Influence of surface pretreatment on the coating quantity and properties of nanostructured octacalcium phosphate on commercially pure titanium

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The influence of surface pretreatment conditions including sodium hydroxide concentrations and cleaning methods on composition, microstructure and properties of the octacalcium phosphate (OCP) coating on commercially pure titanium by rapid biomimetic technique was studied. Layer of amorphous sodium trititanate (Na$_2$Ti$_3$O$_7$) gel was found to form on the surface of alkali pretreated titanium. Using high NaOH concentration and rinsed cleaning method could produce more uniform, but thinner OCP coatings than using low NaOH concentration and rigorous ultrasonic cleaning which resulted in isolated spheroidal microstructure. Regardless of pretreatment conditions, the contents of all OCP coatings were not significantly different and all coatings similarly comprised nano-thick and plate-like OCP crystals vertically grown on the surface of titanium. Contact angles of all coatings similarly decreased to zero compared to value of 76 for uncoated titanium indicating high wettability of all coating surfaces.

Keywords: Biomimetic, Octacalcium phosphate, Bioactive coating, Medical, Alkali pretreatment

The main purpose of surface engineering is to retain the bulk properties of the materials while modifying the surface property by altering the atoms, compounds or molecules chemically or physically or by coating with different materials$^{1-2}$. In biomedical engineering field, bioactive coating has been employed to enhance the bioactivity of the metallic implant and to improve the stability the bone/implant interface resulting in the increase in quality and quantity of the osteointegration. Generally, various bioactive materials for example hydroxyapatite, tricalcium phosphate and glass ceramics which could form direct bonding with bone tissue were employed as bioactive coatings on the titanium implants to increase their bioactivities$^{3-5}$. Typically, coating was done by thermal spraying process which involved the use of extremely high temperature resulting in the phase heterogeneity and coating delamination$^{6}$. Several alternative coating techniques for example electrophoretic deposition, solgel and radio frequency magnetron sputtering have been; thus, investigated to substitute high temperature thermal spraying process$^{7-9}$.

One of the promising techniques for producing such bioactive coating is a biomimetic method which mimics the mineralization process of bone$^{10-12}$. This method is carried out by immersing the sample in an aqueous solution containing calcium and phosphate ions at pH and physiological temperatures. The biomimetic coating process typically employed simulated body fluid solution (SBF) or modified SBF as soaking media$^{13-15}$. The produced coating consisted of minute crystals resembling the biological apatite crystals found in bone. However, this process tended to be slow and needed long soaking times in order of several days or weeks to produce sufficient amount of coatings. Recently, a new accelerated calcium phosphate solution which contained highly supersaturated calcium and phosphate ions was developed for using as media for rapid biomimetic process$^{16}$. It was able to produce octacalcium phosphate (OCP, Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O) coating rapidly in few hours which provided a comparatively faster coating process compared to several days or weeks for typical biomimetic coating. OCP has been postulated as precursor of biological apatite crystals in bone, tooth dentin and enamel. Therefore, it has recently attached interest and was increasingly investigated for using as bone regenerative implant and coating due to its greater resorbability, osteoconductivity and osteoinductivity compared to typical biostable hydroxyapatite$^{17-20}$. In this study, the influence of alkali pretreatment parameters including sodium hydroxide concentration

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and cleaning methods on the composition, microstructure and properties of such rapid biomimetic OCP coating was investigated and compared. The knowledge gained from this study could be employed to further enhance properties of the resulted rapid biomimetic coating for possible use as bioactive coating in the future.

Materials and Method

Coating preparation

Titanium sheet (cpTi grade 2, Pengfa Industry Inc.) was cut into 25 mm diameter discs and cleaned by acetone and deionized water using ultrasonic cleaning bath (Crest CP1100D). The rapid biomimetic coating process was divided into two main steps including alkali pretreatment and coating (Table 1). Alkali pretreatment step was carried out by soaking the discs in sodium hydroxide (NaOH) solutions having concentrations of 1 M, 3 M or 5 M at 70°C for 24 h. The discs were then cleaned by deionized water using either sonicating for 1 h (designated Ultrasonic or S) or gently rinsing (designated Rinse or R). Subsequently, the pretreated samples were subjected to similar coating step by submerging them in the accelerated calcium phosphate solution (Na+ 154 mM, Cl- 201.7 mM, Ca2+ 3.87 mM and HPO42- 2.32 mM; pH 7.3) at 50°C for 6 h using a ratio of surface area to solution volume of 32.74. Finally, the coated samples were taken out, rinsed and dried at room temperature.

