Hydrogel Coating of Nanoporous Particles for Enhanced Protein Stability and Controlled Intracellular Delivery

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Statement of Purpose: Protein therapeutics has recently developed and gained a significant role in many fields of medicine [1]. Proteins are currently used as therapeutic agents and for vaccinations and require repeated administration, making their clinical use often more challenging than that of conventional drugs [2]. Controlled and sustained release of proteins may enhance their therapeutic efficacy and reduce the pain and inconvenience of frequent injections. However, long term release poses a single major issue: protein instability [3]. Proteins are unstable molecules and once injected in the bloodstream they are rapidly degraded and deactivated by specific enzymes [4]. Furthermore the polymeric formulation of most of the delivery system used exposes the protein to harmful conditions that disrupt its integrity and ultimately compromise its therapeutic efficacy [5].

Methods: *Hydrogel coating of nanoporous silicon particles:* Nanoporous silicon particles (NSP) were designed and fabricated as revised elsewhere [6]. Hydrogel coating was performed after protein loading by suspending NSP in warm (40°C) agarose solution (0.05%w) for 15 minutes then cooling the solution at 4°C for 30min. Coating quality and degradation over time were evaluated by scanning electron microscope (SEM). *Protein loading, release and stability analysis:* Bovine serum albumin (BSA) was chosen as a protein probe. BSA was loaded into NSP by suspending and mixing NSP in 25mg/mL BSA (0.3mg/mL BSA FITC-conjugated) aqueous solution (prepared in PBS - GIBCO Invitrogen) for 2 hours. Protein loading efficiency and release were measured by spectrofluorimetry and FACS. The structural integrity of the BSA released after 24 hours from NC and Ag NSP after 10min trypsin treatment was analyzed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-page) using Criterion Tris-HCl Gel (BioRad) in non reduced condition. *In vitro confocal microscopy*: Cellular internalization of NSP and uptake of BSA was observed by confocal microscopy and quantified with Elements (Nikon). Cells were stained with fluorescent phalloidin (actin filaments) and DRAQ5 (nuclei) after fixation. Cellular uptake of BSA-FITC in solution was also evaluated as a control.

Results: Agarose coating was uniform, filled the pores and covered the particles surface completely as assessed by SEM (Figure 1 A and B). Loading efficiency was about 70% (10 ug of BSA were loaded in a million particles). Loading efficiencies of not coated (NC) and agarose coated (Ag) NSP were not significantly different. Spectrofluorimetry and FACS data consistently showed that while the BSA was released from NSP, particles fluorescence decreased accordingly; after 3 days almost 90% all BSA was released and NSP showed minimal fluorescence (~5%).

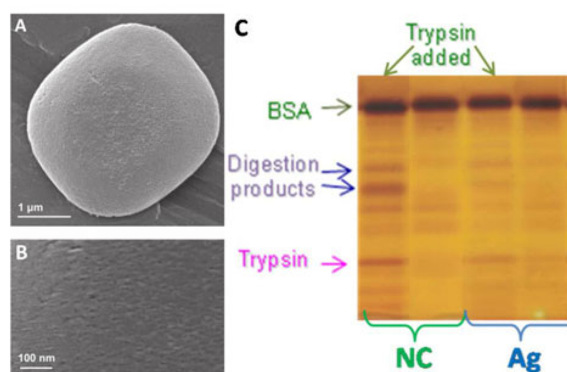

Figure 1. SEM images of agarose coated particles (A, B) and SDS-page of BSA released after 24 hours from NC and Ag NSP with and without trypsin treatment (C).

SDS-page analysis quantified also with ImageJ showed several protein fragments, digestion products (Figure 1C). The samples obtained from NC NSP in presence of trypsin have a higher number and concentration of fragments than the one from Ag NSP.

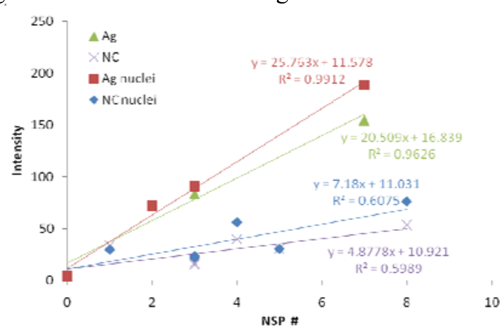

Figure 2. Intracellular fluorescence as function of number of internalized particles.

To quantify the BSA uptake, the average green fluorescence intensity of confocal images of the cytoplasm and within the nucleus of the cell was quantified with Elements (Nikon) and correlated with the number of NSP internalized per cell (figure 2). Data showed a higher uptake of BSA in cells incubated with Ag NSP than with NC NSP.

Conclusions: In this work we successfully developed a composite hydrogel NSP system for improved protein delivery. Agarose coating protects the payload from enzymatic digestion, improves intracellular delivery and increases the accumulation of the protein within the nuclei. We envisioned the use of this versatile delivery system for protein therapeutics in tissue engineering applications and for the administration of antigens in immunization protocols.

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