Bioceramic Nanocrystal Coating via Pickering Emulsion Route: Fabrication of Hydroxyapatite/Biodegradable Composite Microspheres for Injectable Cell Scaffold

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Introduction: There are strong needs for synthesis of biodegradable synthetic polymers in a form of microsphere, because they have found their applications as injectable scaffolds and cell carriers for tissue engineering.

In our previous study [1], hydroxyapatite (HAp)-coated poly(L-lactide) (PLLA) microspheres were successfully prepared from an oil-in-water (o/w) type Pickering emulsion without any molecular surfactants, which has possibilities to cause allergy-like reactions and carcinogenicity. Pickering emulsions are emulsions stabilized with solid particles adsorbed at oil-water interfaces; Inorganic particles such as silica, metals, clays and organic particles such as latex particles and microgels have been used as particulate emulsifiers. In the previous study [1], PLLA was used as a model biodegradable polymer, which has a long half-life time (almost one year). The biodegradation rate of lactide polymer is often controlled by an adjustment of copolymerization ratio.

Herein we describe the fabrication of HAp-nanocrystal-coated poly(L-lactide-co-ε-caprolactone) (PLCL) or poly(L-lactide-co-glycolide) (PLGA) microspheres from an o/w-type Pickering emulsion. In this study, HAp nanocrystals were employed as a particulate emulsifier to prevent flocculation of emulsion droplets/microspheres in the absence of any molecular surfactant as well as to give cell adhesiveness on the microspheres obtained.

Methods: Spherical HAp nanocrystals were prepared with wet chemical process and used after calcination at 800˚C for 1h with anti-sintering agent to prevent calcination-induced sintering among the crystals [2]. Aliquots of centrifugally washed HAp dispersions were mixed with dichloromethane (CH_2Cl_2) solution of PLCL or PLGA at room temperature.

The HAp-nanocrystal-coated microspheres were prepared by *in situ* evaporation of CH_2Cl_2 from the emulsion at room temperature.

Results: Scanning electron microscope (SEM) observation of the HAp nanocrystals prepared with wet chemical process indicated a number-average particle diameter of 50 nm, and a coefficient of variation was calculated to be 23%.

HAp-nanocrystal-stabilized CH_2Cl_2 droplets containing PLCL or PLGA were prepared in aqueous media in the absence of any molecular surfactant. An electrical conductivity measurement indicated that o/w type emulsion was obtained for each case, and high emulsion stability was confirmed after standing for 24 h by visual inspection. As control experiments, homogenizations of CH_2Cl_2 and water, the CH_2Cl_2 solution of polymer and water, and CH_2Cl_2 and the aqueous dispersion of the HAp nanocrystals were conducted. All these control systems failed to prepare stable emulsions. Hence it has been confirmed that the HAp nanocrystals and polymer are clearly necessary toward stable Pickering-type emulsions.

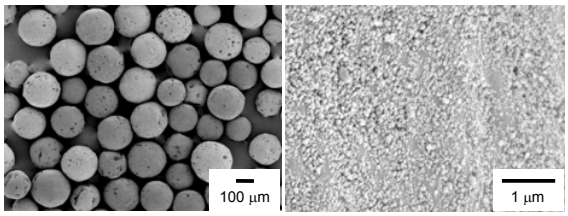

Fig. 1 SEM photographs of typical HAp nanocrystal-coated PLCL microspheres prepared from Pickering emulsion stabilized with HAp nanocrystals.

We speculated the interaction between the HAp nanocrystals in aqueous phase and carbonyl/carboxylic acid groups originated from the polymer in oil phase should be a driving force for the HAp nanocrystals to adsorb to the oil-water interface and to play an important role toward stable emulsions. HAp nanoparticle-coated PLCL and PLGA microspheres were, respectively, fabricated by evaporation of CH_2Cl_2 from the emulsion. SEM studies (Fig. 1) indicated the production of micrometer-sized and near-spherical microspheres, and the diameter was 150-300 μm. The surfaces of the microspheres were uniformly coated with the HAp nanocrystals.

The fabricated microspheres were examined as an injectable cell scaffold for cell-based therapeutic angiogenesis in mice ischemic hind-limbs. After co-injection of bone marrow-mononuclear cells (BMC), avoidance rate of limb necrosis increased statistically and immunocytochemistory showed coexistence of HAp-nanocrystal-coated microspheres and BMCs. In relation to cell sustaining, intramuscular levels of proangiogenic cytokines were also significantly elevated in ischemic tissues treated [3].

Conclusions: We described the use of HAp nanoparticles as particulate emulsifiers to fabricate the HAp-nanoparticle-coated biodegradable microspheres without any molecular surfactants. The microspheres showed an effective cell supporting in ischemic tissues and should be useful for a variety of biomedical applications including cell carrier and cell scaffold.

References: 1) Fujii S. Langmuir 2009;25:9759. 2) Okada M. J Mater Sci. 2006;41:6134. 3) Fukumoto S. Diabetes 2010;59(suppl.1):A187.