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OSSEOINTEGRATION OF DENTAL IMPLANTS IN rhBMP-2 INDUCED BONE AND STEM CELL-BASED INDUCED BONE: A HISTOLOGIC STUDY IN THE RABBIT PERI-IMPLANT DEFECT MODEL

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ABSTRACT

This work pretends to investigate the effects of recombinant human bone morphogenetic protein (rhBMP-2), rhBMP-2 associated to anorganic bovine bone (ABB) and allograft cellular bone matrix (ACBM) on both osseointegration of implants and bone formation in the grafted areas, compared with ABB as a regeneration material in the treatment of bone defects around implants in the rabbit model.

In this study a total of 48 implants will be inserted in the right and left femur of 24 rabbits, after standardized peri-implant defects with 8 X 8 mm of diameter were created. These defects will be regenerated at random, with the following four groups of grafts: group A - rhBMP-2 (n=12); group B - rhBMP-2/ABB (n = 12); group C - ACBM (n = 12) and group D (control group) - ABB (n = 12). Healing will be allowed to progress for 4 and 8 weeks, at which time the rabbits will be euthanized and block sections containing the implants and surrounding bone will be collected for histologic and histomorphometric analysis. Histologic examinations will be conducted using a light microscope and electron scanning microscope. The histomorphometric analysis will be performed to determine the percentage of direct bone contact with the implant surface. A statistical analysis of the results will, also, be performed.

Keywords: bone regeneration, xenograft, anorganic bovine bone, osseointegration, peri-implant defects, rhBMP-2, allograft cellular bone matrix.

INTRODUCTION

Endosseous implants have been used to support single-tooth, partial and complete arch dental reconstruction, and to support maxillofacial reconstruction. Patients with edentulous jaws often present loss of alveolar bone and as a result of an inadequate volume of bone the placement of dental implants might be compromised. Considerable efforts have been made to develop techniques and materials that increase the host bone volume, thus increasing the bone-to-implant contact.

Over the last 3 decades, numerous articles have documented clinical and histologic results of the peri-implant regeneration procedure using a number of various graft materials, with different degrees of success. The xenogenic bone is one of the most widely used scaffolds in regeneration procedures in peri-implant dehiscence (Hsu, 2010), but it requires a long healing period to produce adequate bone formation for implant placement.

Currently, there are promising alternative therapies for repairing bone defect in the peri-implant regeneration procedures such as bone morphogenetic proteins (BMPs) (Wikesjö,

2005) and cell-based bone tissue engineering (Kim, 2009).

Recombinant human (rh) BMP-2 has been assayed in several systems and has been found to have very high osteogenic and osteoinductive activity (Schwartz, 2008). The availability of recombinant BMPs has permitted definitive tests of their osteoinductive activity in a variety of experimental systems, including several animal models of clinically relevant bone defects (Wozney, 1995).

Cell-based bone tissue engineering seems to be a promising alternative therapy for repairing bone defect. Different types of cells have been examined for their potential application in bone regeneration (Arpornmaeklong, 2009), and stem cells are reported to be a suitable cell source for developing engineered tissues.

In this research project we intend to test, in the peri-implant defect model, regeneration materials that are currently at the forefront: rhBMP-2 and Stem Cell-based approaches, contributing to the data concerning these materials.

MATERIAL AND METHODS

Dental implant fixture characteristics

In this study will be used resorbable blast media-coated threaded titanium dental implants (ACE® Surgical Supply Co., Brockton, MA, USA) with a diameter of 3.3 mm and a length of 13 mm.

Xenograft characteristics

In this investigation will be used anorganic bovine bone (ABB) (NuOss^{TM}, cancellous granules 0.25-1.0 mm in size, ACE Surgical Supply Co., Brockton, USA).

Animals

A total of 24 adult rabbits in healthy condition will be used in this study. The experimental protocol was already proposed to Institutional Animal Care for approval.

Surgical procedure

For the surgical procedure, the animals will be anesthetized with sodium thiopental (20-25 mg/Kg intravenously) and maintained on gas anesthesia (1.5% halothane/ O_2 to effect) (Fig1).

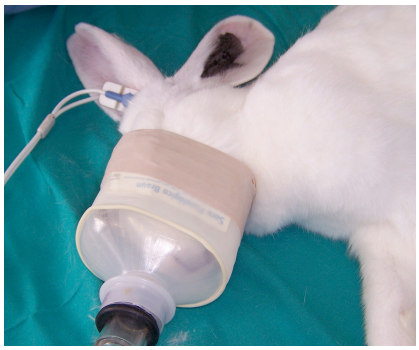

Fig 1. Rabbits maintained on gas anesthesia.

Infiltration anesthsia (lidocaine 2% with epinephrine 1:100000) will be used at the surgical sites. A long-acting opioid (buprenorphine HCL, 0.015 mg/kg subcutaneously every 12h for 5 days) will be used for post-surgery pain control. A broad-spectrum antibiotic (cefazolin sodium, 25 mg/kg intramuscularly daily for 5 days) will be used for post-surgery infection control.

