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The Synthetic Powders Extracted of Ostrich Eggshell and Evaluated the Effect of These Bone Powders on Bone Remodeling and Repairing

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IN this paper we present the bone repairing immediately by ostrich eggshell powder that performed powder by ultrasonic way, lower than bone healing by self healing. The radiological and clinical survey about bone healing by ostrich eggshell powder was compared with the result of self-healing of bone defect in control groups. The study was performed on 10 mix bread dogs in two groups. Two defects on tibia diaphysis bone has been done, that one of them filled by ostrich eggshell powder and another one defect was empty for self-healing as control. Clinical and radiological evaluation of the animal model were done at thirty days and sixty days after beginning of study. Radiologically finding seems to be satisfactory regeneration with acceptable new bone formation due to osteogenesis in the main defect that filled by ostrich eggshell powder at earlier stage and bone healedremodeled defect sites that filled by ostrich eggshell powder at 60th day. Minimalbone reconstruction in the control defects are seen in the 30th and 60th day.Surgical complication in the animal study hind limbs didn't seen. Body weights were normal. Physical examination detected ostrich eggshell particles remnants under the skin was evidenced, Deformity and new bone formation on the tibia bones didn't see. Bone healing by Ostrich eggshell that powdered by ultrasound way was helpful compared with natural bone healing. There may be a significant relationship between particle size and bone oste induction that requires additional testing to determine this relationship. The reaction to ostrich egg shell particles was a non-immunogenic reaction without destructive effects.

Keywords: Radiography, Ostrich, Eggshell Powder, Bone Repair.

Introduction

Patient age, bone segment (femur, tibia), cortical level (metaphyseal, diaphysealand etc.), are important in bone healing [1]. Bone healing duration in the long bones with stroncortex is very important. In a recent study, bone reconstruction in the long bone defects, periosteal callus development, new bone formation, callus tissue composition, during repairing have been evaluated[2]. Maximum periosteal callus size in the 5 week and 9 week after operation are seen [2] and new bone formation or increased

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bone density is reduced by about 10 weeks (9-12 weeks)[2]. No evidence of bone regeneration was observed in 15 days, while in thirty days, bone reconstruction was performed completely on day ninety (group I)[3]. Materials such as allografts, autografts and other alternatives and materials similar to artificial bone grafts have a very important role in orthopedic repair and the biological effects of these materials are necessary for optimal use[4].

Although vascularized and cancellous autograft show optimum skeletal incorporation but some minerals like that biomaterials as natural materials can help to bone regeneration. Egg shell powder has been considered as a new therapeutic solution for bone regeneration [3,4]. Ostrich eggshell consists of three parts such as membranes, calcareous shell, and cuticle. Ostrich eggshell has all the elements in the eggshell and is 2 to 3 mm in diameter and has high strength [3,4]. Ostrich eggshells as useful biowaste contain large amounts of minerals that are used in tissue engineering and repair[3,4]. The purpose of this study is bone osteoprotective by ostrich eggshell powder. Chicken shells of birds, especially ostrich eggshells, are very similar to bone matrices as a bone substitute in restorative medicine. Its main constituents include calcium carbonate (97.4%) and magnesium phosphate (1.9%)[5-7]. This matrix is composed of compounds such as ovo-transferrin, ovoclidin-116, ovalbumin, ovocalixin-32, ovoclidin-17, osteopontin and lysozyme. These materials have the ability to alter the structure of calcite crystals and calcium carbonate deposits. In the present experimental study, the process of bone repair, new bone formation, callus formation and bone regeneration in animal model of tibial bone without filling vacancieswas evaluated and filled with ostrich eggshell powder as much as done by ultrasonic cutting , and then, using clinical, radiological evaluation.

Materials and Methods

Animal models

In this experimental study, 10 adult male dogs of three to four years old with weighing 20 to 30 kg and average body position were performed [8]. The animals were placed in a restricted access room and kept under controlled temperature (22 °C) and light conditions. Cycles of darkness and light [12 hours/12 hours] were provided (animal without free access to food and water). Experimental animals were grouped into two groups of 5 (randomly). The method of conducting animal studies was reviewed and approved by the Research Ethics Committee of AJA Medical University, Tehran, Iran.

