Supplementary Material (ESI) for *Journal of Materials Chemistry*

Supporting Information

Reinforcement of Collagen with Covalently-Functionalized Single-Walled Carbon Nanotube Crosslinkers

*Christa M. Homenick,[1] Heather Sheardown,[2] and Alex Adronov[1]**

[1]Department of Chemistry and the Brockhouse Institute for Materials Research, McMaster University, Hamilton, Ontario Canada

[2]Department of Chemical Engineering, McMaster University, Hamilton, Ontario Canada

E-mail: Adronov@mcmaster.ca [*]

Department of Chemistry

McMaster University

1280 Main St. W

Hamilton, Ontario L8S 4M1

Tel: (905) 525-9140 x23514

Fax: (905) 521-2773

Supplementary Material (ESI) for *Journal of Materials Chemistry*

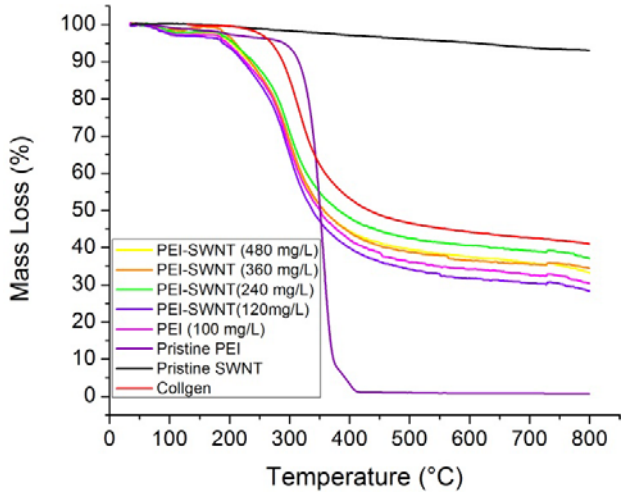

Figure S1. Thermogravimetric analysis of collagen hydrogels crosslinked by PEI-SWNT of various concentrations (w/w).

Use of thermogravimetric analysis to confirm the mass percentage of SWNTs within the collagen matrix was found to be impractical. Although PEI completely decompose when heated above 300°C, both collagen and crosslinked collagen hydrogels were found to not decompose completely, leaving a relatively high amount of residual mass even when heated to 800°C (Figure S1). Considering that the nanotube content within these samples is an order of magnitude lower than the residual mass remaining after heating, it was impossible to quantify the nanotube content with any degree of certainty using this method.

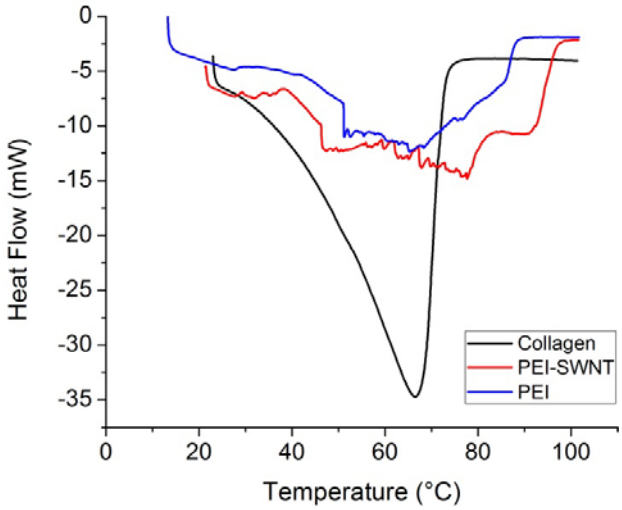

Figure S2. Representative differential scanning calorimetry data comparing collagen before and after crosslinking with either PEI (101.1 mg/L) or PEI-SWNTs (480 mg/L) macro-molecular crosslinking agents.

General procedure for determination of primary amine content using a ninhydrin assay

Procedure adapted from Zhu and co-workers.[1] A 1.0 M sodium acetate buffer was prepared by dissolving 41.16 g of sodium acetate in approximately 400 mL of deionized water. The pH of the resulting solution was adjusted to 5.2 using 1.0 N hydrochloric acid and the volume adjusted to 500 mL with deionized water. The ninhydrin reagent was prepared by charging a 20 dram vial with ninhydrin (0.407 g, 2.28 mmol, 12 eq.), hydrindantin (0.061 g, 0.19 mmol, 1 eq.), sodium acetate buffer (5 mL, 1.0 M) and 15mL DMSO. The assay was administered by charging a 20 dram vial with 0.5 mL of the sample of interest (PEI, PEI-SWNTs or water) and 0.5 mL of the ninhydrin reagent. Subsequently, the vials were capped, gently mixed by swirling and placed in an oil bath at 100°C for 30 minutes. After cooling each vial was charged with 15 mL of a 50:50 ethanol:water mixture and vortexed for 15-30 seconds. The samples that contained SWNTs were then filtered through a 0.2 µm PTFE

membrane to remove the precipitated SWNTs. The absorbance of each solution was then measured and the absorbance at 570 nm was used to calculate the concentration of Ruhemann's Purple in the solution.