The surgical protocol will use the same methodology in relation to our first research (Guerra, 2011). The rabbit legs will be shaved and washed with 70 % ethanol (Fig 2a). The crest of femur will be exposed and the cortical bone will be removed in a 5 mm circular area (Fig 2b).

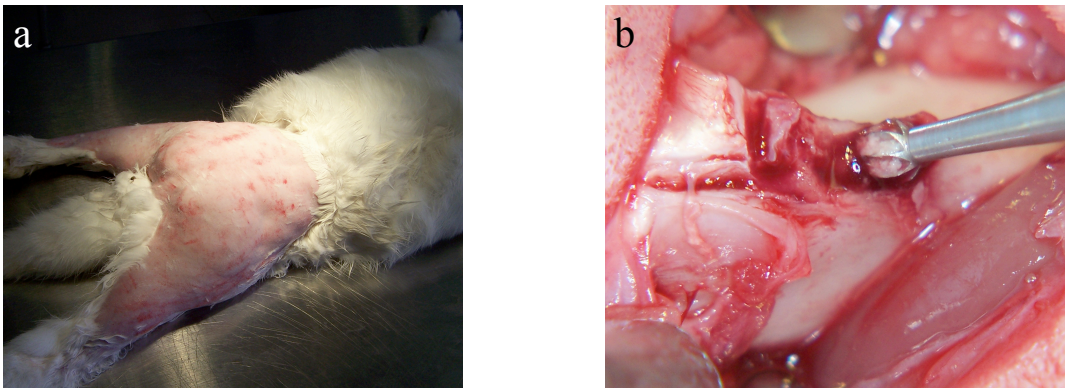

Fig 2. (a) Rabbit legs after being shaved; (b) Exposure of femoral bone.

Trepanation of the implant bed will be carried out according with ACE® protocol. A dehiscence type bone defect with approximately 8 X 8 mm will be created, involving the medial, lateral and anterior wall of the implant bed (Fig 3).

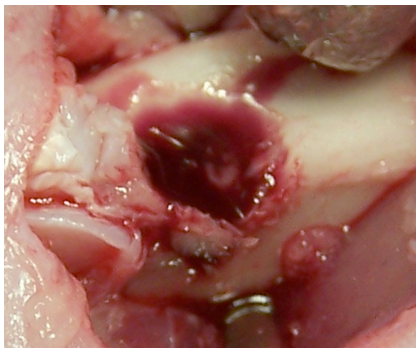

Fig 3. Dehiscence type bone defect.

The implants ACE Surgical, commercially pure titanium resorbable blast media (RBM), with a diameter of 3.3 mm and a length of 13 mm, will be inserted (Fig 4a) so that four threads of the implant will be exposed in the dehiscence defects (Fig 4b). The animals will be divided into 4 groups: in group A the defects will be repaired with rhBMP-2 (n=12); the group B with rhBMP-2/ABB (n=12); the group C with ACMB (n=12); and the group D with ABB (n=12) as a control.

The tissues will be sutured by layers, using a resorbable suture in the periosteum muscle (Fig 5a) and a silk suture in the skin (Fig 5b).

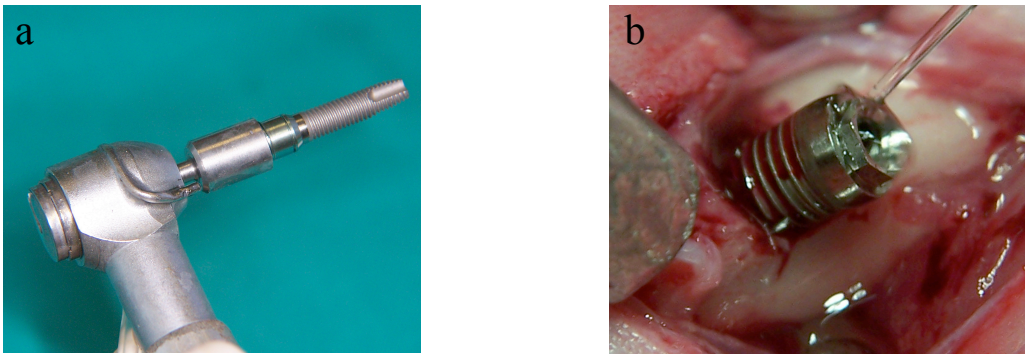

Fig 4.(a) Implant Ace 3.3 mm x 13 mm; (b) Threads of the implant exposed.

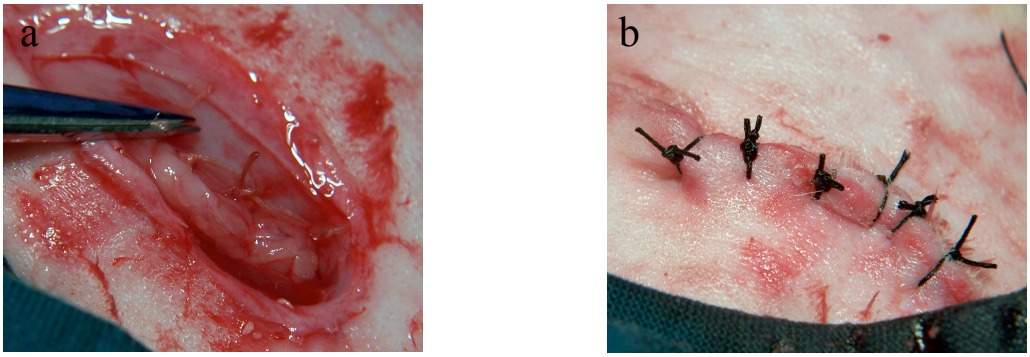

Fig 5.(a) Suture of the periosteum muscle; (b) Suture of the skin.

