week of culture. A representative fluorescently stained hydrogel can be seen in Figure 1. The image shows a large number of viable (green) fibroblasts and no dead (red) cells, suggesting a high degree of viability for cells grown on the hydrogel, indicating the materials do not elicit a cytotoxic effect.

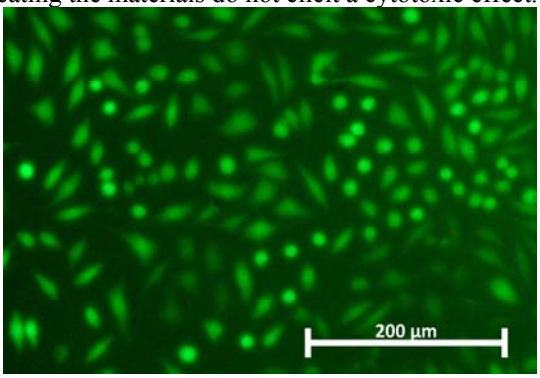

Figure 1. Representative LIVE/DEAD fluorescent image of L929 fibroblasts grown on a gelatin-based hydrogel for 1 week.

A proliferation assay showed the effect of hydrogel composition on cells seeded on the hydrogels after 1 week (Figure 2). The data indicated that the cells were able to proliferate on all of the sample types. The 100 mg/mL cross-linker, 15 wt% gelatin sample type exhibited the most proliferation. Statistical analysis reveals that 15 wt % gelatin hydrogels had significantly higher proliferation than 10 wt% gelatin hydrogels ($p = 0.0259$). There is also a significant trend of increase proliferation for hydrogels made with 100 mg/mL vs. 200 mg/mL cross-linker ($p = 0.0149$). In particular, hydrogels made with 5 wt% and 15 wt% gelatin demonstrated significantly increased proliferation when made with 100 mg/mL or 200 mg/mL cross-linker ($p = 0.0035$ and $p = 0.0373$, respectively).

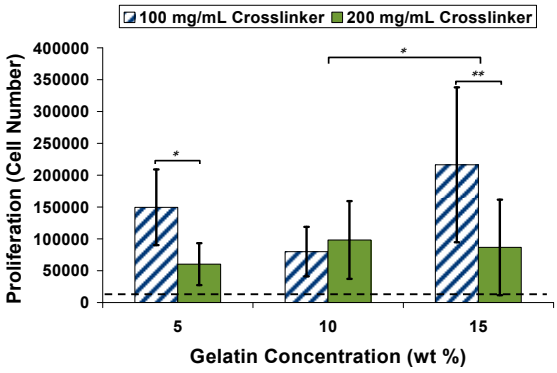

Figure 2. Proliferation data for L929 fibroblasts grown on gelatin-based hydrogels for 1 week.

Conclusions: Our data shows that L929 fibroblasts are able to proliferate and maintain their viability on these *in situ* forming hydrogels. Future studies will include cell infiltration studies, as well as *in vivo* studies to examine the biocompatibility of these hydrogels.

References

[1] Drury, JL. Biomaterials 2003:24:5337 – 4351.