

## Enzymatic pH Control enables Spatially Controlled CaP Deposition onto Micropatterned Surfaces

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**Introduction:** Since bone is comprised of calcium phosphate nanocrystals (70wt%), deposition of calcium phosphate crystals onto implant surfaces has been investigated extensively over the past three decades. Wet-chemical methods such as biomimetic deposition are particularly attractive for coating implants of complex geometry. Biomimetic depositions are carried out at either fixed pH (such as Simulated Body Fluids) or gradually increasing pH, but both of these methods are associated with drawbacks such as low deposition rate or limited control over pH, respectively. In the current study, we propose a different, enzymatic biomimetic coating technique with improved control over pH and increased deposition rates. The enzyme urease was used to decompose urea into CO<sub>2</sub> and basic NH<sub>3</sub>. The goal of the current study was to test if this enzymatic coating technique was sufficiently sensitive to allow for spatial control of calcium phosphate crystals onto micropatterned implant surfaces.

**Methods:** Urease (0.04-1 units/ml) was added to solutions containing CaCl<sub>2</sub> (2.5-12.5 mM), Na<sub>2</sub>HPO<sub>4</sub> (1-5 mM), NaCl (146-730 mM) and urea (100 mM) while adjusting the initial pH at 6.00. A polystyrene (PS) film with 1000 nm wide grooves was added as a substrate for the deposition of CaP. After urease addition, a pH profile was recorded while samples taken from the solution and substrates were studied using cryo-transmission electron microscopy (cryo-TEM) and scanning electron microscopy (SEM), respectively.

**Results and Discussion:** After addition of urease, the pH generally increased until a slight pH drop at ~6.8 (caused by precipitation of CaP, Figure 1A), followed by a subsequent increase in pH up to a plateau value of ~8. The rates of pH increase were carefully controlled by adjusting concentrations of urease as well as calcium and phosphate salts. At pH 6.00 scattered CaP pre-nucleation clusters were observed with cryo-TEM, which densified with increasing pH (Figure 1B). Formation of these dense clusters was observed previously in solutions at a constant (buffered) pH, and our results confirm that such pre-nucleation clusters are also the first step towards precipitation in systems at gradually increasing pH. After the initial pH increase, CaP crystals nucleated at the micropatterned PS surfaces. These nuclei grew steadily until full coverage of the substrate surface. By varying the concentrations of the salts (2.5, 1.0 and 146 mM for CaCl<sub>2</sub>, Na<sub>2</sub>HPO<sub>4</sub> and NaCl respectively) and urease (0.1 U/ml) as well as the time of the reaction (12 hours), spatially controlled deposition of CaP was

observed characterized by confinement of CaP deposition along the ridge tops of the PS films (Figure 1C). AFM characterization indicated that ridges exhibited higher roughness values ( $R_q = 18.3 \pm 5.0$  nm) compared to the grooves ( $R_q = 8.6 \pm 1.3$  nm), thereby causing preferential deposition of CaP crystals along the rough ridge top.

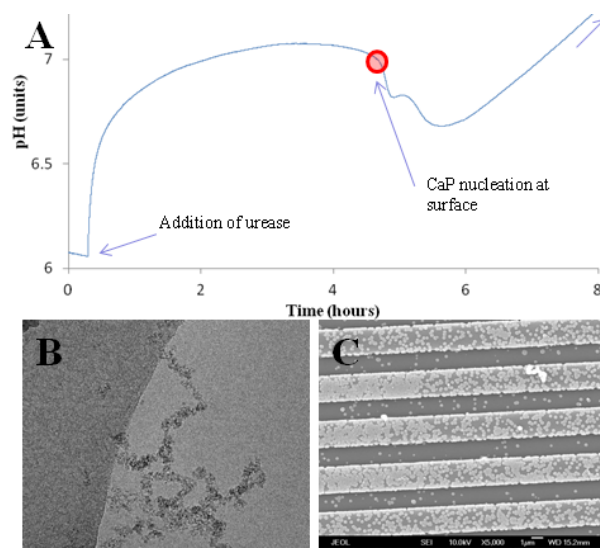


Figure 1: A: pH for sampling cryo-TEM and SEM. B: Cryo-TEM image 7 min after urease addition. C: SEM image of CaP deposited on grooved PS under optimized parameters resulting in spatiotemporally control of the deposition.

**Conclusion:** Enzymatic control over pH allows for careful control over supersaturation of solutions containing calcium and phosphate salts. After optimization of the rate of pH increase, spatially controlled deposition of calcium phosphate onto micropatterned implant surfaces was obtained.