Histologic processing

Healing will be allowed to progress for 4 and 8 weeks, when the animals will be sacrificed. The implants and the surrounding bone will be removed for histologic evaluation.

All the core biopsies obtained will be fixed in 4% neutral formaldehyde with a pH of 7.4, for a 24 hour period. The specimens will be dehydrated in an ascending series of alcohol rinses, respectively 70, 80, 90 and 100% ethylic alcohol, for a period of 96 hours in each alcohol concentration. The blocks will be impregnated with a 75% methyl-methacrylate solution for a period of 72 hours. The samples will be finally placed in a solution containing 800ml/l of methyl-methacrylate (Methyl Methacrylate BDH), 200 ml/l of Plastoid (Plastoid N, Röhm Pharma), and 1 gr/l of Perkadox (Akzo Chemicals BV, Holland). To remove the air bubbles that usually occur in the solution before polymerization, the open bottles will be placed in vacuum equipment for 10 to 15 minutes, then hermetically sealed and placed in GFL water bath equipment (Gesellschaft fur Labortechnik GmbH, Germany) at 37ºC temperature for 48 hours. After polymerization, the specimens will be sectioned on a slow-speed diamond disc (Acuttom, Struers, Danemark) into approximately 150-200 µm thick sections. These undecalcified specimens will be sectioned along the frontal longitudinal axis of the implants. These sections will be processed in accordance with the requirements for light and scanning electron microscopy.

The sections to be observed with the light microscope (Leica DMLB, Switzerland) will be ground and polished to a final thickness of 40 ± 10 µm (P1200, 3M 314, UK), and surface-stained with 2 colorizing techniques: *(1)* hematoxylin (Harris Hematoxylin Acidified, Shandon Sci. Ltd., UK) and eosin (Eosin Y, Shandon Sci. Ltd., UK); *(2)* Solocromo Cianine R.

The 150-200 μm thick sections to be observed with electron scanning microscopy (JEOL JSM-35C, Japan) will underwent careful silica disc polishing, with decreasing granulometries of # 1000 and # 1200. Then the samples will be coated with a thin gold film through cathodic deposition in Ion sputter equipment (Jeol Fine Coat, Ion Sputter JFC 1100, and Japan).

Histomorphometric analysis

The histomorphometric analysis will be performed by one blinded examiner using a microcomputer-based image analysis system.

Five sections will be randomly selected, and in each section two threads will be isolated corresponding to the first thread closest to implant shoulder and the thread in the middle of the bone defect.

Linear measurements will be made from the total perimeter of the two selected threads and from the perimeter of direct bone-to-implant contact (BIC) in that two threads. The percentage of osseointegration between bone and implant surface will be calculated by the ratio between the BIC of selected threads and the total perimeter of selected threads.

Statistical analysis

Summary statistics (means ± SD) based on animal means for the experimental conditions will be calculated using selected sections. Differences between experimental conditions will be analysed using appropriate nonparametric tests.

RESULTS AND CONCLUSIONS

After the healing process, we expect to find osseointegration of all the implants, in all groups. The rhBMP-2 and allograft cellular bone matrix will accelerate the process of bone formation, resulting in a greater amount of regenerated bone and a higher percentage of bone contact with the implant surface, in a shorter period of time.

The cellular component of the allograft cellular bone matrix will potentially expedite the healing process by directing bone formation within the graft material, which may result in a larger quantity of available vital bone at an earlier time point.

Histologic evaluation

Under light microscopy, the two staining procedures, hematoxylin and Solocromo Cianine R,

will allow the distinction between pre-existing and newly formed bone. We expect to find in the three experimental groups (rhBMP-2 group, rhBMP-2/ABB group and ACMB group) a high number of osteocytes and numerous Haversion systems in the newly formed bone, and a pronounced vascularization of the regenerated bone.

The electron scanning microscopy will allow a more detailed analysis of the interfaces between the newly formed bone and the implant surface, and between the grafting material and the newly formed bone. The observations with this type of microscopy will allow the observation of different mineralization stages of the bone matrix.

Histomorphometric analysis

We will calculate the mean percentages of new bone formation in the four groups.

The percentage of bone tissue in direct contact with the implant surface, we expect to be greater in the rhBMP-2 group, in the rhBMP-2/ABB group and in the ACMB group compared to the ABB group.

We, also, expect that in the sections with 8 weeks of implantation time the percentage of bone tissue in direct contact with the implant surface will be superior with respect the samples with 4 weeks of implantation time, in all the groups.

We expect to achieve statistically significant differences between groups at either 4 and 8 weeks ($P>0.05$).

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