Synthesis, Characterization and Lymphatic Trafficking of Polymeric Nanoparticles

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Statement of Purpose: Nanoparticles are currently being designed for use in biomarker detection, diagnostic imaging, and therapeutic drug delivery (Liu Y. Int J Cancer. 2007;120:2527-37). With the development of particles within the 10 to 50 nm size scale, methods for the diagnosis and treatment of metastatic human cancer within lymphatic tissues continue to undergo significant evolution (Yezhelyev M. Nanomedicine. 2009;4:83-103). Our primary interests lie in developing a diverse array of nanoparticles to support the advance of this biomedical application within the field of oncology. Herein, we describe the design and synthesis of one of these nanoparticle systems, which we have evaluated for lymphatic migration in a large animal model.

Methods: Nanoparticles (NPs) were prepared using a miniemulsion polymerization technique, which combines high energy emulsification and base-catalyzed free radical polymerization of an acrylate monomer and crosslinker (Figure 1). Briefly, the monomer and crosslinker were dissolved in an organic solvent which was then added to an aqueous solution of the surfactant sodium dodecyl

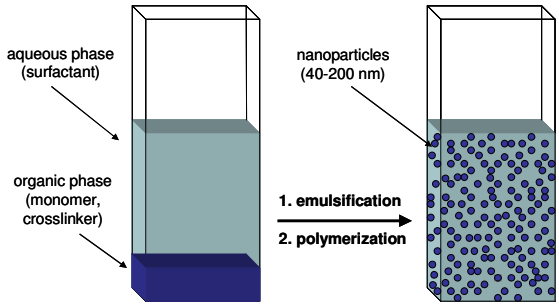

Figure 1. Scheme to synthesize the nanoparticles.

sulfate (SDS). This solution was then sonicated for 30 minutes before polymerization was initiated. Following polymerization, scanning electron microscopy (SEM; Zeiss) was used to characterize the size and shape of the prepared NPs. For the large animal imaging studies, the nanoparticles were fluorescently labeled by encapsulating the near-infrared dye IR-786 to allow for *in vivo* NIR fluorescence tracking. 400 µL of the NP suspension was then injected subcutaneously into the breast tissue lateral to the first nipple of Yorkshire pigs (n=6). 24 hours post-injection, the animals were imaged with the FLARE NIR imaging system (Troyan S. Ann Surg Oncol. 2009;16:2943-52) to identify the location of the nanoparticle IR-786 payload.

Results: Nanoparticles have been synthesized from monomer **1** and crosslinker **2** (Figure 2a) using the miniemulsion polymerization technique described above. Scanning electron microscopy (SEM) micrographs show the spherical shape and smooth morphology of the NPs

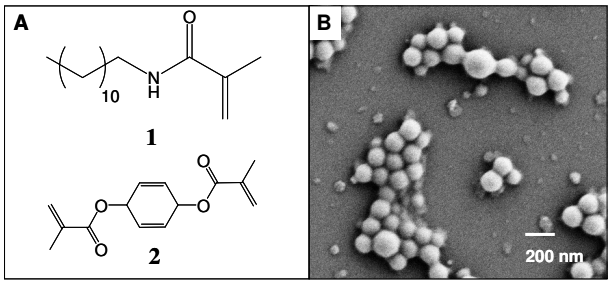

Figure 2. (a) Chemical structures of the monomer, **1**, and crosslinker, **2**, used to prepare the nanoparticles. (b) SEM micrograph of the polymeric nanoparticles.

(Figure 2b). By varying the amount of SDS added to the aqueous phase, we were able to successfully synthesize particles ranging from ~40 to ~200 nm in size. In all large animal imaging experiments, the near-infrared signal from nanoparticles prepared from monomer **1** (~40 nm) was visualized trafficking from the injection site to the draining sentinel lymph node via a discrete lymphatic vessel over a distance of up to 40 cm (Figure 3, invisible NIR fluorescence pseudo-colored lime green and superimposed on the color video for this merged image). Histologic examination was also used to confirm delivery of the IR-786 payload within the lymph node.

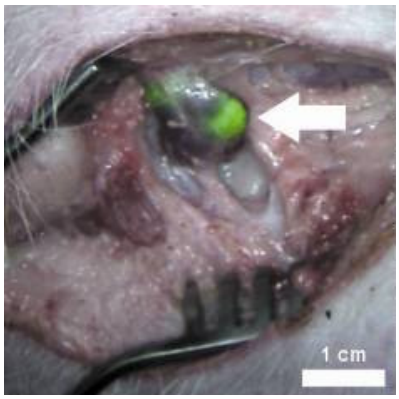

Figure 3. Visualization of NPs loaded with IR-786 NIR dye within an exposed sentinel lymph node (arrow) ~24 cm away from the injection site.

Conclusions: In summary, we have synthesized smooth, spherical polymeric nanoparticles using a mild, base-catalyzed miniemulsion polymerization technique. Although only one monomer system has been discussed, this method will allow different monomers and encapsulants to be easily combined, in addition to enabling us to vary the size of the particles. Initial imaging studies have demonstrated the lymphatic targeting capabilities of these polymeric nanoparticles, suggesting that near-infrared labeled and drug loaded NPs may play a role in future lymphatic therapy.