Smart microspheres for stimuli responsive drug delivery

Rucha V. Joshi and Craig L. Duvall,
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA, 37235-1631

VANDERBILT UNIVERSITY
Advanced Therapeutics Laboratory

pH and Temperature-sensitive Microspheres

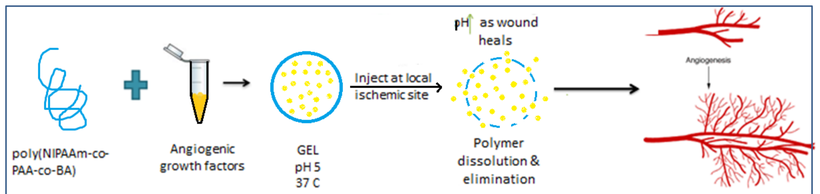
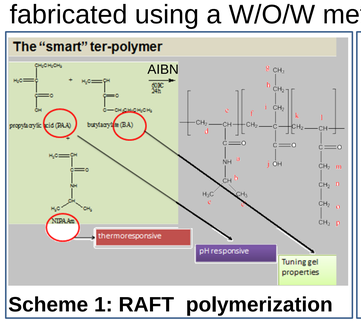


Fig. 1: Overview of the N-P-B project

Bolus delivery of proteins can cause undesirable systemic effects and provide insufficient local concentration for the optimal therapeutic effect. Therefore, platforms to deliver growth factors in an optimized, sustained pattern are necessary. Stimuli-responsive materials, which exhibit sharp physical changes in response to small physical or chemical stimuli, can be designed to take advantage of the low pH environment of ischemic wounds (5.2-7.2). To this end, we present such an "intelligent" temperature and pH responsive microsphere delivery system with the potential for sustained protein release at ischemic sites. The microspheres are designed to undergo gradual dissolution and release their payload at ischemic sites.

Methodology

Polymer synthesis and characterization: A series of random copolymers composed of N-Isopropyl acrylamide (NIPAAm), Propyl acrylic acid (PAA), and Butyl acrylate (BA) was formulated by RAFT polymerization[1] targeting NIPAAm:PAA:BA feed ratios of 80:10:10%, 77.5:10:12.5%, 75:10:15%, and 72.5:10:17.5%. The polymers were characterized by ^{1}H NMR, GPC and temperature controlled absorbance for the composition, molecular weight, polydispersity, and lower critical solution temperature (LCST). **Microsphere fabrication and characterization:** NPB microspheres encapsulating FITC-BSA microspheres were fabricated using a W/O/W method[2] as shown in Fig. 2.



Scheme 1: RAFT polymerization

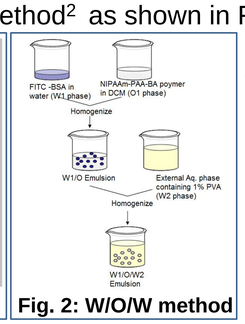


Fig. 2: W/O/W method

The microspheres were then characterized for size, morphology, loading capacity, encapsulation efficiency, and their *in vitro* release profile.

Results

The series of polymers demonstrated increasing BA content, molecular weights close to the target Mn, and polydispersity ~1.1. LCSTs of the polymers are given in table 1, and the behavior is shown in Fig. 3. For microsphere fabrication, the polymer R61 was selected since it showed the most desirable LCST.

Table 1: LCST of polymers at different pHs

Polymer	LCST in °C at pH 6.5	LCST in °C at pH 7.0	LCST in °C at pH 7.4
R41	28	74	>84
R51	<4	47.5	76
R61	<4	32	64

FITC–BSA was then used as a model protein for encapsulation in microspheres prepared from R61 using the w/o/w method. Microspheres were characterized for their size and morphology using SEM as shown in Fig. 4. Yield of the microspheres was found to be 46 %, loading capacity 19.54%, and encapsulation efficiency 89.02 %

pH dependent LCST behavior of R61 polymer

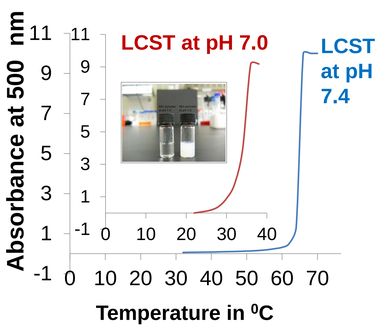


Fig. 3: LCST behavior of the R61 polymer as a function of pH. LCST of 32 °C at pH 7.0 indicated the polymer's tendency to gel at physiological temperature (37 °C) in acidic pH. LCST of 64 °C at pH 7.4 confirmed its solubility at physiologic pH and temperature.

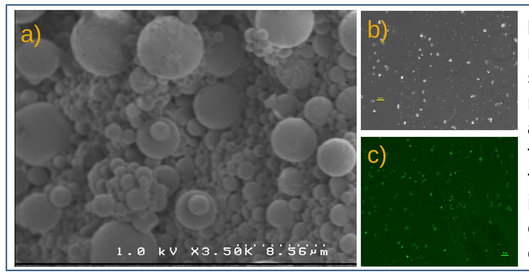


Fig. 4: a) SEM of R61 microspheres. SEM images showed a smooth, spherical morphology of microspheres and their size ranged from 1 to 30 um. b) Optical and c) fluorescent microscopy images of R61 microspheres confirmed the encapsulation of FITC-BSA.

