Comparative Evaluation of Demineralized Bone Matrix and Type II Collagen Membrane versus Eggshell Powder as a Graft Material and Membrane in Rat Model

Abstract

Introduction: The regeneration of supportive periodontal tissues after destructive periodontal disease has become one of the primary objectives of periodontal therapy. Demineralized bone matrix (Osseograft), when used, has shown osteoinductive potential and collagen membrane (GTR) prevents the migration of epithelium into it. The literature showed that eggshell which consists of hydroxyapatite and calcium carbonate shows osteoconductive as well as inductive properties. Eggshell membrane which is made of type X collagen matrix can be used as a barrier membrane. Hence, the aim of the study was to compare the demineralized bone matrix with GTR membrane to eggshell constituents and its membrane as a regenerative material in Wistar rats.

Materials and Methods: A critical size periodontal defect of 1.5 × 6 mm was created on either side adjacent to mandibular incisors after raising the full thickness flap. Osseograft and collagen membrane covered the defect on one side and the eggshell components and its membrane filled the defects. The animals were sacrificed on the 45th day.

Results: Histological evaluation showed intensive new bone formation on both sides of the defect. Inflammation has resolved completely with no signs of eosinophils. Complete defect healing was noted in both the defects. A minimal amount of epithelial entrapment was noted in both the sites. There was no significant difference observed between the comparisons. More connective tissue was found in the test group.

Conclusion: Within the limits, it can be concluded that eggshell powder along with membrane can be used as a potential graft material.

Keywords: DFBM, eggshell graft, eggshell membrane, guided tissue regeneration membrane

Introduction

Periodontitis is one of the most common microbial infections in adults. It is an inflammatory disease which is characterized by the loss of the tooth-supporting structures which leads to apical migration of the junctional epithelium along the surface of the root with progressive destruction of the periodontal ligament and the alveolar bone. The primary objective of regenerative periodontal therapy is to restore the lost periodontal tissues caused by periodontal diseases. To achieve this objective, various techniques and types of graft materials have been used with varying grades of success.[1,2,7]

Autogenous bone grafts, considered as a gold standard graft material due to their excellent biocompatibility and osteogenic properties, were harvested from various intraoral sites. The disadvantage of using the autogenous bone graft was that it needs a second surgical site, unreliable graft incorporation, insufficient graft material, etc.[1,2,7] To prevent this, alternative biomaterials have been investigated and were being used efficiently. Currently, natural bone substitutes, particularly the coral skeleton composed of calcium carbonate (CaCO3), have been used as a bone graft substitutes in the management of periodontal defects.

Avian eggshell with a mineral composition similar to that of coral (95%) has been studied as a bone graft substitute in the oral cavity.[9-11] The eggshell structure is well documented in literature. The organic matrix contains proteoglycans and proteins such as ovocleidin 116, ovotransferrin, ovalbumin, ovoclyxin-32, ovocleidin-17, osteopontin and lysozyme. Calcium, phosphorus, magnesium, and sodium are major inorganic...
constituents of the avian eggshell. The eggshell powder is a safe and biodegradable material that is easy to obtain as well. Clinical and experimental studies have shown a number of positive effects of eggshell powder on bone metabolism. These features qualify eggshell powder as a worthwhile bone substitute candidate. Furthermore, eggshell-derived CaCO3 has been demonstrated to be efficacious as a biocompatible and osteoconductive biomaterial in several experimental animal studies.

In addition to the bone grafts, a space maintaining barrier membrane (Guided Tissue Regeneration, GTR) allows enough time for the migration, proliferation, and maturation of periodontal ligament and bone cells. A wide variety of GTR membranes have been developed which are non-resorbable and bio-resorbable. Each has its own advantages and disadvantages. Expanded polytetrafluoroethylene (e-PTFE), which is one of the non-resorbable membranes has a favourable space maintaining ability and better mechanical strength, although it requires a second surgical procedure for its removal. Therefore, bioresorbable GTR membranes have been increasingly used, which are mainly made from natural biopolymers like collagen and from synthetic polymers such as the polyactic acid (PLA). Collagen membrane has optimal biocompatibility and cell affinity, although it is generally criticized on compromised mechanical property, unpredictable degradation rate, higher cost and technological complexity of the manufacturing process.

