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Silver Doped Hydroxyapatite Coating on Titanium Surfaces and its Effect on Early Bone Response and Osseointegration

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Abstract

The clinical success of dental implants is determined mostly by surface properties of implants and interactions with surrounding tissues. Furthermore, clinical success of dental implants is directed by implant surfaces and bone cell responses that promote osseointegration and long-term stability. Hydroxyapatite (HA)-coated Titanium (Ti) implants have been widely used due to its effect on bone response. However, microbial infection is common. One material known to have anti-microbial properties is silver (Ag). However, the effects Ag has on osteoblastic activity as well as decrease bacterial load has yet to be determined.

In this study, the effect of Ag-doped HA coatings on initial bacterial adhesion and osteoblast cell proliferation, differentiation, and mineralization was investigated. Using a sol-gel process, HA coatings doped with 2 wt % $AgNO_3$ were prepared. Coated surfaces were characterized using Xray diffraction (XRD). The osteoblast cell attachment, differentiation which is measured by alkaline phosphatase activity, and mineralization which is measured by osteocalcin activity were evaluated using human embryonic palatal mesenchyme cells (HEPM), an osteoblast precursor cell line. Initial bacteria adhesion was evaluated using an RP12 strain of Staphylococcus epidermidis (ATCC 35984) and the Cowan I strain of Staphylococcus aureus.

A significant difference in osteoblastic cell attachment over time was observed with 2% Ag-doped HA having the least cell attachment. The use of HEPM cells indicated no significant difference in alkaline phosphatase specific activity or osteocalcin activity among the 2% Ag-doped HA, HA, and Ti surfaces. Furthermore, the in vitro bacterial adhesion study indicated a significantly reduced number of S. epidermidis and S. aureus on Ag-doped HA surfaces when compared to HA and Ti surfaces.

Overall, it was concluded that 2% Ag-doped HA surfaces have similar osteoconductive activity when compared to HA and Ti surfaces. It was also concluded that the doping of HA with Ag minimized the adhesion of bacteria on its surface. Further studies on Ag-doped HA surfaces should involve long and short-term animal studies to evaluate its biocompatibility and ability to induce bone formation for implant stability, as well as bacterial adhesion properties. Furthermore, a decreased concentration of Ag may aid in an increasing osteogenic activity as well as have an antimicrobial effect.

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SILVER DOPED HYDROXYAPATITE COATING ON TITANIUM SURFACES AND ITS EFFECT ON EARLY BONE RESPONSE AND OSSEOINTEGRATION

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ABSTRACT

The clinical success of dental implants is determined mostly by surface properties of implants and interactions with surrounding tissues. Furthermore, clinical success of dental implants is directed by implant surfaces and bone cell responses that promote osseointegration and long-term stability. Hydroxyapatite (HA)-coated Titanium (Ti) implants have been widely used due to its effect on bone response. However, microbial infection is common. One material known to have anti-microbial properties is silver (Ag). However, the effects Ag has on osteoblastic activity as well as decrease bacterial load has yet to be determined.

In this study, the effect of Ag-doped HA coatings on initial bacterial adhesion and osteoblast cell proliferation, differentiation, and mineralization was investigated. Using a sol-gel process, HA coatings doped with 2 wt % AgNO3 were prepared. Coated surfaces were characterized using Xray diffraction (XRD). The osteoblast cell attachment, differentiation which is measured by alkaline phosphatase activity, and mineralization which is measured by osteocalcin activity were evaluated using human embryonic palatal mesenchyme cells (HEPM), an osteoblast precursor cell line. Initial bacteria adhesion was evaluated using an RP12 strain of Staphylococcus epidermidis (ATCC 35984) and the Cowan I strain of Staphylococcus aureus.

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bacterial adhesion study indicated a significantly reduced number of S. epidermidis and S. aureus on Ag-doped HA surfaces when compared to HA and Ti surfaces.

Overall, it was concluded that 2% Ag-doped HA surfaces have similar osteoconductive activity when compared to HA and Ti surfaces. It was also concluded that the doping of HA with Ag minimized the adhesion of bacteria on its surface. Further studies on Ag-doped HA surfaces should involve long and short-term animal studies to evaluate its biocompatibility and ability to induce bone formation for implant stability, as well as bacterial adhesion properties. Furthermore, a decreased concentration of Ag may aid in an increasing osteogenic activity as well as have an antimicrobial effect.

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CHAPTER 1. INTRODUCTION

Osseointegration

The clinical success of dental implants is partly dictated by the surface properties of the implants and their interaction with the host. Furthermore, the clinical success of dental implants is directed by implant surfaces and bone cell responses that promote rapid osseointegration and long-term stability. Selecting materials based on their biocompatibility is an important matter when used for medical devices and dental implants. Adding materials to the surface may modify and affect early onset of osseointegration and bone cell attachment. Osseointegration is defined as a "direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant".[1] When osseointegration occurs, the nonbiologic material is in direct contact with bone, and no rejection or inflammatory response is evident.

