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Original Article

# Biodegradable nanocomposite coatings accelerate bone healing: *In vivo* evaluation

**Mehdi Mehdikhani-Nahrkhalaji[1], Mohammad Hossein Fathi[2], Vajihesadat Mortazavi[3], Sayed Behrouz Mousavi[4], Ali Akhavan[5], Abbas Haghighat[6], Batool Hashemi-Beni[7], Sayed Mohammad Razavi[8], Fatemeh Mashhadiabbas[9]**

[1]Department of Biomedical Engineering, Faculty of Engineering, University of Isfahan, [2]Dental Materials Research Center, Department of Materials Engineering, Biomaterials Group, Isfahan University of Technology, [3]Torabinejad Dental Research Center, Department of Operative Dentistry, [4]Torabinejad Dental Research Center, Department of Endodontics, [5]Dental Materials Research Center, Department of Endodontics, [6]Dental Implant Research Center, Department of Maxillofacial Surgery, School of Dentistry, Isfahan University of Medical Sciences, [7]Dental Materials Research Center, Department of Anatomical Sciences, School of Medicine, Isfahan University of Medical Sciences, [8]Dental Implant Research Center, Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, [9]Department of Oral and Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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**Address for correspondence:**
Prof. Vajihesadat Mortazavi, Torabinejad Dental Research Center, Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, 174673461 Isfahan, Iran.
E-mail: v_mortazavi@dnt.mui.ac.ir

## ABSTRACT

**Background:** The aim of this study was to evaluate the interaction of bioactive and biodegradable poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coatings with bone.

**Materials and Methods:** Sol-gel derived 58S bioactive glass nanoparticles, 50/50 wt% poly (lactic acid)/poly (glycolic acid) and hydroxyapatite nanoparticles were used to prepare the coatings. The nanocomposite coatings were characterized by scanning electron microscopy, X-ray diffraction and atomic force microscopy. Mechanical stability of the prepared nanocomposite coatings was studied during intramedullary implantation of coated Kirschner wires (K-wires) into rabbit tibia. Titanium mini-screws coated with nanocomposite coatings and without coating were implanted intramedullary in rabbit tibia. Bone tissue interaction with the prepared nanocomposite coatings was evaluated 30 and 60 days after surgery. The non-parametric paired Friedman and Kruskal-Wallis tests were used to compare the samples. For all tests, the level of significance was $P < 0.05$.

**Results:** The results showed that nanocomposite coatings remained stable on the K-wires with a minimum of 96% of the original coating mass. Tissue around the coated implants showed no adverse reactions to the coatings. Woven and trabecular bone formation were observed around the coated samples with a minimum inflammatory reaction. PBG nanocomposite coating induced more rapid bone healing than PBGHA nanocomposite coating and titanium without coating ($P < 0.05$).

**Conclusion:** It was concluded that PBG nanocomposite coating provides an ideal surface for bone formation and it could be used as a candidate for coating dental and orthopedic implants.

**Key Words:** Bioactive, biocompatibility, biodegradable, nanocomposite coating, surgery

## INTRODUCTION

Bioactive glasses (BGs) and calcium phosphates (CPs) have numerous applications in the repair and reconstruction of bone. But, as a bulk, they are brittle and relatively weak when compared with common implant metals and alloys and high strength ceramics. Bioactive materials (CPs and BGs) have osteoconductive properties — an ability to serve as a scaffold or template to guide the newly forming bone along its surfaces. Osteoconductive materials allow bone cell attachment, proliferation, migration and phenotypic expression, leading to the formation of new bone in direct apposition to the biomaterial, thus creating a uniquely strong interface. Metal

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implants, primarily titanium (Ti) or Ti alloy, are not bioactive and therefore do not bond directly to bone. Plasma-sprayed hydroxyapatite (HA) coating was developed to combine the strength of the metal and the bioactivity of the HA. However, the plasma-spray methods involve very high temperatures causing the partial transformation of the HA to amorphous calcium phosphate (ACP) and HA (untransformed). It is conceivable that a coating with high ACP/HA could biodegrade prematurely and delaminate before the bone had the opportunity to attach to the implant, thereby causing loosening and eventual implant failure. In addition, because the plasma-spray method is a line of sight method, implants with complex geometry or porosity cannot have a homogeneous coating.[1-3]

BGs are synthetic biocompatible osteoconductive bone substitutes, with bone bonding capacity and documented antibacterial and angiogenesis-promoting properties.[4-12] Our previous study showed that 58S (57.72 $SiO_2$, 35.09 CaO and 7.1 $P_2O_5$ by weight) and 63S (62.17 $SiO_2$, 28.47 CaO and 9.25 $P_2O_5$ by weight) BGs nanoparticles have antibacterial activities. Especially, compared to the 63S BG nanoparticles, the 58S BG nanoparticles showed a stronger bactericidal effect on the studied pathogens with a lower minimum bactericidal concentration. In fact, the antibacterial activity of BGs has been suggested to be based on several factors including high pH levels and osmotic effects caused by the nonphysiological concentration of ions dissolved from glass. pH measurements revealed that the broth containing the 58S BG nanoparticles had higher pH levels as high as 9 when compared with the 63S and 72S BGs nanoparticles, which is the threshold concentration inducing antibacterial activity. The synergic effects of high calcium concentration and alkaline pH level may make the broth containing 58S a good antibacterial agent.[13]

