A radiographic survey of eggshell powder effect on tibial bone defect repair tested in dog

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Abstract. Objective: Nowadays, skeletal system injuries are of major importance. In addition, it is recommended to use materials for hard tissue repair in open or closed fractures. It is important to use complex minerals with a beneficial effect on hard tissue repair, stimulating cell growth in the bone. Materials that could help avoid bone fracture inflammatory reaction and speed up bone fracture repair are of utmost importance in the treatment of bone fractures. Material and Method: Similar to minerals, the inner eggshell membrane consists of carbohydrates, lipids, proteins with high pH, high calcium absorptive capacity and with faster bone fracture repair ability. In the present radiographic survey, eggshell-derived bone graft substitutes were used for bone defect repair in 8 dog tibia, measuring bone density on the day of implant placement and 30 and 60 days after placement. Results: In fact, the result of this study shows the difference in bone growth and misshapen bones between treatment and control sites. Conclusion: Cell growth was adequate in treatment sites and misshapen bones were less frequent here than in control sites.

Key Words: radiography, eggshell powder, bone repair.

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Introduction

A complication-free and fail-safe healing process in defect sites is of vital significance when considering the reconstruction of cranial, craniofacial and oral defects associated with congenital malformation, surgical oncology and traumatic injury (Lew et al 1997). For this purpose, allogeneic and alloplastic bone substitutes, as well as autogenic bone grafts, have been used for a very long time. Moreover, in the last decade, the concept of guided bone regeneration (GBR) has become widely accepted, since they play important roles in reconstructive surgery. In experimental studies, the calvarial defect model has been regarded as the most selective experimental model for bone regeneration due to the poor blood supply and the membrane structure resembling the mineralized bone matrix and it is mainly composed of calcium carbonate (97.4%), magnesium phosphate (1.9%) and tricalcium phosphate (0.7%). Moreover, the calcified eggshell contains an organic matrix, accounting for 2% of the total eggshell weight. This organic matrix contains several proteins and proteoglycans, such as ovocleidin-116, ovotransferrin, ovalbumin, ovocalyxin-32, ovocleidin-17, osteopontin (OPN), and lysozyme, some of them being able to modify the morphology of calcite crystals and the precipitation of calcium carbonate (Pines et al 1995; Panheleux et al 1999). OPN plays an important role in calcification, as it increases osteoblast adhesion onto the matrix and binds to hydroxyapatite (Pines et al 1995; Li-Chan&Nakai 1998). Understanding the biological effects and resorption kinetics of autograft, allograft and synthetic bone substitute materials is necessary for their optimum use, since they play important roles in reconstructive surgery. In experimental studies, the calvarial defect model has been regarded as the most selective experimental model for bone regeneration due to the poor blood supply and the membrane structure precluding spontaneous healing (Dupoirieux et al 2001). In the present study, the process of bone healing was assessed in the experimental dog model of tibial bone defect without filling, on the one hand, and filled with either same size eggshell granules specifically ostrich eggshell, has recently been introduced as a bone substitute candidate in reconstructive surgery (Dupoirieux et al 2001; Dupoirieux 1999) and in jaw cyst repair (Baliga et al 1998), as a result of its thickness, suitable for preparing particular materials of different size and of its inorganic phase, mainly consisting of calcite crystals (Feng et al 2001). It closely resembles the mineralized bone matrix and it is mainly composed of calcium carbonate (97.4%), magnesium phosphate (1.9%) and tricalcium phosphate (0.7%). Additionally, the calcified eggshell contains an organic matrix, accounting for 2% of the total eggshell weight. This organic matrix contains several proteins and proteoglycans, such as ovocleidin-116, ovotransferrin, ovalbumin, ovocalyxin-32, ovocleidin-17, osteopontin (OPN), and lysozyme, some of them being able to modify the morphology of calcite crystals and the precipitation of calcium carbonate (Pines et al 1995; Panheleux et al 1999). OPN plays an important role in calcification, as it increases osteoblast adhesion onto the matrix and binds to hydroxyapatite (Pines et al 1995; Li-Chan&Nakai 1998). Understanding the biological effects and resorption kinetics of autograft, allograft and synthetic bone substitute materials is necessary for their optimum use, since they play important roles in reconstructive surgery. In experimental studies, the calvarial defect model has been regarded as the most selective experimental model for bone regeneration due to the poor blood supply and the membrane structure precluding spontaneous healing (Dupoirieux et al 2001). In the present study, the process of bone healing was assessed in the experimental dog model of tibial bone defect without filling, on the one hand, and filled with either same size eggshell granules
or commercially available DBM, on the other hand, using clinical, radiological and densitometric methods.

