日本財団 The Nippon Foundation 助成事業

# 財団法人　日中医学協会

## 2011年度共同研究等助成金報告書－在留中国人研究者－

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財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書


中国人研究者氏名：　郝　佳　㊞
指導責任者名：　春日井　昇平
所属部署名：北京朗瑞口腔　门部　　職名：医員
所在地：北京朝阳区广顺北大街31号夏都家园
電　　話：　03 3872 4869　　内線：


**1．助成金額：　60万　円**

**2．研究テーマ**

**ビスフォスフォネート（BP）局所投与による、骨粗鬆モデルラット脛骨骨髄内に埋入した薄膜ハイドロキシアパタイト（HA）コーティングインプラント骨性結合への効果**

**3．成果の概要**

**BP局所投与した薄膜HAインプラントを骨粗鬆症モデルラット脛骨内へ埋入した際に、インプラント周囲骨の骨量を増加させる効果があること。また、ＢＰを局所投与した際は材料表面の細菌接着に影響ないことを証明した。**

**4．研究業績**

（1）学会における発表　　無・(有)（学会名・演題）

1. バイオインテグレーション学会・第２回学術大会,
2. ISTA 2011 Anual Congress

演題：

Bacterial adhesion behavior and bone formation effect of Zoledronic Acid (ZOL) immobilized hydroxyapatite implants.

（2）発表した論文　　無・(有)（雑誌名・題名）

Enhanced osteoblast and osteoclast responses to a thin film sputtered hydroxyapatite coating. J Mater Sci Mater Med. 2011 Jun;22(6):1489-99. Epub 2011 May 13.

5．指導責任者の意見（指導責任者がご記入・ご捺印ください）

我々の研究室は，チタン表面にハイドロキシアパタイト（HA）を薄膜コーティングしたインプラントを開発しておりますが，彼女はこのインプラントの骨性結合の向上を確認し，その有効性と安全性を示してきました．本研究では，このHAコーティングとビスフォスフォネート（BP）の局所投与を組み合わせることにより，骨結合をさらに強化するインプラントの有効性を証明しました．

指導責任者署名　春日井 昇平　印

—日中医学協会助成事業—

# ビスフォスフォネート局所投与による、骨粗鬆モデルラット脛骨内に埋入した薄膜ハイドロキシアパタイト(HA)コーティングインプラント骨性結合への効果

研究者氏名　郝　佳
中国所属機関　北京朗瑞口腔门诊部
日本研究機関　東京医科歯科大学
指導責任者　教授　春日井 昇平
共同研究者名　黒田　真司

**要 旨（Abstract）**

Bisphosphonates are well known drugs that can inhibit bone resorption and normalize the high rate of bone turnover that characterizes osteoporosis. Recently, hydroxyapatite (HA) has been used as bisphosphonates local delivery system to enhance peri-implant bone formation, and the results are generally encouraging. In the present study, a thin film HA coating with strong adhesion and bioactive microstructure prepared by radio frequency (RF) magnetron sputtering technique was used as bisphosphonate carrier. Microbial adhesion and the accumulation of pathogenic biofilms are considered to play major roles in the pathogenesis of peri-implantitis and implant loss. In addition, bisphosphonate-related osteonecrosis of the jaw (BRONJ) has become a big concern lately. A recent study reported that zoledronic acid (ZOL) promoted the adherence of streptococcus mutans to hydroxyapatite and the proliferation of oral bacteria. The purpose of the present study is to find out a coating concentration which can improve peri-implant bone formation but minimize bacterial adhesion. Custom made sputtered HA coated titanium cylinders were used as the substrate materials for ZOL application. There are four groups: (1) control group (without ZOL treatment); (2) Low dose group (0.5 μg/implant); (3) medium dose group (2μg/implant); (4) high dose group (10μg/implant). Each implant was inserted in the medullary cavity of a femur from the intercondylar notch. After 2 weeks healing, animals were sacrificed and femora were harvested for micro-CT and histology analysis. Bacteria were cultured on the samples with different amount of ZOL, and analyzed with the Live/Dead BacLight bacterial viability kit. We found out that the low dose and medium dose groups showed significantly higher bone implant contact than the control and high dose groups. There was also a significantly larger peri-implant bone volume in the low dose group than in the control and high dose groups, which was consistent with the result of mineral apposition rate. In addition, no significant difference in bacterial adhesion was observed among groups. The results indicated that the ZOL released from the sputtered HA coating stimulated peri-implant bone formation at relatively low dose (0.5 μg and 2μg). Furthermore, the bacterial adhesion to the HA implant was not affected by the application of ZOL.

