Silicon addition to hydroxyapatite increases nanoscale electrostatic, van der Waals, and adhesive interactions

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Abstract: The normal intersurface forces between nanosized probe tips functionalized with COO-terminated alkanethiol self-assembling monolayers (SAM) and dense, polycrystalline Si-substituted synthetic hydroxyapatite (SiHA) and phase pure hydroxyapatite (HA) were measured via a nanomechanical technique called chemically specific high resolution force spectroscopy. A significantly larger van der Waals component was observed for the SiHA compared to HA; Hamaker constants ($A$) were found to be $A_{SiHA} = 44 \pm 38 \ \text{zJ}$ and $A_{HA} = 18 \pm 19 \ \text{zJ}$. Using the Derjaguin-Landau-Verwey-Overbeek (DLVO) approximation which assumes linear additivity of the electrostatic double layer and van der Waals components, and the Poisson-Boltzmann surface charge model for electrostatic double layer forces, the surface charge per unit area, $\sigma (\text{C/m}^2)$, was calculated as a function of position for specific nanosized areas within individual grains. SiHA was observed to be more negatively charged than HA with $\sigma_{SiHA} = -0.024 \pm 0.013 \ \text{C/m}^2$, 2× greater than $\sigma_{HA} = -0.011 \pm 0.006 \ \text{C/m}^2$. Additionally, SiHA has increased surface adhesion ($0.7 \pm 0.3 \ \text{nN}$) over HA ($0.5 \pm 0.3 \ \text{nN}$). The characterization of the nanoscale surface variations of surface forces of SiHA and HA could lead to improved understanding of the initial stages of bone-biomaterial bonding.

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INTRODUCTION

The development of enhanced synthetic materials for use in orthopedic implants to replace lost or damaged human bone is a continual goal of biomaterials research. Studies have indicated the importance of soluble silicon to bone mineralization and regeneration, and recently silicon has been incorporated into one of the most promising synthetic bone implant materials, hydroxyapatite (HA, Ca$_5$(PO$_4$)$_3$OH). HA is considered bioactive, meaning that an interfacial bond between the implant and the surrounding bone forms. Si-substituted HA (SiHA) has shown markedly enhanced \textit{in vitro} apatite formation in simulated body fluid (SBF), increased \textit{in vitro} cell proliferation and creation of focal points of adhesion, as well as \textit{in vivo} bone ingrowth and remodeling. In this research, we employ dense, polycrystalline 0.8 weight (wt.\%) SiHA as a model system and focus on the measurement and understanding of nanoscale surface interactions, i.e. their magnitude, functional form, spatial distribution, and deconvolution into various constituents such as electrostatic double layer and van der Waals, all of which are expected to play a critical role in the physiochemical processes that take place upon implantation of a bioactive biomaterial into a bony site such as adsorption of ions and biomolecules, formation of calcium phosphate (apatite) layers, and cellular interactions.

The formation of apatite layers when HA-based biomaterials are implanted into a bony site is considered essential for the creation of a strong bond with the surrounding tissue. The literature suggests that negative surface functional groups provide preferential sites for nucleation of an amorphous calcium phosphate layer, through the higher adsorption of positive calcium ions, resulting in a positive layer that will attract the phosphate groups leading to the formation of the apatite layer. Negatively charged self-assembled polymer monolayers were found to produce the highest growth rate of precipitated apatite from simulated body
fluid, and electrically polarized, negatively charged ceramic surfaces have been shown to exhibit increased apatite layer formation, cell adhesion, and osteobonding versus non-poled HA samples.

Our previous work on (unsubstituted) dense, polycrystalline HA employed microfabricated cantilever force transducers with nanosized probe tips (end radii, $R_{tip} < 100$ nm) chemically functionalized with negatively charged carboxy-terminated self-assembling monolayers (COO$^-$-SAMs) to measure the net (repulsive) surface interaction in aqueous solution within individual HA grains as a function of nanoscale position. At pH 5.6 and an ionic strength (IS) of 0.01M, the local nanoscale surface charge per unit area, $\sigma_{HA}$ (-0.0037 to -0.072 C/m$^2$) and corresponding zeta potential, $\xi_{HA}$ (-18 to -172 eV), were estimated from fits of this nanomechanical data to a Poisson-Boltzmann based electrostatic double layer surface charge model. This analysis showed that HA was negatively charged, thought to be due to preferential concentration of PO$_4^{3-}$ groups to the top few nanometers of the surface, and that $\sigma$ and $\xi$ were spatially dependent, possibly associated with the exposed crystal plane or facet. The magnitude of these values were consistent with microelectrophoretic measurements on similar samples yielding $\xi_{HA} = -50 \pm 5$ eV (pH 7.4, IS=0.0001M).

