An Injectable Collagen-Genipin Gel for the Treatment of Spinal Cord Injury

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Statement of Purpose: A significant barrier to regeneration after spinal cord injury (SCI) is the formation of a cystic defect impeding cellular repopulation of the lesion site.[1] Injectable hydrogels provide a minimally invasive approach to bridge this defect by providing a provisional matrix with mechanical properties similar to the native tissue. Hydrogels can also serve as delivery vehicles for therapeutic agents such as growth factors[2] and stem cells to address additional factors of growth inhibition after SCI. The goal of this study was to characterize and investigate the use of a thermo-responsive soluble collagen (Col) gel incorporating genipin (Gen), a plant extract with additional therapeutic benefits[3], as an *in situ* covalent cross-linker. Gelation, mechanical, degradation, and cytotoxicity tests were performed. Additionally, a novel *in situ* assay to predict gel stiffness was investigated.

Methods: Rat-tail type I Col (BD Biosciences) and Gen (Wako Chemicals) were used at 2 mg/ml and 0-2 mM, respectively. Thermal gelation of the collagen solutions was induced at 37°C. Absorbance: Col-Gen solutions (n=8), were plated into 96-well plates and measured at 595 nm using a Perkins-Elmer Wallac 1420 spectrophotometer. Rheological testing: Col-Gen solutions (n= 3-4), were gelled in a rheometer using a cone and plate geometry (40 mm, 2 °) at 37°C using a constant stress at 0.1 Pa at a frequency of 1 rad/s. (Preformed gels were similarly tested using a 8 mm parallel plate geometry). Degradation assay: 0.5 ml Col-Gen gels (n=3) in 2 ml cryotubes were exposed to 1 ml of 0.1 % type I collagenase (163U/mg) for 5.5 hours after a set cross-linking time. The dry weights of the scaffolds were compared to controls treated with 1x PBS buffer. Mesenchymal stem cells (MSC): Col-Gen solutions (n=2-4) containing goat or pig MSCs were plated at 400,000 cells/ml in 24-well plates containing pre-warmed cell culture medium. Medium was replaced at regular intervals to remove un-reacted Gen. Fluorescent live/dead assays were performed on 3 sections/gel.

Results: The addition of Gen to Col resulted in improved gelation characteristics including a decreased gel time (G'=G") and linear increases in storage modulus (G') over the first 1500 seconds (Table 1). G' also rapidly approached a value on the order of spinal cord tissue.[4]

Table 1: Rheological evaluation of Col-Gen gels at 37°C.

Gen(mM)	$t_{gelation}$ (s)	G'_{1500s} (Pa)	Rate of increase (Pa/s)
0	51.1 ± 1.5	20.8 ± 0.9	$2.9 \times 10^{-3} \pm 0.4 \times 10^{-3}$
0.25	50.4 ± 0.9	31.5 ± 5.7	$8.5 \times 10^{-3} \pm 2.5 \times 10^{-3}$
0.5	49.4 ± 0.7	33.8 ± 4.7	$10.2 \times 10^{-3} \pm 2.8 \times 10^{-3}$
1	47.0 ± 4.6	45.1 ± 5.9	$17.4 \times 10^{-3} \pm 3.1 \times 10^{-3}$

Mean ± Std. Dev.

Results of degradation studies show that Gen increased degradation resistance. After 24 hours of cross-linking, Col-Gen gels exposed to a collagenase assay retained 23% (0.25 mM), 44% (0.5 mM), and 74% (1mM) of their original weight. Gels without Gen were fully degraded within half of the full assay time.

Gen reacted with primary amines to produce cross-links, which absorbed and fluoresced at 595/630 nm.[5] The Gen-induced absorbance of Col gels was found to be a rapid *in situ* predictor of its mechanical properties.

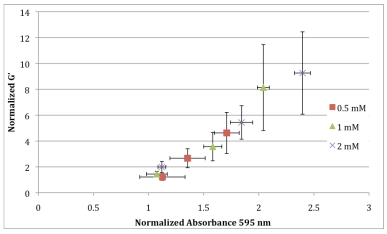

Figure 1. Correlation of absorbance and mechanical properties in Col-Gen gels. Normalized to Col. (n=3-6)

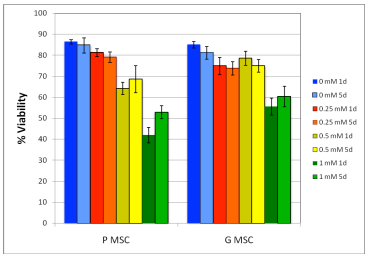

Figure 2. Pig and goat MSC viability assay

Cell viability assays at 1 and 5 days demonstrated that Col-Gen gels up to 1 mM could successfully encapsulate and sustain stem cells for transplantation.

Conclusions: The addition of Gen to soluble Col provides substantial improvement and control over gelation, as well as the mechanical and degradative behavior of the gel. Col-Gen gels can potentially undergo gelation rapidly enough to be confined within the lesion site and create a provisional stroma to bridge the cystic defect resulting from SCI. Studies on MSCs show that Gen can maintain cell viability for several days, and thus serve as a cell delivery vehicle at concentrations of 1 mM. Future studies will involve translation of these findings to *in vivo* models to determine the efficacy of the gel for SCI treatment.

Acknowledgments: Dept. of Veterans Affairs and NSF (DM).

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