Polyesters based on poly (lactic acid), poly (glycolic acid) and poly (lactic-co-glycolic acid) (PLGA) are found as the best biomaterial with regard to design and performance. Biocompatibility of monomer is considered as the foundation for biocompatibility of degradable polymer systems, not the polymer itself.[14] Even though PLGA is extensively used and represents the gold standard of degradable polymers, increased local acidity due to its degradation can lead to irritation at the site of polymer implant. Agrawal and Athanasiou have introduced a technique in which basic salts are used to control the pH in the local environment of PLGA implant.[15] The feasibility of lactide/glycolide polymers as excipients for the controlled release of bioactive agents is well-proven and they are the most widely investigated biodegradable polymers for drug delivery.[16] Hollinger showed the osteogenic potential of PLGA.[17] Furthermore, it has been proposed that the use of PLGA in the composite coating provides a locking mechanism between the coating and the juxtaposed bone with time. The composite coatings of HA and $TiO_2$ in PLGA is produced by Sol-gel method. This study demonstrated increased adhesion of osteoblast-like cells on this coating compared with conventional plasma-spray techniques.[18] Our previous study showed excellent attachment and viability of human adipose-derived stem cells (hASCs) on the poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) nanocomposite coating. Therefore, PBGHA nanocomposite coating provides an ideal surface for the stem cells attachment, viability and proliferation.[19] This study aimed at preparation and *in vivo* evaluation of novel bioactive and biodegradable PBGHA and PBG nanocomposite coatings as candidates for dental and orthopedic implant applications.

## MATERIALS AND METHODS

### 58S BG nanoparticles synthesis

Starting materials used in this preparation were of analytical grade tetraethyl orthosilicate (TEOS), triethyl phosphate, $Ca(NO_3)_2.\ 4H_2O$ (Aldrich), ethanol and hydrochloric acid (Merck). All materials were used intact without further purification. The composition of studied BG belongs to the $SiO_2$-CaO-$P_2O_5$ system with 58S (57.72 $SiO_2$, 35.09 CaO and 7.1 $P_2O_5$ by weight) composition. BG nanopowders were prepared using the Sol-gel technique. Ethanol was used as a dispersant to obtain the nanopowders. The chosen volume ratio of ethanol to TEOS was two. Proper amounts of deionized water (15 ml), 2N hydrochloric acid (2.5 ml) and TEOS (20.49 ml) were dissolved in ethanol and stirred at room temperature for 30 min. Triethyl phosphate (2.08 ml) was then dissolved into the acid silica sol. After 20 min of stirring, $Ca(NO_3)_2.4H_2O$ (13 g) was added. The solution was stirred for an hour longer. The reaction mixture was transferred to a large teflon container, which was then placed in an oven for aging at 60°C for 54 h. The aged gel was transferred into another teflon vessel which was placed inside an especially designed drying chamber (cylindrical steel chamber capped with an aluminum foil with holes in it) with a proper amount of water. In the next step, the whole chamber was placed in an oven at 130°C for 54 h. Finally, the dried gel nanopowders were calcined at 600°C for 1 h.

### Preparation of the nanocomposite coatings for bioactivity and degradation studies

The solvent casting process was applied to coat the substrates. Commercially pure Ti (Grade 2), was cut into pieces 20 mm × 10 mm in size and used as the substrate. These substrates were ultrasonically cleaned first in acetone for 20 min, then in a 70% ethanol solution for 20 min and finally in distilled water for 15 min. HA nanoparticles (Aldrich, USA) and the 58S BG nanoparticles were dispersed ultrasonically in 20 ml of chloroform (Merck, Germany) and added to PLGA (0.1 g/ml in $CHCl_3$) (RESOMER® RG 502H, PLGA; 50/50 wt% poly (lactic acid)/poly (glycolic acid); inherent viscosity = 0.20 dl/g (25°C; 0.1 in $CHCl_3$)) with stirring. Solutions with one ratio of the components (PLGA:nanoparticles = 90:10 by wt%) were prepared to coat the specimens by the dip-coating procedure. The dip-coating process was repeated three times for each sample. The substrates were soaked in the solutions at a speed of 2 cm/min. After each dip-coating step, samples were dried for 1 min and the procedure was repeated. Both the PBGHA and PBG nanocomposite coatings were prepared by the same method. Solutions with the ratio of the components (PLGA:nanoparticles 90:10 by wt%) were prepared to coat the specimens by the dip-coating procedure. In the PBGHA nanocomposite coating equal amounts of HA (5 wt%) and BG (5 wt%) nanoparticles were used. The solvent was then allowed to evaporate at the room temperature (21°C) for 24 h. Scanning Electron Microscopy (SEM, Phillips XL 30) and Atomic force microscopy (AFM, Bruker, Nanos 1.1, Germany) were used to study the microstructure, morphology and surface roughness of the nanocomposite coatings. X-ray diffraction (XRD, Philips X' Pert-MPD System with Cu kα wavelength of 1.5418 Å) technique was utilized to determine the composition of the coatings.