Titanium

An example of a material that is frequently used is titanium (Ti), due to its biocompatible nature. Ti has the ability to resist corrosion in aggressive body fluids due to the stable oxide layer of about 3-5nm thick. Ti's oxide layer is produced because of its high reactivity,[2] and breakdown of this oxide layer is only known to happen in highly disruptive conditions, which does not often occur in the oral cavity.[3,4] As such, Ti has been successfully used in medical devices, which includes dental and orthopedic implants. However, the Ti oxide layer does not allow for direct contact of the Ti and possible harmful metallic ions with the living host.[1] Therefore, the Ti oxide layer allows

for an implant to be accepted by host tissues, and eventually bone will grow around the implant.

Hydroxyapatite

Hydroxyapatite (HA) is another important material used for implants because it is very similar to calcium phosphate (CaP) of teeth and bones. HA has an osteoconductive property that permits bone growth and direct bonding of bone to the implant surface.[5–9] Previous evidence in a number of animal studies exemplified HA binding to bone.[10–12] Studies showed that a gradual mineralization process occurs where there is bone healing around Ti implants, as opposed to bone-conducive healing.[13,14] However, despite its excellent bone conductive property and biocompatibility, HA is a ceramic and is brittle in bulk form. In situations where load bearing implants are needed, such as in dental and orthopedic applications, the poor mechanical property of bulk HA thus renders the material useless for such applications. As a result, HA is a material of interest in early bone formation that has been used as a surface coating for Ti.[15]

Hydroxyapatite and Titanium

The development of a HA coating is based on the reasoning of combining HA's bioactivity with Ti's strength. Combining HA with Ti has shown to form a strong bond between synthetic materials and bone.[16-17] Furthermore, HA coatings have been reported to promote early bone formation around implants, and promote the deposition and differentiation of mesenchymal cells into osteoblasts on HA-coated implant surface.[19] Ability of osteoblast adhesion onto the surface is due to the increased adsorption and

production of proteins. In addition, a study has indicated HA-coated implants to have a higher percentage of bony contact after six weeks implantation as opposed to commercially pure Ti screws.[20] Histologically comparing osseous apposition to HA-coated implants and Ti implants has demonstrated mineralization of bone directly on HA surfaces with no fibrous tissue layer formation. However, a predominately fibrous tissue interface was observed on Ti implants, with only minimal areas of direct bone contact.[21] In addition, in an animal study HA-coated implants showed an increased coronal bone growth that was not observed with Ti implants.[22] Maintaining a bony osseous crest is essential clinically because it may prevent peri-implant saucerization and subsequent pocket formation.[23-24] Other histometric studies in animal models have also exemplified that bone adapts in much less time to HA-coated implants than to Ti implants.[25-26]

Limitations

However, there are limitations of HA and Ti in regards to healing and inflammation, and further investigations of antimicrobial materials are essential. Wounds can often influence an increase in a micro-organism population. This in turn, can affect the natural healing process and increase the rate of infection. There is a concern about enhanced susceptibility to bacterial colonization on HA-coated surfaces as compared to non-coated Ti implants. When implants are placed clinically, there are instances where bacteria can become a problem. Implant-associated infections account for nearly 50% of the estimated 2 million infections in the United States per year.[27] Certain implants, like external fixation pins, have infection rates of up to 100%.[28-30] There are high complication rates with these infections, which places a huge weight on patients, health

care professionals, and the health care setting. A prolonged hospital stay, increased morbidity and mortality, and an increased financial load usually occurs to patients with infected implants.[31] Bacteria on implant surfaces is known to cause inflammation around the implants which can decrease the implant's stability. For example, one study showed that the implantation of HA-coated implants had a higher intense inflammatory response as opposed to other implant materials.[32] The HA coatings have been suggested to be more susceptible to bacterial colonization compared to Ti implants or natural teeth.[33] As a result of the roughened HA surfaces, enhanced plaque growth on HA-coated implant surfaces is suggested, and therefore may contribute to peri-implantitis.[34]

Silver

One material known to have such antimicrobial effects is silver (Ag). One in vivo study examined the antimicrobial efficacy and potential side-effects of a Ag-coated megaprosthesis.[35] The Ag group showed significantly lower infection rates than the titanium group. In addition, there were no pathologic or histological changes evident. Ag has shown in a number of previous studies to have an antibacterial effect.[36-38] As the Ag ion concentration was increased to 37°C, an increase in the rate of bacteria killed was reported.[36] Ag could decrease the rate of infection and decrease antimicrobial activity in dental implants. Ag is believed to be effective against a wide range of micro-organisms, and therapeutic effects are seen in low concentrations.[39-40] Ag is known to have antibacterial activity and a non-cytotoxic effect in stimulated body fluids.[41] Furthermore, Ag can inhibit the growth of gram-positive and gram-negative bacteria.[42] As a result of Ag's antimicrobial effects, many medical devices such as catheters[43-44] and wound

dressings[45] contain Ag. In addition, the use of Ag-coated catheters in patients was shown to have reduced bacteria infection in comparison to non-coated catheters.[46] The mentioned studies have provided strong evidence that Ag in medical implants can aid in reducing bacterial infection.