Here, we apply the nanomechanical technique of chemically and spatially specific high resolution force spectroscopy to dense, polycrystalline 0.8 wt.% SiHA at pH 7.4 and IS=0.01M. Silicon incorporation into an HA lattice (SiHA) has been shown to result in a more negative $\xi_{SiHA} = -71 \pm 5$ eV (pH 7.4, IS=0.0001M, measured by microelectrophoresis) compared to HA, possibly due to substitution of PO$_4^{3-}$ by SiO$_4^{4-}$. In this paper, in addition to an electrostatic double layer component, a marked shorter range attractive component, presumed to be van der Waals interactions, was observed for SiHA that was minimal for the unsubstituted HA. Hamaker constants ($A$) for the 0.01M data were estimated from the distance of cantilever
jump-to-contacts and verified by both this method and also fits to the inverse square power law\(^{28}\) to data taken at high IS (1M) where the majority of electrostatic double layer forces were screened out. Using these estimated values of \(A\), the Derjaguin-Landau-Verwey-Overbeek (DLVO) approximation which assumes linear additivity of the electrostatic double layer and van der Waals components,\(^{29}\) and the Poisson-Boltzmann surface charge model for electrostatic double layer forces\(^{25}\), \(\sigma_{\text{SiHA}}\) and \(\xi_{\text{SiHA}}\) were calculated as a function of position for specific nanosized areas within individual grains. These values were compared to control experiments on dense, polycrystalline, phase pure HA conducted with the same probe tip to avoid variations due to probe tip geometry.

Such investigations of nanoscale surface properties have great potential to contribute important information relevant to the molecular origins of HA biocompatibility. Furthermore, examining how the incorporation of silicon affects such nanoscale properties as electrostatic repulsion, van der Waals interactions, and morphology of precipitated layers will be critical to the optimization, development, and design of new HA-based biomaterials.

**MATERIALS AND METHODS**

**A. Sample Preparation and Characterization**

HA and SiHA were synthesized using an aqueous precipitation chemical route between calcium hydroxide and orthophosphoric acid. For the case of SiHA, silicon tetraacetate was added to the reaction as a source of silicate ions. A detailed protocol is described elsewhere\(^{2,30}\). Phase purity of the precipitated material used in this study was verified through X-ray diffraction (XRD) using a Philips PW1710 X-ray diffractometer (PANalytical Inc., Almelo, The Netherlands). Data were collected between 25° and 40° \(2\theta\) using a step size of 0.02° and a count time of 2.5 seconds. Phase identification was accomplished by comparing the peak positions of
the diffraction patterns with ICDD (JCPDS) standards. X-ray fluorescence confirmed the incorporation of 0.8 wt.% Si in the HA lattice and stoichiometry (Ca/P or Ca/(Si+P) ratio ~1.67) using a Philips PW1606 spectrometer. FTIR spectra on sintered powders were obtained using a System 2000 FT-IR/NIR FT-Raman with a resolution of 4 cm\(^{-1}\) and by averaging 100 scans.

Dense (>98% of the theoretical density, 3.13 g/cm\(^3\)), polycrystalline HA and SiHA pellets (~1 cm in diameter) were prepared by compacting the as-prepared powders and isostatically pressing them at a pressure of 150 MPa prior to sintering in air at 1200 °C for 2 hours, using a ramp rate of 2.5 °C/min. The sintered pellets were visualized using a environmental Scanning Electron Microscopy (ESEM, Phillips ESEM-FEG, XL30) in low vacuum mode, using an off axis gaseous secondary electron detector and a 10 kV operating voltage. Image analysis was performed using the Leica QWIN image analysis software package. The grain size of the sintered samples was determined by drawing around the boundaries of individual grains manually. The equivalent circular diameter of the grains (\(G_{gr}\)) was determined from the measured area of each grain according to the following equation:

\[
G_{gr} = \left( \frac{\text{Area}}{\pi} \right) \times 2 \quad (1)
\]

Wettability of the HA and SiHA pellets was assessed via contact angle measurements with deionized (DI) water (Video Contact Angle System 2000, AST Inc.) as a function of time allowed to sit on the surface. The temporal evolution of three separate water drops (at different positions) on a HA and SiHA sample were measured, giving \(n=6\) angles (right and left angle of each drop) measured at each time point.

