Reactive Oxygen Species Degradable Thermo-Responsive Hydrogels for *In Situ* Drug Delivery to Cell Therapies

Mukesh K. Gupta, John R. Martin, Tianwei Shen, Craig L. Duvall*
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37235, USA.

Statement of Purpose: Injectable, *in situ* forming, biodegradable hydrogels are effective for encapsulation and delivery of drugs/cells in a variety of biomedical applications[1]. Poly(N-isopropylacrylamide) (PNIPAAM) is a commonly used lower critical solution temperature (LCST) polymer, but direct use of homo-PNIPAAM for thermo-gels is limited by syneresis, lack of biodegradability, and lack of a specific mechanism for drug loading and release. ABC triblock polymer-based micelles can be designed that form robust thermoresponsive hydrogels[2], and here we have applied this approach, for the first time, for formation of biodegradable thermo-gels amenable to cell and drug delivery. The hydrogels are formed from a novel poly(propylene sulfide-*block*-(N,N-dimethylacrylamide)-*block*-N-isopropylacrylamide) poly(PS-b-DMA-b-NIPAAM) ABC triblock polymer that assembles into micelles in aqueous solution that are hydrophobically crosslinked through PNIPAAM blocks into 3-D gels upon heating (**Fig 1A**). PPS undergoes a hydrophobic to hydrophilic phase change upon exposure to reactive oxygen species (ROS)[3]. With the current design, PPS forms ROS-sensitive hydrophobic nano-domains that can be preloaded with hydrophobic drugs and provide sustained, ROS-dependent drug delivery following *in situ* hydrogel formation *in vivo*. Hydrophilic DMA serves as the middle "B" block to ensure formation of non-syneresing, cytocompatible hydrogels. Herein, we present initial validation of this novel hydrogel.

Methods: Two different triblock polymers, (PPS_{50}-b-$PDMA_{150}$-b-$PNIPAAM_{150}$ (PDN150) and PPS_{50}-b-$PDMA_{250}$-b-$PNIPAAM_{150}$ (PDN250)) were synthesized using a combination of anionic and RAFT polymerization. Formation of the triblock polymers was confirmed by ^{1}H NMR and GPC. The size of micelles was measured using DLS. The LCST behavior of the terpolymers was determined using temperature-controlled UV-vis spectrophotometric absorption and rheometry, with temperature being changed at 1 °C/min. Nile Red was utilized as a model molecule to measure oxidation-dependent hydrogel degradation and small molecule release following treatment with the oxidant H_2O_2. NIH3T3 fibroblasts were utilized to confirm hydrogel cytocompatibility and the ability of *in situ* curcumin delivery to protect against oxidative stress-induced death.

Results: PPS_{50}-b-$PDMA_{150}$-b-$PNIPAAM_{150}$ (PDN150) and PPS_{50}-b-$PDMA_{250}$-b-$PNIPAAM_{150}$ (PDN250)) showed controlled molecular weight by ^{1}H NMR and GPC. In aqueous solutions at room temperature, the polymers formed stable ~50 nm micelles with hydrophobic PPS cores surrounded by hydrophilic PDMA−PNIPAM coronas as confirmed by DLS. PDN polymers undergo reversible sharp sol to gel transition above LCST (**Fig 1A, D**) at concentrations at or above 2.5 wt%. Temperature-dependent rheomery (*G′* and *G″*) on 5 wt% PDN150 showed sharp gelation at 29 °C and a lack of syneresis (**Fig 1B**). Spectrophotometric measurements of LCST also confirmed thermo-gelling in a range desirable for *in situ* hydrogel formation (**Fig 1C**). The ROS degradability of PDN250 hydrogels (5 wt%) was confirmed by overnight incubation with 0.5M (supraphysiologic) H_2O_2 (**Fig 1D**). The PDN250 hydrogels also showed sustained *in vitro* release of Nile red at rates dependent on H_2O_2 concentration (**Fig 1E**). The hydrogels were also compatible with fibroblast growth, and encapsulation with hydrogels loaded with the antioxidant curcumin reduced cytotoxicity due to H_2O_2 treatment (**Fig 1F**).

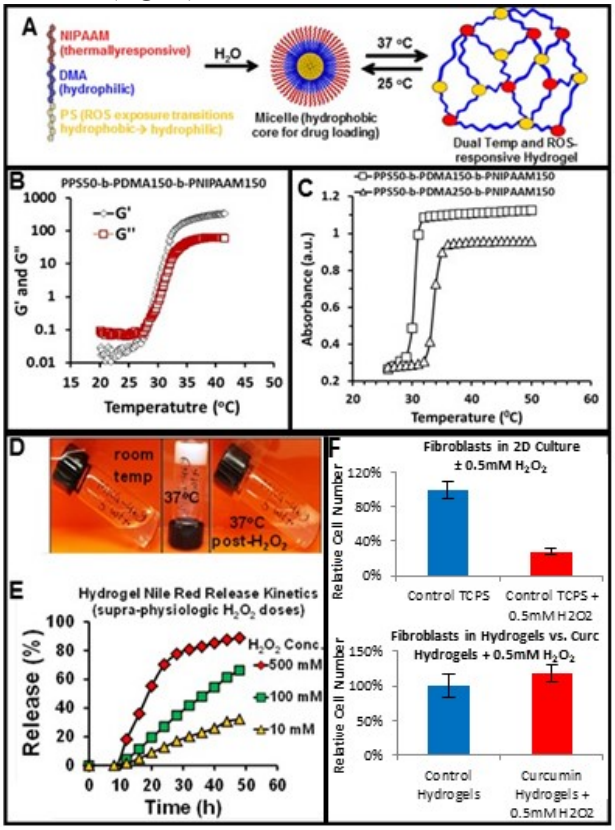

Figure 1. (A) Schematic demonstration of hydrogel formation. **(B)** Temperature-dependent dynamic shear moduli (*G′* and *G″*) for 5 wt% PDN150. **(C)** LCST measurement of PDN150 and PDN250 by *UV*-vis spectroscopy. **(D)** Hydrogel formation at 37 °C and destabilization after overnight incubation with 0.5 M H_2O_2. **(E)** H_2O_2 concentration dependent sustained drug release. **(F)** Hydrogels loaded with the antioxidant curcumin reduced H_2O_2- induced cytotoxicity

Conclusions: Here, we present a new ROS-degradable, thermo-responsive ABC triblock polymer hydrogel compatible with cell encapsulation and ROS-dependent degradation / drug release mechanism. This platform has numerous applications for enhancement of cell therapies.

References: 1. Li Y. Tomás, H Chem Soc Rev.2012; 41:2193-2221., **2.** Zhou,C. J Am Chem Soc. 2012;*134:* 10365-10368. **3.** Napoli A. Nat Mat, 2004; 3:183