Evaluation of 3D Hybrid Alginate/Single Wall Carbon Nanotube Tissue Scaffolds in Terms of Process and Cytocompatibility

Yildirim, E.D., Yin, X., Ko, F.K., Guceri, S., Sun, W.
Drexel University, Philadelphia, PA 19104

***Abstract*-Three-dimensional hybrid hydrogel tissue scaffolds of calcium alginate/single-walled carbon nanotubes (SWCNTs) composite were fabricated by solid freeform fabrication process in a multi-nozzle biopolymer deposition system. The scaffolds' morphological, structural, mechanical and cytocompatibility properties were characterized. Except for a few areas, SWCNTs were evenly dispersed in the fabricated alginate scaffolds, and SWNTs were well aligned on the hydrogel surfaces from the SEM pictures. Raman spectroscopy showed that the structure of the Alginate/SWNT samples had been altered by the nanotube reinforcement. Microtensile test proved the reinforcement effect of SWNT to the mechanical strength of Alginate. Cell culture experiment for Rat Heart Endothelial Cell (RHEC) showed the cytocompatibility and biological enhancement of SWNTs to the Alginate substrate. In this manner, this type of biomaterial and scaffold possesses a good potential for future use in tissue engineering applications.**

I. INTRODUCTION

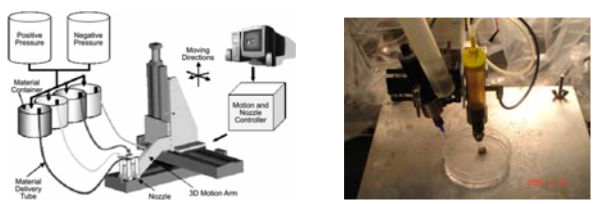
As a new approach in tissue engineering scaffold design, the application of nanophase matrix materials has the potential to improve cellular tissue engineering behavior [1]. This presentation reports our study on fabrication and cellular responses of hybrid alginate/carbon nanotube scaffolds encapsulated with Rat Heart Endothelial Cells (RHEC) and integrating. Preliminary results show good cytocompatibility and enhanced cell proliferation for encapsulated endothelial cells in freeform fabricated alginate/carbon nanotube composite scaffolds.

II. MATERIALS AND METHODS

High-purity single-walled carbon nanotubes were produced by the high-pressure carbon monoxide (HiPco) process, and then purified by a multistage purification method to reach a purity of up to 97 mol % [2].

In the experiments, the aseptic SWCNT mixed calcium alginate hydrogel 3D scaffolds encapsulated with Rat Heart Endothelial Cells (RHEC) were fabricated by a proprietarily developed multi nozzle deposition system through rapid prototyping process in a sterile environment.

Multi nozzle depositing system is capable of extruding biopolymer solutions and living cells for freeform construction of three-dimensional tissue scaffolds, and it has been used to successfully deposit three-dimensional calcium alginate hydrogel scaffolds. [42] During the fabrication process, we put the mixed SWCNT - sodium alginate solution into one nozzle, and put 5% calcium chloride solution into another nozzle. When the solutions from the two nozzles were extruded and met, the alginate anion and the calcium cation were crosslinked, thus the hydrogel was formed. The air pressure of the pneumatic valve was 8.0 psi. The nozzle inner diameter and the nozzle traveling speed we used were 330 µm and 10 mm/s, respectively. And the cross-linking time for each layer was 6 sec. Images and schematic view of the multi nozzle system is given in Figure1.



a b

Fig 1. Schematic Diagram of Multi-nozzle Deposition System (a) schematic of deposition system (b) in-situ dual nozzle cross-linking

Each scaffold was built in 10 layers and had a porosity of about 70%. The alginate concentration was 1.5% (w/v), and the CNT concentration was 1% (w/w). The cell number for each scaffold was approximately 250,000. The images of the fabricated scaffolds for both pure alginate and alginate/SWCNT composite are given in Figure 2.