**B. High Resolution Force Spectroscopy**

High resolution force spectroscopy experiments were performed using both one and three-dimensional Molecular Force Probes (Asylum Research, Inc. Santa Barbara CA). The three dimensional version has the additional capability to image and perform nanomechanical
measurements with nanometer-scale spatial sensitivity. An Au-coated, Thermomicroscopes, V-shaped, Si₆N₄ cantilever probe tip (cantilever spring constant, k ~0.05 N/m as measured individually by a thermal vibration method) was chemically functionalized with the carboxyl-terminated self-assembling monolayer (COOH-SAM) 11-mercaptoundecanoic acid (HS-(CH₂)₁₀-COOH, Aldrich - used as received) as described previously. The same cantilever probe tip was used to image and nanomechanically probe several distinct positions within a variety of grains on both HA and SiHA samples in 0.01M ionic strength trihydroxymethyl aminomethane ((CH₂OH)₃CNH₂, Tris buffer) solution (pH=7.4). The probe tip end radius, Rₜₐₚ, was measured by scanning electron microscopy to be ~70 nm. Experiments were also carried out in 1M, pH7.4 Tris buffer solution with a different probe tip of Rₜ₉ ~90 nm. Surface forces, F, were measured as a function of tip-sample separation distance (henceforth referred to as "distance", D) on approach (i.e. probe tip advancing towards the sample surface at a constant rate) and retract (i.e. probe tip moving away from the sample surface at a constant rate) at a z-piezo displacement rate of 2 μm/s. Ten nanomechanical experiments were carried out at each sample site location. For the approach data, F-D curves for each position were averaged, and standard deviations reported. For the retract data, the maximum attractive adhesive force and the corresponding distance of adhesion of each individual retract curve were recorded and averaged for each position. Images presented are contact mode normal cantilever deflection (scan rate of 1 Hz), which is reflective of surface topography; light areas correspond to regions of high topography and darker areas to regions of lower topography. Surface topographical analysis was performed on the corresponding height images within a ~200 × 200 nm square area around each position tested nanomechanically. Tests for statistical significance were carried out on data using unpaired Student’s t-tests.

C. Theoretical Predictions
Following Derjaguin-Landau-Verwey-Overbeek (DLVO) theory,\textsuperscript{29} the total net interaction force measured on approach was assumed to be a linear summation of an attractive van der Waals component and a repulsive electrostatic double layer component as follows:

\[ F_{\text{total}}(D) = F_{\text{electrostatic}}(D) + F_{\text{vdw}}(D) \] (2)

The non-retarded van der Waals force approximately follows an inverse square power law:\textsuperscript{28}

\[ F_{\text{vdw}} = AR_{\text{TIP}}/(6D^2) \] (3)

where \( F_{\text{vdw}} \) is the van der Waals force between a sphere of radius \( R \) (assumed to be equal to the probe tip radius, \( R_{\text{TIP}} \)) and a planar surface separated by a distance \( D \) and \( A \) is the Hamaker constant. An average Hamaker constant was estimated for the 0.01M data at each position from the jump-to-contact separation distances (\( D_{\text{jump-to-contact}} \)) as follows:

\[ D_{\text{jump-to-contact}} = \left( \frac{AR_{\text{TIP}}}{3k} \right)^{1/3} \] (4)

The resulting values of \( A \) were confirmed by fits of high IS (1M) nanomechanical data (where electrostatic double layer forces are largely screened out) to equation (3), as well as the jump-to-contact method. \( F_{\text{electrostatic}} \) was approximated for each position by a Poisson-Boltzmann-based formulation which describes the electrostatic double layer interaction between a planar surface and hemispherical probe tip both with a constant surface charge per unit area in an electrolyte solution.\textsuperscript{25} A numerical method, known as the Newton method on finite differences, was used to solve the nonlinear Poisson-Boltzmann equation.\textsuperscript{32} The surface charge per unit area of the probe tip, \( \sigma_{\text{COO}^-} \), was fixed in the simulations and estimated to be \( \sim 0.018 \text{ C/m}^2 \) as determined by control experiments using a COO\(^-\)-SAM probe tip versus a COO\(^-\)-SAM planar substrate and fitting the data to the same electrostatic double layer theory described above. \( R_{\text{TIP}} \), \( A \), and the solution ionic strength were also fixed parameters and \( \sigma_{\text{SIHA}} \) or \( \sigma_{\text{HA}} \) was the only free fitting variable. Five individual F-D curves per position were fit for \( D > 5 \text{ nm} \) to exclude any possible
short range non-DLVO components of the interaction. $\sigma_{\text{SiHA}}$ or $\sigma_{\text{HA}}$ were averaged for each position and reported with standard deviations. Once the full nonlinear Poisson-Boltzmann equation is fit to the experimental data, the surface potential was calculated by setting $D=0$ and solving the Poisson-Boltzmann equation. The surface potential calculated in this way is equivalent to zeta potential.$^{33}$