Characterizations

Weight change of the samples after passing through pretreatment (\(W_p\)) and coating (\(W_c\)) steps of the rapid biomimetic coating process were measured by a precision balance (Mettler Toledo XS204) having a resolution of 0.0001 g. Coating thickness was measured by a stylus profilometer (Bruker Dektak XT) having a vertical resolution of 1 Å and using a scanning speed of 100 μm min\(^{-1}\). Due to the rough surface of the coating consisting of peak and valley responses, the arithmetic mean of the mean peak vertical height and the mean valley vertical height was considered as an average vertical height since it gave equal consideration to peak and valley responses. In the measurement, the stylus was scanned across the coated and non-coated area on the same sample at the scanning distance of 3 mm. Coating thickness was then determined from the difference in the arithmetic mean of mean peak and valley vertical heights of the coating area and that of the uncoated titanium surface. Surface roughness values (\(R_a\)) of the samples were measured by an atomic force microscope (Seiko SPA 400) using dynamic force microscopy (DFM) mode and a scanning speed of 0.25 Hz. Surface wettability was measured by a contact angle technique using a goniometer (Rame-Hart Instrument Model 200) and deionized water was employed as a dropping liquid. Phase composition of the surface of the samples was evaluated by X-ray diffractometer (Rigaku TTRAX III) in thin film mode using a step angle of 0.02° and speed of 5° min\(^{-1}\). JCPDS files were used to identify the peaks of main components of the samples. Functional groups of the samples were analyzed by FT-IR imaging microscope (Perkin Elmer Spectrum Spotlight 300) using micro attenuated total reflectance (micro-ATR) technique. Coating adhesion was evaluated by performing a scratch test using a nano mechanical tester (Ti Premier, Hysitron) with a spherical sapphire probe (500 μm ball radius). The sample was scratched by the indenter with a linearly increasing normal load from 0 to 800 mN using a loading rate of 0.89 mN s\(^{-1}\) and scanning rate of 11.1 μm s\(^{-1}\). The coating adhesion strength was estimated from the critical load when discontinuities appeared on the curve of normal force and lateral force. Microstructure and morphology of the samples were studied by a scanning electron microscopy (SEM, JEOL JSM-5410) and a transmission electron microscope (TEM, JEOL JEM-2010). In the case of SEM, all the samples were gold sputtered prior to the observation. In the case of TEM,

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the coatings were scrapped off, dispersed in ethanol and sonicated for 15 min and dropped on continuous carbon film grids prior to observation.

Statistical analysis
The differences in properties amongst samples were analyzed using an analysis of variance (ANOVA) and Tukey post hoc testing. A value of \( p < 0.05 \) was considered significantly different.

Results and Discussion

Phase composition and functional groups
Figures 1a and 1b show the XRD patterns of the titanium surfaces after passing through alkali pretreatment step and coating step, respectively. In the case of alkali pretreated surfaces, the low intensity and broad diffraction peaks at 20 of 24.2°, 28.5° and 48.5° were observed. These broad peaks were similar to the XRD peaks reported previously and could possibly be assigned to sodium trititanate (Na\(_2\)Ti\(_3\)O\(_7\)) (JCPDS card # 72-0148). These peaks intensity tended to increase with increasing NaOH concentration, but no significant difference was observed when using different cleaning methods. In the case of coated samples, the main characteristic peaks of octacalcium phosphate (OCP) at 20 of 4.7°, 9.4°, 9.7° and 16.0° (JCPDS card # 26-1056) were clearly seen. The peaks at 20 of 17.2°, 26° and 28.9°-34.1° were also observed and could be assigned to OCP (JCPDS card # 26-1056) or hydroxyapatite (HA)(JCPDS card # 09-432) since they are generally overlapped. Upon examination of magnified portion of XRD patterns (Fig. 1c), a broad peak at around 20 of 10.7° and a very low magnitude at around 20 of 13.5° was observed. The former broad peak was not thought to give any useful information and might be resulted from the noise of the measurement. The latter peak could be assigned to OCP. All these peaks were similarly detected for all samples regardless of the concentration of pretreatment sodium hydroxide solution or cleaning technique used. Figures 2a and 2b show FT-IR spectra of alkali pretreated samples and coated samples, respectively. All pretreated samples similarly showed broad bands of absorbed water at 3700–2400 cm\(^{-1}\) and 1658 cm\(^{-1}\). The intensity of these bands increased with increasing NaOH concentrations employed. For similar NaOH concentration, pretreated samples which were cleaned by rinsing, showed greater band intensities than those of samples that were cleaned by ultrasonic. In the case of coated samples, all samples displayed main spectra bands at 1023-1126 cm\(^{-1}\) corresponding to the \( \nu_3 \) mode of PO\(_4\)\(^{3-}\). In addition, HPO\(_4\)\(^{2-}\) bands at 861 cm\(^{-1}\), \( \nu_1 \) mode of PO\(_4\)\(^{3-}\) band at 962 cm\(^{-1}\), OH bands at 3700-2400 cm\(^{-1}\) and \( \nu_2 \) mode of H\(_2\)O band at 1658 cm\(^{-1}\) were observed. These were typically the characteristic bands of OCP or HA. However, a closer look at the magnified spectra, especially in the \( \nu_3 \) PO\(_4\)\(^{3-}\) region (Fig. 2c), indicated two more bands at ca. 1195 and 916 cm\(^{-1}\).