Syntheis of dimethyoxy poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (M108)

Adapted from the procedure by Nace and co-workers,[2] a 100 mL round bottom flask was charged with a Teflon stirbar, flame dried and then back filled with argon. The flask was charged with 60% NaH powder in oil (1.655 g, 41.4 mmol, 99 eq.). The oil was subsequently extracted with 10 mL of hexanes (3 times). A second 100 mL round bottom flask was flame dried, back filled with argon and subsequently charged with poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (6.00 g, 0.414 mmol, 1eq.), also known as Pluronics F108, and 20 mL of dry dichloromethane. The two flasks were connected with a cannula and the polymer solution was then transferred into the NaH reaction flask utilizing positive nitrogen pressure. The reaction flask was placed into an ice bath and stirred. The flask was then charged with methyl idodide (2.6 mL, 5.873 g, 41.4 mmol, 99 eq.) and stirred continuously overnight. The reaction was quenched with 25 mL of methanol and 25 mL of water followed by extracting the product with 150 mL of dicholoromethane (3 x 50 mL). The organic layer was dried over magnesium sulphate, filtered and concentrated to a pale yellow oil by evaporation. The oil was dissolved in a minimal volume of dichloromethane, precipitated in 400 mL cold hexanes, and subsequently filtered and dried as a white powder. Yield: 3.95g, 83.4%. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 3.71 – 3.75 (m, 4H, -C*H*$_2$-OCH_3), 3.56 – 3.71 (m, 498, (C*H*$_2$-C*H*$_2$-O)$_n$), 3.46 – 3.56 (m, 45H, O-C*H*HC*H*(CH_3)-O), 3.33 – 3.41 (m, 17H, O-CH*H*CH(CH_3)-O), 1.05-1.15 (dd, *J* = 5.5, 5.5 Hz,

62H, O-CHHCH(CH_3)-O), 3.35 (s, 6, -CH_2-OCH_3).

Preparation of M108-stabilized SWNT solution

A 250 mL glass jar was charged with 200 mg of pristine SWNTs and 100 mL of M108 (0.690 mM) and subsequently sonicated for two hours in an ice bath. The black solution was then centrifuged for 20 minutes at 5000 rpm. The supernatant was decanted from the precipitate, sonicated for an additional hour and centrifuged again for 20 minutes at 5000 rpm. The supernatant was again decated from the precipitate to yield the stable SWNT solution.

Detailed Experimental for Chemically Crosslinking Type I/III Bovine Collagen

Synthesis of a Collagen Hydrogel with 1.1% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (1.25 mL, 600 mg/L), NaOH (10 μL, 1.0 N), distilled water (25 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.9% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (1.0 mL, 600 mg/L), distilled water (0.25 mL), NaOH (15 μL, 1.0 N), distilled water (20 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.7% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (0.75 mL, 600 mg/L), distilled water (0.50 mL), NaOH (20 μL, 1.0 N), distilled water (15 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.5% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (0.50 mL, 600 mg/L), distilled water (0.75 mL), NaOH (25 μL, 1.0 N), distilled water (10 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.2% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (0.25 mL, 600 mg/L), distilled water (1.0 mL), NaOH (30 μL, 1.0 N), distilled water (5 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0% w/w of SWNTs: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), distilled water (1.25 mL), NaOH (35 μL, 1.0 N), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with PEI (101.1 mg/L): Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI(1.25 mL, 101.1 mg/L), NaOH (10 μL, 1.0 N), distilled water (25 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with PEI (80.88 mg/L): Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI (1.25 mL, 80.88 mg/L), NaOH (15 μL, 1.0 N), distilled water (20 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with PEI (60.66 mg/L): Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI (1.25 mL, 60.66 mg/L), NaOH (20 μL, 1.0 N), distilled water (15 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with PEI (40.44 mg/L): Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI (1.25 mL, 40.44 mg/L), NaOH (25 μL, 1.0 N), distilled water (10 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with PEI (20.22 mg/L): Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI-SWNT (1.25 mL, 20.22 mg/L), NaOH (30 μL, 1.0 N), distilled water (5 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with M108: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), M108 (1.25 mL, 0.690 mM), NaOH (30 μL, 1.0 N), distilled water (5 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.5% w/w SWNTs in M108: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), SWNTs (0.38 mL, 798 mg/L in 0.690 mM M108), M108 (0.87 mL, 0.690 mM), NaOH (30 μL, 1.0 N), distilled water (5 μL), EDAC/NHS solution (200 μL).

Synthesis of a Collagen Hydrogel with 0.5% w/w SWNTs and PEI in M108: Collagen (0.66 mL, 100 mg/mL), HCl (50 μL, 1.0 N), PEI/SWNTs (0.38 mL, 798 mg SWNT/L and 76.9 mg PEI/L in 0.690 mM M108), M108 (0.87 mL, 0.690 mM), NaOH (30 μL, 1.0 N), distilled water (5 μL), EDAC/NHS solution (200 μL).

References

(1) Tian, H. Y.; Xiong, W.; Wei, J. Z.; Wang, Y.; Chen, X. S.; Jing, X. B.; Zhu, Q. Y. *Biomaterials* **2007**, *28*, 2899.
(2) Mai, S. M.; Booth, C.; Nace, V. M. *Eur. Polym. J.* **1997**, *33*, 991.