Novel injectable thermo-responsive complexes for prolonged nitric oxide delivery

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Statement of Purpose: Nitric oxide (NO) plays an important role in several biological processes. In the cardiovascular system, NO synthesized by endothelial cells can prevent thrombus formation, promote endothelial cell proliferation, and inhibit vascular smooth muscle cell proliferation and migration. Therefore, polymers that release NO may be useful for the prevention of restenosis. However, controlled and localized delivery of NO to the vasculature has been challenging [1]. Furthermore, it is desirable to develop therapeutic delivery systems that take advantage of minimally invasive techniques. We developed a water soluble NO releasing thermo-responsive and phase transitioning biomaterial capable of slowly releasing NO. Herein, we report the synthesis and characterization of poly(citric acid-co-polyethylene glycol-co-glycerol 1,3-diglycerolatediacrylate-co-N-isopropylacrylamide) copolymer (CPN55).

Methods: Citric acid-PEG prepolymer (CPEGD) containg double bonds was prepared via polycondensation of citric acid, PEG (400) and glycerol 1,3-diglycerolate diacrylate at 130 °C [2]. CPEGD was then polymerized with N-isopropylacrylamide (NIPA) via free radical polymerization in 1.4-dioxane at 70 °C . The resulting copolymer (CPN55) was characterized by ^{1}H NMR and spectrophotometry. CPN55, a mixture of CPEGD and PNIPA, and PNIPA were mixed with NO-treated protamine sulfate (PS) and nitrite release was monitored using the Griess reaction.

Results: ^{1}H NMR showed that CPN55 had a prepolymer to PNIPA ratio of 1:1. Lower critical solution temperature (LCST) was 33 °C and 32°C for CPN55 and PNIPA, respectively (Figure 1). CPEGD did not exhibit phase transition. CPN55 reversibly and quickly formed a solid at 37 °C (Figure 1). For all samples, after mixing with PS, homogenous solutions were formed at 25°C. At 37 °C, PNIPA/PS and CPEGD/PNIPA/PS mixture formed solids and released NO for 18 hours. Solidified CPN55/PS released NO for up to 5 days at 37 °C (Figure 2).

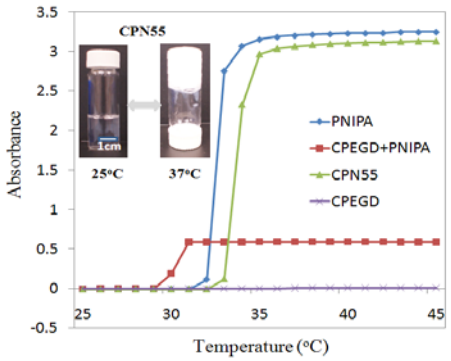

Figure 1. Phase transition of CPEGD, PNIPA, CPN55 and CPEGD/PNIPA mixture in aqueous solution as measured using a Jasco-815 CD spectrophotometer at 550 nm.

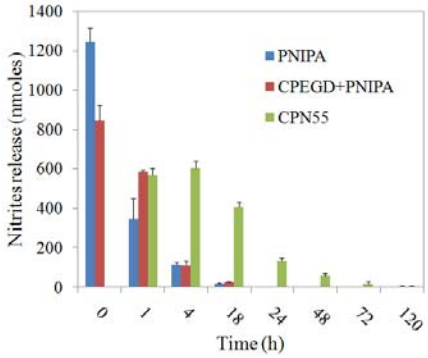

Figure 2. NO release of PNIPA, CPN55 and CPEGD/PNIPA mixture in PBS solution at 37 °C.

Conclusions: A water soluble copolymer with reversible phase transition between room and body temperature was synthesized. When the copolymer is mixed with a NO donor, NO release was significantly prolonged.

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References: (1) Acharya G. Adv Drug Del Rev. 2006;*58*:387-401. (2) Zhao HC. J Appl Polym Sci. 2009;114:1464-70.