Ostrich Eggshell powder (OEP) preparation

The method of preparing ostrich egg shell was that first, the ostrich egg was broken in front of the air chamber and after discarding the albumin and its yolk, the shells were washed several times with serum. Then, the outer and inner shell membranes were removed using forceps. Next, the ostrich eggshell was removed by hand, washed and dried at 25 °C (24 hours). After crushing and pulverizing the shells, the packed in separate sheetsand then sterilized with ethylene oxide (for one hour) (AX-400/ Izmir/ Turkey) [9]. In this study, after preparing Ostrich Eggshell powder, ultrasonic method was used to create the powder.

Experimental Animals and Surgical Technique

The dogs were anesthetized with isoflurane. They were then operated on under sterile conditions. Caudomodial procedure was performed through 1-1.5 cm incisions to the inner part of the tibia. After removing the muscle around each bone to separate the tibia, osteotomy was performed using an 8 mm wide swinging saw. Then, 1 mg of ostrich eggshell powder was inserted and filled at the osteotomy site. The other site of the tibia underwent osteotomy only and was considered a natural healing controller. 10 densitometry was performed by LC NDT FV-2009 Viewer and densitometer prob. Surgical incision was made by placing the subcutaneous tissue in a simple continuous pattern [2-0 polyglactin 910] and the discontinuous pattern of the skin [0-2 nylon], ketoprofen [1 mg/kg/24 hours/3 days]. It is prescribed as an anti-inflammatory and/or analgesic medication. Radiographs of the specimens were obtained on days 0, 30, 60, and 90 of the test, [Trophy CCX Digital, Croissy-Beaubourg, France], exposed to 45 kV, 2.4 MAs.

Results

Radiographs were used to analyze the density of bone repair. Implant site (Part A) and control site (Part B) in X-ray images obtained after surgery (Fig. 1).

X-ray image on the 30th day of tibia after surgery is specified in the first group (Fig. 2).

X-ray image of the tibia sixty days after surgery is evident in the second group (Fig. 3).

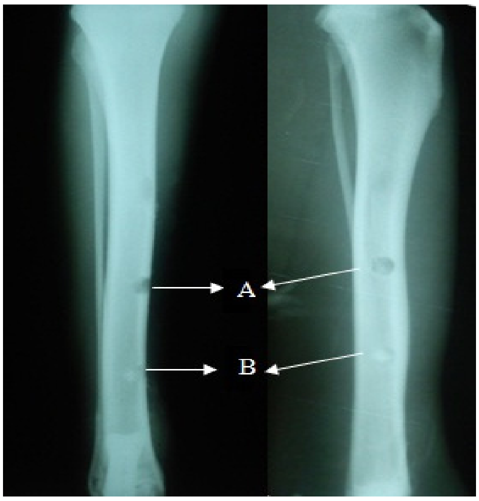

Fig. 1. Postoperative radiograph of the canine tibia on the day of the surgery.

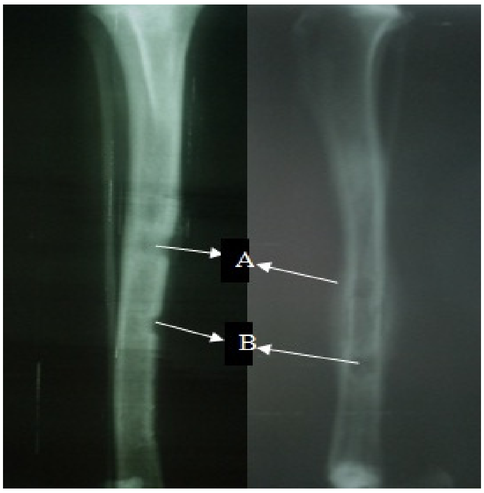

Fig. 2. Postoperative radiograph of the canine tibia 30 days after treatment.

Fig. 3. Postoperative radiograph of the canine tibia 60 days after treatment.

TABLE 1. Densitometry results and p values. ***Effective (p<0.05), *Non-Effective (p< 0.01).