Fig. 1 — XRD patterns of samples (a) after alkali pretreatment step, (b) after coating step, (c) enlarged patterns of samples after coating step between 20 of 4° and 15°
Figures 3a and 3b show the microstructures of samples after pretreatment step and coating step, respectively. Pretreated surfaces comprised rough nanotextures while untreated titanium displayed smooth surface (image not shown). The sizes of these rough nanostructures generally increased with increasing NaOH concentrations. For similar NaOH concentration, cleaning by rinsing produced larger nanotextures than those of ultrasonic cleaning. In the case of coated samples, all coatings were porous consisting of sharp and interconnected plate-like crystals vertically grown on the surfaces of titanium. Using ultrasonic cleaning produced isolated spheroids on the titanium surfaces when pretreating with 1 M and 3 M NaOH concentration, but changed to uniform platelet crystals when using 5 M NaOH. In contrast, samples which were cleaned by rinsing showed isolated spheroids only when pretreating with 1 M NaOH, but displayed uniform platelet crystals when using 1 M and 5 M NaOH concentrations. TEM analysis (Fig. 4) also confirmed that the shape of crystals in all coatings were nano-thick platelets having smaller thicknesses compared to their widths and lengths. The crystal widths and lengths were of various sizes and could not be measured accurately (ranging 1.7-5.2 μm). However, the crystal thicknesses in each coating samples were not significantly different and were approximately 65.1 nm excepting for 5R-C sample which had the greater crystal thickness (Table 2).

Weight changes and coating properties

Figure 5 shows the influence of changing NaOH concentrations and cleaning methods on weight changes after alkali pretreatment ($W_p$) and after coating ($W_c$). $W_p$ of 1R-P, 1S-P and 3S-P samples were limited and not significantly different from that of untreated titanium. Increasing NaOH concentrations increased $W_p$ regardless of cleaning methods used. At similar NaOH concentration, cleaning by rinsing showed greater $W_p$ than ultrasonic cleaning. In the case of coated samples, $W_c$ were much greater than $W_p$, but did not significantly alter with changing NaOH concentrations or cleaning methods. Table 2 shows the coating thicknesses of samples as measured by a profilometer. Samples pretreated with 1 M NaOH had significantly greater coating thicknesses than those of using higher concentrations. For the same concentration of 1 M, sample cleaned by rinsing showed greater coating thicknesses than sample cleaned by ultrasonic (8.7 μm vs 6.3 μm), but not statistical significant. Regardless of cleaning methods, pretreating with other NaOH concentrations did not produce any significant changes in coating thicknesses (4.3-5.1 μm).

Figure 6 shows the typical AFM images of the samples after passing through pretreatment and coating steps. It could be seen the rough nanostructures were
Fig. 3 — SEM images showing microstructure of alkali pretreated titanium surface and rapid biomimetic coating using different NaOH concentration and cleaning methods
formed as a result of coating process. Figure 7 shows the measured surface roughness of pretreated samples and coated samples. In the case of alkali pretreated samples, mean roughness ($R_a$) of the samples varied between 83.1-184.2 nm depending on the pretreatment conditions. Only samples which were cleaned by rinsing showed significantly greater roughness than that of untreated titanium. In addition, the roughness of rinsed samples were also greater than those of ultrasonic cleaned samples excepting when using the highest NaOH concentration where the roughness of ultrasonic cleaned sample (5S-P) was comparable to rinsed samples which employed lower NaOH concentrations (1R-P and 3R-P). In either cleaning methods employed, using different NaOH concentrations did show significant influence on the roughness values for each group. In comparison to alkali pretreated samples, roughness of the coated samples (561.6-1152 nm) was much higher. Coated samples pretreated with 1 M NaOH concentration had greater surface roughness than samples pretreated with higher NaOH concentrations. For similar NaOH concentrations, no influence of cleaning methods on coating roughness of the samples was observed. Regardless of alkali pretreatment conditions, contact angles of all coatings similarly decreased to zero compared to value of 76 of uncoated titanium (Table 2) indicating an increase in a wettability of all samples after coating process.