**RESULTS**

FTIR was used to identify the molecular groups present on the SiHA and to assess the alteration in the hydroxyl and phosphate bands (Fig. 1). Peaks were observed for both samples at 568, 600, 960, 1043, and 1008 cm$^{-1}$ corresponding to PO$_4^{3-}$ groups and at 630 cm$^{-1}$ corresponding to the OH$^-$ group. For SiHA, additional peaks were observed at 498 and 884 cm$^{-1}$ corresponding to the SiO$_4^{4-}$ group. We see that silicon content leads to a decrease in the intensity of the band at 630 cm$^{-1}$ that corresponds to the OH$^-$ group.$^6$ This is consistent with the substitution mechanism proposed where some phosphate groups (-3) are replaced by silicate groups (-4), leading to the loss of some OH groups in order to maintain the charge balance.$^2$ The introduction of silicon in the structure of HA also influences the bonds and symmetry modes of the phosphate groups, as can be seen by the intensity ratio between the phosphate bands at 960 (?1) cm$^{-1}$ corresponding to the symmetric stretch and 1043 (?3) cm$^{-1}$ that corresponds to the asymmetric stretch. The ratio changes from 0.440 for HA to 0.444 for SiHA. This result is in agreement with previously reported data.$^2$

ESEM images (data not shown) show HA has significantly larger ($p < 0.001$) grains ($5.2 \pm 2.1 \, \mu\text{m}$, $n=100$) than SiHA ($2.6 \pm 1.3 \, \mu\text{m}$, $n=100$). This observation confirms that silicon interferes with grain growth, which is likely to occur if the silicon was preferentially located at the grain boundaries and therefore diminished grain boundary movement during grain growth.$^3$
Contact angle measurements showed SiHA to have an instantaneous contact angle (65 ± 2°, n=6) significantly lower than HA (75 ± 2°, n=6)\(^b\) (p < 0.001). As the water droplet remained on the surface, the contact angle for both samples decreased at a rate of 0.11°/second for SiHA and 0.13°/second for HA as shown in Fig. 2. This decrease is possibly due to absorption of water into the crystal lattice, forming a hydrated surface layer, as has been observed previously.\(^{34}\)

A contact mode deflection image of the SiHA and HA surfaces in aqueous electrolyte solution (pH=7.4, IS=0.01M) along with numerical labels for the specific locations where each series of nanomechanical experiments was carried out is shown in Figs. 3A and B. The probe locations are grouped by crystalline facet where a facet was defined as a distinct topographical face whose area had a relatively constant slope. Fig. 3C shows typical height profiles of the SiHA and HA surfaces with the baseline slope of underlying grain subtracted. For both the SiHA and HA sample, the average linear root-mean-squared (RMS) roughness of height profiles (after subtraction of the baseline slope of the grain) through all positions probed was < 1 nm. Therefore, within experimental resolution, the surfaces of both samples had similar roughness and the scale of surface features was much smaller than the scale of the probe tip. For comparison, the radius of the maximum probe tip-surface interaction area at the maximum interaction distance (i.e. D=15 nm) was ~200 nm.\(^{35}\)

Fig. 4 shows typical individual force and force/radius versus distance curves in aqueous electrolyte solution (pH=7.4, IS=0.01M) using a COO\(^-\)-SAM probe tip (\(R_{\text{TIP}} \sim 70\) nm). Both curves exhibit a nonlinearly increasing net repulsive force (as indicated by the positive value) with decreasing separation distance for D < 15 nm and jump-to-contacts; for SiHA position 1 of Fig. 3A, \(D_{\text{jump-to-contact}} = 2.46\) nm and for HA position 1 of Fig. 3B, \(D_{\text{jump-to-contact}} = 1.04\) nm. The average \(D_{\text{jump-to-contact}}\) for SiHA (n=76 experiments from all 13 positions) was found to be equal