**Materials and methods**

**Preparation of animal models**

This experimental study was conducted on adult male mixed breed dogs (n=8) aged 3 to 4 years and weighing 20 to 30 kilograms. The animals were randomly divided into 2 groups. The research protocol was approved by the Research Ethics Committee of the College of Veterinary Medicine, Shahrekord Branch, Islamic Azad University. All the experiments were carried out in accordance with the rules of the Institutional Animal Care and Use Committee (IACUC). The study design and the parameters for tissue reaction evaluation were in accordance with the ISO 10993-6 (1994) standard.

**Eggshell powder (ESP) collection and preparation**

Eggshell powder was obtained from White Leghorn hen eggs by breaking the eggshell opposite the air chamber, discarding the albumen and yolk and washing them thoroughly with distilled water several times. The outer and inner shell membranes were carefully removed with a forceps. Then, the eggshell was manually extracted, rinsed and dried at 25°C for 24 h. After being crushed, the sheets were individually packed and sterilized with ethylene oxide for 1 hour at room temperature. (AX-400, Axis, Izmir, Turkey) It has been suggested that the methods used for the harvesting and sterilization of graft materials had a strong impact on their osteogenic capacity (Dupoirieux et al 1994).

**Experimental Animals and Surgical Technique**

In order to assess the effect of eggshell powder-derived graft substitutes on bone healing (Adam 2004), the present study was conducted on adult male mixed breed dogs (n=8, 2-3 years, 23-23.5 kg), divided into 2 groups. The isoflurane anaesthetized dogs underwent surgery under sterile conditions. In this study, the caudomedial approach to the medial part of the right tibia was performed via 1–1.5 cm incisions. After removing the surrounding musculature to isolate the tibia, an 8-mm wide osteotomy was performed using an oscillating. Further, 1 mg of eggshell powder was implanted to the site of osteotomy. The fixation of the implant was accomplished using two simple discontinuous periosteal sutures (2-0 nylon) of the cranial and caudal face of the proximal fragment. The other site of the tibia was only subjected to osteotomy and served as negative control for natural healing. Densitometry (manufacture by MedicalExpo) was performed using the LC NDT FV-2009 Viewer.

Radiological examination detected minimal bone regeneration at the periphery of the bone defect site in the control group, 1 month after surgery. Bone formation displayed centripetal progression from the entire circumference of the defect. In the experimental groups, small particles were absorbed more excessively than the larger ones. Bone regeneration was considerable in both groups when compared to the first group in the first month of the experiment. Bone regeneration significantly increased within the implanted site in the second group, in the second month of the experiment, when compared to the control group.

**Clinical Results**

All animals healed without any complications and gained body weight. Physical examination detected eggshell particle remnants under the skin, with no major displacement in bone defect and relatively smaller than the initial size of the particles.

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**Radiological Results**

The results of radiography and densitometry and the p values in this study are shown in Table 1. The data were analyzed using SPSS software (version 17. SPSS Inc., USA) and P value was calculated using Chi-square and fisher’s exact tests to fine any significant relationship. P value less than 0.05 was considered statistically significant.

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In the present study, 8-mm critical size defects were created in the tibial bone of dogs, as bone lesions above this critical size become scarred rather than regenerated, leading to the emergence of a cavity (Bauer & Muschler 2000; Lindholm & GAO 1993). Such defects have been traditionally treated with bone graft substitutes. When using a bone graft, the wound is expected to heal and new bone to form (Bodner 1996), further replacing the bone defect. Graft materials obtained from other sources have been introduced as a possible solution. Chicken eggshell has been recently introduced as a candidate for bone substitution in maxillofacial surgery (Dupoirieux et al 2000; Dupoirieux 1999). The chicken eggshell has a high mineral content (over 97%) and its mineralization is known as being the most rapid and with the largest amount of calcium deposition (Lavelin et al 1999). Previous researchers (Dupoirieux et al 2000; Dupoirieux et al 1995; Dupoirieux 1999; Durmus et al 2003) have focused on the material’s biological behavior, biocompatibility, binding ability and resorption kinetics. However, previous studies have mostly taken into consideration the possible roles of the eggshell inorganic matrix as a tissue scaffold. Bone formation and bone remodelling are controlled by non-collagenous proteins of the bone matrix. These low-molecular weight polypeptides determine the crystal structure and mechanical strength of the material by modulating crystal nucleation in the bone and other extra cellular mineralized tissues. (Addadi and Weiner 1985) Osteocalcin (OCN), osteopontin (OPN) and bone sialoprotein (BSP) were found in the non-collagenous proteins of the bone matrix. Since OPN increases osteoblast adhesion onto the matrix and binds to hydroxyapatite, it plays important roles in bone formation and bone remodeling (Dominguez et al 2000; Lavelin et al 1999; Glimcher...
References


Acknowledgments

We would like to express my appreciation to the Department of Radiology and Surgery of the College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran, for supporting this paper. A special thanks to Dr. Shahriar Adibi, who helped us with the initiation of the present research.


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Citation


Editor Ştefan C. Vesa

Received 16 March 2015

Accepted 12 May 2015

Published Online 3 December 2015

Funding None reported

Conflicts/Competing Interests None reported