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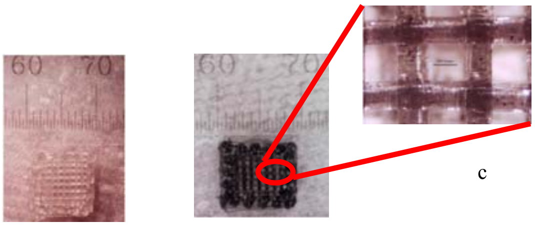
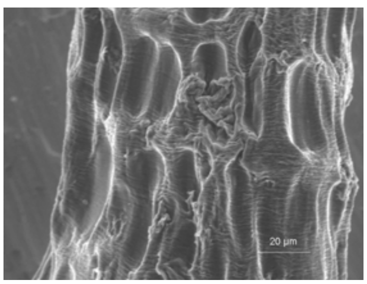


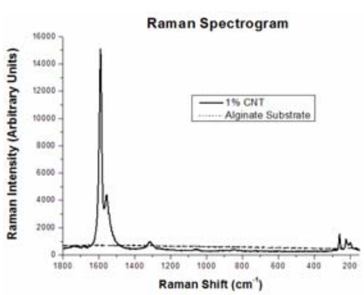
a b c

Fig 2. Representative images of 3D Scaffolds
(a) Pure alginate (b) alginate/SWCNT composites
(c) strut morphology of alginate/SWCNT composites

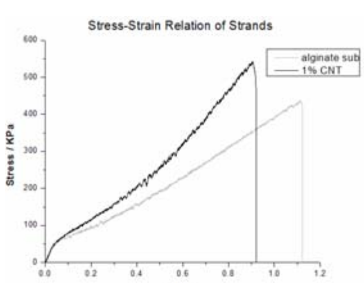
The scaffolds' morphological, structural and mechanical properties were characterized (Figure 3). CNTs were evenly dispersed in the fabricated scaffolds, and they were well aligned on the hydrogel surfaces from the SEM picture. Raman spectroscopy showed that the structure of the Alginate/SWCNT samples had been altered by the nanotube filling. Microtensile test proved the reinforcement effect of SWCNT to the mechanical strength of alginate.



(a) SEM micrograph



(b) Raman Spectrogram



(c) Tensile Stress-Strain Relation

Fig 3. Characterization of Alginate/SWCNT scaffold

III. RESULTS AND DISCUSSION

The alamarBlue Assay was used to measure quantitatively the cell viability and proliferation at Day 0, 3 and 7 after seeding. The data normalized to Day 0 number are shown in Figure 4. We can see that RHEC in the 1% SWCNT sample continuously proliferated at a fast and constant rate for up to one week, and at Day 7 its number reached more than six times as many as the number of Day 0. This rate was higher than that of pure alginate sample. Thus we can see that this type of purified SWCNT has a good biocompatibility and can enhance RHEC proliferation. This phenomenon could be explained by the altered interactions of proteins in the SWCNT/Alginate scaffold [3], SWCNT's high flexibility helpful for cell proliferation and migration, and the similarity of dimension between SWCNT and RHEC which is beneficial for cell growth.

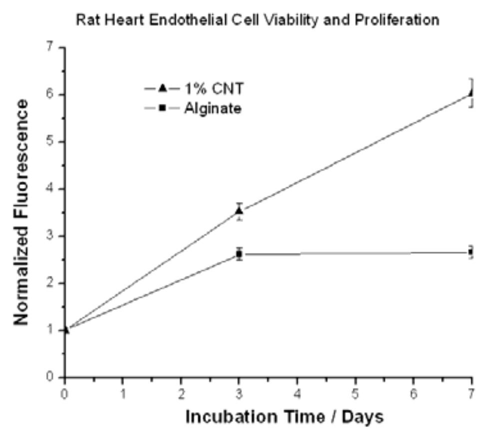


Fig 4. RHEC Viability and Proliferation

CONCLUSION

Three-dimensional hybrid alginate/SWCNT tissue scaffolds encapsulated with endothelial cells were fabricated by solid freeform fabrication process in a multi-nozzle biopolymer deposition system. The preliminary studies revealed a promising potential of this type of biomaterial in the enhancement of scaffold structural, mechanical and biological properties for vascular tissue engineering application.

ACKNOWLEDGEMENT

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