**Key words**

Implant, hydroxyapatite, bisphosphonates, bone formation, bacteria.

## 緒　言( Introduction)

Bisphosphonates (BP) are well known drugs that can inhibit bone resorption and normalize the high rate of bone turnover that characterizes osteoporosis. BP, such as zoledronic acid (ZOL), have a high affinity for both natural and synthetic hydroxyapatite (HA), and their powerful anti-resorptive effects in osteoporosis were recognized through directly blocking osteoclastic proliferation and activity, or indirectly acting on osteoclasts via osteoblasts. Considering the undesirable effects such as gastrointestinal ulceration and osteonecrosis of the jaw, local application of BPs, a direct targeting at osteoclasts to be controlled, seems more effective. Recently, HA has been used as a local delivery system for bisphosphonates to enhance peri-implant bone formation, and the results are generally encouraging [1, 2]. However the HA coating in the previous studies was produced by a plasma spray technique which has been reported to result in a non-uniformity in coating density and poor adhesion between the coating and substrates [3]. The inflammatory response which in turn induces implant loosening is a biological consequence of the coating debris. In the present study, a thin film HA coating with strong adhesion and bioactive microstructure prepared by a radio frequency (RF) magnetron sputtering technique [4] was used as an alternative bisphosphonate carrier. Microbial adhesion and the accumulation of pathogenic biofilms are considered to play major roles in the pathogenesis of peri-implantitis and implant loss. In addition, bisphosphonate- related osteonecrosis of the jaw (BRONJ) has become a big concern lately. A recent study reported that ZOL promoted the adherence of streptococcus mutans to hydroxyapatite and the proliferation of oral bacteria. The purpose of the present study is to find out a coating concentration which can improve peri-implant bone formation but minimize bacterial adhesion.

## 対象と方法 (Materials and Methods)：

### Preparation of implants

Custom made Radio frequency (RF) sputtered HA coated titanium cylinders, measuring 1 mm in diameter and 15 mm in length, were used as the substrate materials for ZOL. Briefly, all the titanium cylinders were sandblasted by fluorapatite crystal and then subjected to an acid etching treatment. Sputtering was carried out to produce an average thickness of 1.1μm. Subsequently, a hydrothermal treatment was performed at a temperature of 120□ in an electrolyte solution containing calcium and phosphate ions for 20h. The surface roughness (Ra) was determined using a surface measurement tester (SURFCOM 130A). The average roughness of the sputtered HA coating was 1.5μm. These implants were sterilized and subjected to different amount of ZOL, including (1) control group (without ZOL treatment); (2) Low dose group (0.5 μg/implant); (3) medium dose group (2 μg/implant); (4) high dose group (10 μg/implant)

### Animal and surgical procedures

Twelve 24-week-old female Wistar rats were randomly assigned into four groups explained above. Each implant was inserted in the medullary cavity of a femur from the intercondylar notch. After 2 weeks healing, animals were sacrificed and femora were harvested for micro-CT and histology analysis.

### Micro CT analysis

X-ray imaging was performed by a micro-CT scanner (InspeXio; Shimadzu Science East Corporation, Tokyo,Japan) with a voxel size of 20 mm/pixel. Tri/3D-Bon software (RATOC System Engineering Co. Ltd, Tokyo, Japan) was used to make a 3D reconstruction from the obtained set of scans. Out of the entire 3D data set, the region of interest (ROI) was defined as the 100 slices from 3 mm below the growth plate and limited to a semi-ring of 2.0 mm diameter from the implant axis. Bone volume within the region of interest was calculated.

**Histological evaluation**

To obtain non-decalcified sections, samples were dehydrated in ascending gradient of ethanol, and then embedded in polyester resin (Rigolac-70F, Rigolac-2004, Nisshin EM Co.,Tokyo, Japan). The sections at approximate 3 mm below the growth plate were cut (Exakt, Mesmer, Ost Einbeck, Germany) in the horizontal direction and ground to a thickness of about 200 μm. The sections were finally stained with 0.1% toluidine blue, and observed under a light microscope. Bone implant contact (BIC) was quantified by a computer image analyzer (Image J, National Institute of Health, U.S.A).

