Evaluation of Hollow HA Microspheres as a Device for Controlled Release of Proteins

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Introduction: Carrier materials for controlled delivery of proteins such as growth factors and drugs commonly consist of biodegradable synthetic and natural polymers. Hydroxyapatite (HA), composed of the same elements as the main mineral constituent of bone, is biocompatible and produces no systemic toxicity or immunological reactions. In this study, hollow HA microspheres with a mesoporous shell wall were prepared by a novel room temperature glass conversion process and evaluated as a device for controlled release of a model protein, BSA, into a medium of PBS or PEG hydrogel.

Methods: Li_2O-CaO-B_2O_3 glass microspheres (106–150 μm) were prepared by conventional methods and converted to hollow HA microspheres by reacting the glass microspheres in an aqueous phosphate solution, as described previously.[1] Some HA microspheres were also heat treated (1–24 h at 600–900°C) to modify the microstructure of the shell wall (porosity; pore size). The microspheres were loaded with a solution of BSA (66 kDa), or FITC-labeled BSA in PBS (5 mg/ml), and the release kinetics into PBS or a PEG hydrogel were measured using a micro-BCA reagent or a fluorescence.

Results and Discussion: The as-prepared HA microspheres had a hollow core with a diameter equal to 0.6 the external diameter, high surface area (~100 m^2/g), and a mesoporous shell wall (pore size ≈ 13 nm) (**Fig. 1a**). The shell wall consisted of two distinct layers: a less porous surface layer and a more porous inner layer. Heating the HA microspheres for 5 h at 600°C resulted in coarsening of the surface layer, but little change in the microstructure of the inner layer (**Fig. 1b**). A dense shell wall with little surface porosity was obtained after heating for 5 h at 900°C (**Fig. 1c**).

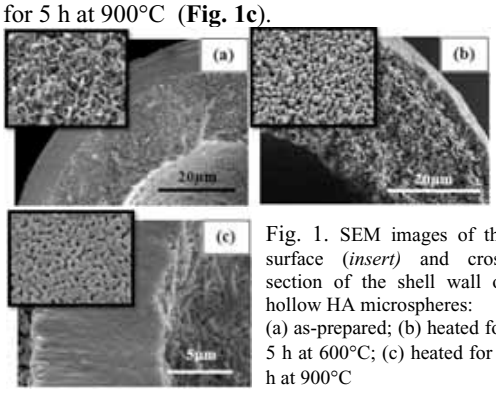

Fig. 1. SEM images of the surface (*insert)* and cross section of the shell wall of hollow HA microspheres: (a) as-prepared; (b) heated for 5 h at 600°C; (c) heated for 5 h at 900°C

The release of BSA from the as-prepared microspheres into PBS solution (**Fig. 2(a)**) was initially rapid, then slowed considerably, with 95% of final amount released within 24 h. Approximately 40% of the total BSA initially loaded into the microspheres was released into the PBS. The BSA release from the HA microspheres heated for 5 h at 600°C was markedly different: the total amount of BSA released was far higher, and the duration of the release increased markedly. Sustained release occurred over 7–14 days. Approximately 30% of the total BSA loaded into the microspheres was released after 14 days. For the HA microspheres heated for 5 h at 900°C, release of the BSA was limited, due to the dense shell wall.

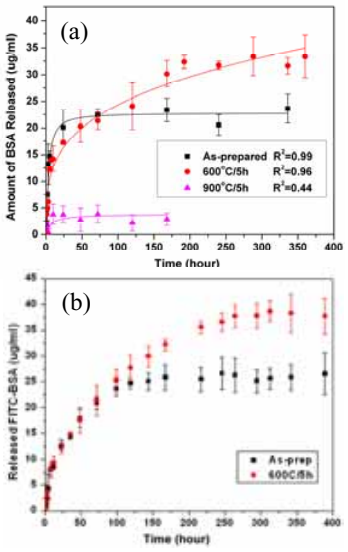

Fig. 2. Release of BSA from hollow HA microspheres (as prepared or heat treated) into a medium of (a)PBS; (b)PEG hydrogel.

The release of BSA from the HA microspheres into a PEG hydrogel (**Fig. 2b**) was used to simulate protein release into a soft tissue. Except for an increase in the duration of release from the as-prepared HA microspheres, the release into the PEG is qualitatively similar to that into the PBS (**Fig. 2a**). When compared to the release into the PBS, the duration of the release is creased from 1–2 days to 4–5 days. For both groups of microspheres, the release kinetics into the PEG are similar during the first 4-5 days, which indicates that diffusion into the PEG might be the rate controlling process (rather than diffusion through the shell wall. Later, release from the HA microspheres heated for 5 h at 600°C) continued, and ceased after 10–14 days. This longer duration of release is presumably related to the higher amount of BSA initially loaded into these heat treated microspheres.

Conclusions: The present results show promising potential for the application of hollow HA microspheres as a novel inorganic biocompatible device for controlled local delivery of proteins such as growth factors and drugs. It was shown that release of BSA can be controlled by the microstructure of the hollow HA microspheres (porosity; pore size of the shell wall), and by the surrounding medium (PBS versus PEG hydrogel). Sustained release of BSA into PBS or PEG was achieved over 7–14 days by modifying the microstructure of the as-prepared HA microspheres by a suitable heat treatment (5 h at 600°C).

References:

1. H. Fu *et al*. J Am Ceram Soc. 2010 :93:3116-3123.

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