Defects	Positive controls				Treatment using biomaterial			
Days	0	30	60	p value	0	30	60	p value
Group 1	3.90	3.90	3.70	0.01*	3.90	3.20	2.50	0.001***
Group 1	3.90	3.90	3.70	0.01*	3.90	3.20	2.50	0.001***
Group 1	3.80	3.80	3.60	0.01*	3.80	3.10	2.50	0.001***
Group 1	3.85	3.35	3.70	0.01*	3.85	3	2.50	0.001***
Group 2	3.90	3.90	3.70	0.01*	3.90	3	2.50	0.001***
Group 2	2.90	3.90	3.70	0.01*	2.90	3.10	2.50	0.001***
Group 2	3.80	3.80	3.60	0.01*	3.80	3	2.50	0.001***
Group 2	3.85	3.85	3.70	0.01*	3.85	3.11	2.50	0.001***

The radiographs in this study consist of craniocerebral and lateral views. The results of radiography and densitometry and p values in this study are specified in Table 1.

Clinical Results

Surgical complication in the animal study hind limbs didn't seen. Body weights were normal. Physical examination detected ostrich eggshell particlesremnants under the skin was evidenced, Deformity and new bone formation on the tibia bones didn't see,

Radiological Results

Radiological results seem to be mild bone regeneration at the around of the bone defect sites in the control groups,at 30th day after surgery and moderate bone regeneration at 60th day after surgery are seen. In the study groups by OEP, Bone regeneration was significant in the control group in the first month. Bone regeneration in the implanted group was also significantly increased in the second month of the study compared to the control group.

Discussion

Critical size defects of 8 mm occurred in the tibia of dogs because bone lesions larger than 8 mm become wounds instead of regenerating, leading to cavities 4,11. The most common way to treat a bone defect is to replace the lesion with a bone graft. Time to use a bone graft, researchers expect the wound to heal and repair the bone defect with the newly formed bone[12]. Bonding materials extracted from other sources have also been introduced as therapeutic solution. Ostrich eggshell has also been considered as a bone replacement option in maxillofacial surgeries [3,9]. Egg shell contains more than 97% minerals. Egg shell minerals are the fastest and highest amount of calcium deposition [13]. Previous research shows that it is possible and practical to use eggshell powder from different breeds of chicken as a bone graft 3,5,9,10. The use of ostrich eggshell powder is good due to its size, strength and assembly, so it has excellent mechanical properties for bone grafting [14]. These shells were used as bone repleasment in maxillofacial reconstructive surgery in animal experimental models and good results have been obtained [3,9,10,15]. Researchers have focused on the biological behavior of such materials, biocompatibility, binding ability and resorption kinetics [3,9,10,15]. Possible roles of the egg shell mineral matrix have been reported as tissue scaffolds [16]. Studies have shown that new bone regeneration and formation are controlled by non-collagenous bone matrix proteins [4,17]. Materials such as osteopontin, osteocalcin and non-collagenous bone matrix proteins contain sialoprotein protein [17]. Because osteopontinare causes osteoblast adhesionto the bone tissue matrix and binds it to hydroxyapatite, plays an important role in bone formation and bone regeneration [13, 18-20]. 21000 Da protein in proteins in egg shell solution play an important role in increasing calcium transport through IECs

(Intestinal epithelial cells)21. Eggshell organic matrix proteins modify calcite morphology and precipitation during chicken shell formation. In addition, chicken osteopontin is an egg shell matrix protein. It also contains transforming growth factor-beta1and progesterone is also isolated from egg shell powder, each of which plays an important role in bone repair eggshell [6, 22, 23, 25].

Mann and Seidler proteins have shown different types of C proteins such as lectin as the main components of the calcified matrix of the egg shell[26]. Calbinidine is synthesized by the uterus overnight during egg shell production. Egg shell is an absorbable implant due to the presence of calbindin [3,5,9,13, 18]. The researchers stated that the eggshell is an absorbable substance, but with a size-dependent degradation kinetics. 75 particles were completely absorbed after two months, while one hundred and fifty to three hundred particles were gradually absorbed during the fourth month. It was found that 50 egg shells were not detected after one month of implantation in laboratory animals [5,9,10]. In recent study, some bioactive glass such as CaO–SrO–P2O5–SiO2 was obtained by the sol-gel processing method, evaluated by X-ray diffraction technique, the results showed that they are non-toxic and revealed good biocompatibility [27]. After three months of treatment, no encapsulation, inflammation was observed. Larger egg shell particles are partially absorbed and smaller particles are moderately absorbed[10]. The results and statistical analysis showed that systemic delivery of lovastatin and simvastatin increased serum calcium, mineral density and expression of osteogenic bone genes[28].