### Scratch test

Figure 8 shows the critical loads of coated samples resulted from a scratch test. In general, coated samples pretreated with 1 M NaOH concentration had significantly lower critical load than samples pretreated with higher NaOH concentrations. Critical
loads of coated samples pretreated with 3 M and 5 M NaOH concentration were not significantly different. For similar NaOH concentrations, no influence of cleaning methods on critical loads of the samples was observed.

Resorbable or biodegradable materials have attracted interest in the field of tissue engineering since they can be substituted by new tissue over time through the process of tissue remodeling. Several calcium phosphates have been reported to biodegrade in vivo and could be associated with their solubility at physiological pH. Octacalcium phosphate (OCP) was one of such soluble calcium phosphates. It was considered to be chemically closed to hydroxyapatite and could transform to hydroxyapatite both in vitro and in vivo. In vivo biodegradability, osteoconductivity and osteoinductivity of OCP were observed and shown to be better than those of hydroxyapatite. OCP was also reported to
participate in the early stage of biomineralization process of bone and teeth\textsuperscript{26-28}. In this study, all the coatings could be indexed to OCP from XRD diffraction peaks at 20 of 4.7°, 9.4°, 9.7° and 16.0° which corresponded to (0 1 0), (0 2 0), (1 1 0) and (-1 0 1) planes of OCP respectively. The peaks at 20 of 17.2°, 26° and 28.9°-34.1° could be indexed for (-1 1 1), (0 0 2) and (-4 0 2) planes of OCP or (-1 2 1), (0 0 2) and (2 1 1) planes of hydroxyapatite (HA) since they are closed and overlapped. However, crystallographic data are not always conclusive in terms of phase identification. Vibrational spectroscopy such as FTIR can be used as complimentary tool for gaining more insight on the very specific vibrational features of ions. Two distinct bands at approximately 1195 cm\textsuperscript{-1} and 916 cm\textsuperscript{-1} of FT-IR spectrum were detected. These bands were specifically assignable to HPO\textsubscript{4}\textsuperscript{2−} ions in the OCP lattice configuration, but not for hydroxyapatite. These differences could be used to distinguish between OCP and HA by IR spectroscopy\textsuperscript{29}. Furthermore, measurement of d-spacing of the crystals from TEM (images not shown) showed the values along the crystal width plane of approximately 3.8 Å and along the crystal thickness plane of approximately 9.3 and 18.8 Å\textsuperscript{0}. These values corresponded well to (0 2 0), (1 1 0) and (0 1 0) diffraction planes of OCP. Therefore, the main phase of coating formed by the rapid biomimetic coating process in this study was considered to be OCP. It is well known that precipitated calcium phosphate in aqueous solutions mainly include several types of phases. Although HA formation in SBF has frequently been reported, OCP formation in similar environments has also been observed. HA was reportedly more thermodynamically stable than OCP. However, OCP has been regarded as precursors of HA or the metastable phases of calcium phosphate since it was kinetically favorable than that of HA\textsuperscript{30-32}. The nucleation rate of OCP was, thus, substantially higher than that of HA. HA would not form directly from nucleated, OCP could grow without transforming into HA as long as the driving force for OCP growth was greater than that for HA formation. Thermodynamical analysis of the precipitation condition also indicated that OCP crystals could grow even in a basic solution at 37°C, so long as the driving force to precipitate OCP was larger than that to form HA\textsuperscript{33}. Although studies of calcium phosphate formation in simulated physiological environments have not yet fully identified specific conditions for forming specific calcium phosphate phases, some parameters were observed to influence the formation of OCP. To maintain the driving force of OCP precipitation, it was reported that keeping the solution in a static condition either with or without constant calcium ions supply was important to precipitate OCP over a wide range of calcium/phosphate ratios\textsuperscript{34-36}. The ions present in the solution were also thought to play an important role in the kinetics of calcium phosphate phase formation\textsuperscript{37}. The chemistry of substrate surface was another factor that could affect the OCP formation\textsuperscript{38,39}. In this study, the coating step was carried out in the static condition by using buffered ACS solution. The use of ACS solution which had greater supersaturation than that of SBF and contained different ion species from SBF were also thought to favor rapid nucleation and precipitation of OCP. This nucleation and precipitation of OCP could acidify the solution as the pH of the ACS solution was observed to decrease from the initial value of 7.3 to 6.8 during coating process. Subsequently, the ACS solution then quickly became under saturated due to reduction of calcium ions which, together with the decrease in pH, caused the solution to be less supersaturated with respect to OCP. Ultimately, the nucleation of OCP was limited and crystal growth became the main mechanism of OCP formation. The transformation of grown OCP plates to HA was difficult due to the slow kinetics of reaction.