Synthetic bioresorbable membranes show comparatively poor biocompatibility and their acid degradation products might result in an inflammatory response. Considering the above disadvantages, an ideal barrier membrane has to be developed which will have good biocompatibility and the space-making ability for clinically predictable periodontal tissue regeneration.

The natural eggshell membrane is a non-calcifying bi-layered membrane between the egg albumin and the inner surface of the eggshell. It is composed of water-insoluble interwoven protein fibers that highly cross-linked by a lot of disulfide bridges. It has been discovered to contain types I, V, and X collagens, osteopontin, sialoprotein, sialic acid, lysozyme, ovotransferrin, uronic acid, clusterin, etc. Zhao reported that bone marrow stromal cells (BMSCs) could grow and proliferate well on natural ESM and then concluded that ESM had the potential to be used as a bone tissue engineering scaffold. These studies indicated that ESM has great biocompatibility, bi-layered fibrous topology and the ability to enhance the healing of damaged tissues, thus suggested that it can be a suitable candidate for GTR membrane.

Materials and Methods
Selection of subjects
All Wistar rats weighing between 180–300gms were selected for the study. A total sample size of five was chosen based on the study using G power software. The study is of split-mouth design and the use of animals for experimentation was approved by the animal ethical committee of Saveetha University, Chennai, holding the number SU/CLR/14/2017. The study was divided into two groups, in which one group will receive DMBM and GTR membrane and the other group receives eggshell powder and its membrane as a regenerative material.

Surgical procedure
A critical size periodontal defect of 1.5 × 6 mm was created on either side adjacent to mandibular incisors after raising the full thickness periosteal flap. Demineralised freeze-dried bone matrix and second generation collagen membrane covered the defect on one side and the eggshell powder and its membrane filled the defect on the other side. The membranes on both sides were stabilized and sutured with 6-0 Vicryl suture. Postoperative analgesics and antibiotics were given.

The animals were sacrificed on the 45th day postoperatively. The jaw bones were removed and en bloc biopsies were taken from the operated sites. All histological procedures were carried out in a histopathological lab. The obtained specimens along with the soft tissues were taken and sectioned for histopathological evaluation.

Preparation of hen’s eggshell as a graft material
Chicken eggshell was prepared based on the previous study. The eggs obtained were rinsed thoroughly with distilled water several times. The contents of the egg are poured out. The inner and outer eggshell membrane are carefully separated using forceps and stored for further process. The shells were then crushed to a particle size of 1mm diameter. The eggshell powder is then rinsed thoroughly with distilled water with continuous agitation for one hour at 37°C. It is further packed and autoclaved at 136°C for 18 mins to remove the spores and other bacterial contamination.

Preparation of eggshell membrane
After extraction of the membrane from the walls of the egg, the membrane is washed thoroughly with 1% acetic acid solution for 10 mins, rinsed with distilled water and dried at 37°C for 24 hours. The dried membrane which were obtained as sheets were packed and sterilized with ethylene oxide.

Results
The results of postoperative healing were eventful during the experimental period. There was no foreign body or allergic reactions to the materials. Histological evaluation showed intensive new bone formation on both the sides of the defect which indicates the activity of osteoblasts, but more bone formation was noted in the control group [Figure 1]. Considering inflammation, there were
no signs of any inflammatory mediators like eosinophils noted. The defect was completely filled and healing was noted in both the defects. Most connective tissue was observed surrounding the defect in the control test group rather than the control group [Figure 2]. A minimal amount of epithelial entrapment was noted in both the groups. This entrapment resulted in more voids in the control group. There was no significant difference observed between the comparisons when epithelial entrapment was considered. Most connective tissue was found in the test group. Bone particles were gradually resorbed and replaced by new bone.