### *In vitro* bioactivity evaluation of the nanocomposite coatings

The assessment of *in vitro* bioactivity was carried out by soaking the samples in simulated body fluid (SBF) in sterilized polyethylene containers maintained at 37°C. The SBF experiment protocol was presented elsewhere.[20,21] The samples were soaked in SBF for three different periods (7, 14 and 30 days). In order to provide more favorable conditions for apatite deposition, the solution was renewed every 2-3 days. Hence, the exchange of SBF leads to better simulation of the *in vivo* conditions, making the assay more precise and reliable. Next, the samples were removed from the solution, rinsed gently, first with pure ethanol, then with deionized water. Finally, they were dried at the room temperature for 3 h. The formation and growth of apatite layer on the samples were verified by SEM and XRD.

### The coatings' degradation studies

In order to study the effect of BG and HA nanoparticles on the degradation of the nanocomposite coatings, *in vitro* degradation tests were carried out in phosphate buffer saline (PBS; pH 7.4) at 37°C. Each sample was placed in a vessel containing 50 ml of PBS and incubated for periods up to 60 days. The ratio of the sample mass to the PBS solution volume was selected 6 mg/ml. The pH of the PBS solution was monitored every 2 days. The medium was changed every week. At each time interval (14, 30 and 60 days), samples were removed from the solution, washed with distilled water and air-dried overnight. Changes in the surface morphologies of the coatings during *in vitro* degradation were evaluated by SEM. In order to evaluate the effect of nanoparticles on the degradation of nanocomposite coatings, the degradation of PLGA coating was investigated, simultaneously.

### Animal implantation test

The experimental animal study was approved by the school of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. Titanium screws (diameter 1.5 mm, length 6 mm) were purchased from Synthes, Switzerland. A total of 60 screws were divided into three groups. 20 screws were coated with PBGHA nanocomposite coating, 20 screws were coated with PBG nanocomposite coating (PLGA:nanoparticles = 90:10 by wt%) and 20 screws were used without coating. Samples were sterilized by exposure to UV light for 2 h followed by soaking overnight in 70% ethanol according to standard techniques. The screws were implanted in the tibia of 16 white mature male New Zealand rabbits (Razi Vaccine & Serum Research Institute). The rabbit's ages were ranged from 8 to 10 months and the weights were ranged from 3 to 3.5 kg. The animals were kept in separate stainless steel cages that allowed some movement, therefore their legs were load bearing before and after the surgical placement of implants. All surgeries were performed under sterile conditions in an animal operation room. The animals were anesthetized with an intramuscular injection of ketamine (10 mg/kg). The local nerve supplies of the internal surface of the tibia were further blocked with 0.5 mL of 2% lidocaine. All operations were performed in standard surgical sterile

conditions. For each of the right and left tibia, an incision was made at the medial side of the tibia. Bone preparations of 6 mm depth were done under profuse sterile physiological saline cooling using careful drilling with low rotary speed (500 rpm). The screws were placed inside the bony preparation to the full-length unicortically. The fascia and muscles were sutured by a resorbable suture and the skin was sutured by a silk suture. After surgery, animals were injected subcutaneously with 3rd generation cephalosporin antibiotic once per day for 5 days at a dose of 20 mg/kg body weight. In addition, analgesic diclofenac sodium was injected intra-muscularly once a day for 2 days at a dose of 5 mg/kg bodyweight. After 30 and 60 days, the animals were sacrificed by an intravenous overdose of pentobarbital. The implants, together with the surrounding bone and soft-tissues, were removed and were fixed in 10% buffered formaldehyde solution (pH 7.4) at 4°C for 1 day. The implants were removed and they were decalcified in a mixture of formic acid and sodium citrate at 4°C for 6 days. Then, the samples were embedded in paraffin, decalcified in nitric acid , cut into 5 μm serial sections and stained with hematoxylin and eosin (H and E). All of the sections showing mini-screws space were evaluated. Histologic evaluation include: Type of bone (Trabecular, lamellar and woven bone), bone formation percentage (histomorphic analysis by IHMM, VER.1, sbmu, Iran) and the connective tissue (fibrosis, granulation tissue, normal bone marrow [BW]) formation. Histological evaluations were made under a light microscope (E400, Olympus, Japan) at ×40, ×100 and ×200 magnifications. The pathologist was blinded to the procedure.

Twenty commercially available Kirschner wires (K-wires) (Synthes, Switzerland) made of stainless steel with a diameter of 0.8 mm were coated with PBGHA and PBG nanocomposite coatings. Total coating mass was determined with an electronic micro-balance (Sartorius AG, Gottingen, Germany, readability 0.01 mg). Then, without previous drilling, K-wires were incorporated proximally into rabbit tibiae (white mature male New Zealand rabbits.) as intramedullary rods and they were immediately explanted. After explantation and removal of adherent bone and BW, the loss of coating mass (denoted LCM) was determined gravimetrically.

The non-parametric paired Friedman test was used to analyze differences between the amount of bone formation for the three samples (PBGHA and PBG coated implants and Ti without nanocomposite coating) on the same experimental day while the non-parametric Kruskal-Wallis test was used to compare the results between the different experimental days. For all tests, the level of significance was $P < 0.05$.