Generally, titanium is covered with a passive oxide layer which can dissolve in NaOH solution and forms an amorphous sodium titanate gel layer containing Ti–OH groups on the surface after alkali pretreatment step\textsuperscript{40}. Several types of sodium titanate could be formed depending of the pretreatment conditions including Na\textsubscript{2}Ti\textsubscript{3}O\textsubscript{11}, Na\textsubscript{2}Ti\textsubscript{3}O\textsubscript{7}, Na\textsubscript{2}Ti\textsubscript{6}O\textsubscript{13} and Na\textsubscript{2}TiO\textsubscript{3}\textsubscript{21,41-43}. These hydrogels are hydrated by nature and Na\textsuperscript{+} ions in the gel layer can be continuously released when soaking in biomimetic solution causing the increase in degree of supersaturation, ion activity and pH of the soaking solution. All of these lead to the induction of calcium phosphate nucleation on the titanium surface. In this study, XRD and FT-IR analysis indicated that sodium trititanate (NaTi\textsubscript{2}O\textsubscript{3}) was mainly formed on titanium surface after alkali pretreatment step. The amount of this amorphous gel layer (as indicated from W\textsubscript{p}) increased with increasing NaOH concentration. This was corresponding to the increase in the intensity of OH bands at 3700-2400 cm\textsuperscript{-1} and H\textsubscript{2}O band at 1658 cm\textsuperscript{-1} of FT-IR spectra with
increasing NaOH concentration. The increase was possibly due to the hydrated nature of the sodium trititanate gel layer. Furthermore, the increase in the size of nanotexture on the pretreated titanium surface as observed by SEM and the increase in surface roughness values with increasing NaOH concentration could also indicate such correlation between gel layer content and NaOH concentration. This corresponded well to previous reports that the amount of gel layer increased with increasing concentration of NaOH solution, treatment time, and treatment temperature.

Cleaning step after pretreatment was reported to be a crucial step to yield a crack-free biomimetic coating. Ultrasonic and rinsing were two main cleaning techniques that were frequently employed, but no comparative study was previously reported. In this study, different cleaning methods were found to affect the residual amount of this amorphous gel layer on the titanium surface after cleaning. From weight change after pretreatment, FT-IR, SEM and surface roughness results, the amounts of sodium trititanate gel layer on samples which were cleaned by rinsing were greater than samples which were cleaned by ultrasonic. Since sodium titanate gel layer formed by alkali pretreatment was noted to be mechanically unstable and required heat treatment to convert it into a more stable form, the use of ultrasonic cleaning which was a more rigorous process than rinsing could destroy greater amount the gel layer. Therefore, the contents of residual amorphous gel layer of ultrasonic cleaned samples were lower and possibly less uniform. The combination of using high NaOH concentration and cleaning by rinsing was; thus, favorable to yield high content of sodium trititanate on the titanium surface.