\(^b\) This contact angle for HA is larger than measured previously\(^{12}\) because the value reported here was an instantaneous value while the previously reported value was taken ~60 seconds after placing the water droplet on the surface.
to 2.64 ± 0.82 nm, corresponding to an average Hamaker constant (calculated using equation (4)), $A_{SiHA}$ of 44 ± 38 $zJ$ with a range from 3 - 103 $zJ$. This value can be compared to that of pure HA where the average $D_{jump-to-contact}$ for HA ($n=46$ experiments from all 13 positions) for HA was 1.94 ± 0.70 nm, yielding an average $A_{HA}$ of 18 ± 19 $zJ$ with a range from 2 - 32 $zJ$, which is statistically smaller than that of SiHA (p<0.001). The magnitudes of Hamaker constants calculated by equation (4) were confirmed by additional nanomechanical experiments performed in pH 7.4, 1M Tris buffer aqueous solution using a COO$^-$-SAM probe tip ($R_{tip}$ ~90 nm) (Fig. 5). Since the majority of $F_{electrostatic}$ is screened out at this high salt concentration, the net force at $D>5$nm was assumed to be primarily van der Waals. The averaged approach force versus distance curves ($n=20$ experiments at 4 positions each) were fit to equation (3) and yielded an average $A_{SiHA} = 70 ± 14 zJ$ with a range from 60 - 90 $zJ$, which is somewhat higher than $A_{SiHA}$ calculated from the $D_{jump-to-contacts}$ at 0.01M but within the same range. Additionally, an average $A_{SiHA}$ was calculated for the 1M data from the jump-to-contact distances and found to be 62 ± 49 ($n=22$ experiments from all 4 positions) which is similar to the value found through fitting the averaged curves to equation (3) and demonstrates self-consistency of the two methods used to estimate $A$. It was not possible to calculate $A_{HA}$ from the 1M data as only non-DLVO repulsive forces were observed for $D<5$nm. Hence, all subsequent comparisons to electrostatic double layer theory (at 0.01M IS) were carried out for $D>5$ nm, where <1% of the force were non-DLVO. Fig. 6A is plot of the average Hamaker constants versus position and the corresponding histogram for this data is shown in Fig. 6B. Unpaired Student’s t-tests comparing the average Hamaker constant for different facets, show that HA possesses significant differences in 7 out of 10 comparisons (p<0.01) and for SiHA 6 out of 10 comparisons are statistically different (p<0.01).
**Fig. 7A** and **B** show a typical averaged force and force/radius versus distance curve for the COO⁻-SAM probe tip versus the SiHA (position 1 of **Fig. 3A**) and HA (position 1 of **Fig. 3B**) samples respectively in aqueous electrolyte solution (pH7.4, 0.01M), each plotted with ± one standard deviation. The jump-to-contact regions are smoothed out compared to the individual curves (e.g. **Fig. 4**) by the averaging process. The standard deviation remains relatively constant and low in the longer range repulsive region (D > 5 nm) and is observed to increase with decreasing separation distance at shorter distance ranges (D < 5 nm) due to variability in the adhesive jump-to-contacts. **Fig. 8** shows the averaged force versus distance curves for the COO⁻-SAM probe tip versus the SiHA and HA samples at selected (for data clarity) sample locations labeled in **Fig. 3**. While the data presented here is all from a single probe tip-sample combination, experiments using different probe tips and samples showed the trends described here to be highly consistent.

Typical individual force and force/radius versus distance curves with example theoretical fits showing the van der Waals and electrostatic components are given in **Fig. 9**. At D = 5 nm, which is one Debye length away from the surface, the electrostatic component accounted for ~98% of the net force for the HA sample and ~86% of the force for the SiHA sample. The average $\sigma_{\text{SiHA}}$ for all positions calculated from the theoretical fits to the force versus distance curves was found to be $-0.024 \pm 0.013 \text{ C/m}^2$ ($n=64$ experiments from all 13 positions) while for $\sigma_{\text{HA}}$ it was found to be $-0.011 \pm 0.006 \text{ C/m}^2$ ($n=65$ experiments from all 13 positions) (the latter value being consistent with experiments on similar samples reported previously).\(^{12,c}\) Using the non-linear Poisson-Boltzmann equation, surface potentials, equivalent to zeta potentials, were calculated. For SiHA, a potential of $-87 \pm 55 \text{ eV}$ was obtained, while for HA a potential of $-49 \pm 28 \text{ eV}$ was obtained. Unlike more standard zeta potential measurements, however, the average

\(^c\) In our previous work,\(^{12}\) the van der Waals component was not included in theoretical fits for unsubstituted HA, but due to its small magnitude, inclusion of it resulted in a minimal different in the estimated surface charge density (<5%).
σ_{SiHA} and σ_{HA} can be calculated for each nanosized surface position and compared to surface features such as crystalline facet (Fig. 10A). The effect of sample surface slope on the surface charge density was investigated through geometrical calculations in previous work and shown to be inconsequential in the ranges measured.\textsuperscript{12} A histogram corresponding to the data in Fig 10A is given in Fig. 10B and shows that the SiHA has a bimodal distribution with the first peak approximately equivalent to that for HA. Unpaired Student’s t-tests comparing the average surface charge density magnitudes on different facets, show that HA possesses significant differences in 7 out of 10 comparisons (p<0.01) and for SiHA 4 out of 10 comparisons are statistically different (p<0.01).

