HYDROGELS INCORPORATING SINGLE WALLED CARBON NANOTUBES FOR CARDIAC CELL TISSUE ENGINEERING

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INTRODUCTION: A common cardiac defect, Tetralogy of Fallot, generally requires surgical placement of a patch or baffle across the right ventricular outflow tract (RVOT) in an area that consists of myocardial tissue. Various types of biocompatible polymers or decellularized matrices have been attractive candidates for cardiac patch applications, including Dacron, Gore-Tex®, and autologous or bovine pericardium[1-2]. However, these constructs have significant drawbacks in cardiac patching, including the absence of growth potential, loss of mechanical strength over time, lack of conductivity and inability to grow with the patient as well as an increased risk of infection and aneurysm. We have developed a multi-layered scaffold manufactured by forming a gelatin-chitosan hydrogel around a self-assembled polycaprolactone core[3] that can be invaded by cells, and degraded over time. In this study, single-walled carbon nanotubes (CNT) were incorporated with gelatin/chitosan composite hydrogels. We hypothesized that dispersion of CNT in gelatin-chitosan solution would improve the conductivity of hydrogels as well as cardiac cell function. We tested this by measuring the toxicity of various concentrations of CNT, then evaluated the beating rate and conduction velocity of CNT-loaded gels populated with neonatal rat ventricular myocytes.

MATERIALS AND METHODS: CNT were dispersed in a 0.5 M acetic acid and chitosan (2% w/v) solution using a sonicator for 5 min, then mixed with gelatin. Blended solutions were used to measure the length and concentration of CNT using UV absorption spectroscopy, NIR emission spectra and NIR microscopy. Then, CNT dispersed solutions were formed into disk shaped scaffolds using a Teflon mold, followed by lyophilization at -30°C for 24 hours. Formed matrixes (Fig. 1a) were neutralized using 100% ethanol and rehydrated using phosphate buffered saline (PBS). Effects of different concentrations of CNT on porosity, compressive modulus, degradation, spontaneous beating rate, and viability of neonatal rat ventricular myocytes (NRVM) were analyzed to optimize the CNT concentration. The conduction velocity of NRVM-containing gels was measured using a voltage sensitive dye (Di-8-Anepps). α-actinin and cx-43 expression levels of NRVM cultured on CNT-hydrogels were also analyzed using immunofluorescence (IF) microscopy and Western Blot.

RESULTS: CNT incorporated hydrogels had a hive like porous characteristic similar to hydrogels without CNT. NIR microscopic analysis showed the presence of individually dispersed CNT through the hydrogel solution (Fig. 1b). Compressive modulus increased with increasing concentration of CNT ($p < 0.05$; n=3). The incorporation of any CNT reduced the degradation rate significantly. However, there was no significant effect of CNT concentration on the degradation rate. NRVM in CNT-hydrogels had ~80% cell viability after 7 days in culture, similar to tissue culture plastic (TCP) (Fig. 1c) at concentrations up to 63 ppm. However, gels with 125 ppm or higher concentrations of CNT had significantly deceased viability (Fig. 2a) ($p < 0.05$; n=10). The spontaneous beating frequency of NRVM increased significantly with CNT (> 40ppm; $p < 0.05$; n=10). NRVM cultured on CNT-hydrogel had a faster conduction velocity (Fig. 2b) ($p < 0.05$; n=5). Western Blot and IF analysis showed that NRVM cultured in CNT-hydrogel had higher expression levels of α-actinin ($p < 0.05$; n=5).

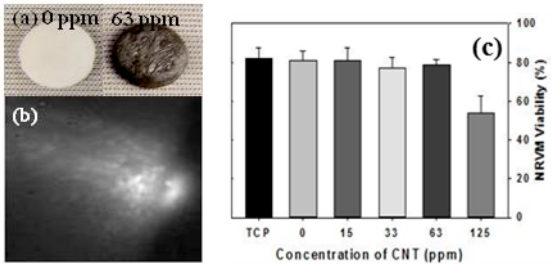

Figure1. (a) CNT dispersed hydrogel, (b) NIR microscopic view (CNT=bright dots) dispersed solution (c) NRVM viability.

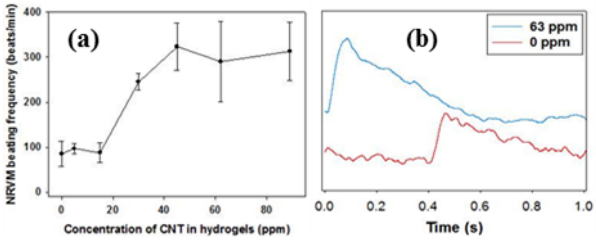

Figure2. Effects of different concentration of CNT on (a) NRVM beating rate and (b) conduction velocity (showing significantly lower delay at 12mm from a pacing signal).

CONCLUSIONS: This study demonstrates that CNT incorporated into a gelatin-chitosan hydrogel at levels of 63 ppm or lower is not cytotoxic, and leads to improved cardiac cell electrical connection and response and increased sarcomeric protein. The best results in terms of minimum concentration of CNT, cell spreading and viability and functions as a cardiac patch resulted from concentrations of 63 ppm CNT. In summary, this novel hydrogel shows significant potential for use of cardiac patch to repair RVOT defects.

REFERENCES: [1]Jenkins, K. J. et al., Circulation 2007, 115 (23), 2995-3014. [2]Kochupura, P. et al., Circulation 2005, 112 (9), I144-I149. [3]Pok S, et al.. Acta Biomater. 2013;9:5630-42.