Discussion

In this study, chicken eggshell membrane was shown to be a good interposition material and chicken eggshell powder to be an effective graft material when used in the rat mandible experimental osteotomy. Eggshell membrane discouraged epithelial migration thereby helping in osteoblast proliferation and neovascularization.

Various materials based on calcium carbonate have been widely used in many studies as a bone graft material and showed varying results.\(^{[13-18]}\) In this study, chicken eggshell was used as a graft material due to its various biological properties like chemical nature and biocompatibility which are closely related to the structure of the host bone.

A one-year study done on the resorption kinetics on eggshell showed that particle size plays a crucial role in determining the complete resorption and kinetic degradation capacities.\(^{[10]}\) Similarly, in evaluating the ostrich eggshell powder in rabbits, it was reported that smaller the particle size, the less is the bone formation and vice versa.\(^{[14]}\) This is in agreement with our study where larger particle size was used and a good amount of bone formation has resulted.

Major expectations when a new graft material used, was its healing and induction of new bone formation. The results from the present study did not show any inflammatory signs nor necrosis of bone, which is in agreement with another study.\(^{[31]}\) Hence, it suggests the eggshell powder is biocompatible as a graft material. Considering the osteoinductive nature of the eggshell graft material, the eggshell graft material showed a similar amount of regenerated bone on defects site when compared to an osteoinductive material used as a control which supports the results of another study.\(^{[31]}\) These findings are inconsistent with the results of other studies.\(^{[10,13,14,18]}\)

Conclusion

Within the limits of our study, it can be concluded that eggshell powder along with membrane can be used as a potential graft material for intra-bony defects. Further studies are needed to assess the applications of the materials in clinical settings.

Acknowledgments

I would like to acknowledge Dr Selvanathan for helping me in handling the animal models.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Orthop Relat Res 2000;3711:10-27.
2. Brunsvoeld MA, Mellonig JT. Bone grafts and periodontal regeneration. Periodontol 2000 2000;1993:180-91.
3. Cochran DL, Jones A, Heijl L, Mellonig JT, Schoolfield J, King GN, et al. Periodontal regeneration with a combination of enamel matrix proteins and autogenous bone grafting. J Periodontol 2003;74:1269-81.
4. Gupta R, Pandit N, Malik R, Sood S. Clinical and radiological evaluation of an osseous xenograft for the treatment of infrabony...
Kavarthapu and Malaiappan: Evaluation of eggshell powder and its membrane as an effective graft and barrier material in animal model

5. Orsini M, Orsini G, Beniloch D, Aranda JJ, Sanz M. Long-term clinical results on the use of bone-replacement grafts in the treatment of intrabony periodontal defects. Comparison of the use of autogenous bone graft plus calcium sulfate to autogenous bone graft covered with a bioabsorbable membrane. J Periodontol 2005;76:1601-22.

6. Baliga M, Davies P, Dupoirieux L. Powdered eggshell in the treatment of intrabony periodontal defects with bone grafts. Periodontol 2000 2000;22:88-103.

7. Wang HL, Greenwell H, Fiorellini J, Giannobile W, Offenbacher S, Salkin L, et al. Periodontal regeneration. J Periodontol 2005;76:1601-22.

8. Dragoo MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. I. Wound healing 2 to 8 months. J Periodontol 1973;44:599-613.

9. Panheleux M, Bain M, Fernandez MS, Morales I, Gautron J, Arias JL, et al. Organic matrix composition and ultrastructure of eggshell: A comparative study. Br Poult Sci 1999;40:240-52.

10. Dupoirieux L, Pourquier D, Neves M, Téot L. Resorption kinetics of eggshell: An in vivo study. J Craniofac Surg 2001;12:53-8.

11. Dupoirieux L, Pourquier D, Souyris F. Powdered eggshell: A pilot study on a new bone substitute for use in maxillofacial surgery. J Craniofac Surg 1995;23:187-94.