The retract force versus distance curves for the COO\textsuperscript{-}-terminated SAM probe tip vs. HA and SiHA were also analyzed; 100% of the SiHA retract curves showed adhesion while 91% of the HA retract curves showed adhesion. The average adhesion distance for SiHA was 3.6 ± 4.8 nm (number of F-D curves for all 13 positions, n=130) and for HA it was 4.3 ± 5.5 nm (number of F-D curves for all 13 positions, n=126) which are statistically the same (p<0.01). The average adhesion force for SiHA was 0.7 ± 0.3 nN (number of F-D curves, n=126 for all 13 positions), which is statistically larger than for HA which was 0.5 ± 0.3 nN (number of F-D curves, n=123 from for 13 positions) (p<0.01). The adhesion forces for HA showed more positional dependence (Fig. 11A), 8 out of 10 facet average comparisons statistically different (p<0.01), than SiHA where only 1 out of 10 facet average comparisons were statistically different. Fig. 11B shows the corresponding histogram for the data in Fig. 11A. For HA, there appears to be a positive correlation (p<0.01) between average Hamaker constant and adhesion force for each position (Fig. 12A, R\textsuperscript{2} = 0.75, number of data points, n=13 positions) and a negative correlation between average surface charge density and adhesion force for each position (Fig. 12B, R\textsuperscript{2} = 0.74, number of data points, n=13 positions), while minimal correlation is observed for SiHA.
DISCUSSION

One major result of this work was the observation of an increased attractive component on approach for SiHA compared to HA. This was shown by increased jump-to-contact distances at 0.01M (Fig. 4) and the large difference between samples observed at 1M (Fig. 5) where the HA has a net repulsive intersurface interaction and the Si-HA has a net attractive intersurface interaction. This attraction was attributed to van der Waals forces and the average Hamaker constant of SiHA (46 zJ) was found to be ~2.5 × greater that of HA (18 zJ). The magnitudes of the van der Waals values here are intermediate compared to typical ceramics in aqueous solution which range from 1.6 to 94 zJ. Initial contact angle measurements are consistent with SiHA having a higher surface energy than HA, which is related to the Hamaker constant. A contribution to this could come from the fact that Si is more easily polarized than P due to lower atomic number with the same number of electron shells. Another possible contribution is the effect the introduction of silicon into the HA structure has on the symmetry modes of the phosphate groups. The intensity ratio between the phosphate bands symmetric stretch (1043 (?3) cm$^{-1}$) and asymmetric stretch at 960 (?1) cm$^{-1}$ changes from 0.440 for HA to 0.444 for SiHA. This indicates the phosphate groups in SiHA have less symmetry, which could increase their polarizability. Both HA and SiHA show spatial variation in the Hamaker constant which could be due to crystal structure and varied number of substituted SiO$_4^{4-}$ groups. Variation in Hamaker constant could play a role in the bone-biomaterial bonding.

Consistent with previous results, SiHA was observed to be more negatively charged than HA with $\sigma_{\text{SiHA}}$ (-0.024 C/m$^2$) 2× greater than phase pure HA (-0.011 C/m$^2$). In terms of surface or zeta potential, $\xi_{\text{SiHA}} = -87 \pm 55$ eV is 1.8× greater than $\xi_{\text{HA}} = -49 \pm 28$ eV. This may be attributed to the substitution of some PO$_4^{3-}$ groups by SiO$_4^{4-}$ groups. Although FTIR results in air indicate that the substitution mechanism leads to the loss of OH$^-$ groups in order to maintain
charge balance, charge balance is not maintained in fluid. The zeta potential predicted by nanomechanical measurements were, surprisingly, extremely similar to the measured microelectrophoretic values on similar samples $\xi_{\text{SiHA}} = -71 \pm 5 \text{ eV (pH7.4, IS}=0.0001\text{M})$ and $\xi_{\text{HA}} = -50 \pm 5 \text{ eV (pH 7.4, IS}=0.0001\text{M})$ given the difference in ionic strength, sample type, dependence on particle size and shape,\textsuperscript{38,39} and fundamental differences between the two methods. Typically, $\xi$ measured by microelectrophoretic and other techniques are expected to be lower than fitted potentials for nanomechanical data ideal (smooth) surfaces\textsuperscript{40} because of the potential drop in the immobilized liquid layer close to the surface since the slip plane where zeta potential is measured is further from the surface than the Stern surface where Poisson-Boltzmann theory begins.\textsuperscript{41} The surface charge densities calculated in this paper via F-D curves are effective charges at the Stern surface since electrostatic double layer theory is valid only within the diffuse double layer.\textsuperscript{33} This should resemble what a biomacromolecule or cell feels inside the diffuse double layer approaching a biomaterial surface.

The Hamaker constant and surface charge of SiHA had slightly less of a surface positional dependence than HA in that there were fewer statistical differences between facet averages (Fig. 6 and 10). The results for HA are consistent with previous research showing the surface charge to vary with nanoscale position on the surface\textsuperscript{12}. Variation is associated with exposed crystal plane, each of which has a different arrangement of the charged ions making up the HA lattice, since different facets in the same grain have different surface charges.\textsuperscript{12} In this experiment, it was observed that there was less of a correlation between surface charge and crystal plane or facet for SiHA. This could be accounted for by substitution of SiO\textsubscript{4}\textsuperscript{4-} groups into random PO\textsubscript{4}\textsuperscript{3-} lattice locations which could decrease the overall surface charge inconsistently within crystal planes. This is also consistent with the bimodal distribution in that the more negatively charge
peak could be associated with areas of higher SiO$_4^{4-}$ concentration and the peak aligned with HA likely is associated with areas lower in SiO$_4^{4-}$ concentration.