Coating Processes

Another problem with HA coatings is the current process that is used in the deposition, namely plasma-spraying process. It has been reported that plasma-sprayed HA exhibited variation in bond strength at the coating-metal interface, non-uniformity in coating thickness between vendors, alteration in structural and chemical properties, non-uniformity in coating density.[47-56] In addition to these problems, plasma-spraying is a line-of-sight coating process and thus is not very useful in complex implant surfaces, such as beaded surfaces. As such, numerous experimental coating processes have been investigated, including electrophoretic deposition, high velocity oxy-fuel (HVOF) deposition, and radio frequency sputter deposition.[56] Each of these experimental coating processes has resulted in improved coating properties, such as adhesion strength. However, one solution-based coating process known as the sol-gel technique is being proposed in this study. The sol-gel coating process is a low temperature deposition process that permits the operator to maintain control over the chemical composition and structure of the coating as well as coating on complex structures.[57,58,59] In addition, the sol-gel procedure is economical as well as less time consuming.[60]

Significance of Study

In addition to the selection of a coating process to ensure successful implantation, the composition of the coatings are also critical for osseointegration. There have been studies examining surface modifications of implants and bone activity. However, none have studied the effects of Ag-doped HA coatings applied to dental implants on early bone response. It has never been determined whether Ag can affect bone remodeling as well as early onset of osseointegration. Therefore, Ti implants with coatings of Ag-doped HA should be carefully examined in order to have a better understanding of bone response to various implant surfaces. Data generated from this study will provide important information on the effect of Ag-doped HA coatings on osteoblast response in vitro. In addition, this study will give useful information on the optimum surface treatment required for maximum implant stability and aid in providing a foundation for future in vivo and clinical studies with Ag. This essential information will contribute to the development of an ideal dental implant surface as well as reduce the number of implant failures. This, in turn, will minimize time, trauma, and implant and surgery costs for the patient.

Aims and Hypotheses

The overall purpose of this study was to investigate the biological response to Ag-doped HA-coated Ti surfaces produced using a sol-gel deposition process. In this study, molecular and structural properties of the coatings were evaluated. In addition, the response of osteoblast cells as well as Staphylococcus aureus on Ag-doped HA coatings were evaluated. As such, the three specific aims for this study were the following:

- Specific Aim 1: To characterize the coatings produced using the sol-gel coating process.
- Specific Aim 2: To determine attachment, differentiation, and mineralization of osteoblast cells on Ag-doped HA coatings.
- Specific Aim 3: To determine the attachment of bacteria on Ag-doped HA coatings.

In order to fulfill each of the above aims, the following hypotheses were tested:

- Hypothesis 1: No chemical and structural changes will be observed with the coatings produced using the sol-gel coating process.
- Hypothesis 2: Osteoblast attachment, differentiation, and mineralization will be affected by the doping of Ag on HA coatings.
- Hypothesis 3: Bacteria attachment on Ag-doped HA will be reduced when compared to HA coatings and Ti surfaces.

CHAPTER 2. METHODOLOGY

Titanium Substrates

Commercially pure grade 2 Ti from Metal Samples, Munford, AL (14 mm diameter and 2 mm thick) was used in this study. The Ti surfaces were prepared by wet grinding with 240, 400 and 600 grit silicon carbide paper. Surface roughness measurement using Surtornic 3 profilometer (Taylor-Hobson, UK) indicated a R_a value of 0.37 ± 0.01 μm. These surfaces were ultrasonically degreased in acetone and ethanol for 10 minutes each, with deionized water rinsing between applications of each solvent. A passivation procedure was conducted by exposing the Ti samples to a 40% volume nitric acid solution at room temperature for 30 minutes (ASTM F86-76). After each surface treatment, the Ti samples were rinsed with deionized water and air-dried. After drying, these Ti samples were either used as controls, coated with HA or Ag-doped HA. All coated and non-coated samples were then UV sterilized for 24 hours prior to cell culture experiments.

Coatings

In this study, HA and 2% Ag-doped HA sol were used as coatings on Ti implant surfaces. Silver nitrate ($AgNO_3$) was chosen as a precursor for silver because of the easy decomposition of nitrates during heating. $AgNO_3$ was weighed so that Ag ion occupied 2.0% of all cations (Ag and Ca) and dissolved with calcium nitrate tetrahydrate [$Ca(NO_3)_2{\cdot}4H_2O$]. The HA sol was prepared by reacting calcium nitrate tetrahydrate [$Ca(NO_3)_2{\cdot}4H_2O$] (Aldrich) with the triethyl phosphite [$(OC_2H_5)_3P$] (Fluka) in methyl

alcohol using correct amounts to obtain the stoichiometric Ca/P ratio of 1.67 for HA. This was followed by the addition of 0.03 mol of HCl and 0.1 mol of DCCA (Drying Control Chemical Additive). All work was performed in argon atmosphere. The mixed solution was then aged at 80ºC for 12 hours. The aged solution was filtrated through a 0.2 syringe filter, and aged again at 50ºC for 7 days. After aging, HA or Ag-doped HA coatings was then spin-coated on Ti surfaces at 3500 rpm for 30 seconds. The coated-Ti surfaces were immediately dried at 70ºC for 12 hours and then heat-treated at 650ºC for 3 hours. The HA without Ag doping was used as controls in this study. All samples were autoclaved prior to materials characterization and all culture experiment.

