Tunable Dual Growth Factor Delivery Using Multilayered Microparticles with Controllable Degradation Kinetics

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Statement of Purpose: There is an increasing need to control the type, quantity and timing of growth factors released by various delivery systems during the healing process of tissues in order to maximize the efficiency. However, most growth factor delivery systems have been limited to the controlled release of a single growth factor, which fails to mimic the natural process of tissue healing. Tissue repair and healing is orchestrated by numerous bioactive proteins with precise spatial and temporal control. Thus, sophisticated delivery systems offering the ability to deliver multiple growth factors with distinct kinetics and high bioactivities are desirable. We have recently developed a series of approaches to control release kinetics of multiple, biologically active growth factors. Mechanisms for dual release have included controlling growth factor-mineral affinity, and creating multilayered mineral coatings on biomaterials. Here we focus on describing multilayered mineral coated microparticles (MPs) for dual growth factor delivery. We hypothesized that bone morphogenic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF), bound on different mineral coating layers, would have distinct release kinetics. Moreover, we reasoned that the release behaviors of both growth factors could be further tailored by changing the intrinsic properties (e.g. dissolution kinetics) of the mineral coatings.

Methods: HAP microparticles(HAMPs) were incubated in modified simulated body fluid (m-SBF) to create the first layer of mineral coating by incubating 10 mg of HAMPs in 10 mL m-SBF with varying $NaHCO_3$ concentrations at pH 6.8 and 37°C. The mineral coated HAMPs were then lyophilized and used for growth factor binding. 5.0 mg of the mineral coated HAMPs were incubated in 1.0 mL 1 μg/mL BMP-2 in PBS at 37°C for 4h to allow the equilibrium binding of BMP-2 on first mineral layer. The BMP-2 bound MPs were then incubated in 10 mL m-SBF for 5 days with daily m-SBF refresh to generate the second layer of mineral coating. The double layered MPs were then incubated with 1.0 mL PBS containing 100ng VEGF for 4h at 37°C to facilitate the binding of the second growth factor (Fig.1-A). The morphology of both layers of mineral coating was examined by scanning electron microscopy (SEM). The composition of coating on MPs was analyzed by both FT-IR and X-ray diffraction (XRD). To study protein binding efficiency and release kinetics of BMP-2 and VEGF, $_{125}$I-BMP-2 and $_{125}$I-VEGF were used as tracers. An *in vitro* protein release study was performed by soaking the dual growth factors bound HAMPs in 1.0 mL Tris buffer saline (TBS, pH 7.4) at 37°C. At each predetermined time point, the amount of growth factor released was measured by counting the radioactivity in the release medium.

Results: Formation of mineral coating was confirmed by showing a nano-porous, plate-like layer continuously covering the HAMPs. FT-IR and XRD spectra indicate coating formed on HAMPs surface is a carbonated hydroxyapatite mineral (cHAP). Furthermore, by incorporating different ions into the mineral coating, the morphology of the coating can be easily changed from plate-like structure (carbonate incorporated) to needle-like structure (fluoride incorporated) (Fig. 1-B&C). The BMP-2 binding efficiency on the first layer was about 55% of the initial BMP-2 amount in the solution. Only about 15% of the bound BMP-2 was lost during the formation of second layer which is mainly attributed to the "burst" release of this protein during second m-SBF incubation. The binding efficiency of VEGF on the second layer increased to about 70%, perhaps due to the increase of coating surface area from the second layer of mineral coating. Two distinct release profiles of the incorporated growth factors were observed *in vitro*: while VEGF bound on the second layer showed a typical two-phase release profile with fast release in first two weeks and more sustained release in the next five weeks, the release of BMP-2 exhibited a near zero-order release profile with no "burst" release. At the end of the release period (50 d), about 70% of the initially bound VEGF was released, while only less than 20% of the BMP-2 was released (Fig. 1-D). Notably, the release kinetics of both growth factors could be further tailored by changing the intrinsic properties of the mineral coating. The fluoride doped mineral coating significantly slowed down the release of BMP-2 while incorporation of carbonate into coatings could accelerate its release.

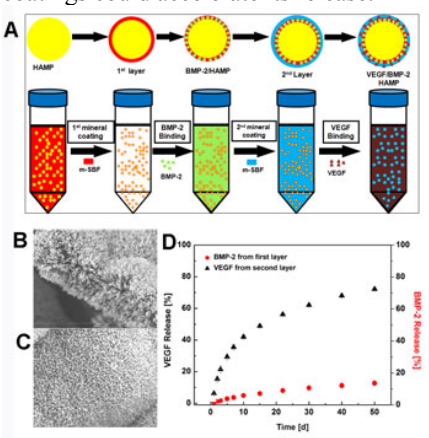

Figure 1. Schematic of dual release HAMPs fabrication (A) SEM image of the 1st layer (B) SEM image of the 2nd layer (C) Representative dual growth factor release profiles (D)

Conclusions: We developed multilayered, mineral coated MPs that can serve as an adaptable dual growth factor delivery system. Growth factors could be released with controllable kinetics based on the intrinsic properties of the mineral coating layers. This injectable microparticle based delivery platform might have numerous applications in the field of tissue engineering and regenerative medicine.