

Original Research Article

Evaluation of the activity of hen eggshell graft in experimentally induced mandibular defects in rabbits: pilot study

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ABSTRACT

Background: For the treatment of the cranial and maxillofacial bone defects autogenic and/or allogenic bone grafts and alloplastic materials have been used for a long time. In the last decade, avian eggshell powder has been suggested as a bone substitute candidate in reconstructive surgery. The purpose of this study was to investigate the beneficial effects of particulate hen eggshell grafting on the healing of experimentally induced mandibular defects.

Methods: The study was conducted on 10 adult local rabbits. Two cavities of 4mm diameter have been created in the lateral surface of the mandible. One of the cavities was filled with particulate hen eggshell graft (test one), while the other hole was left for normal healing (control one). On the 8 weeks, the rabbits have been sacrificed and defective regions have been extracted.

Results: All animals showed normal wound healing. No foreign body reaction was observed, hen eggshell grafts were resorbed, integrated with bone at 8 weeks. There were no differences between control and chicken eggshell groups in new bone formation. At 8 weeks, histological analysis of specimens showed different amounts of woven bone contained osteocytes and covered by lining of osteoblasts.

Conclusions: Within the limitations of this study, it was concluded that hen eggshell powder is a worth-while bone substitute because it is a safe, cheap, and easily available material.

Keywords: Bone defects, Bone graft material, Eggshell powder

INTRODUCTION

Bone graft materials that are presently used in bone defects are autogenous bones, allogeneic bones, xenogeneic bones, and alloplastic materials.¹ Ideally, the graft material is required to have the ability to facilitate osteogenesis, stability when implanted with the graft, low risk of infection, ready availability, low antigenicity, and a high level of reliability.² Autograft is considered the gold standard for its biocompatibility, capability of osteogenesis, osteoinduction, and osteoconduction. However, the use of autografts has shown increased treatment time, surgical complications, pain and dysfunction at the harvested site, high cost, and limited

bone availability for the graft.³ On the other hand, allogenic and xenogenic bone graft may lead to immunologic reactions, infections, and improper fibrous healing.⁴ Recently avian eggshell powder has been suggested as a bone substitute.⁵⁻¹⁰ The mineral matrix of the avian eggshell, specifically ostrich eggshell, has been introduced as a bone substitute candidate in reconstructive surgery because that it has a reasonable thickness suitable for preparing different sized particulate material and its inorganic phase is mainly consisted of calcite crystals.⁹⁻¹² This study evaluated the histological responses to hen eggshell when used as graft on the healing of experimentally induced mandibular defects.

METHODS

Preparation of hen eggshell graft

Hen eggshells were purchased from a local market. The eggs were rinsed with sterile distilled water. After the egg's contents were poured, the outer and inner shell membranes of the eggs were carefully removed with forceps. The shells were crushed and sorted into particles of 300-500 μm by means of a standard sieve. They were immersed in 5% sodium hypochlorite and then in a sodium hydroxide solution to remove organic components. The grafts were subsequently washed with sterile water and sterilized by autoclaving at 136°C for 18 minutes.

Experimental animals

Ten adult local male rabbits with a mean weight of 2.3 ± 0.42 kg were used as the animal model. Experimental protocols were approved by Al Andalus university Committee of Animal Research.

Surgical procedures

All surgical procedures were performed under general anesthesia with a combination of 35 mg/kg intramuscular ketamine and 5mg/kg subcutaneous xylazine. Local anesthesia, consisting of 2% lidocaine with 1:100,000 epinephrine was infiltrated into the lateral surface of the mandibular body. The surgical site was shaved, prepared with 10% povidone-iodine solution, and draped to maintain aseptic conditions. A 1.5cm dissection was performed through the subcutaneous and muscle layers. The periosteum was carefully elevated to expose the lateral aspect of the mandibular body. Two intra bony cavities were made under a constant irrigation of sterile saline for each rabbits. The diameter of each cavity was 4mm. One of the cavities was filled with particulate chicken eggshell graft (test one), while the other hole was left for normal healing (control one) (Figure 1). The wound was closed in layers, using 4-0 Vicryl sutures. Postoperative analgesic included buprenorphine (0.3mg intramuscular).

