# Effect of functional end groups of silane self assembled monolayer surfaces on apatite formation, fibronectin adsorption and osteoblast cell function.

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**Statement of Purpose:** The effect of calcium phosphate (Ca-P) surfaces of bioactive ceramics and glasses on the adhesion, proliferation, differentiation, and extracellular matrix formation of cells of the osteoblast lineage has been documented (1, 2). The terminal groups of silane self-assembled monolayers can induce and nucleate Ca-P surfaces on substrates, since these surfaces possess varying capabilities for the heterogeneous nucleation and growth of hydroxyapatite (3). This study investigates the effect of calcium phosphate precipitates formed on functionalized surfaces using SAMs on protein adsorption, cell attachment, cell proliferation and alkaline phosphatase activity (ALP). The SAM chemistry produces highly controlled surfaces with –OH, –COOH and $–NH_2$ -terminated groups. We hypothesize that Ca-P coating is controlled by the SAM functional end group and that the function of biomolecules and bone cells, will be enhanced on –OH and –COOH terminated SAMs, and further enhanced by Ca-P precipitates. We focus on the interaction of biomolecules and cells with the composite SAM - Ca-P surfaces. We investigate the effect of these Ca-P mineralized surfaces on fibronectin adsorption, attachment, proliferation and alkaline phosphatase activity of osteoblast-like cells.

**Methods**: Three SAMs, 3-amino propyl- triethoxysilane (APTES), 3-triethoxysilylpropyl succinic anhydride (TESPSA) and 3-glycidoxypropyl trimethoxy- silane (GPTMS) were grafted onto the silicon oxide surfaces for use as the template to induce apatite mineralization under argon atmosphere. Surface induced mineralization process was used to coat the SAMs with Ca-P by immersion in a supersaturated solution, which simulates the electrolyte content of physiological fluids complemented with various concentrations of electrolytes, comparable to the human blood plasma (4). Protein coverage on the Ca-P coated surfaces was determined by adsorbing different concentrations of fluorescent labeled Fn on the surfaces under physiological conditions and using spectrophotometer readings. Human osteoblast cells (MC3T3-E1) seeded at $10^4$cells/cm$^2$ was used to investigate the attachment and proliferation on the surfaces and the percentage of attached cells was calculated. ALP activity was determined by enzymatic conversion of 4 (para)-nitrophenylphosphate (pNPP) to 4-nitrophenol (pNP). Absorbance of pNP was measured at 430 nm. Analysis of the cytoskeleton organization was done by selective labeling of F-actin of the MC3T3-E1 cells seeded on the substrates using phalloidin 548 at 4 $^oC$. Confocal microscopy was used to scan extent of cell spreading and cytoskeleton formation on the substrates.

**Results / Discussion:** The $–NH_2$ surface showed the highest protein coverage (monolayer) of 229.7 ng/cm$^2$, while the least Fn density of 146.4 ng/cm$^2$ was obtained on the –OH surface, in 2.5 µg/mL Fn concentration. The Fn surface density isotherm shows a rapid increase followed by a slower growth, consistent with conventional adsorption isotherms. The % cells attached were evaluated in comparison to the number of cells/cm$^2$ initially seeded on the substrates. Up to 70 % increase in cell attachment was seen on the –COOH and –OH surfaces after 24 h cell culture (Fig. 1). It was observed that cells barely proliferated on non-apatite-coated surfaces. ALP activity was greatly enhanced on Ca-P coated compared to the non-coated surfaces. Confocal microscopy images show that cells on Ca-P coated substrates exhibited comparatively higher fluorescence intensity than on non-coated surfaces. The fluorescence intensity of cells seeded on the biomineralized surface show full actin development and formation after 36 h of cell attachment. Cytoskeleton organization of MC3T3-E1 cells is enhanced on Ca-P coated surfaces.

**Conclusions:** The results consistently demonstrate that the functions of osteoblast-like cells and biomolecules are largely influenced by Ca-P coatings. This work further indicates the potential for the use of Ca-P coatings in hard tissue repair therapies.

## References

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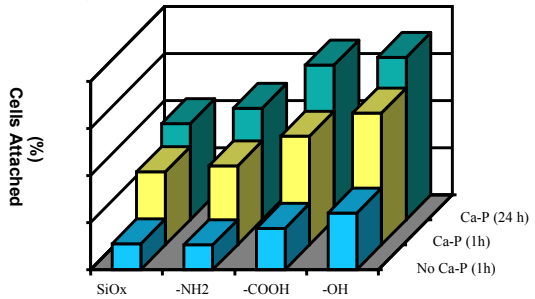


**Fig. 1: Increased cell attachment (expressed as % of number of cells initially seeded) after 1 h incubation (seeding $10^4$ cells/cm$^2$) was observed on the Ca-P precipitates on the silanes compared to the non-coated surfaces. Cell attachment was significantly higher on OH and COOH surfaces. There was a slight increase in cell attachment after a longer duration (24 h) of incubation.**

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