Interestingly, bone formation was lower in control sites, whereas the sites of implantation had relatively higherbone density rates, with a greater osteogenic effect. There might be a strong relationship between particle size and osteoinduction that needs further experiments in order to be determined. The reaction to ostrich eggshell particles was a non-immunogenic reaction without any detrimental effects. In the present study, it was found that the density of planting sites was higher than the control sites. Resorption occurred at slower from control sites. However, none of the control sites were completely degraded at the end of the experiment. Interestingly, bone formation was lower at control sites, while bone density was relatively higher at implant sites. There may be a significant relationship between particle size and bone oste induction that requires additional testing to determine this relationship. The reaction to ostrich egg shell particles was a non-immunogenic reaction without destructive effects.

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Conflict of interest

The author declares that he has no conflict of interest.

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References

1. Fischgrund, J., Paley, D. and Suter, C. Variables affecting time to bone healing during limb lengthening. *Clin. Orthopaedics Related Res.,* **301**, 31-37 (1994).
2. Larsson, S., Kim, W., Caja, V.L., Egger, E.L., Inoue, N. and Chao, E.Y. Effect of early axial dynamization on tibial bone healing: a study in dogs. *Clin. Orthopaedics Related Res.,* **388**, 240-451 (2001).
3. Dupoirieux, L., Neves, M. and Pourquier, D. Comparison of pericranium and eggshell as space fillers used in combination with guided bone regeneration: an experimental study. *Journal of Oral and Maxillofacial Surgery,* **58**(1),40-46 (2000).
4. Bauer, T.W. and Muschler, G.F. Bone graft materials: an overview of the basic science. *Clinical Orthopaedics and Related Res.*, **371**, 10-27 (2000).
5. Dupoirieux, L., Pourquier, D., Picot, M. and Neves, M. Comparative study of three different membranes for guided bone regeneration of rat cranial defects. *Int. J. Oral Maxillofacial Surg.,* **30**(1), 58-62 (2001).
6. Panheleux, M., Bain, M., Fernandez, M., Morales, I., Gautron, J. and Arias, J. Organic matrix composition and ultrastructure of eggshell: a comparative study. *British Poultry Sci.*, **40**(2), 240-252 (1999).
7. Mine, Y. and Zhang, J.W. Comparative studies on antigenicity and allergenicity of native and denatured egg white proteins. *J. Agricultural Food Chem.*, **50**(9), 2679-2683 (2002).

8. Yadegari, M., Karimi, M., Sheishi, P.G., derakhshan, S. A radiographic survey of eggshell powder effect on tibial bone defect repair tested in dog. *HVM Bioflux,* **7**(4), 392-396 (2015).

9. Dupoirieux, L. Ostrich eggshell as a bone substitute: a preliminary report of its biological behaviour in animals–a possibility in facial reconstructive surgery. *British J. Oral Maxillofacial Surg.*, **37**(6), 467-471 (1999).

10. Durmuş, E., Celik, I., Ozturk, A., Ozkan, Y. and Aydin, M. Evaluation of the potential beneficial effects of ostrich eggshell combined with eggshell membranes in healing of cranial defects in rabbits. *J. Int. Med. Res.,* **31**(3), 223-30 (2003).

11. Lindholm, T. and Gao, T., editors. Functional carriers for bone morphogenetic proteins. *Annales Chirurgiae et Gynaecologiae Supplementum,* **4**, (1993).

12. Bodner, L. Effect of decalcified freeze-dried bone allograft on the healing of jaw defects after cyst enucleation. *Journal of Oral and Maxillofacial Surgery,* **54**(11), 1282-1286 (1999).

