ROS-Responsive Microspheres for Local, On Demand Delivery of Antioxidant Curcumin to Ischemic Tissues

Christopher E. Nelson, Rucha V. Joshi, Skylar C. Haws, Kristin M. Poole, Mukesh K. Gupta, Melissa C. Skala, Craig L. Duvall.
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN

Statement of Purpose: Elevated reactive oxygen species (ROS) are characteristic of inflammatory disease sites such as peripheral arterial disease (PAD). In this setting ROS cause oxidative stress that causes local cell and tissue damage.[1] Here, we have synthesized ROS-responsive poly(propylene) sulfide (PPS) microspheres encapsulating the antioxidant and anti-inflammatory molecule curcumin. Upon exposure to ROS, PPS is converted into poly(sulfoxide) and poly(sulfone)[2] releasing the drug cargo. This delivery system was designed to leverage this phase change for ROS-dependent, "on demand" release of curcumin, a polyphenol that acts as NF-κB antagonist[3]. Current studies evaluated ROS-dependent release and biological therapeutic performance *in vitro* and *in vivo*.

Methods: PPS was polymerized via thioacyl group transfer (TAGT) polymerization[4] and used to fabricate microspheres using a modified O/W emulsion solvent evaporation method[5]. Curcumin loaded (Cur-PPS) and "blank", unloaded microspheres (PPS) were prepared similarly. Microspheres were characterized for size and morphology by SEM. To quantify drug loading, microspheres were dissolved in DMSO, centrifuged, and measured by fluorescence (ex:488nm, em:535nm). *In vitro* release profiles of curcumin were measured by incubating microspheres on transwell inserts with varying concentrations of H_2O_2 or 3-morpholinosydnonimine (SIN-1) at 37ºC under constant shaking. Samples were collected at regular time intervals and evaluated by fluorescence. Cell viability was determined by incubating NIH-3T3 fibroblasts with 2mM H_2O_2 in the presence of blank microspheres, curcumin alone, or curcumin loaded microspheres. Animal studies were approved by the animal care and use committee of Vanderbilt University and in adherence to NIH guidelines. Hind-limb ischemia (HLI) was used as a model for PAD by ligating the right femoral artery and vein. Microspheres were injected at a dose of (14.2mg/kg PPS, 29.3 mg/kg curcumin) and empty microspheres were used as a control. Mice were monitored intravitally by Laser Doppler Perfusion Imaging (LDPI) and hyperspectral hemoglobin saturation. After 7 days, mice were anesthetized and the gastrocnemius was dissected and incubated with an ROS-sensitive dye[6] and imaged with an IVIS (ex 670, em 700).

Results: PPS polymers (M_n = 17.7 kDa, PDI = 1.36) formed homogeneous microspheres with diameter = 1.33±0.55µm (**Fig 1A**i, scale =10µm). The loading capacity was found to be 49% (w/w) and the encapsulation efficiency was 40%. The relatively low encapsulation efficiency is compensated for by the very low cost of curcumin. When incubated in H_2O_2, there was a concentration dependent release noted over 8 weeks where 0mM H_2O_2 released <20% and 50mM H_2O_2 released 95% by 32 days (not shown). When incubated in physiologically relevant concentrations of SIN-1 (a generator of the radical peroxynitrite), a concentration dependent and an 'on-demand' release was noted over a sustained timeframe (**Fig 1A**, green bars = + SIN-1, white = no SIN-1). Cell survival was improved 4.5 fold from NT controls by co-addition of curcumin microparticles in NIH3T3 fibroblasts incubated with 2mM H_2O_2, (**Fig 1B**). In mice with surgical induction of HLI, LDPI demonstrated significant improvement in the curcumin-PPS microparticle group relative to the PPS control after 7 days (**Fig 1C**) and hemoglobin saturation measured by hyperspectral imaging showed a similar trend (not shown). Excised gastrocnemii from the ischemic limbs demonstrated a significant decrease in ROS for the curcumin loaded PPS microspheres (**Fig 1D**). Taken together these data suggest curcumin delivery improves perfusion and reduces ROS in the ischemic hind limb.

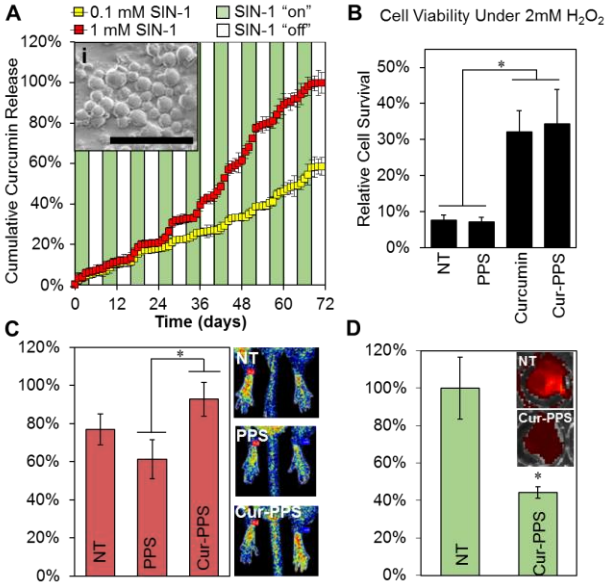

Figure 1. A) On demand release of curcumin from PPS microparticles treated with SIN-1 B) Cur-PPS enhanced cell survival under ROS. C) Cur-PPS showed trend toward improved perfusion of in HLI model of PAD. D) Cur-PPS suppressed ROS in the ischemic PAD limb. * $p < 0.05$

Conclusions: Cur-PPS microspheres deliver curcumin on demand over several weeks *in vitro* under physiologically relevant concentrations of peroxynitrite, improve cell survival when exposed to ROS, reduce the presence of ROS at sites of PAD, and show a trend toward increasing recovery from hind limb ischemia. PPS microspheres present a promising platform for the sustained, on demand delivery of therapeutics to sites of chronic disease associated with inflammation and oxidative stress.

References: [1] Dopheide et al. Atherosclerosis. 2013; 229(2):396-403 [2] Napoli et al. Nat Mat 2004;3(3):183-9 [3] Joe et al. Crit Rev Food Sci Nutr 2004;44(2):37-111 [4] Gupta et al. J Control Rel 2012;162(3):591-8. [5] Shahani et al. Cancer Res 2010;70(11):4443-52. [6]Kundu et al. Angew Chem Int Ed. 2009;2:299-303