Cytocompatiliby and Optimization of Cardiomyocytes on Poly Lactic-Co-Glycolic Acid Carbon Nanofiber Materials

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Statement of Purpose: In recent years, various techniques have been developed to promote cardiomyocyte cell growth around dead tissue after a myocardial infarction [1], but one area that has been largely omitted to date is the exploration of nanotechnology (or materials with one dimension less than 100nm) in cardiovascular applications. This might be due to concerns over the consequences of carbon nanotubes entering the human body. The objective of the present research was to determine cardiomyocyte function and optimize such function on poly lactic-co-glycolic acid (50:50 (PLA:PGA); PLGA) with greater amounts of carbon nanofibers (CNFs) for myocardial tissue engineering applications.

Methods: Purified carbon nanofibers (CNFs) (99.9% by weight %, Catalytic Materials, MA) with diameters of 100 and 200 nanometers were sonicated in 20 ml of chloroform at 20W for 30 minutes. Two pellets of PLGA (50:50 PLA:PGA wt.) (Polyscience Cat #23986) were diluted in a 50 ml flask with 30 ml of tetrahydrofuran and sonicated in a water bath below 30 ^{0}C for thirty minutes.

After the PLGA and CNF solutions were prepared, various PLGA:CNF weight percent ratios were created (100:0, 75:25, 50:50, 25:75, 0:100) by adding the appropriate amount of CNF to PLGA in 20 ml disposable scintillation vials. The weight ratios were measured using a laboratory balance. When the appropriate ratios were reached, each composite material was sonicated at 10W for 20 minutes each. 1 ml of the appropriate PLGA:CNF via solution was placed onto the glass substrate and then placed into an oven below 50 ^{0}C for 15 minutes. Each composite film was then vacuum dried at -20 inches of Hg gauge pressure for 48 hours to allow the THF and chloroform to evaporate.

All samples and controls were sterilized using ultraviolet light for 24 hours prior to cells seeding. Human cardiomyocytes (Celprogen, Cat #36044-15) were seeded in human cardiomyocyte stem cell culture complete growth media with serum (Celprogen, Cat #M36044-15S) at a cell concentration of 3.5 x 10^4 cells/cm^2 for the cell adhesion assay and 1.5 x 10^4 cell/cm^2 for the cell proliferation assay. Cells were seeded into 12-well human cardiomyocyte stem cell culture extra-cellular matrix plates (Celprogen, Cat #E36044-15-12Well) with PLGA:CNF samples ratio, and 22 mm diameter microscope cover slips as controls. Samples were incubated for 4 hours for the cell adhesion assay and 1, 3, and 5 days for the proliferation assay under standard incubation conditions (at 5% CO_2 95% humidified air and 37^{0}C, changing the media ever other day).

Results: Scanning electron microscopy showed that the CNFs were uniformly dispersed within the PLGA material and that more CNFs were observed for the higher CNF ratio samples.

Results of this study also provided evidence that increasing the CNFs weight ratio in PLGA increased conductivity of the samples with 100:0 [PLGA:CNF; (wt:wt)] having zero conductivity. Clearly, the increased conductivity of the composite was due to the presence of more conductive CNFs within the sample.

After 4 hours of culture, the results of this study showed for the 200nm CNFs that the 25:75 ratio [PLGA:CNF; (wt:wt)] had the highest cardiomyocyte density whereas the lowest density was on the 100:0 ratio [PLGA:CNF; (wt:wt)]. For 100nm CNFs the 50:50 ratio [PLGA:CNF; (wt:wt)] had the highest cardiomyocyte density whereas the lowest density was also on the 100:0 ratio [PLGA:CNF; (wt:wt)].

Results from the present study also showed that the same trend observed during the 200nm and 100nm CNFs adhesion assay was present during the proliferation assay (specifically, the 25:75 sample ratio for 200nm and 50:50 sample ratio for 100nm [PLGA:CNF; (wt:wt)] had the highest cardiomyocyte density and the 100:0 sample ratio [PLGA:CNF; (wt:wt)] had the lowest cardiomyocyte density after 1, 3, and 5 days.

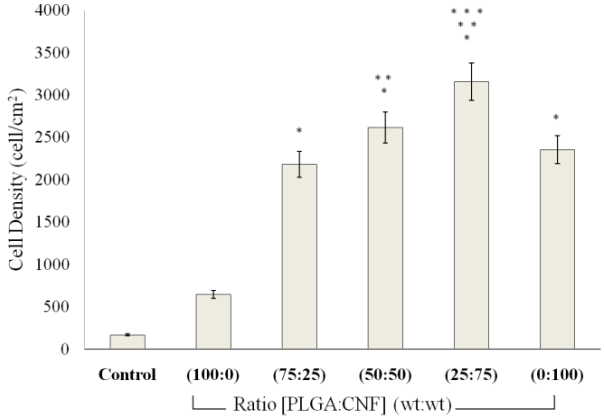

Figure 1. Cardiomyocyte adhesion after 4 hours on the materials (PLGA with 200nm CNF) of interest to the study. Seeding density = 3500 cells/cm^2. Data are mean density values ± s.d. n=3. Control was a glass substrate. * $p < 0.05$ compared to 100:0 ratio. ** $p < 0.05$ compared to 75:25 ratio. *** $p < 0.05$ compared to 50:50 ratio.

Conclusions: Cytocompatibility properties of PLGA can be improved through the addition of CNFs for myocardial tissue engineering applications with (25:75 PLGA:CNF) weight ratios showing the most promising results for future applications. Future directions will look at the cytotoxicity of CNFs to cardiomyocytes and electrical stimulation will be performed.

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References:

[1] S. Cohon, J. Leor. Sci. Am. **291**(5): 45-51.