Microfluidic-based generation of protease-degradable PEG-MAL microgels for protein delivery

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Statement of Purpose: Controlled delivery of proteins is a highly promising strategy to treat pathological conditions [1]. A major roadblock to effective protein therapeutics is the lack of biocompatible and injectable carriers that deliver proteins with high bioactivity and suitable release profiles. The maleimide-functionalized poly(ethylene glycol) (PEG-MAL) hydrogel system is a modular platform that incorporates protease-degradable or non-degradable cross-links, bioactive ligands, and therapeutic growth factors allowing significant material tunability [2]. Integration of this hydrogel system with microfluids facilitates the formation of highly monodisperse, covalently cross-linked microgels not accessible using previously reported techniques [3]. The objective of this study is to establish a robust and flexible protein delivery platform with tunable dosing and release profiles by designing, via microfluidics, protease-degradable PEG-MAL hydrogel microparticles with different crosslinkers and encapsulated proteins.

Methods: Microfluidic devices with flow focusing geometry and 200 µm nozzles were prepared by casting PDMS on silicon masters. Two different solutions of macromer 5% PEG-4MAL (20 kDa) and crosslinker (GPQ (W), VPM or GPQ (A) peptides) were prepared in PBS at a PEG-4MAL:crosslinker molar ratio of 2:1. The pH of both solutions was fixed at 6.5 and 4.5 respectively, without modification of the molar ratio, to control the kinetics of the gelation after contact of both phases in the microfluidic device. AlexaFluor488-conjugated IgG was encapsulated in the microgels. IgG protein was dissolved in 5% PEG-4MAL solution at 100 µg/ml and was posteriorly mixed, with the corresponding crosslinker solution in the microfluidic device before being encapsulated by macromer droplet formation. The protein release was evaluated by enzymatic degradation via collagenase I and fluorescence imaging.

Results: Protease-degradable PEG-MAL hydrogel microparticles have been produced via microfluidic techniques by using different protease-degradable crosslinkers (VPM, GPQ(W), and GPQ(A)). Populations of particles with tight size distributions, with a diameter range of 380±50, 434±69 and 401±44 µm, were obtained respectively with the different crosslinkers. AlexaFluor488-conjugated IgG was successfully encapsulated in the microgels as shown in Fig. 2. The protein release from the different microgels was evaluated along the time and amount of released protein depended on the crosslinker used (Fig.2C).

Conclusions: We have established a new platform to produce protease-degradable PEG-MAL microgels, via microfluidic techniques, for protein encapsulation; where the hydrogel degradation rate and subsequently the protein release can be easily controlled.

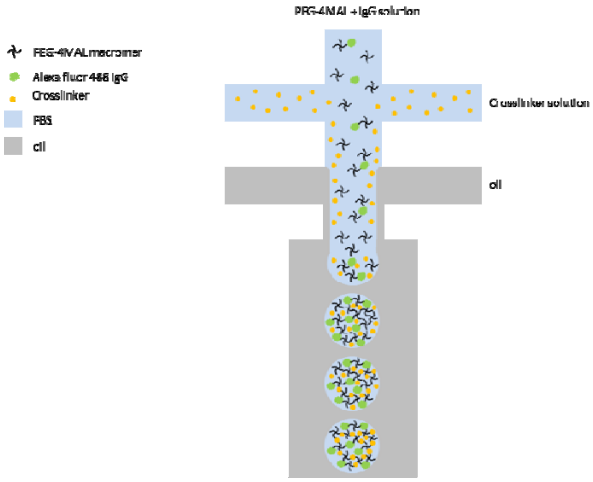

Fig. 1: Schematic representation of the protein encapsulation via microfluidics on a flow focusing device. The solution of macromer PEG-4MAL and protein is mixed with the crosslinker phase before the droplet formation. After droplet formation, peptide containing free cysteines reacts with the PEG-4MAL macromer via Michael-type addition reaction to produce biodegradable crosslinked microgels.

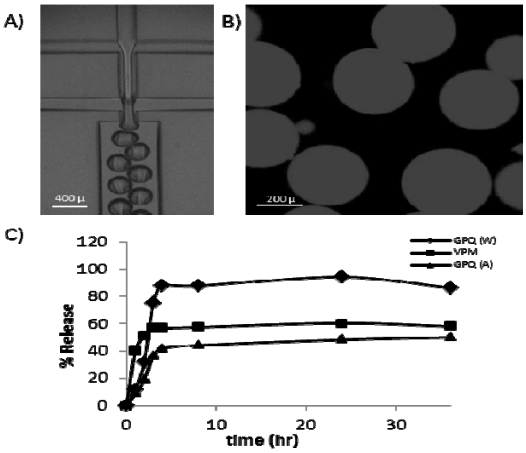

Figure 2: Protein encapsulation and release. a) Droplets with tight size distributions are generated in the microfluidic device. b) Confocal image of the encapsulated AlexaFluor488-conjugated IgG. c) Protein release from the different microgels by enzymatic degradation via collagenase I.

References:
[1] Yasko et al. J. Bone.Joint. Surg. 1992,74,659
[2] Phelps et al. Adv. Mater. 2012,24,64
[3] Tumarkin et al. Chem. Soc. Rev. 2009,38,2161.

Funding: This work is supported by Marie Curie Fellowship Program.