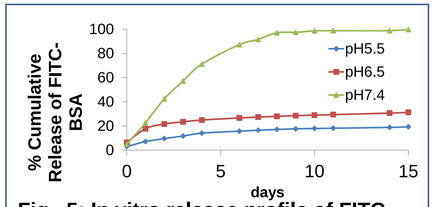


Fig. 5: In vitro release profile of FITC – BSA from R61 microspheres

In vitro release experiments performed in PBS of pH 5.5, 6.5 and 7.4 indicated that there was a burst release as microspheres were solubilized faster at pH 7.4. A more sustained release was observed at pH 5.5 and 6.5 over span of 15 days.

Conclusion

Microspheres that are soluble at pH 7.4 at 37 °C but are in a physical gel form in the conditions of intermediate acidity (pH 5-7) at 37 °C were successfully formed using a water-in-oil-in-water emulsion method that can facilitate spatio-temporal control of protein delivery to ischemic environments.

References

1. J. Garbern et al., Biomacromolecules, **2010**, 11, 1833-1839
2. Jae Hyung Park et al, Molecules **2005**, 10, 146-161
3. Komal Shahani et al., Cancer Res; **2010**, 70 (11), 4443-4452

Acknowledgement: Advanced Therapeutics Laboratory, Combinatorial Biomaterials & Biointerface Laboratory and the Department of Biomedical Engineering

ROS-sensitive Microspheres

Reactive oxygen species (ROS) are prevalent in inflammatory disease sites, such as arthritic joints, where activated macrophages are present. ROS presents a viable trigger for "smart", environmentally-triggered delivery of anti-inflammatory drugs. Toward this end, we have fabricated ROS-sensitive microspheres from poly(propylene) sulfide (PPS) using an O/W emulsion method.

PPS is converted from its hydrophobic form into a more hydrophilic poly(sulfoxide) and poly(sulfone) form upon exposure to ROS. Here, we encapsulated curcumin, a natural anti-inflammatory and anti-oxidant drug, into injectable PPS microspheres capable of "on-demand" anti-oxidant delivery.

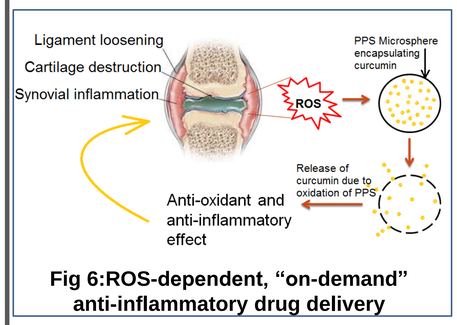
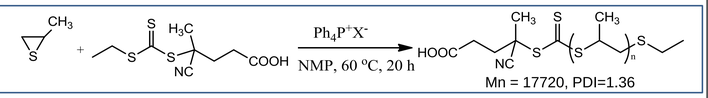


Fig 6:ROS-dependent, "on-demand" anti-inflammatory drug delivery

Methodology

Polymer synthesis and characterization: PPS was synthesized as shown in scheme 2 and characterized for Mn and PDI by GPC.

Scheme 2: PPS synthesis



Microsphere synthesis and characterization: PPS Microspheres encapsulating curcumin were prepared by a modified O/W method[3] and were characterized for size and morphology by SEM. Curcumin encapsulation was confirmed by fluorescent microscopy. *In vitro* release profile was obtained by exposing the PPS microspheres to 0 M, 0.5 mM and 0.5 M hydrogen peroxide for 31 days.

Results

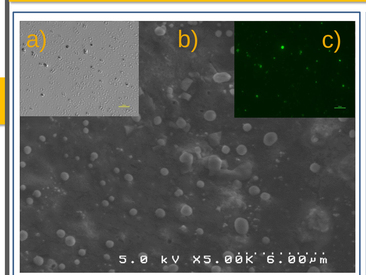


Fig. 7: a) Optical b) SEM & c) fluorescent microscopy images of the PPS microspheres encapsulating curcumin.

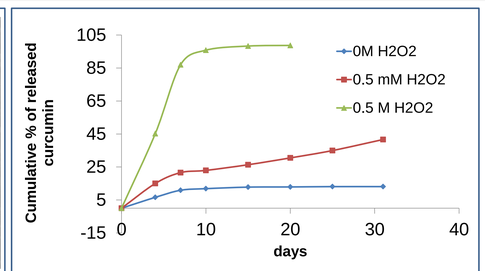


Fig. 8: In vitro release profile of curcumin from PPS microspheres at 0M, 0.5 mM and 0.5M H_2O_2 concentration. More sustained release is seen at 0.5 mM H_2O_2 exposure.

Conclusion

ROS-sensitive microspheres were successfully prepared from PPS and demonstrated H_2O_2 dependent release of curcumin.