The Effect of Ion Content and pH on Plasmid DNA Delivery from Dissolving Mineral Coatings

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Statement of Purpose: Biomimetic fluids such as simulated body fluid (SBF) have been used to study mineral growth on a variety of biomaterials. The resulting coatings also have potential applications in biomolecule delivery. The intrinsic properties of calcium phosphate (Ca-P)-based mineral coatings, including binding and release of DNA (e.g. in chromatography), dissolution, and re-precipitation, suggest that they may be used as controllable carriers for DNA delivery. We hypothesized that plasmid DNA (pDNA) would bind within Ca-P coatings formed via a biomimetic process, and that they pDNA release rate would be dependent on Ca-P mineral composition and structure and the solution conditions surrounding Ca-P minerals.

Methods: A set of mineral growing solutions which contains 2, 3.5, and 5 × of Ca^{2+} and PO_4^{3-} concentrations of standard SBF solution was used to grow Ca-P mineral on pre-hydrolyzed poly(lactide-co-glycolide) films. After 10 d incubation at 37 °C, the composition and structure of mineral coatings were analyzed by scanning electron microscopy, Fourier-transformed infrared spectroscopy, and X-ray diffractometry. Ca-P mineral coatings were immersed in two set of solution solutions: (1) solutions containing 0, 0.3, 0.6, and 1 × of physiologic Ca^{2+} and PO_4^{3-} concentrations at pH 7.4; (2) solutions with varying pH in the range from 4.0 to 7.4 with physiologic Ca^{2+} and PO_4^{3-} concentrations. The dissolved Ca^{2+} and released pDNA amount were measured by Arsenazo III (MP Biomedicals, Solon, OH) and Quant-iT Picogreen dsDNA assay kit (Invitrogen, Carlsbad, CA) respectively.

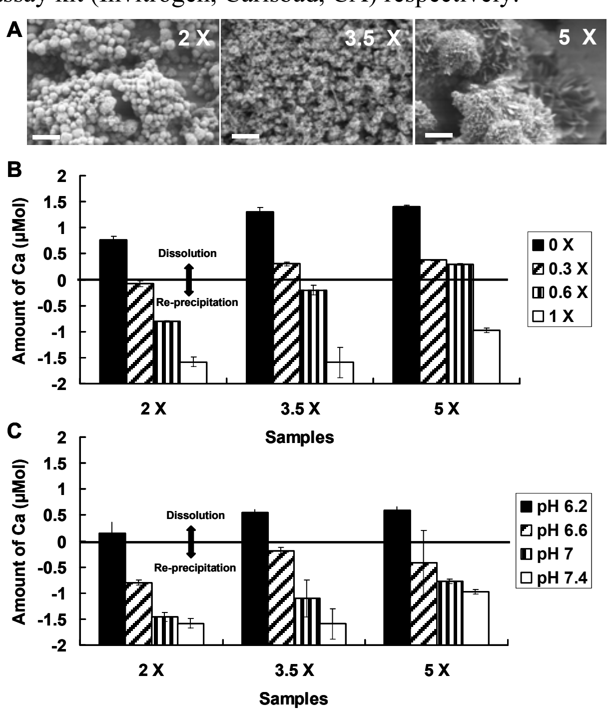

Figure 1. Morphologies of Ca-P mineral coatings formed in SBF solutions (A). Dissolution and re-precipitation of Ca-P mineral coatings are dependent on Ca^{2+} and PO_4^{3-} concentrations (B) and pH (C) in various dissolution solutions.

Results: The morphologies of mineral coatings grown on PLG films were dependent on the Ca^{2+} and PO_4^{3-} concentrations in SBF solutions and spherulitic mineral microstructure formed with different spherulite size in each condition (Fig. 1A). The amount of Ca^{2+} ions released from mineral coatings was decreased with increasing Ca^{2+} and PO_4^{3-} concentrations or pH in dissolution solutions. In addition, the Ca^{2+} release rate from Ca-P minerals formed in 5 × SBF was higher than that from minerals formed in 2 × and 3.5 × SBF solutions (Fig. 1B, C).

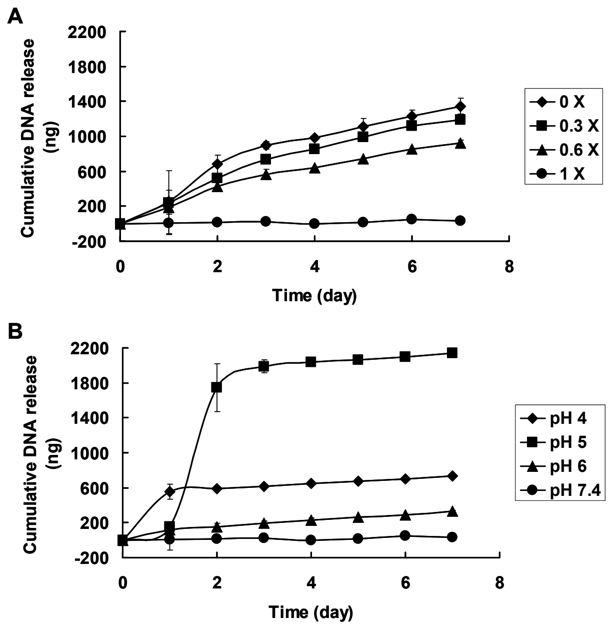

Figure 2. pDNA release from Ca-P mineral coatings formed in 2 × SBF solution is dependent on Ca^{2+} and PO_4^{3-} concentrations (A) and pH (B) in various dissolution solutions.

The release rates of pDNA were also increased with lower Ca^{2+} and PO_4^{3-} concentrations or pH in dissolution solutions (Fig. 2A, B), similar to the Ca^{2+} release trends. This result indicates that the release rates of pDNA correlated with the mineral dissolution rates.

Conclusions: Mineral stability was strongly dependent on intrinsic mineral properties and the surrounding solution environment, which contained varying ion content and pH. The mineral stability directly influenced the release rates of pDNA from mineral coatings. Therefore, mineral coatings could be designed to control the release rate and the amount of pDNA released in response to surrounding solution environments.