SiHA was observed to have a larger attractive adhesion force ($0.7 \pm 0.3$ nN) than HA ($0.5 \pm 0.3$ nN). The magnitude of the adhesion forces observed for both HA and SiHA are typical of multiple noncovalent interactions. The adhesion forces measured are likely not hydrophobic interactions since both HA and SiHA become more hydrophilic over time in aqueous solution, as seen in the contact angle measurements. The adhesion forces for HA showed more positional dependence and there appears to be some correlation between increased Hamaker constant and surface charge with increased adhesion force for small $A$ and $\sigma$ as shown in Fig.10. Since both $A$ and $\sigma$ increase for SiHA, this correlation is only seen in the HA data meaning that the SiHA has larger and more consistent surface adhesion. The larger adhesion forces observed for SiHA could be due to its larger Hamaker constant versus HA and could play a role in the bone-biomaterial bonding. The greater attractive van der Waals force for SiHA could help overcome the electrostatic double layer repulsion of osteoblasts with negatively charged cell membrane surfaces (e.g. surface potentials of MC3T3-E1 mouse osteoblasts suspended in physiological saline were measured through ultrasonic attenuation spectroscopy to vary from -29.4 to -52.4 mV at pH 7.3 - 7.5$^{42}$) and the net negative charge of most serum proteins.$^{43}$ In general, adhesion interactions have contributions from surface forces such as van der Waals, surface charge, and hydrophobicity as well as surface topology, which can be difficult to deconvolute.

As described in this paper, the magnitude and spatial distribution of nanoscale surface characteristics such as surface charge, Hamaker constant, and adhesion are markedly different between SiHA and HA. It is expected these characteristics play an important role in the physiochemical processes that take place upon implantation of a bioactive biomaterial into a bony site. In addition to surface forces measured in this paper it should be reiterated that HA did
have larger grains (5.2 ± 2.1 µm) than SiHA (2.6 ± 1.3 µm), likely due to silicon interference in grain growth.\(^3\) The decreased grain size, and therefore increased number of triple grain junctions in SiHA, increases its solubility, which could also affect its bioactivity.\(^{44,45}\) Bioactivity is likely determined by a combination of nano- and microscale properties. Isolating the effects of each, in theory, could be determined by a general methodology whereby samples are prepared of varying microstructure (e.g. grain size) but constant nanomechanical and structural features, which in practice is nontrivial.

**CONCLUSIONS**

In this paper, we have employed the technique of chemically and spatially specific high resolution force spectroscopy to measure the net nanoscale surface interactions of dense, polycrystalline 0.8 wt.% Si-substituted HA at specific nanosized positions within individual grains in aqueous electrolyte solution. We have shown that silicon incorporation into an HA lattice results in increased nanoscale attractive van der Waals interactions and an increased negative charge surface charge density relative to unsubstituted HA. Additionally, SiHA was found to have increased surface adhesion less dependant on surface position than HA, possibly due to increased van der Waals interactions. The markedly enhanced *in vitro* apatite formation in simulated body fluid (SBF),\(^6-8\) increased *in vitro* cell proliferation and creation of focal points of adhesion,\(^9\) as well as *in vivo* bone ingrowth\(^10\) and remodeling\(^11\) of SiHA over pure HA may be due in part to these nanoscale surface characteristics.