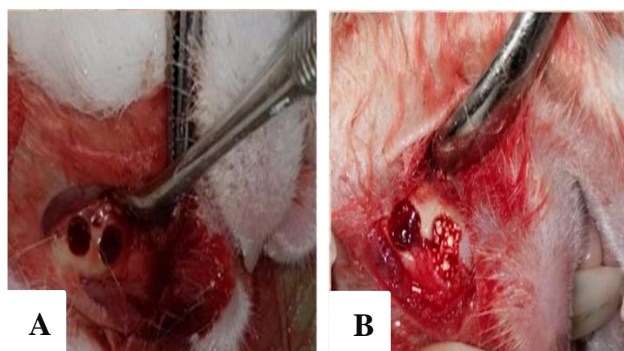


Figure 1: A) Intraoperative photograph, B) Test and control cavities.

Specimen preparation

On the 8 weeks, the rabbits were sacrificed by an intravenous over dose of pentobarbital sodium. Bone segments on which experiment has been done were extracted and kept in a 10% neutral buffered formalin solution for at least 3 days. The specimens were then decalcified in the formic acid solution. When sufficiently soft, tissue samples were processed and embedded in paraffin for histological examination. Standard 4-5mm sections were prepared and transferred onto slides for each block of tissue. All slides were stained with haematoxylin and eosin and evaluated using a light microscope.

RESULTS

All animals showed normal wound healing. No wound infection was encountered in all experimental animals and no fibrous encapsulation was observed in all specimens. There were no foreign body reaction, inflammatory response, or infection at all of the groups, chicken eggshell grafts were resorbed, integrated with bone at 8 weeks. There were no differences between control and hen eggshell cavities in new bone formation. At 8 weeks, histological analysis of specimens showed different amounts of new bone contained osteocytes and covered by lining of osteoblasts surrounding by loose connective tissues. Particles graft were completely resorbed and bone trabeculae occupied the defect site providing that the defect healed entirely (Figure 2).

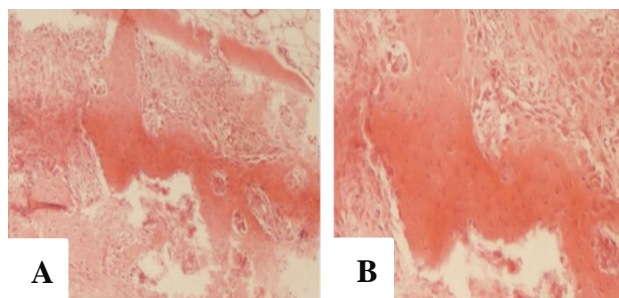


Figure 2: Histologic analyses of 8 weeks biopsy sample. A) x 40, B) x 100: (H&E staining).

DISCUSSION

The purpose of this study was to investigate the beneficial effects of particulate hen eggshell grafting on the healing of experimentally induced mandibular defects. In an earlier study by Dupoirieux on the use of chicken eggshell as bone substitute in maxillofacial surgery, he concluded that the eggshell is biocompatible and suggested its use for filling limited bone defects in non weight-bearing areas.¹⁰ A 1999 follow-up study by the same author using ostrich eggshell as onlay graft on rabbit mandibles showed similar results.¹¹ In the present study, 4 mm sized defects were created in the mandible of rabbits. There were no foreign body reaction,

inflammatory response, or infection. and the defect healed entirely. There were no differences between control and hen eggshell groups in new bone formation. Previous researches have concluded that the eggshell was a resorbable implant.^{5,8,11} In the present study, we used 300-500 µm particle sized material and the hen eggshell grafts were resorbed, integrated with bone at 8 weeks. Some authors have suggested that particles were resorbed in a size dependent manner.^{10,11} Durmuş et al, reported that smaller and rapidly resorbed powder particles had a low level of bone formation, whereas larger particles seemed to have more osteogenic effects on the sixth month of the experiment period.¹³ Dupoirieux et al, found that the 50µm particles of ostrich eggshell were not detected radiologically in any of the animals at the first month, the 75 µm particles were completely resorbed after 2 months, whereas the 150 to 300 µm particles were progressively resorbed at the fourth month.¹⁴ Controversial results have been reported for the osteoproduative activity of ostrich eggshell implants. Baliga et al, suggested that hen's eggshell powder was not an osteoinductive material, but it enhanced bone regeneration from the defect margins Dupoirieux et al.^{10,13,15} have also suggested that ostrich eggshell implants had no osteoinductive effect in the thigh muscles of rats and confirmed that long-term studies should be done to clarify ostrich eggshell particle's possible role in maxillofacial surgery. Similarly, Durmuş et al, have reported limited bone regeneration on the 90th day after grafting with ostrich eggshell-derived graft materials and eggshell membranes.¹⁶

CONCLUSION

Within the limitations of this study, it was concluded that hen eggshell powder is a worth-while bone substitute because it is a safe, cheap, and easily available material.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Committee of Animal Research of University of Al Andalus, Syria

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