## RESULTS

### Characterization of the nanocomposite coatings

Figure 1 shows the SEM micrographs of the prepared nanocomposite coatings. An even nanoparticle distribution was observed along with

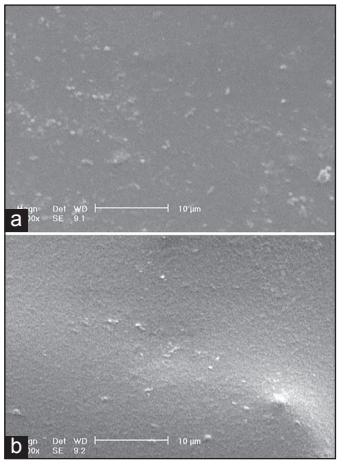


**Figure 1:** Scanning electron microscopy micrographs of the poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coatings (a) PBGHA (b) PBG

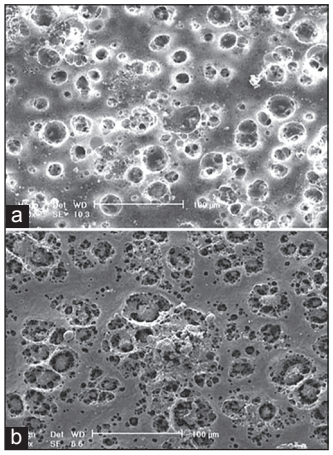


**Figure 2:** Scanning electron microscopy micrographs of the poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coatings after sterilization. Nanoparticles were exposed on the surface and the coating exhibited macropores. (a) PBGHA (b) PBG

some nanoparticle aggregates. Figure 2 shows SEM micrographs of PBGHA and PBG nanocomposite coatings after sterilization. Nanoparticles were exposed on the surface after sterilization because the PLGA was decomposed during sterilization in ethanol. Furthermore, this coating exhibited macropores after sterilization. XRD was used to provide clues to phases that existed in the nanocomposite coatings [Figure 3]. XRD pattern of the prepared PBGHA nanocomposite coating showed the peaks belonging to Ti and HA and an amorphous pattern is related to the PLGA and BG. XRD pattern of the PBG nanocomposite coating showed the peaks belonging to Ti and an amorphous pattern is related to the PLGA and BG. Quantitative measurements of root mean square (RMS) roughness and surface area obtained using AFM [Table 1 and Figure 4] showed more surface roughness of the PBGHA nanocomposite coating than the PBG nanocomposite coating after sterilization.

**Table 1: Surface roughness/area of substrates and nanocomposite coatings**

| Substrates | RMS ± SD (nm) | Surface area ± SD ($\mu m^2$) (projected area is 100 $\mu m^2$) |
|---|---|---|
| PBGHA nanocomposite coating | 1407±207 | 2788.593±547 |
| PBG nanocomposite coating | 299±97 | 1867.710±245 |
| Titanium | 131±48 | 105±3.2 |

SD: Standard deviation; PBGHA: Poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite; PBG: Poly (lactide-co-glycolide)/bioactive glass; RMS: Root mean square

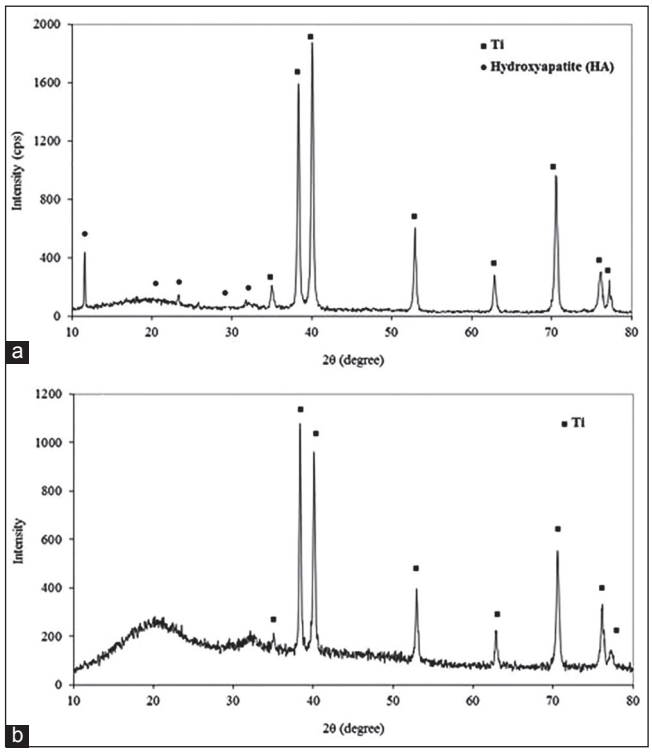


**Figure 3:** X-ray diffraction pattern of the poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coatings (10 wt% nanoparticles). (a) PBGHA nanocomposite coating: The peaks belong to both titanium plus hydroxyapatite. An amorphous pattern belongs to the PLGA and bioactive glass (BG) (b) PBG nanocomposite coating: The peaks belong to titanium. An amorphous pattern belongs to the PLGA and BG