After subjecting different alkali pretreated titanium samples to coating step to induce rapid biomimetic coating on the titanium for 6 h, it was found that the coating could be formed on all titanium surfaces. It was shown previously that the simulated body fluid or other coating solution could become cloudy due to the self-crystallization or self-precipitation of calcium phosphate crystals depending on the compositions or conditions employed which could affect the coating performance. In this study, the accelerated calcium phosphate solution was observed to be clear without precipitation during preparation step and remained clear during the coating step. The coating amounts, as indicated by $W_c$, were seen to be similar regardless of pretreatment parameters. However, using low NaOH concentration and ultrasonic cleaning in the pretreatment step tended to induce the formation of isolated spheroidal structure whereas using high NaOH concentration and rinsed cleaning produced a uniform and distributed platelet structure. The spheroidal structure was also observed to have greater thickness or height than those of distributed platelet structure. The differences in these coating structures could be associated to the content of amorphous gel content resulted from using different pretreatment parameters. It was reported previously that the amount of coating formation was related to the content of sodium titanate gel layer which would increase with increasing alkali concentration, time and temperature. Higher content of gel layer on titanium surface was reported to lead to a faster rate of coating formation since more Na$^+$ ions could be released which in turn increased the degree of supersaturation. However, the correlation might be not straightforward and depended on the processing conditions employed. It was shown that the reproducibility of the gel layer and coating formations was poor due to the difference in the titanium surface conditions. Recently, a more systematic study reported that the rate of apatite formation and microstructure were not significantly influenced by different amount of Na$^+$ ions or gel layer content resulted from alkali pretreatment step. In this study, the rapid biomimetic coating content was not observed to increase with increasing gel layer content resulting from different alkali pretreatment step. The differences resulted from using different alkali pretreatment parameters were only found for microstructures, roughness and thicknesses of the coatings. The use of ultrasonic cleaning resulted in a low content and non-uniform gel layer on the surface since calcium phosphate crystals would preferentially nucleate in certain areas with sufficient amount of residual gel layer and resulted in isolated spheroidal structure. Increasing NaOH concentration increased the content of gel layer initially formed and also increased the chance of residual sodium trititanate gel layer on the surface of titanium after cleaning. In the case of rinsed cleaning, greater amount and more uniform gel layer remained on the surface which could induce uniform nucleation throughout the surface resulting in a uniform and distributed platelet coating structure. Furthermore, since only nucleation could be formed in some areas in spheroidal structure, the crystal could grow to a greater height or thickness than those of uniform platelet structure as observed. Previously, a variety of OCP morphologies resulted from
precipitation could be obtained by adjusting the pH value and temperature to yield long plate-like, thin plate-like or aggregated spheres containing plate-like crystals\(^{51}\). The presence of inorganic ions (CO\(_3^{2-}\), Mg\(^{2+}\) and F\(^-\)) also could influence the crystal shape to be flake-like, rectangular or plate-like morphologies\(^{52}\). Morphology of OCP crystals grown on a cation selective membrane at different phosphate concentration and pH changed from ribbon-like to plate-like and flake-like with increasing pH from 6.0-7.4\(^{53}\). Although the morphology could be varied, OCP crystals were generally in the form of thin sheet morphology. In this study, OCP appeared as plate-like or platelet crystals and were mainly grown perpendicular to the surface of the titanium. During the rapid biomimetic coating process, nucleation of OCP crystals started to occur all over the surface of titanium after submerging the alkali-treated titanium sheet in the ACS solution. OCP crystals then grew with a flower-like shape in all three directions at short coating periods. At long coating times, the growth and spread of crystals was restricted along x- and y-directions on the surface of titanium due to the steric effect. The crystals were; thus, forced to grow along z-direction which was perpendicularly oriented to the surface as observed.

Recently, scratch test was shown to be more suitable to evaluate the delamination of the coatings than pull out test and has been widely used to evaluate the adhesion strengths of ceramic coatings\(^{54}\). Critical loads which coating failures appeared were generally used to compare the adhesion strength of coatings. In this study, critical loads of all coated samples were in the ranges of 232-435 mN depending on the pretreatment conditions. These values were in the similar ranges of HA coating produced by sol-gel and microplasma sprayed techniques\(^{54,55}\). However, it should be noted that values of critical load also depended on several intrinsic and extrinsic parameters and might be comparable when all influencing factors remained constant\(^{57}\). Using 1 M NaOH concentrations resulted in lower values of critical loads for both cleaning methods than using 3 M and 5 M NaOH concentrations. These might be related the differences in coating thicknesses resulting from using different NaOH concentrations. Thicker coating of 1R-C and 1S-C samples might deflect less under load and gavelower critical loads than those of 3R-C, 5R-C, 3S-C and 5S-C\(^{58}\).

**Conclusions**

Octacalcium phosphate was found to be the main composition of all coating and consisted of nano-thick platelet as primary crystal morphology. Phase composition, coating content, crystal morphology and surface wettability of rapid biomimetic coatings were not significantly influenced by varying alkali pretreatment parameters. In contrast, microstructures, thicknesses, critical loads and roughness of the coatings were affected by the different pretreatment parameters employed. The combination of using high NaOH concentration and rinsed cleaning produced coating which was more uniform, but had lower thicknesses and roughness than using low NaOH concentration and rigorous ultrasonic cleaning which produced isolated spheroidal microstructures.

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