Collagen-Carbon Nanotube Composite Biomaterials

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Introduction: The overall goal of this work is to produce novel composite biomaterials consisting of the structural protein Type I collagen reinforced with single-walled carbon nanotubes (SWNT). Collagen is used widely as a biomaterial and as a scaffold in tissue engineering, and living cells can be incorporated directly into collagen matrices. Carbon nanotubes have high potential as the reinforcing phase of composite materials due to their very high aspect ratio and remarkable strength. In addition, SWNT can be electrically conductive. This study examined the effect of SWNT loading on the conductivity of cell-seeded collagen-SWNT composite biomaterials. Such electrically conductive biopolymers have potential in regenerative medicine and biosensor development.

Methods: Collagen-SWNT composites were created by combining a solution of carboxylated single-walled nanotubes, culture medium, fetal bovine serum and acid-solubilized bovine collagen Type I. Human dermal fibroblast cells (HDFb) were added to the mixture, and constructs were gelled by neutralization with NaOH and temperature elevation. Initial cell and collagen concentrations were 1.0 million cells/mL and 2.0 mg/mL, respectively. SWNT concentrations were 0 wt% (control), 0.8 wt%, 2.0 wt%, and 4.0 wt%. Compaction of the constructs was tracked over 7 days. Cell viability was measured using fluorescent vital dyes and confocal microscopy at days 3 and 7. Construct conductivity was measured on days 3 and 7 using a bioimpedance spectrum analyzer and a two-point silver chloride electrode.

Results/Discussion: Fig. 1 shows HDFb-seeded collagen-SWNT constructs after 7 days in culture with differing initial SWNT loadings. Nanotube loading made the constructs visually darker and we confirmed previously that SWNT are incorporated into the collagen matrix (see [Macdonald 2005]). The right-hand panel in Fig. 1 shows the bioimpedance electrode being applied to a construct.

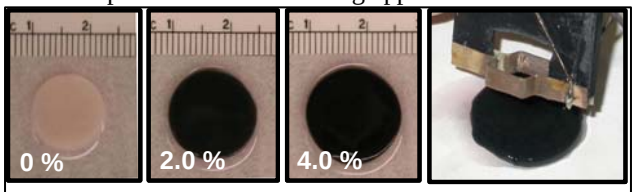

Fig. 1: Collagen-SWNT constructs at day 7 in culture, and conductivity test (right panel). Scale bars in cm.

Fig. 2A shows compaction of the constructs over time in culture. Material compaction was vigorous and the constructs were only around 1-4 % of their original volume by day 7. However, compaction was significantly inhibited at higher SWNT loadings (2% and 4%), relative to control. Gel compaction in protein-based matrices is a cell-mediated remodeling response, and these results suggest that the embedded cells were able to remodel the collagen-SWNT matrix but that the presence of SWNT attenuated this process.

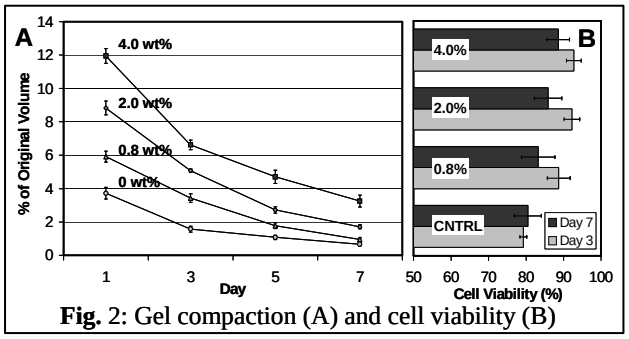

Fig. 2: Gel compaction (A) and cell viability (B)

Fig. 2B shows cell viability in collagen-SWNT constructs at day 3 and 7 in culture. Viability was high at both time points, and there was no statistically significant effect of SWNT incorporation on cell viability. These results suggest that cells are not harmed by the presence of nanotubes in the matrix. In spite of high viability, our preliminary results (not shown) show that HDFb number may be lower in SWNT-containing constructs, which may explain the decrease in gel compaction.

Fig. 3 shows the electrical conductivity of collagen-SWNT composites at day 7, normalized for the thickness of the sample. Increased nanotube loadings led to increased conductivity, with the 4% SWNT constructs exhibiting a two-fold increase over controls. Conductivity in both the 2% and 4% composites was significantly greater than in the control constructs.

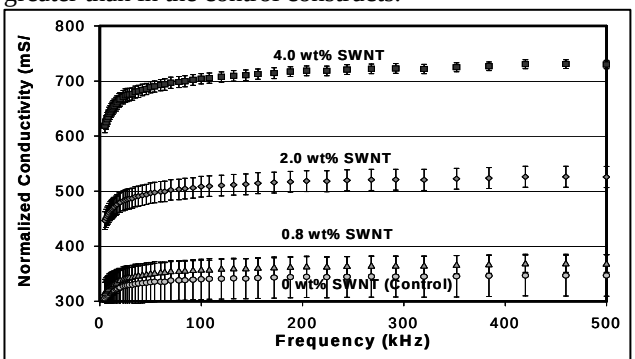

Fig. 3: Electrical conductivity of collagen-SWNT materials at day 7.

Conclusions: This study has shown that SWNT can be incorporated into collagen protein matrices by a simple modification of the established process for producing collagen hydrogels. Such constructs retain their ability to compact via cell-mediated remodeling but SWNT loading modulates this process. Cell viability is not adversely affected by SWNT in the matrix. Electrical conductivity of the composite biomaterials increases with SWNT loading level, and we have demonstrated a 2-fold increase in conductivity in this study. Collagen-SWNT composites have potential as a new class of fiber-reinforced protein matrix, with possible applications in regenerative medicine and biosensor development.

References: Macdonald RA et al, *J. Biomed. Mater. Res. A*, 74(3):489-496, 2005.