## *In vitro* bioactivity evaluation

SEM analysis shows the effect of PBGHA and PBG nanocomposite coatings on HA deposition during increasing immersion times in SBF. It was observed that PBGHA and PBG nanocomposite coatings were able to nucleate more HA formation on their surfaces compared with Ti substrate. After 7 and 14 days, apatite deposits were observed and detected by XRD on the nanocomposite coatings but was not detected on the Ti substrates. After 30 days, HA deposits covered all the surface of PBGHA and PBG nanocomposite coatings [Figure 5a and b]. XRD patterns of the PBGHA and PBG nanocomposite coatings surfaces before and after 7 days immersion in SBF are shown in Figure 6a and b. As it was observed, after 7 days immersion in SBF, the diffraction peaks of apatite deposits formed on the surface of the prepared nanocomposite coatings were

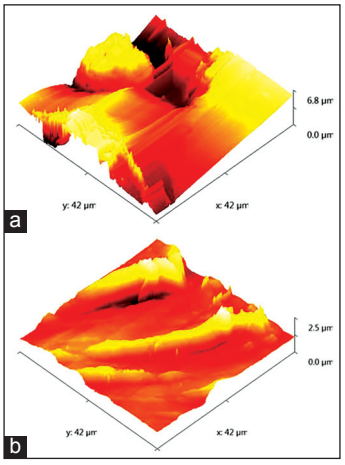


**Figure 4:** AFM analysis of the (a) poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and (b) poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coating. Quantitative measurements of root mean square roughness and surface area showed more surface roughness of the PBGHA nanocomposite coating than the PBG nanocomposite coating after sterilization

sharp. As it was observed, HA deposits on PBGHA nanocomposite coating has finer than the HA deposits on PBG nanocomposite coating [Figure 5a and b].

## Degradation studies

Figure 7 shows morphological changes of the PBGHA and PBG coatings before and after degradation in PBS. Results showed that the morphology of the sterilized coatings before degradation was porous. After degrading for 14 days, more pores could be seen on the surface of the coatings. Pores were formed by PLGA degradation. During degradation, the dissolution of nanoparticles and their aggregates could create pores of similar dimension to them and their aggregates and some of the nanoparticles were exposed on the surface. Nanoparticle aggregates dissolution made pores with greater dimensions than pores formed by pure PLGA degradation. After 30 days, the PLGA coating showed more pore formation and it was observed that it swelled in some parts and lost its adhesion to the substrate. PBGHA nanocomposite coating considerably degraded after about 60 days immersion in PBS.

## Histological findings

After 30 days, PBG nanocomposite coating induces more bone formation than PBGHA nanocomposite coating [Table 2]. Newly formed woven bone tissue was observed in the periosteal and endosteal region as indicated by deep red immature bone. Osteoblasts were lining the woven bone trabeculae and some lamellar segments were presented. The implants coated with PBGHA and PBG nanocomposite coatings showed more bone formation in the medullary cavity compared with that of the Ti implants without nanocomposite coating ($P < 0.05$) [Figure 8]. After 60 days, the healing process had proceeded. PBG nanocomposite coating showed more bone formation than PBGHA nanocomposite coating ($P < 0.05$). Titanium screws without coatings showed considerably less bone formation than PBGHA and PBG nanocomposite coatings ($P < 0.05$) [Table 2].

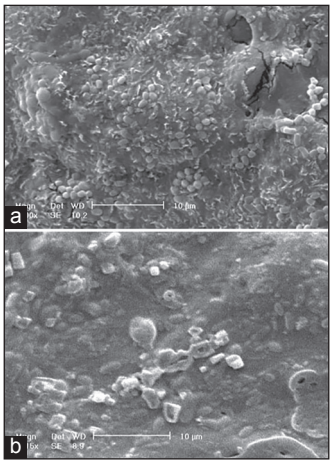


**Figure 5:** Scanning electron microscopy micrographs of the (a) poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (PBGHA) and (b) poly (lactide-co-glycolide)/bioactive glass (PBG) nanocomposite coating (10 wt% nanoparticles) after immersion for 30 days in simulated body fluid. After 30 days, hydroxyapatite deposits covered all the surface of PBGHA and PBG nanocomposite coatings

**Table 2: Histomorphometric analysis**

| Substrates | Bone formation mean (%) |
|---|---|
| PBGHA nanocomposite coating (30 days) | 41.18±1.55 |
| PBGHA nanocomposite coating (60 days) | 86.92±4.45 |
| PBG nanocomposite coating (30 days) | 62.69±2.96 |
| PBG nanocomposite coating (60 days) | 90.9±2.73 |
| Titanium (30 days) | 3.05±0.703 |
| Titanium (60 days) | 70.9±4.04 |

PBGHA: Poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite; PBG: Poly (lactide-co-glycolide)/bioactive glass