13. Lavelin, I., Meiri, N. and Pines, M. New insight in eggshell formation. *Poultry Sci.*, **79**(7), 1014-1017 (2000).

14. Feng, Q, Zhu, X, Li, H. and Kim, T. Crystal orientation regulation in ostrich eggshells. *J. Cry. Growth,* 233(3), 548-554 (2001).

15. Dupoirieux, L., Pourquier, D. and Souyris, F. Powdered eggshell: a pilot study on a new bone substitute for use in maxillofacial surgery. *J. Cranio-Maxillofacial Surg.,* **23**(3),187-194 (1995).

16. Durmuş, E., Celik, I., Aydın, M.F., Yıldırım, G. and Sur, E. Evaluation of the biocompatibility and osteoproductive activity of ostrich eggshell powder in experimentally induced calvarial defects in rabbits. *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* **86**(1),82-89 (2008).

17. Addadi, L. and Weiner, S. Control and design principles in biological mineralization. *Angewandte Chemie International Edition in English*, **31**(2), 153-169 (1992).

18. Dominguez-Vera, J., Gautron, J., Garcia-Ruiz, J. and Nys, Y. The effect of avian uterine fluid on the growth behavior of calcite crystals. *Poultry Sci.*, **79**(6), 901-907 (2000).

19. Gautron, J., Bain, M., Solomon, S. and Nys, Y. Soluble matrix of hen's eggshell extracts changes in vitro the rate of calcium carbonate precipitation and crystal morphology. *British Poultry Sci.*, **37**(4),853-866 (1996).

20. Glimcher, M.J. Mechanism of calcification: Role of collagen fibrils and collagen-phosphoprotein complexes in vitro and in vivo. *The Anatomical Record.*, **224**(2), 139-153 (1989).

21. Daengprok, W., Garnjanagoonchorn, W., Naivikul, O., Pornsinlpatip, P., Issigonis, K. and Mine, Y. Chicken eggshell matrix proteins enhance calcium transport in the human intestinal epithelial cells, Caco-2. *J. Agricultural Food Chem.,* **51**(20), 6056-6061 (2003).

22. Pines, M., Knopov, V. and Bar, A. Involvement of osteopontin in egg shell formation in the laying chicken. *Matrix. Biol.*, **14**(9), 765-771 (1995).

23. Nakano, T., Ikawa, N. and Ozimek, L. Extraction of glycosaminoglycans from chicken eggshell. *Poultry Sci.,***80**(5), 681-684 (2001).

24. Castagnola, P., Bet, P., Quarto, R., Gennari, M. and Cancedda, R. cDNA cloning and gene expression of chicken osteopontin. Expression of osteopontin mRNA in chondrocytes is enhanced by trypsin treatment of cells. *J. Biolog. Chem.*, **266**(15),9944-999(1991).

25. Schaafsma, A., Pakan, I., Hofstede, G., Muskiet, F., Van Der Veer, E. and De Vries, P. Mineral, amino acid, and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. *Poultry Sci.*, **79**(12), 1833-1838 (2000).

26. Mann, K. and Siedler, F. Ostrich (Struthio camelus) eggshell matrix contains two different C-type lectin-like proteins. Isolation, amino acid sequence, and posttranslational modifications. *Biochimica Et Biophysica Acta (BBA)-Proteins and Proteomics,* **1696**(1), 41-50 (2004).

27. Solgi, S., Shahrezaee, M., Zamanian, A., Jafarzadeh Kashi, T., Raz, M. and Khoshroo, K. Sol-gel synthesis and characterization of SiO2-Cao-P2O5-SrO bioactive glass: in vitro study. *Key Engineering MaterialsTrans. Tech. Publ.*, **631**,30-35(2015). doi:10.4028/www.scientific.net/KEM.631.30

28. Shahrezaee, M., Oryan, A., Bastami, F., Hosseinpour, S., Shahrezaee, M.H. and Kamali, A. Comparative impact of systemic delivery of atorvastatin, simvastatin, and lovastatin on bone mineral density of the ovariectomized rats. *Endocrine,* **60**(1),138-150 (2018).