Light-triggered Phase Transitions in Nanofiber-Nanorod Composites

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Statement of Purpose: The complex developmental cycles of growth factor availability are difficult to recapitulate using current growth factor-releasing biomaterials, because release is limited to degradation and diffusion-dependent kinetics and most materials exhibit an initial 'burst' release[1]. Hydrogels based on N-isopropylacrylamide (NIPAM) have been widely investigated because of their lower critical solution temperature (LCST) transition at ~32°C, but research on fibrous scaffolds, useful as tissue engineering substrates, of poly(NIPAM) is sparse[2]. In this study, we demonstrate the synthesis of a novel photocrosslinkable NIPAM-based macromer and its processing into an insoluble nanofibrous scaffold. By incorporating gold nanorods (NRs) during electrospinning, we demonstrate the use of near infrared light to trigger the LCST transition. This opens up possibilities for triggered molecule delivery from scaffolds useful in regenerative medicine.

Methods: NIPAM, hydroxyethyl methacrylate (HEMA) and tert-butyl acrylate (TBA) were polymerized via reversible addition-fragmentation chain transfer (RAFT) polymerization, with azobisisobutyronitrile (AIBN) as initiator and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (TTCA) as a chain transfer agent. After purification of the product, the hydroxyl groups on HEMA were functionalized with acrylate groups –to allow chemical crosslinking– by the addition of acryloyl chloride in the presence of triethylamine (TEA). Trifluoroacetic acid (TFA) was added to the purified product to cause acidolysis of the TBA into acrylic acid (AAc), increasing the hydrophilicity/LCST of the final macromer, poly(NIPAM-*co*-AAc-*co*-HEMA-acrylate) (PNHAA) (Figure 1). The macromer was characterized by GPC and ^{1}H NMR. PEGylated gold nanorods (AuNRs) were prepared as in Hribar *et al*[3]. Nanofibrous scaffolds of PNHAA with incorporated AuNRs were produced by dissolving macromer, nanorods and the photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) in methanol, and electrospinning the solution onto a rotating mandrel. Scaffolds were collected and crosslinked by exposure to 365nm UV light (EXFO) under nitrogen. Swollen AuNR-containing scaffolds were irradiated with an 808nm laser (OEM Laser Systems) to induce transition. Cellular viability and adhesion to PNHAA scaffolds was determined by DAPI and phalloidin staining of cells seeded for 3 days. Scaffolds were imaged by SEM either dry or post-wetting after being vacuum-dried from PBS at 20°C and 50°C. 8mm×8mm squares from scaffolds at 20°C were measured after deswelling at 50°C.

Results: Macromers synthesized via RAFT polymerization exhibited low polydispersity (PDI < 1.25) and sufficient molecular weight for electrospinning (M_w ~ 23 kDa). The final monomer ratios (83% NIPAM, 14% HEMA, 3% AAc) were comparable to feed ratios. Scaffolds were successfully electrospun from methanol along with AuNRs, and crosslinked by UV light. Swollen samples exhibited good stability in PBS, with no visible changes after 1 week of immersion.

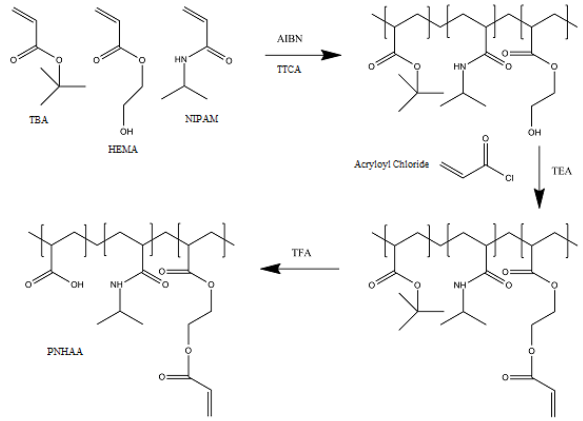

Figure 1. Reaction scheme for PNHAA synthesis.

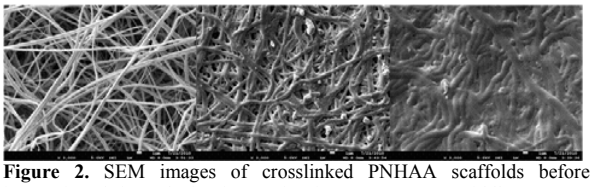

Figure 2. SEM images of crosslinked PNHAA scaffolds before immersion (left) and after immersion in PBS at 20°C (middle) or 50°C (right) and drying. Scale bar (small) = 1μm.

Scaffold areas of excised squares decreased from 64±2mm^2 to 15.7±0.58mm^2 within 10 sec of transfer from 20°C to 50°C, and 5 cycles of swelling and deswelling were performed with no change in temperature response. SEM images confirmed the microscopic stability of the scaffolds, and the mean fiber diameters were 500±137nm, 905±227nm, and 1472±288nm respectively for dry, 20°C PBS, and 50°C PBS samples (Figure 2). The increased diameter is presumed to be due to increased chain bundling within fibers with transition. Upon laser irradiation for 10 min in solution, the temperature recorded near the scaffold surface increased by 15°C, and scaffold opacity increased. Furthermore, cells were viable and adherent on the PNHAA scaffold as compared to a TCPS control (results not shown).

Conclusions: Nanofibers created from the polymer described herein demonstrate significant morphological differences above and below the polymer LCST, as the scaffold holds water below the LCST and expels water above. The reversible transition may be harnessed for controlled release of growth factors, since water-soluble factors can be expelled from the fibers by heating. Ongoing studies are being performed to investigate the differential release of model proteins above and below the LCST, and to demonstrate triggered release of protein by laser-induced heating of scaffolds above 37°C.

References: (1) Tayalia P. Adv. Mater. 2009, 21 (32-33): 3269-3285. (2) Kretlow J.D. Adv. Drug Deliv. Rev. 2007, 59 (4-5): 263-273. (3) Hribar KC. Small. 2009. 5 (16): 1830-1834.