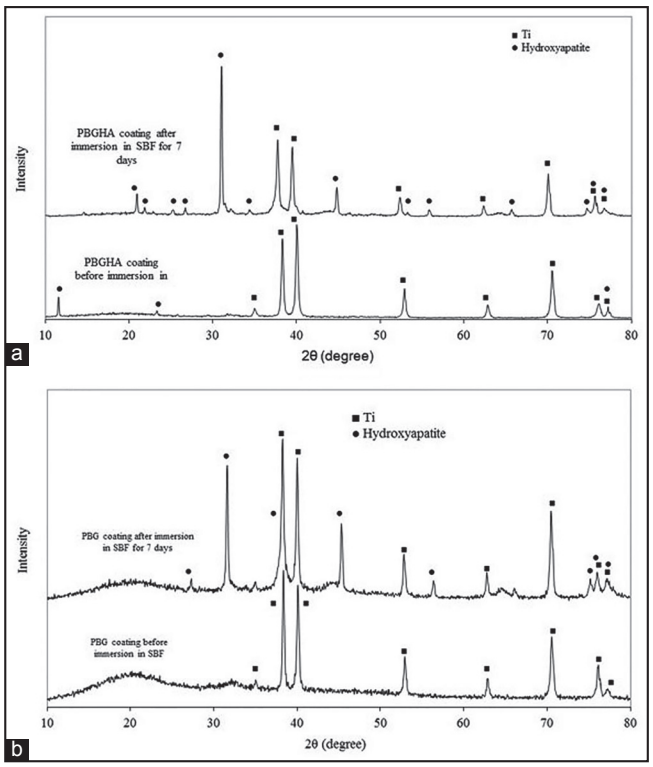


**Figure 6:** X-ray diffraction of the (a) poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite and (b) poly (lactide-co-glycolide)/bioactive glass nanocomposite coatings (10 wt% nanoparticles) before and after immersion in simulated body fluid for 7 days

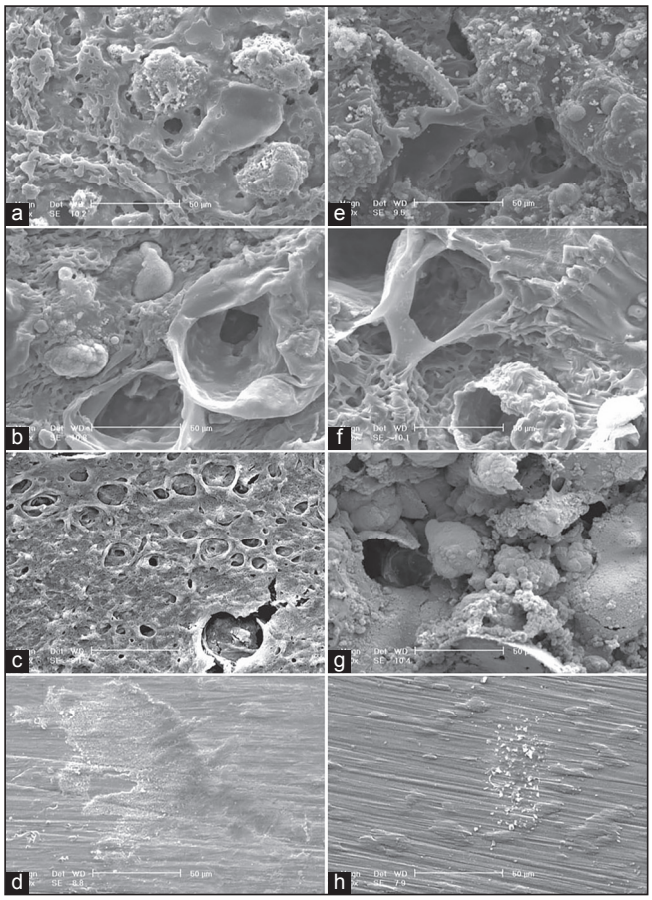

**Figure 7:** Scanning electron microscopy micrographs of the poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite nanocomposite coatins (10 wt% nanoparticles) (a, b, c and d) and poly (lactide-co-glycolide)/bioactive glass nanocomposite coating (e, f, g and h) after degradation for 7 days (a, e), 14 days (b, f), 30 days (c, g) and 60 days (d, h), bar = 50 μm

In all sections, osteoblasts synthesized lamellar bone on woven bone surfaces and then built up tissue deposition circumferentially around and toward the central vessel.

### PBGHA and PBG nanocomposite coatings adhesion to K-wires

After implantation, LCM averaged 3.88 ± 0.21% for PBGHA nanocomposite-coated K-wires and 3.55 ± 0.32% for PBG nanocomposite coating. Thus, about 96% of the both nanocomposite coatings mass remained attached to the K-wires during intramedullary implantation. No adhesive failure between the coating and the substrate was observed and LCM was mainly due to abrasion with cohesive failure within the nanocomposite coating itself. Therefore, they remained attached on the implants surfaces during intramedullary implantation.