Materials Characterization

All coatings were characterized prior to beginning of study. In order to evaluate the structure of HA coatings, x-ray diffraction was used. Triplicate coatings were analyzed using a x-ray diffractometer using Cu K_ radiation. HA coatings (without Ag doping) were used as controls. The energy used was 40 KeV and 30 mA. Data was collected from 5 degrees to 95 degrees two theta at 0.1 degree per minute scan rate. Peak reflections were identified by matching to the standard synthetic HA (JCPDS # 9-0432).

Osteoblast Cell Culture Study

The osteoblast cell culture study was performed with the ATCC CRL 1486 human embryonic palatal mesenchyme cell line (HEPM), a precursor of osteoblast cells. The cells were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, L-ascorbic acid, _-glycerophosphate, 2.5 x 10^{-9}M 1,25 $(OH)_2$ vitamin D_3, and a 1%

antibiotic/antimycotic mixture of penicillin, streptomycin and fungizone. Experiments were conducted in a 5% CO_2 humidified incubator at 37°C, and the medium was changed twice a week. Sterilized HA (controls), Ti (controls), and Ag-doped HA surfaces were placed at the bottom of a well of 24-well plate. Samples were subsequently assayed for cell attachment, osteocalcin activity, and alkaline phosphatase activity. Details of the assays were as follows:

Cell Attachment

At 30, 60,120, and 180 minutes after seeding, cells attached on each surface were evaluated. A 200 µl cell suspension (44,000 cells) were seeded on each surface. At each time point, triplicate samples from each group were assayed by removing the media and washing the samples twice with PBS. The total volume of PBS used for each wash was 200 µl. The removed media and washed solution were saved and recorded as total volume. The number of unattached cells in the removed media and wash solution were subsequently determined using a Beckman Z2 Coulter Counter. Cells attached on each surface were then measured by subtracting the unattached cell concentration from the initial cell concentration seeded. Mean attachment cell concentrations between the different surfaces were statistically analyzed using the ANOVA, with the differences compared using the Tukey's procedure. The alpha level for data analysis will be set at 0.05, and differences were considered significant if $P < 0.05$.

Alkaline Phosphatase Specific Activity

Alkaline phosphatase (ALP) activity was quantified by rinsing (twice) the cells with PBS, followed by exposing the cells to 0.2% Triton-X-100 solution in order to permeabolize the cell membranes. The cells were then subjected to three freeze/thaw cycles, ultrasonically homogenizing the samples for 15 minutes, and centrifuging to remove large cellular debris. The resulting supernatant was assayed for the release of p-nitrophenol from p-nitrophenyl phosphate (pH = 10.2) and its specific activity calculated. 50 µl of each sample and standard was then pipetted into a 96-well plate and 50 µl of p-nitrophenyl phosphate substrate was added, followed by incubation in the dark at room temperature for 1 hour. After stopping the reaction using 50 µl of 1 M NaOH, and the absorbance was read at 405 nm using a microplate reader. ALP activity of cells from each surface was then calculated from prepared standards (Sigma, St. Louis, MO). Significant differences in ALP were statistically evaluated using ANOVA with Student Newman Kuels procedure as the post hoc test for evaluation of significant differences at $P < 0.05$.

Osteocalcin Activity

The production of osteocalcin in this study was measured using a commercially available Mid-Tac Human Osteocalcin EIA Kit (Stougton, MA). On the day of the assay, the samples were thawed at room temperature. The samples (25 µl) or human osteocalcin standard (25 µl) were then added to microtiter plate provided with the kit. This was followed by the addition of osteocalcin antiserum (100 µl) and allowed to incubate at 37°C for 2.5 hours. The solution was then aspirated and the plate was washed 3 times with 0.3 ml of phosphate buffered saline. After washing, 100 µl of Streptavidin-

Horseradish Peroxidase reagent was added to all wells, gently mixed, and allowed to incubate at room temperature for 30 minutes. The medium was again gently aspirated and the plate washed 3 times with 0.3 ml of phosphate buffer saline. 100 µl of 3,3', 5,5' Tetramethylbenzidine and hydrogen peroxide solution (1:1) mixture was then added to all wells and incubated in the dark for 15 minutes. A sulfuric acid solution (100 µl) was added to all wells to stop the reaction. Absorbance was then immediately read at 450 nm using a microplate reader. Osteocalcin concentrations were determined from a standard curve using standards supplied with the kit. Significant differences in protein levels was statistically evaluated using ANOVA with Student Newman Kuels procedure as the post hoc test for evaluation of significant differences at $P < 0.05$.