**References**


Figure 1: FTIR spectra of dense, polycrystalline HA and 0.8 wt.% SiHA.
Figure 2: Contact angle measurements of a SiHA and HA sample surface ($n=6$ for each time point corresponding to droplets at 3 different sample positions including a right and left angle for each droplet). The water droplets were allowed to sit on the surface and contact angle measurements were made over time.
Figure 3: Contact mode AFM deflection images taken with COO$^-$-SAM functionalized probe tip in aqueous solution ($R_{\text{tip}} \sim 70 \text{ nm}, k \sim 0.042 \text{ N/m, IS}=0.01 \text{M, pH}=7.4$) showing specific positions (×'s) probed and labeling of facets on A) SiHA and B) HA surfaces. C) Typical height profiles along black dashed lines in images A and B (corresponding to position 2) with baseline slope of facet subtracted out.
Figure 4: Typical individual force and force/radius versus distance curves on approach taken in aqueous solution (pH=7.4, IS=0.01M, R\textsubscript{tip} \sim 70 nm, k \sim 0.042 N/m) on approach for a COO-SAM probe tip versus A) SiHA (position 1 labeled in Fig. 3A) demonstrating jump-to-contact (D\textsubscript{jump-to-contact} = 2.46 nm) and B) HA (position 1 labeled in Fig. 3B) demonstrating jump-to-contact (D\textsubscript{jump-to-contact} = 1.07 nm).
Figure 5: Force and force/radius versus distance curves on approach taken in aqueous solution (pH=7.4, IS=1M, $R_{tip} \sim 90$ nm, $k \sim 0.053$ N/m) for a COO$^-$-terminated SAM functionalized probe tip vs. HA and SiHA surfaces. Averaged data (number of individual curves per each average curve, $n=20$) at three different positions. Hi-lo bars represent one standard deviation.
Figure 6:  A) Averaged Hamaker constant for each position (corresponding to those labeled in Figs. 3A and B) of SiHA and HA samples calculated from jump-to-contact distances of individual approach force versus distance (number of individual curves per sample position, $n \sim 5-10$).  B) Frequency histogram of Hamaker constant for each position for HA and SiHA.
Figure 7: Average force versus distance curves on approach with standard deviations for one sample position (number of individual curves per average curve, \( n=10 \)) for a COO-SAM functionalized probe tip versus A) SiHA (position 1 labeled in Fig. 3A) and B) HA (position 1 labeled in Fig. 3B) surfaces in aqueous solution (pH=7.4, IS=0.01M, \( R_{tip} \sim 70 \) nm, \( k \sim 0.042 \) N/m). add arrows to the left.
Figure 8: Averaged force and force/radius versus distance curves on approach (number of individual curves per each average curve, \( n=10 \)) for a COO-SAM functionalized probe tip versus HA and SiHA surfaces in aqueous solution (pH=7.4, IS=0.01M, \( R_{\text{TIP}} \sim \text{nm} \), \( k \sim 0.042 \text{ N/m} \)) each for a different sample location shown in Fig. 3A and B.
Figure 9: Typical individual force and force/radius versus distance curves on approach with theoretical fits showing the predicted net force, as well as the individual van der Waals and electrostatic components for a COO$^-$-SAM functionalized probe tip ($\sigma_{\text{COO}^-} = -0.0125$ C/m$^2$) versus A) SiHA ($\sigma_{\text{SiHA}} = -0.018$ C/m$^2$) and B) HA ($\sigma_{\text{HA}} = -0.009$ C/m$^2$) surfaces in aqueous solution (pH=7.4, IS=0.01M, $R_{\text{TIP}}$ ~70nm, k ~0.042 N/m, Hamaker constants were fixed to the values calculated from the jump-to-contacts: $A_{\text{SiHA}} = 42$ zJ, $A_{\text{HA}} = 2.8$ zJ).
Figure 10:  A) Averaged surface charge density for each position (corresponding to those labeled in Figs. 3A and B) of SiHA and HA samples calculated by fitting individual approach force versus distance curves for each position to the Poisson-Boltzmann-based electrostatic double layer surface charge model using the following fixed parameters: $\sigma_{\text{COO}^-} = -0.0125$ C/m², $\text{IS}=0.01$ M, $R_{\text{TIP}} \sim 70$ nm, and $A_{\text{SiHA}}$ or $A_{\text{HA}}$ calculated from the jump-to-contact distances at each location (number of individual curves fit per sample position, $n=5$). B) Frequency histogram of average positional surface charge density for each position of HA and SiHA.
Figure 11:  A) Averaged adhesion force for each position (corresponding to those labeled in Figs. 3A and B) of HA and SiHA samples (IS=0.01M, pH=7.4, k ~0.042 N/m, \( R_{\text{TIP}} \) ~70 nm, (number of individual curves per sample position, \( n=10 \)).  B) Frequency histogram of average adhesion force for each position of HA and SiHA.
Figure 12: A) Average adhesion force versus average Hamaker constant for each position shown in Figs. 3A and B (number of experiments per position used for Hamaker constant calculation, $n \sim 10$, $n \sim 10$ for adhesion calculation) for SiHA and HA (pH=7.4, IS=0.01M, $R_{tip} \sim 70$ nm, $k \sim 0.042$ N/m) showing a correlation factor for HA data of $R^2 = 0.75$ and for SiHA data $R^2 = 0.13$. B) Average adhesion force versus surface charge for each position (number of experiments per position used for surface charge calculation, $n=5$, $n \sim 10$ for adhesion calculation) shown in Figs. 3A and B for SiHA and HA (pH=7.4, IS=0.01M, $R_{tip} \sim 70$ nm, $k \sim 0.042$ N/m) showing a correlation factor for HA data of $R^2 = 0.74$ and for SiHA data $R^2 = 0.27$. 