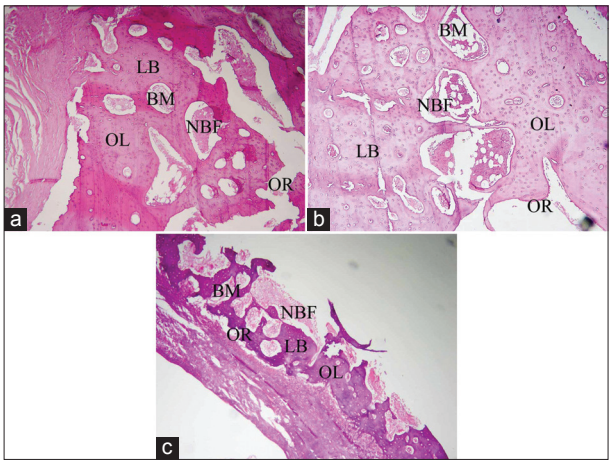


**Figure 8:** Histological analysis of titanium (Ti) screws coated with poly (lactide-co-glycolide)/bioactive glass/hydroxyapatite (a) and poly (lactide-co-glycolide)/bioactive glass (b) nanocomposite coatings and Ti screws without nanocomposite coating (c) implanted in rabbits tibia after implantation for 30 days. Newly formed woven bone, lamellar bone segments, osteoblastic rim, bone marrow and osteocyte lacunae can be observed. Samples were stained with H and E, (×40)

## DISCUSSION

Due to the aging of the population the need of orthopedic and oral bone-anchored implants increases almost considerably every year. However, the number of revision surgeries also grows. Many of the researches have been performed on optimizing implant lifespan. Therefore, different techniques have been used to improve surface wettability, bulk composition and surface topography.[22]

Since the discovery of the osteointegration by Brånemark *et al*. and Schroeder *et al*. and its applicability presented by Adell *et al*., implants have been created with many new designs and different surfaces in the hope of developing and facilitating both technique and results.[23-25] It is known that osteointegration has to do with the close contact of the newly formed bone with the implant surface. The attempts to increase the osteointegration and osteogenesis range from the improvement in the implant material and design to the application of ceramic coatings in its surface. This technique is used to explore the ceramic osteoconductive properties, taking necessary care, during the surgical procedures for installation of the implants. The search for biocompatible materials with osteoconductive properties to be used as implant surface coatings is old.[26-28] Titanium is the best biocompatible material with its remarkable corrosion resistance to make

dental implants. It was confirmed that the CP ceramics coatings make the implant surfaces more bioactive and accelerating the appositional bone growth.[29] However, these coating treatments at high temperatures seem not to be favorable for the bone biological repair.[30,31] A study by Sato *et al*. showed enhanced osteoblast adhesion on hydrothermally treated HA/titania/PLGA Sol-gel Ti coatings.[18]

Coatings can modify the surface properties of surgical-grade biomaterials to achieve improvements in performance, reliability and biocompatibility.[32] For example, Sol-gel derived HA coating with pores could be beneficial on the load bearing implants.[33] In another study, it was showed that the alkaline phosphatase activity of the osteoblast-like cells on the HA/$TiO_2$ double layer was expressed to a higher degree than that on the $TiO_2$ single coating and pure Ti surfaces.[34] The deposition of nanoparticles onto the Ti surface was performed to impart nanofeatures to a Ti dental implant. Sol-gel transformation techniques achieve deposition of nanometer-scale CP accretions to the implant surface. Owing to their resultant atomicscale interactions, the accretions display strong physical interactions. In a modified approach, Nishimura and colleagues recently demonstrated a directed approach to assembly of $CaPO_4$ nanofeatures on dual acid-etched cpTi implant surfaces. The deposition of discrete 20-40 nm nanoparticles on an acid-etched Ti surface led to increased mechanical interlocking with bone and the early healing of bone at the endosseous implant surface in a rat model.[35]

In the present study, the PBGHA and PBG nanocomposite coatings were prepared on the Ti substrates (plates and screws). Our hypothesis suggested that the presence of the 58S BG and HA nanoparticles could be effective in enhancing the biological behavior of the surface.

The presence of the BG and HA nanoparticles in the nanocomposite coatings could have a synergistic effect for increasing the rate of bone formation. Therefore, both BG and HA nanoparticles were used in the PBGHA nanocomposite coating preparation. Furthermore, PBG nanocomposite coating was prepared to evaluate the effect of BG nanoparticles on the bone formation *in vivo*. Previous study showed that nanoparticles content in the nanocomposite coatings could not exceed 10 wt% due to the formation of non-uniform coating on the substrates. For nanoparticle contents of 15 wt% and especially 20 wt%, many particle aggregates were observed throughout the specimen.[19]

BG and HA nanoparticles were exposed on the surface after the nanocomposite coatings sterilization process because the PLGA decomposed during sterilization in ethanol. Furthermore, these coatings exhibited macropores after sterilization. BG nanoparticles showed more tendencies to form aggregates than HA nanoparticles. Quantitative measurements of RMS roughness and surface area obtained using AFM [Table 1 and Figure 4] showed more surface roughness of the PBGHA nanocomposite coating than the PBG nanocomposite coating after sterilization. Porous structure of the nanocomposite coatings could provide a suitable surface topography for cell adhesion. Actually, porous structure of the nanocomposite coating surfaces is capable of accommodating tissue ingrowth.[36]