Bacteria Study

In this study, an RP12 strain of Staphylococcus epidermidis (ATCC 35984) and the Cowan I strain of Staphylococcus aureus were used as model bacteria strain to evaluate the in vitro antimicrobial properties of Ag-doped HA coatings. The bacteria were grown overnight in Trypticase soy broth (TSB), washed in PBS, and adjusted to an OD at 530 nm of 0.1. 2ml of the bacteria (10^7 colony forming units (CFU)) per tube were added to 50 ml sterilized tubes containing Ti (control), HA-coated (control), or Ag-doped HA-coated disks and were incubated at 37°C for 3 hours. Loosely attached cells were washed off by rinsing the disks with PBS. After rinsing, the disks were then put into a new tube containing 5 ml of PBS, and were vigorously vortexed for 30 seconds. The numbers of CFU in the buffer were determined by plating dilutions on THB agar plates.

Significant differences were statistically evaluated using ANOVA with Student Newman Kuels procedure as the post hoc test for evaluation of significant differences at $P < 0.05$.

CHAPTER 3. RESULTS

Materials Characterization

X-ray diffraction pattern of AgHA coating is depicted in Figure 1. Peaks attributable to HA were identified, indicating the transformation of amorphous to crystalline coatings following the post deposition heat treatment at 650 °C for 3 hour.

Cell Attachment

Figure 2 shows osteoblastic cell attachment to different surfaces after 30 minutes, 60 minutes, 120 minutes, and 180 minutes. After 180 minutes, there was a significantly less osteoblastic cell attachment seen with 2% Ag-doped HA, when compared to Ti and HA surfaces ($P < 0.05$).

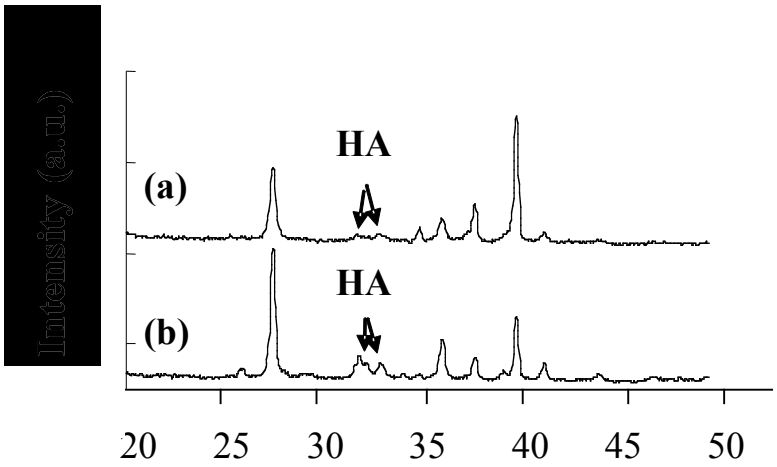

Figure 1. Characteristic x-ray diffraction pattern of (a) HA and (b) AgHA coatings, confirming the crystallinity of HA formed on the surfaces. HA peaks marked by the arrow indicate transformation due to heat treatment.

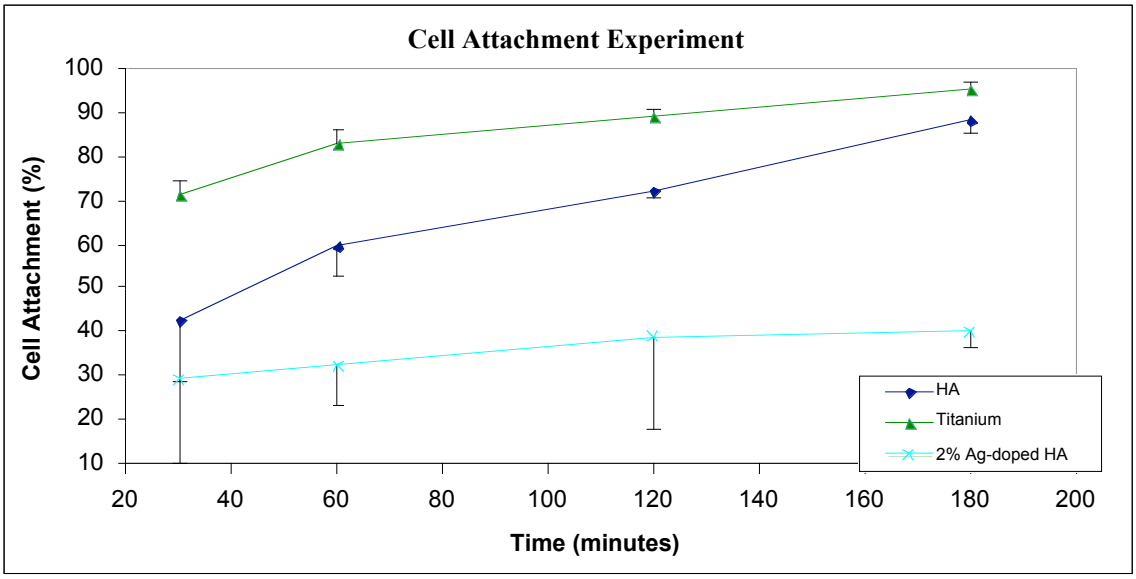

Figure 2. Osteoblastic cell attachment to different surfaces.

