

The Effects of Carbon Nanofiber Wettability on Macrophage Functions

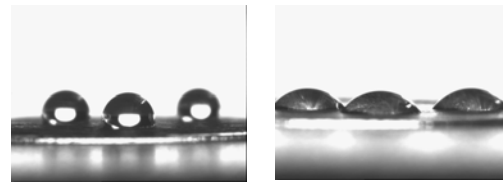
Young Wook Chun and Thomas J. Webster

Division of Engineering and Department of Orthopaedics, Brown University, Providence, RI 02912

Statement of Purpose: Nanomaterials (such as carbon nanotubes (CNTs), carbon nanofibers (CNFs), etc.) have been considered as novel materials for numerous biomedical applications from drug delivery to biosensor applications. However, there has been concern over the immune responses to such nanomaterial scaffolds since they are increasingly being investigated for numerous tissue engineering applications (such as for the bone and nervous system) due to their highly desirable mechanical and conductivity properties. Traditionally, the surface chemistry of carbon nanotubes has been modified through various functionalization strategies to increase their biocompatibility properties. Importantly, modulating surface tension (i.e., wettability) of carbon nanotubes or nanofibers without resorting to extensive chemical functionalization can potentially reduce immune responses mediated by macrophages. To this end, the research presented here demonstrates the effects that carbon nanofiber wettability has on macrophage functions to improve their use in regenerative medicine.

Methods: All carbon nanofibers (hydrophobic and hydrophilic) obtained from Applied Sciences, Inc. (Cedarville, OH) were first rinsed in deionized water. While hydrophobic carbon nanofibers were dissolved by chloroform and sonicated, hydrophilic carbon nanofibers were soluble in ethanol and were also sonicated for better dispersion. Hydrophobic carbon nanofibers were placed on glass discs, but polyurethane was used to enhance the attachment of hydrophilic carbon nanofibers before placing hydrophilic carbon nanofibers on glass discs. A drop shape analysis system (DSA-10, Kruss, Germany) with analysis software was used to determine contact angles for both hydrophobic and hydrophilic CNF scaffolds (Pho and Phi, respectively). TIB186 macrophages (ATCC – need number, passage number between 5 and 15) were used since macrophages are present 24 hours after biomaterial implantation [1]. They were cultured in RPMI 1640 medium (ATCC) with 10% (v/v) FBS and grown under 95% air and 5% CO₂ for up to 48 hours. 5,000cells/cm² were seeded onto CNF scaffolds. Pro-inflammatory cytokines (TNF-α) were measured through ELISA kits (Quantikine; R&D Systems, Minneapolis, MN). In addition, co-stimulatory surface antigens (CD80 and CD86) were stained with FITC and visualized by fluorescence microscopy (Leica Microsystems Ltd., Switzerland). All experiments were conducted at least 6 times (N=6). Glass and titanium (Ti) were used as controls.

Results: As expected, there was a difference in contact angles between hydrophobic (Fig. 1a) and hydrophilic carbon nanofibers (Fig. 1b). This is due to the existence of hydrocarbons on the hydrophobic carbon nanofibers whereas hydrophilic carbon nanofibers did not have such hydrocarbons (as they were pyrolytically stripped).



(a) Hydrophobic (b) Hydrophilic

Fig. 1. Contact angles on (a) hydrophobic and (b) hydrophilic carbon nanofibers. Contact angles on hydrophobic scaffolds were 151.8±4.2° while contact angles on hydrophilic scaffolds showed 23.9±4.3°. As a result of these differences in surface energy and chemistry, different amounts of pro-inflammatory cytokines were secreted by macrophages on the carbon nanofibers samples (Fig. 2).

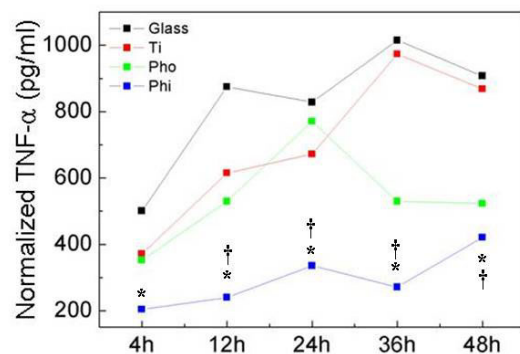


Fig. 2. TNF-α released from macrophages on each carbon nanofiber substrate. Glass and titanium (Ti) were used as controls. Pho and Phi represent hydrophobic and hydrophilic carbon nanofiber scaffolds, respectively. *p<0.001 (compared to released TNF-α by Pho at the same time point), †p<0.001 (compared to released TNF-α at the previous time point). Data are mean±SEM; N=6.

Hydrophilic carbon nanofibers stimulated a less pro-inflammatory response from macrophages compared to hydrophobic carbon nanofibers. As well as cytokines released from macrophages, surface antigens (CD80, 86) on macrophages were expressed more on hydrophobic compared to hydrophilic carbon nanofibers (Fig. 3)

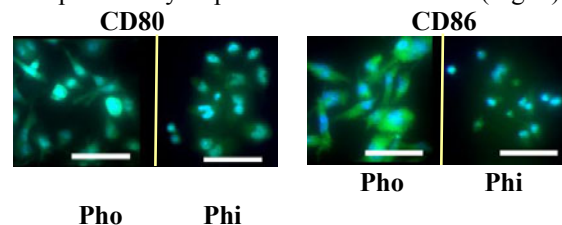


Fig. 3. Immunofluorescent images of the expression of CD80 and CD86 on macrophages on Pho and Phi .

Oral Presentation

Pho and Phi represent hydrophobic and hydrophilic carbon nanofiber scaffolds, respectively. After 24 hours, CD80 and CD86 were expressed more on macrophages cultured on hydrophobic compared to hydrophilic carbon nanofibers. Scale bars = 100µm

Conclusions: This study demonstrated that the wettability and change of chemistry of carbon nanofibers mediated macrophage responses. Such results continue to provide promise for the use of hydrophilic carbon nanofibers as implantable devices.

Acknowledgements: The authors would like to thank the Coulter and Hermann Foundation for funding.

Reference: [1] Luciana P *et al.* Titanium particles and surface-bound LPS activate different pathways in IC-21 macrophages. 2006. *J Biomed Mater Res Part B: Appl Biomater* 79B:66-73