PBGHA and PBG biodegradable nanocomposite coatings could be effective especially at the early stages to induce bone formation. In fact, a biodegradable coating will degrade as new bone grows and sometime after surgery it will degrade entirely. In degradation studies it was supposed that nanoparticles dissolution could form pores in the nanocomposite coatings. Furthermore, degradation of PLGA could lead to expose more nanoparticles and their aggregates on the surface and consequently, increase the size of the pores in the nanocomposite coating. During degradation, the dissolution of nanoparticles could create pores of similar dimension to the original nanoparticles, which will facilitate oligomer diffusion in the composite. This enhancement in oligomer diffusion through the sample surface, together with the buffering effect of dissolution compounds, could reduce the autocatalysis degradation of PLGA. The swelling of the nanocomposite coating could become more homogeneous without the formation of a surface shell found in PLGA coating alone.[19] PBGHA nanocomposite coating considerably degraded after about 60 days of immersion in PBS. Our previous study showed that the 58S BG nanoparticle was effective at buffering; producing a higher $Ca^{2+}$ ion concentration and pH value in the dissolution medium.[13] This buffering effect could dominate the degradation rate of the nanocomposite. Furthermore, nanoparticles degradation made some pores in the nanocomposite coating which could reduce the autocatalytic mechanism of degradation by diffusing the acidic degradation products out of the coatings.

Observation of the samples during the degradation process confirmed that the presence of BG nanoparticles in the PLGA matrix provides a uniform degradation without any swell formation.

Bioactivity test showed that PBGHA and PBG nanocomposite coatings provides a suitable surface for bone-like apatite precipitation formation. Furthermore, it was showed that after 7 days, samples with pure PLGA coating induced the deposition of bone-like apatite.[19]

In our previous research, we used hASCs to evaluate their attachment and viability on the prepared PBGHA[19] and PBG (not published) nanocomposite coatings containing 10 wt% nanoparticles. The results showed excellent attachment and viability of hASCs on the PBGHA and PBG nanocomposite coatings. Furthermore, PBG nanocomposite coating showed more hASCs attachment than PBGHA nanocomposite coating. AFM analysis of the nanocomposite coatings showed more roughness of PBGHA nanocomposite coating than PBG nanocomposite coating. However, there were more cells attached and proliferated on the PBG nanocomposite coating than PBGHA nanocomposite coating. In fact, it was showed that BG nanoparticles have a positive effect for cell capture. Animal experiments revealed a superior response to BG particles with small grain size range (300-355 μm) compared to HA granules. Osteoconductive bone formation starting from the wall of the defects was observed around the BG particles more than around HA particles.[37] It was reported that the existence of BG particles could enhance cell attachment and spreading. The composites with BG particles were hydrophilic, which could induce a more wettable surface than pure poly (L-lactic acid).[38]

Different test methods have been developed to study coating stability. However, these *in vitro* tests mainly focus on single stresses such as bending forces and tensile, shear, or compression loads without mimicking the *in vivo* situation, where forces act in a complicated manner.[39] ASTM C-633-01, for instance, is a standard test to evaluate adhesion and cohesion strength of inorganic coatings.[40] For inorganic coating, bonding agents with epoxy resins and organic solvents are applied to fix the substrate coating to the loading fixture. Penetrating bonding agents may interact with biodegradable polymer coatings such as PBGHA and PBG nanocomposite coatings, invalidating mechanical stability or even dissolving the polymer. Therefore, stability testing is therefore recommended for every specific application. In this research, stability of the PBGHA and PBG nanocomposite coatings were compared by an *in vivo* test. The effect of HA and BG nanoparticles on the nanocomposite coatings was evaluated. PBGHA and PBG nanocomposite coatings proved great stability on K-wires during intramedullary implantation (LCM of PBGHA and PBG nanocomposite coatings was determined 3.88 and 3.55 present, respectively). About 96% of the PBGHA and PBG nanocomposite coatings mass remained attached to the K-wires which presented high stability and adhesion on K-wires.

After 30 days of implantation in rabbit tibiae, the PBGHA nanocomposite coating showed less bone formation (41.18%) than the PBG nanocomposite coating (62.69%). PBGHA nanocomposite coating has more RMS roughness than PBG nanocomposite coating. Interfacial characteristics of biomaterials, such as wettability, chemical composition, electric charges, surface roughness and porosity play a vital role in tissue reactions.[41] In this study, the main role of chemical composition on tissue response was confirmed. BG nanoparticles may induce more bone formation than HA nanoparticles. Therefore, BG presence in the PBG nanocomposite coating could enhance bone formation. After 60 days of implantation, more than 85% bone formation was observed for both nanocomposite coatings (86.92 and 90.9% for PBGHA and PBG nanocomposite coatings, respectively). Therefore, such nanocomposite coatings especially PBG nanocomposite coating could provide an optimum surface for bone formation.

## CONCLUSION

Bioactive and biodegradable PBGHA and PBG nanocomposite coatings on Ti implants can provide an ideal surface for bone formation. This *in vivo* study confirmed that BG nanoparticles induce more bone formation than HA nanoparticles. Although PBG nanocomposite coating has less roughness than PBGHA nanocomposite coating, bone regeneration was occurred more rapidly on the PBG nanocomposite coating. These findings confirmed that the surface composition plays a vital role in tissue response.

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