

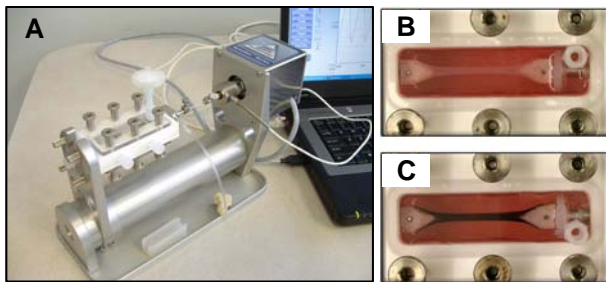
# Directional Conductivity in Protein-Nanotube Biomaterials through Strain-Induced Matrix Alignment

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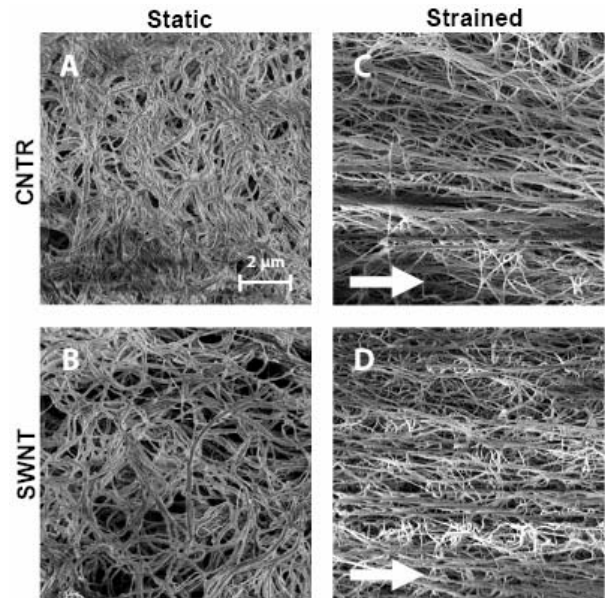
**Introduction:** Our laboratory has been developing composite biomaterials that combine the self-assembly features of structural extracellular matrix (ECM) proteins with the unique mechanical and electrical properties of single-walled carbon nanotubes (SWNT). Our goal is to create electrically conductive biopolymers for use as scaffolds in tissue engineering and other applications. In the present study, composite biomaterials incorporating fibroblast cells, collagen Type I, fibrin and carboxylated SWNT were created, and their properties were compared to similar control constructs without SWNT. In addition, cyclic mechanical stimulation was used to align the matrix and create anisotropy in the constructs.

**Methods:** Constructs were created by combining human dermal fibroblast cells (HDFb), carboxylated SWNT, culture medium, fetal bovine serum, acid-solubilized bovine collagen Type I, and fibrin. Collagen fibrillogenesis was initiated by raising the pH and temperature, and fibrin polymerization was initiated by adding thrombin. Initial cell, protein, and SWNT concentrations were 1.0 million cells/mL and 2.0 mg/mL, respectively. SWNT loading was 2.0 wt% and control (no SWNT) constructs also were prepared. Constructs were cultured for 3 days under either static conditions, or in a mechanical strain bioreactor (Figure 1) in which alignment of the matrix was stimulated by application of 8% cyclic strain for three 12 h periods over three days. Construct conductivity was measured at day 3 using a bioimpedance spectrum analyzer and a two-point silver chloride electrode.



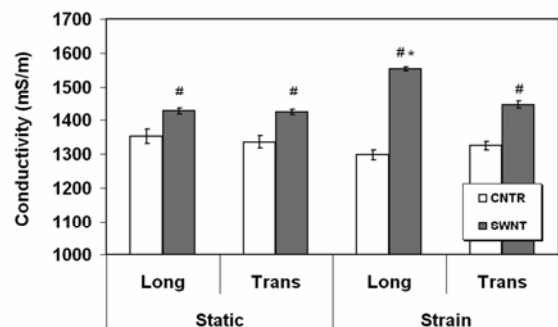
**Figure 1:** A) Image of stimulator and bioreactor chamber assembly. B) and C) show details of bioreactor chamber with control and SWNT-loaded constructs, respectively.

**Results:** All constructs underwent cell-mediated gel compaction to 15-20% of their initial volume, which was not affected by SWNT loading. Mechanical strain increased the rate of compaction, and strained constructs were significantly more compacted than unstrained controls by day 3. Cell viability and morphology were similar in both control and SWNT-loaded constructs, but unstrained samples exhibited a more stellate appearance with more numerous cellular projections. Application of mechanical strain caused clear alignment of both the cells and matrix in the direction of the applied strain, as shown in Figure 2.



**Figure 2:** SEM images of control and SWNT-loaded constructs under both static and strained conditions.

Bioimpedance measurements, shown in Figure 3, demonstrated that SWNT loading increased the electrical conductivity of composite constructs. Importantly, mechanically-induced alignment of the matrix/SWNT caused a further increase in electrical properties, as shown by the higher conductivity values along the axis of elongation. This effect was not observed in control constructs.



**Figure 3:** Electrical conductivity of control and SWNT-loaded constructs under static and strained conditions.

**Conclusions:** These results demonstrate that SWNT can be used to augment the electrical properties of 3D protein hydrogels, and that anisotropy in the matrix further enhances these properties. In the present study, we harnessed the self-assembly and mechanically-induced reorganization of 3D protein matrices to obtain anisotropy in SWNT-collagen-fibrin matrices. Directionally conductive engineered tissues may have application in a variety of fields, including stem cell biology, cardiac and neural tissue engineering, and biosensor development.

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