On-demand drug delivery from self-assembled nanofibrous gels: A new approach for treatment of proteolytic disease

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Introduction: The Holy Grail of drug delivery is an autonomous system that titrates the amount of drug released in response to a biological stimulus, ensuring the drug is released only when needed at a therapeutically relevant concentration.[1] One of the hallmarks of rheumatoid arthritis, for example, is its variable disease activity consisting of exacerbations of inflammation punctuated by periods of remission.[2,3] This presents significant challenges for matching localized drug delivery with disease activity. We report the development of an injectable self-assembled nano-fibrous hydrogel, from a generally recognized as safe (GRAS) material, which is capable of encapsulation and release of agents in response to specific enzymes that are significantly up-regulated in a diseased state including matrix metalloproteinases (MMP-2 and MMP-9) and esterases. We show that these self-assembled nano-fibrous gels can withstand shear forces that may be experienced in dynamic environments such as joints, can remain stable following injection into healthy joints of mice, and can *in vitro*

Methods: *Release kinetics*: DiD-encapsulating Ascorbyl-palmitate (**Asc-Pal**) self-assembled gel fibers (200 µL) were suspended in PBS (800 µL), and either matrix-metalloproteinase-2 (MMP-2), MMP-9, or lipase enzyme (100 ng/mL) was added followed by incubation at 37ºC. At each time-point, an aliquot (10 µL) from the supernatant above the fibers was dissolved in DMSO (90 µL), and the released DiD was quantified by UV-vis spectroscopy at the characteristic wavelength of 655 nm. After withdrawing each aliquot, the incubation medium was replenished with PBS. To examine the potential for on demand drug release, enzyme-containing media was removed after a 4-day incubation and replenished with PBS. After 7-days, fresh enzyme was added.

Stability of self-assembled gel fibers in mice: Self-assembled fibers were re-dispersed in PBS solution, and injected using a 27 gauge needle into the ankles of Balb/C mice. Animals were sacrificed after 8 weeks and thick 20-30 µm cryosections were obtained.

Results: **Asc-Pal** has been identified to form self-assembled nanofibrous hydrogels with the ability to encapsulate hydrophobic drugs or model dye (DiD), and to disassemble in response to enzymes that are typically upregulated in proteolytic diseases including MMPs and esterases. We have demonstrated the ability of self-assembled gel fibers to release an encapsulated payload in response to the enzymes that are expressed within arthritic joints. Following a 4-day incubation with enzyme containing media that triggered disassembly of fibers, media was replaced with phosphate buffer saline (PBS) and incubated at 37°C (without enzyme), which halted the disassembly of fibers and the release of dye. After a subsequent 7-day incubation with PBS, enzymes were added to the suspended fibers, triggering disassembly and the release of the encapsulated dye (Fig. 1). These results clearly suggest that Asc-Pal self-assembled fibers respond to proteolytic enzymes that are present within arthritic joints and release encapsulated agents in an on-demand manner.

To investigate the stability of fibers in the absence of inflammation, fibers were injected into the joints of healthy mice using small-bore (27G) needles. Eight weeks post-implantation mice ankles were sectioned and imaged with optical and fluorescence microscopy to observe the presence of fibers. Images of tissue-sections (Fig. 1) revealed that DiD-encapsulating fibers were present, suggesting the potential for long-term hydrolytic stability of the fibers *in vivo*.

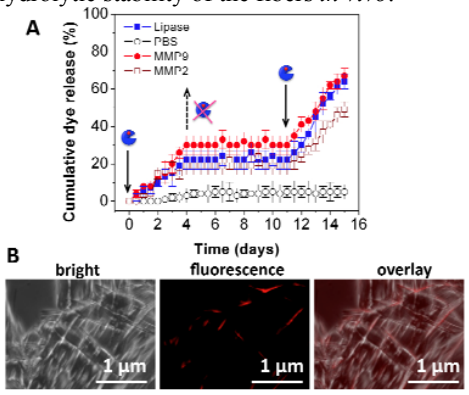

Figure 1. A) DiD release from Asc-Pal self-assembled gel fibers in response to enzymes at 37ºC *in vitro*. B) Fluor. optical microscope images of harvested ankles of healthy mice eight weeks following local injection.

Conclusions: Nanofibrous self-assembled hydrogels formed from the GRAS-agent **Asc-Pal** can encapsulate model drugs, and disassemble in response to proteolytic enzymes, yet are hydrolytically stable in healthy mice joints. Therefore, these hydrogels may be useful for delivery drugs in an inflammation dependent manner. This approach should have broad applications for localized treatment of proteolytic disease.

References:

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