Alkaline Phosphatase Specific Activity

Figure 3 shows the ALP specific activity by osteoblast precursor cells on different surfaces after 3-day, 6-day, 9-day,and 12-day culture. By the 12th day, no significant difference with ALP specific activity was observed on Ag-doped HA surface when compared to Ti and HA surfaces ($P > 0.05$).

Osteocalcin Activity

Figure 4 shows the osteocalcin activity by osteoblast precursor cells on different surfaces after 3-day, 6-day, 9-day, and 12-day culture. By the 12th day, no significant differences were found among the different surfaces ($P > 0.05$). However, by the 12^{th} day Ti had the highest osteocalcin activity, when compared to HA and Ag-doped HA.

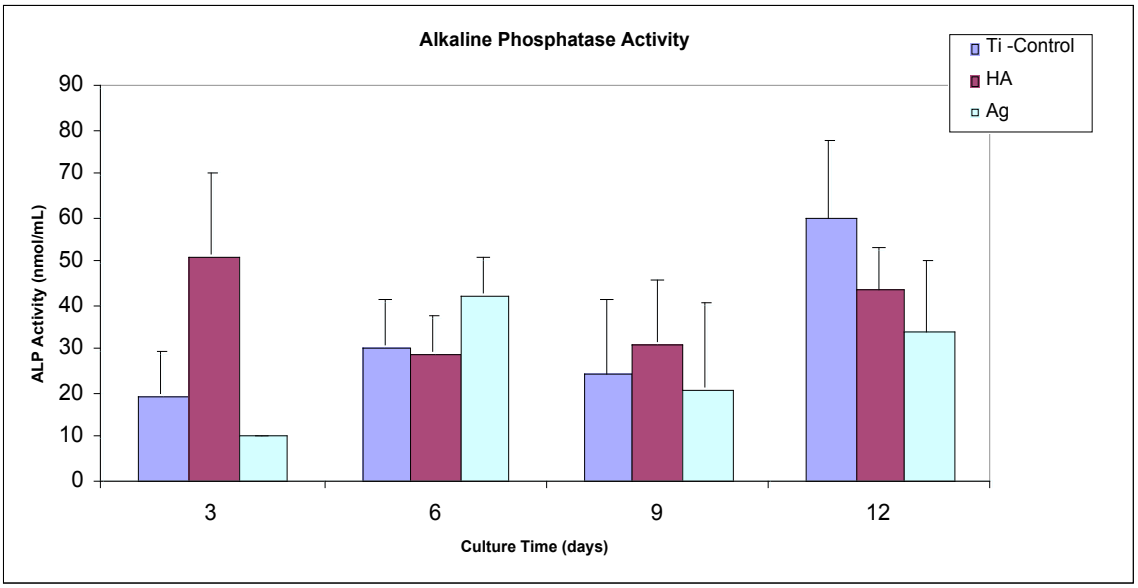

Figure 3. Alkaline phosphatase activity on different surfaces.

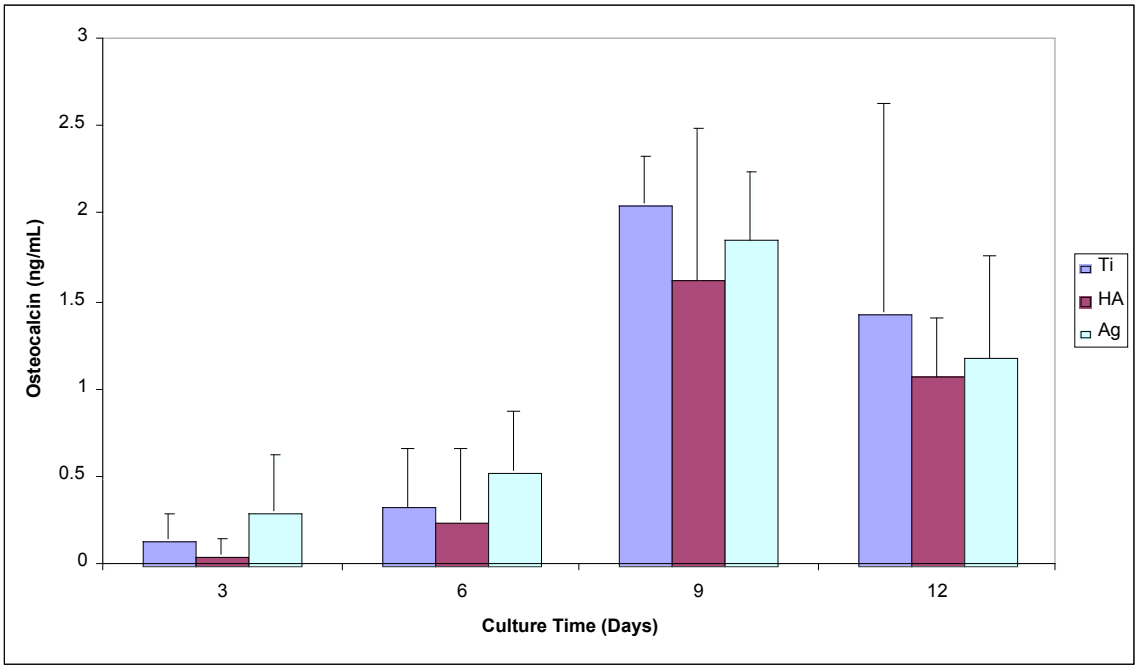

Figure 4. Osteocalcin activity on different surfaces.

