In Vitro Osteoblast Responses to Silver-doped Hydroxyapatite Sol-gel Coatings

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Purpose: Infection after implant placement is a significant rising complication. In order to reduce the incidence of implant-associated infections, several biomaterial surface treatments have been proposed. The anti-bacterial properties of silver (Ag) are well-known and have been recently used in a variety of medical applications. However, little is known about the effect of Ag on bone-implant interactions. As such, the goal of this study was to investigate the role of Ag-doped hydroxyapatite (HA) sol-gel coatings on *in vitro* bone cell activity.

Materials and Methods:

Sol-gel coatings: Grade 2 titanium (Ti) disks (15 mm diameter and 2.0 mm thick) were wet ground to 600 grit, ultrasonically degreased in acetone and ethanol, and passivated using a 40% volume nitric acid solution at room temperature for 30 minutes. The passivated Ti surfaces were then coated with HA sol. HA sol was prepared by reacting calcium nitrate tetrahydrate [$Ca(NO_3)_2.4H_2O$] with methyl alcohol to produce calcium precursors. Phosphorus precursors were also prepared by reacting triethyl phosphite [$(OC_2H_5)_3P$] in 0.03 ml acetic acid (CH_3COOH). The two precursors were then mixed and 0.1 mol of DCCA (Drying Control Chemical Additive) was added to the mixture. All reactions were carried out in argon atmosphere. The mixed solution was then aged at 40ºC for 24 hours, followed by filtration with a 0.2 μm-syringe filter, and a final aging at 40ºC for 4 days to produce the HA sol.

Using the similar sol process, Ag-doped HA sol was produced by mixing the calcium and phosphorus precursors with silver nitrate ($AgNO_3$) powders and 0.1 mol DCCA. In this study, the 1 wt % and 1.5 wt % $AgNO_3$ (AgHA1.0 and AgHA1.5) were used for doping.

The prepared HA sol and Ag-doped HA sol were then coated on passivated Ti surfaces by spin coating at 5,000 rpm for 50 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 gas sterilized prior to materials characterization and all culture experiment.

Materials characterization: The structure of the coatings was analyzed using an x-ray diffractometer. Wettability of the coatings was measured using static contact angles. Using a goniometer, water droplets were captured using a video and transferred to a computer for angle measurement.

Human osteoblast cell culture: Samples were placed in 24 well plates and seeded with 40,000 human embryonic palatal mesenchyme (HEPM) cells per sample. One ml of the Dulbecco's Modified Eagle Medium containing 3% fetal bovine serum, 1% antibiotic-antimycotic solution, 50 μg/ml ascorbic acid and 4 mM β-glycerophosphate was then added per well. The cells were incubated at 37˚C in a humidified atmosphere of 95% air and 5% CO_2, and the culture media were changed every three days. On days 6, 12 and 15, the supernatants were removed and surfaces were washed twice with a phosphate buffered solution. The cells were then be lysed using 0.5 ml 0.2% Triton-X-100 solution and stored at -20˚C until assayed.

Double-stranded DNA (dsDNA) Assay: Cell proliferation was measured by quantify dsDNA via PicoGreen assay.

Alkaline phosphatase (ALP) specific activity assay: Osteoblast differentiation was analyzed by measuring alkaline phosphatase activity. Briefly, cell lysate collected was assayed using an alkaline phosphatase (ALP) detection kit, and absorbance was read at 405 nm using a microplate reader. Samples were compared to a standard curve provided by the manufacturer, and the ALP specific activity was calculated by normalizing the ALP activity to dsDNA produced from each sample.

Statistical analysis: Significance in contact angle, cell proliferation, and ALP activity were analyzed by one-way ANOVA with Tukey's multiple comparison procedure. Significance levels were set at $p<0.05$.

Results:

In this study, XRD analysis of all surfaces indicated peaks corresponding to HA. Contact angles for AgHA surfaces were observed to be significantly lower when compared to HA surfaces.

The use of HEPM cells indicated no significant difference in dsDNA production between all surfaces. Additionally, as shown in Fig 1, no differences in ALP specific activity were observed between HA and AgHA1.0 surfaces. However, a significantly less ALP specific activity was observed on AgHA1.5 surface when compared to HA and AgHA1.0 surfaces after 12-day and 15-day culture ($P<0.05$).

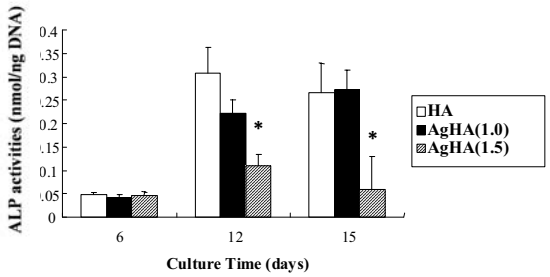

Fig 1. ALP specific activity by osteoblast precursor cells on different surfaces over time.

Conclusion: It was concluded that HA coatings doped with 1 wt% Ag has similar biological activity as HA, with respect to bone cell proliferation and differentiation.