In Vitro Cytotoxicity of Single-Walled Carbon Nanotube/Poly(Propylene Fumarate) Nanocomposites

Xinfeng Shi, Balaji Sitharaman, Quynh P. Pham, Jared L. Hudson, Lon J. Wilson, James M. Tour, Antonios G. Mikos
Rice University, Houston, TX 77005

Introduction: Recently, single-walled carbon nanotubes (SWNTs), functionalized SWNTs (F-SWNTs), and ultra-short SWNTs (US-tubes) have shown significant mechanical reinforcements to poly(propylene fumarate) (PPF) polymer, making these nanocomposites promising materials for use as injectable scaffolds for bone tissue engineering.[1-3] However, the biocompatibility of these novel materials is still unknown. In this study, we investigated the *in vitro* cytotoxicity of different forms of these three nanocomposites: uncrosslinked components, crosslinked networks, and degradation products.

Materials and Methods: SWNTs, F-SWNTs, US-tubes, and PPF were synthesized and nanocomposites were fabricated and thermally crosslinked as previously described. [1-3] Extracts of unreacted components and crosslinked networks were prepared by incubating the samples with culture media (0.1mg nanotubes/ml media and 3 cm^2 network surface/ml media) at 37 ^oC for 24 h. Degradation products of the crosslinked networks were obtained by grinding and placing 1 g crosslinked sample in 50 ml 1N NaOH solution with stirring at 60 ^oC for one week, followed by pH adjustment to 7.4 with HCl and filtration. ATCC CRL1764 rat fibroblast cell line was used in this study. Cell viability studies were carried out by incubating cells with gradedly diluted extracts of different materials at 37 ^oC for 24 h. Afterwards, cells were stained by a LIVE/DEAD reagent, quantified using a fluorescent microplate reader, and imaged with a confocal microscope. For cell attachment studies, cells were seeded on network surfaces and incubated for 24 h. Then, attached cells were quantified by measuring enzymatically lifted cells with a Coulter Multisizer, or imaged under a confocal microscope after LIVE/DEAD staining.

Results and Discussion: Before crosslinking, PPF macromers exhibited a known dose-dependent toxic effect, however, no cytotoxicity was observed for all three carbon nanotubes (Figure 1A) There was no significant difference in cell viability of extracts from crosslinked networks among pure polymer and all nanocomposites (Figure 1B). Furthermore, all crosslinked networks showed no adverse effects on cell viability, where the minimum viability was 92 ± 7%. For cell attachment, there was also no significant difference in adherent cell number on network surface between nanocomposites and PPF polymer or tissue culture polystyrene (positive control, data not shown). All networks exhibited similar cell attachment as illustrated in figure 2, in which the nanocomposite surface was covered by live and spread fibroblasts (green color) with only a few dead cells (red color). All degradation products displayed similar dose-dependent cytotoxic response, which is partly due to the increased osmolarity by NaCl and can be overcome once diluted with media (Figure 3).

Conclusions: Excellent cell biocompatibility was observed for nanocomposites of PPF and different forms of SWNTs, which paves the way for them as novel biomaterials for bone replacement.

References:

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2. Shi X., et al. Biomacromolecules 2006; 7: 2237-2242.
3. Sitharaman B., et al. J Biomater Sci Polym Ed, submitted.

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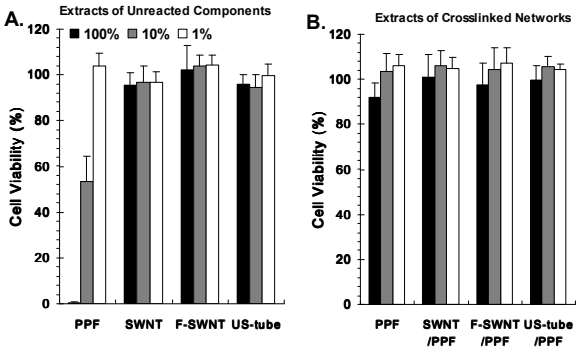

Figure 1. Cell viabilities of extracts of unreacted components (A) and crosslinked networks (B) gradedly diluted with culture media (10% = 1 ml extract in 9 ml media).

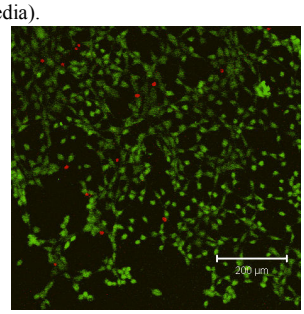

Figure 2. Confocal microscopy image of cells seeded on US-tube nanocomposite for 24 h and stained with a LIVE/DEAD fluorescent reagent. The scale bar corresponds to 200 µm.

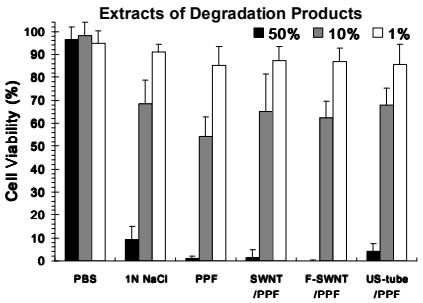

Figure 3. Cell viabilities of extracts of degradation products gradedly diluted with culture media (PBS was used as a positive control and 1N NaCl as a blank control).