Bacteria Study

Figure 5 shows the results of viable bacteria adhering to the different surfaces when exposed to S. aureus. Bacterial adhesion was significantly reduced for S. aureus on Ag-doped HA surfaces when compared to HA and Ti surfaces ($P < 0.05$).

Figure 6 also shows the results of viable bacteria adhering to the different surfaces when exposed to S. epidermidis. Bacterial adhesion was also significantly reduced for S. epidermidis on Ag-doped HA surfaces when compared to HA and Ti surfaces ($P < 0.05$).

Overall, the Ag-doped HA surface was significantly better in reducing bacterial adhesion for both S. aureus and S. epidermidis, as compared to the HA and Ti surfaces.

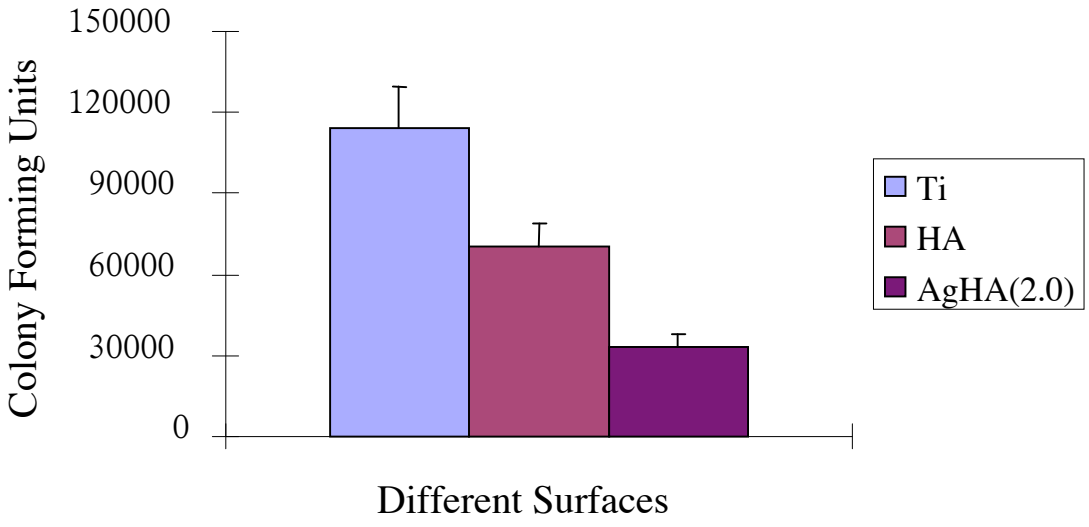

Figure 5. Attachment of S. aureus to different surfaces.

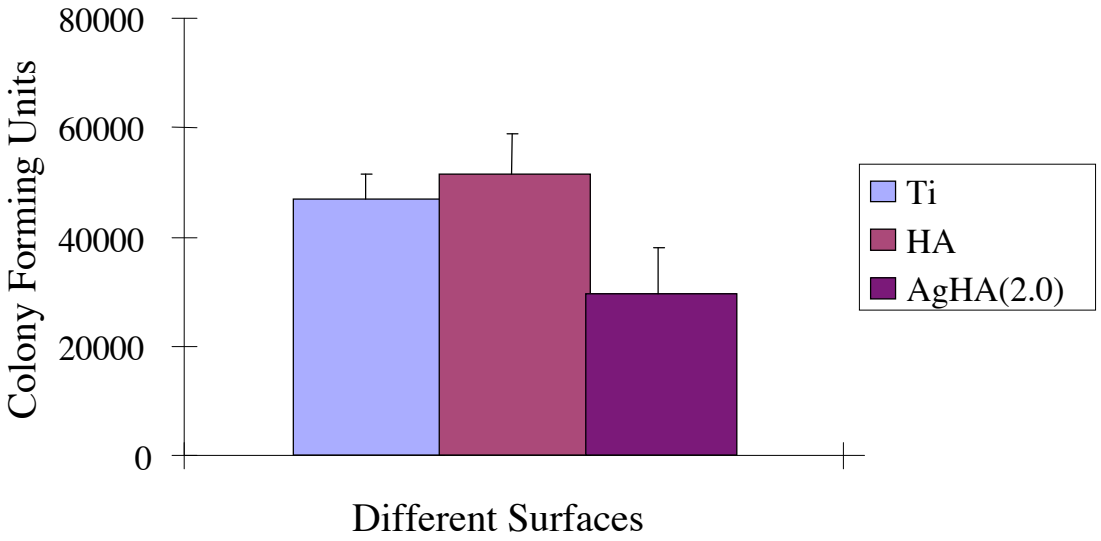

Figure 6. Attachment of S. epidermidis to different surfaces.