**Measurement of mineral apposition rate (MAR)**

Inter label distance was measured (Image J, National Institute of Health, U.S.A) and the value was divided by the time interval (7 days) between administrations of two vital markers.

**Bacteria growth**

Bacteria were cultured on the samples with different amount of ZOL, and analyzed with the Live/Dead BacLight bacterial viability kit.

## 結果（Results）：

Micro-CT analysis revealed considerable difference among different groups (Fig. 1). Low dosage (0.5 μg/implant) and medium dosage (2 μg/implant) groups had striking effects on increasing the peri-implant bone volume when compared with the control group ($p<0.05$). By contrast, the high dosage group (10 μg/implant) could not induce a greater restoration in the bone volume.

In all the histological sections, no delamination of the HA coating was noted. The low dosage (0.5 μg/implant) and medium dosage (2 μg/implant) groups showed significantly higher BIC than the control and high dosage (10 μg/implant) groups (Fig 2). Furthermore, the MAR in The low dosage (0.5 μg/implant) was also significantly higher than those of other groups (Fig 3.)

**考察（Discussion and conclusions）：** The results indicated that the ZOL released from the sputtered HA coating stimulated peri-implant bone formation at relatively low dose (0.5 μg and 2μg), which is even less than the previous study by using plasma spray HA coating. This might be due to the small crystallite size (around 100nm) of the sputtered thin film HA, which was supposed to increase the effectiveness of ZOL absorption. Furthermore, the bacterial adhesion to the HA implant was not affected by the application of ZOL. A long-term in vivo study should be performed to test coating degradation.

## 参考文献（References）：


1. Wermelin K et al., Acta Orthopaedica 2007; 78:385-392.
2. Peter B et al., J Biomed Mater Res A 2006; 76:133-143.
3. Kangasniemi I et al., J Biomed Mater Res 1994;28:563.
4. Ozeki K et al., Biomed Mater Eng 2003;13:451.

Fig 1. Bone volume ($10^8$ µm$^3$)

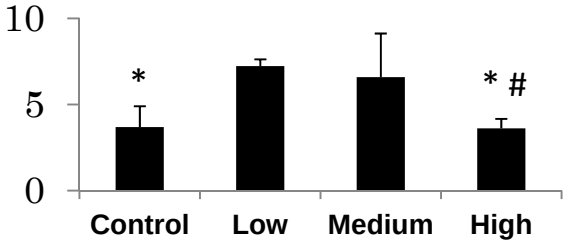



Fig 2.Bone implant contact (%)

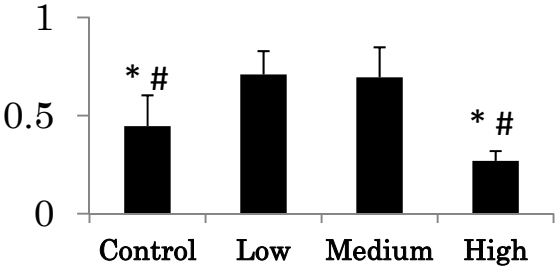
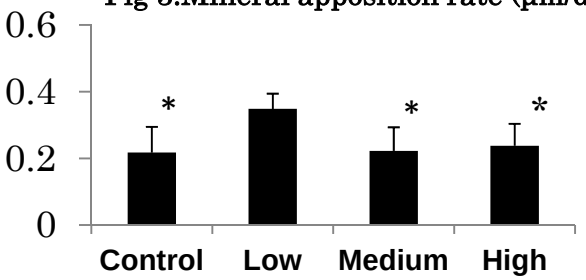



Fig 3.Mineral apposition rate (µm/day)




Data was expressed as mean ±SD (n=3).
* p<0.05 vs. low dosage group;
#p<0.05 vs. medium dosage group (One way ANOVA-LSD test).

注： This study was preseted in the バイオインテグレーション学会・第２回学術大会 and ISTA 2011 Anual Congress. The preliminary data of the present study was published in J Mater Sci Mater Med. 2011 Jun;22(6):1489-99. Epub 2011 May 13.

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