12. Baliga M, Davies P, Dupoirieux L. Powdered eggshell in the repair of cystic cavities of the jaw. Preliminary study. Rev Stomatol Chir Maxillofac 1998;99 Suppl 1:86-8.

13. Dupoirieux L. Ostrich eggshell as a bone substitute: A preliminary report of its biological behaviour in animals – A possibility in facial reconstructive surgery. Br J Oral Maxillofac Surg 1999;37:467-71.

14. Durmuş E, Celik I, Aydin MF, Yıldırım G, Sur E. Evaluation of the biocompatibility and osteoprotective activity of ostrich eggshell powder in experimentally induced calvarial defects in rabbits. J Biomed Mater Res B Appl Biomater 2008;86:82-9.

15. Dupoirieux L, Pourquier D, Picot MC, Neves M. Comparative study of three different membranes for guided bone regeneration of rat cranial defects. Int J Oral Maxillofac Surg 2003;30:58-62.

16. Durmuş E, Celik I, Ozturk A, Ozkan Y, Aydin MF. Evaluation of the potential beneficial effects of ostrich eggshell combined with eggshell membranes in healing of cranial defects in rabbits. J Int Med Res 2003;31:223-30.

17. Naaman NB, Ouhayoun JP. Bone formation with discs or particles of natural coral skeleton plus polyglactin 910 mesh: Histologic evaluation in rat calvaria. Int J Oral Maxillofac Implants 1998;13:115-20.

18. Park JW, Bae SR, Suh JY, Lee DH, Kim SH, Kim H, et al. Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: A pilot study. J Biomed Mater Res A 2008;87:203-14.

19. Gottlow J. Guided tissue regeneration using bioresorbable and non-resorbable devices: Initial healing and long-term results. J Periodontol 1993;64:1157-65.

20. AlGhamdi AS, Ciancio SG. Guided tissue regeneration membranes for periodontal regeneration – a literature review. J Int Acad Periodontol 2009;11:226-31.

21. Villar CC, Cochran DL. Regeneration of periodontal tissues: Guided tissue regeneration. Dent Clin North Am 2010;54:73-92.

22. Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: Morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96:1-1.

23. Liao S, Wang W, Uo M, Ohkawa S, Akasaka T, Tamura K, et al. A three-layered nano-carbonated hydroxyapatite/collagen/PLGA composite membrane for guided tissue regeneration. Biomaterials 2005;26:7564-71.

24. Tsai WT, Yang JM, Lai CW, Cheng YH, Lin CC, Yeh CW, et al. Characterization and adsorption properties of eggshells and eggshell membrane. Bioresearch Technol 2006;97:488-93.

25. Nakano T, Ikawa NI, Ozimek L. Chemical composition of chicken eggshell and shell membranes. Poult Sci 2003;82:510-4.

26. Wong M, Hendrix MJ, von der Mark K, Little C, Stern R. Collagen in the egg shell membranes of the hen. Dev Biol 1984;104:28-36.

27. Arias JL, Carrino DA, Fernández MS, Rodríguez JP, Dennis JE, Caplan AI, et al. Partial biochemical and immunochemical characterization of avian eggshell extracellular matrices. Arch Biochem Biophys 1992;298:293-302.

28. Chien YC, Hinke MT, McKee MD. Avian eggshell structure and osteopontin. Cells Tissues Organs 2009;189:38-43.

29. Hinke MT, Gautheron J, Panheleux M, Garcia-Ruiz J, McKee MD, Nys Y, et al. Identification and localization of lysozyme as a component of eggshell membranes and eggshell matrix. Matrix Biol 2000;19:443-53.

30. Chien YC, Hinke MT, McKee MD. Ultrastructure of avian eggshell during resorption following egg fertilization. J Struct Biol 2009;168:527-38.

31. Uraz A, Gultekin SE, Senguven B, Karaduman B, Sofuoglu IP, Pehlivan S, et al. Histologic and histomorphometric assessment of eggshell-derived bone graft substitutes on bone healing in rats. J Clin Exp Dent 2013;5:e23-9.