CHAPTER 4. DISCUSSION

Overview

Ti has been widely used as implant materials because of their highly biocompatible properties with relatively low modulus, good fatigue strength, formability, machinability, and corrosion resistance.[61-64] In addition, HA has been added to Ti surfaces as a coating material on orthopedic and dental implants. Adding HA to Ti has allowed combining the strength of the metals with the bioactivity of the ceramics. HA has helped promote more rapid osseointegration; this includes direct bony growth and early mineralization at the interface, as well as equivalent or higher bone implant bond strengths and percentage bone contact at the bone-implant interface compared to uncoated Ti implants.[60,65-67]

Furthermore, the sol-gel process has shown to be an economical and time-saving process.[60] The sol-gel procedure allows for control of composition and coating thickness as well as the ability to coat large or complex surfaces.[57-59] In addition, the water-based sol-gel technique producing HA coating was shown to be dense and have a high bonding strength (37–44 MPa) between the coating and substrate.[57,68,69]

Bacterial infection and inflammation at the site of implant placement may impede healing of the soft and hard tissues, therefore eventually leading to chronic peri-implantitis which has been reported to be one of the major etiological agents causing late implant failures.[70,71] Due to this insight, a biomaterial that has enough strength and bioactivity, as well as anti-bacterial properties is worth investigating. One such material that has these mentioned properties is Ag. An important feature of Ag is its wide spectrum of antibacterial susceptibility and low incidence of resistance.[72,73]

It was hypothesized that HA thin film doped with Ag would not only enhance tissue compatibility, but also promote the inhibition of bacterial adhesion on the implant surface. In this study, characterization of Ag-doped HA film prepared using water-based sol gel technique was produced.

As observed in the x-ray diffraction analysis of the coatings, no Ag peaks were observed. The lack of Ag peaks from x-ray diffraction analysis was attributed to the low concentration of Ag used in the coatings. As expected, all HA and Ag doped HA peaks matched standard synthetic HA (JCPDS # 9-0432), indicating the presence of HA coatings.

Additionally, the effect of Ag-doped HA was also evaluated using the human embryonic palatal mesenchyme cells (HEPM), an osteoblast precursor cell line. Osteoblastic cell attachment with 2% Ag-doped HA was significantly less when compared to Ti and HA surfaces. However, this suggested that cell attachment for 2% Ag-doped HA was observed even if it was significantly less. It was also observed that the Ti surfaces had significantly more osteoblastic cell attachment than the HA surfaces. It is possible that the Calcium and Phosphorous released from the sol-gel coating on the HA surfaces impeded cell attachment. One study suggested that additional Phosphorous concentration in the cell culture media was suggested to slow down osteoblast differentiation and mineralization and affect HA dissolution behavior.[74]

It was observed that there was no significant difference on differentiation and mineralization of osteoblast precursor cells on 2% Ag-doped HA surface when compared to the HA and Ti surfaces. This suggested that the osteoconductivity of AgHA was not

reduced even though 2% Ag-doped HA surface had less osseous activity than the HA and Ti surfaces.

Significant differences on the results of bacterial adhesion studies on Ti, HA, and Ag-doped HA surfaces were observed. The Ag-doped HA surface was observed to significantly reduce the number of viable adhering bacteria of tested strains which included S. aureus and S. epidermidis. This suggested that the HA coating doped with 2% Ag has an anti-bactericidal adhesion effect. Attachment of bacteria to implanted biomaterial surfaces is an important step in the pathogenesis of infection. Therefore, it is essential to eliminate as many infectious bacteria as possible. This work demonstrated that the Ag-doped HA coatings play an important role in preventing or minimizing initial bacterial adhesion.

Future Research

Future studies on Ag-doped HA surfaces will involve the long and short-term animal studies to evaluate its biocompatibility and its ability to induce bone formation for implant stability as well as bacterial adhesion properties. Furthermore, a change in the concentration for Ag may aid in an increased osteogenic activity as well as have an antimicrobial effect. For example, 1% or 0.5% Ag may be used in future studies.

Conclusion

In this study, a significant difference in osteoblastic cell attachment over time was observed with 2% Ag-doped HA having the least cell attachment. In addition, no

significant differences of the ALP specific activity or osteocalcin activity were observed over time, although there was less activity for 2% Ag-doped HA. Changing the composition affected cell responses, which can explain the reason for the lower attachment levels, ALP and osteocalcin activity. However, the Ag doping did not entirely prevent osteoblast cells from attaching and differentiating. Furthermore, the in vitro bacterial adhesion study indicated a significantly reduced number of S. epidermidis and S. aureus on 2% Ag-doped HA surfaces when compared to HA and Ti surfaces. . Overall, it was concluded that 2% Ag-doped HA surfaces have similar osteoconductive activity when compared to HA and Ti surfaces. In addition, it was also concluded that the doping of HA with Ag minimized and prevented the adhesion of bacteria on its surfaces.

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