Original Research

Characterization of eggshell as a bio-regeneration material

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Abstract

Objective. The objective of this study was to identify and summarize the characteristic features of eggshell for regeneration purpose in oral surgery procedures.

Methods. A review of literature was undertaken based on the PubMed database. A search to reveal the current state of knowledge and the current uses of the eggshell as a biomaterial was performed. The characteristics of the materials, the specific use, the procedure and the outcome were extracted from the articles.

Results. The materials have been found to be used in humans, animals, and *in vitro* studies. There is a wide use regarding oral surgery especially in experimental models. There have also been attempts to enhance certain properties and improve the capabilities of eggshell as a biomaterial. There is yet a commercial product to be developed and approved for human use.

Conclusions. Eggshell can be an important biowaste which can be of use in guided bone regeneration procedures, but it has not yet entered the commercial phase and approval through official regulation channels.

Keywords: egg shell, bone regeneration, oral surgical procedures

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Background and aim

The modern approach to dental medicine and especially dental implantology is nowadays the *restitution ad integrum* concept. The current concept and focus of medicine are shifting to prevention rather than treatment. Nevertheless, in Eastern European countries this is still not the case [1]. More and more patients require services of complete dental rehabilitation and require fixed solution without any use of pink ceramics or other artificial prosthetic solution. Extensive bone resorption of the alveolar bone such as is seen in figure 1 and figure 2 can lead to the loss of possibility of dental implant placement and a very difficult rehabilitation. This often leads to the need of alveolar bone augmentation procedures which come with their own risk and possible negative sequelae.

In this context the biomaterial industry is thriving, and the research is more and more focused on seeking the gold standard for specific regeneration procedures [2]. There are certain requirements concerning the ideal regeneration material: biocompatibility, ease of use, bone induction and bone conduction properties, to name just a few [3]. Additionally, a low cost may be also required, as well as antibacterial properties and viable cells that produce bone [4].

GBR (guided bone regeneration) is a method that divides two discrete areas in which only osteogenic cells are permitted to populate a bone defect [5]. Membranes can be resorbable or non-resorbable and semi-permeable materials like titanium and polytetrafluoroethylene (PTFE) are employed in these operations. The fact that they require surgical intervention for removal is their major drawback.

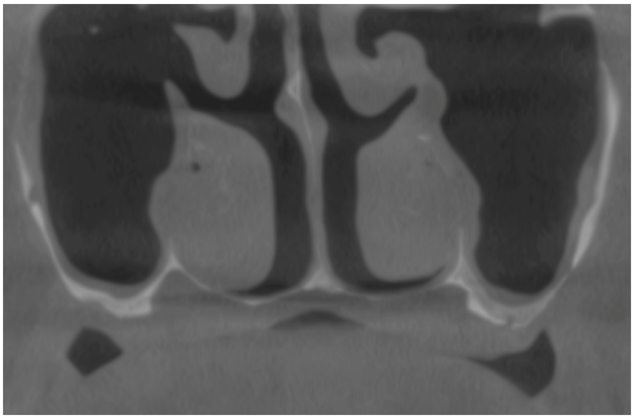

Figure 1. Resorbed alveolar bone of the maxilla in which bone augmentation is needed, coronal section CBCT image (Courtesy of Dr Horia Opris).

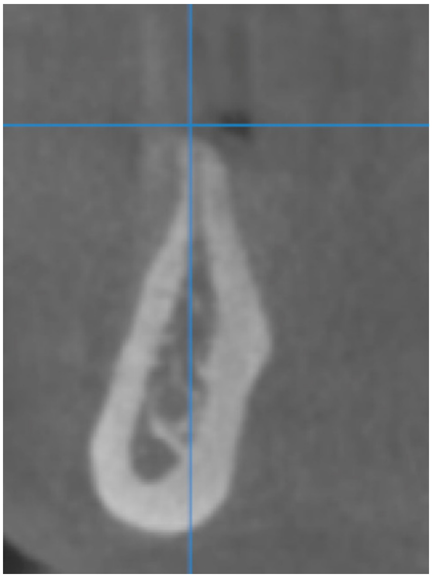

Figure 2. Narrow alveolar ridge in the mandible, cross section CBCT image (Courtesy of Dr Horia Opris).

The quest for a new biomaterials has since long been a struggle and the main commercially available and clinically validated products often use xenografts, allografts, or alloplastic materials [6].

Xenografts must be specifically processed to exclude a host reaction after implantation. Furthermore, they provide a shell, a matrix in which the host bone fills in the first healing phase with blood clot which in turn will form the primary bone and the mature bone subsequently. In contrast, the alloplastic materials have been proven to act rather as a filler material which holds up a certain volume for the body to heal. They do not actually offer a matrix for the bone to heal. New materials have been developed in the attempt to address these flaws. Due to the shortage of functional osteoblasts, their success is restricted [4]. Because of the presence of microorganisms and saliva, as well as the mastication taking place there, the oral cavity is a unique habitat. When looking for a good biomaterial to employ, it is critical to examine all these factors [5].

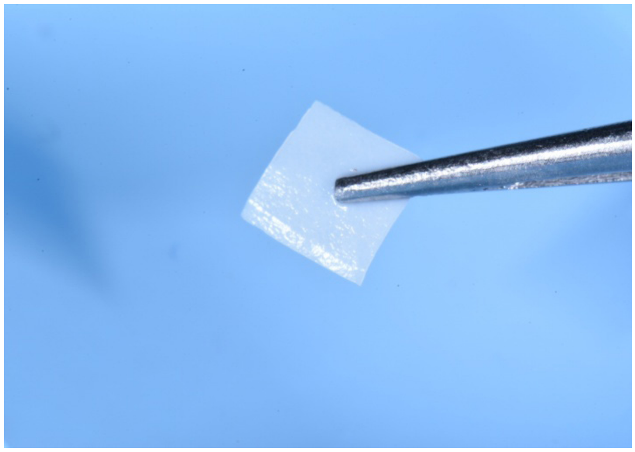

Figure 3. Eggshell membrane developed for research purposes (Courtesy of Dr Horia Opris).

For nutritional supplements, eggshell is a significant source of calcium [7]. It has been found to prevent bone loss in osteoporosis patients and postmenopausal women [8]. In animal models, it is also employed as a matrix for bone development. In figure 3 an eggshell membrane prepared for use in an experimental rat model can be seen [9]. Recent research has attempted to incorporate nanohydroxyapatite into a variety of substances to further improve its properties [10].

The search for a cost-efficient, readily available material has often considered the eggshell. It has plenty of characteristics that from the first glance recommend it for bone regeneration: high concentration of calcium carbonate, it is readily available in large quantities, relatively easy to exploit.

The purpose of this study was to research the literature and review it to find studies regarding the properties, features and biomechanics of the eggshell as a bio-regeneration material. The aim of this article was also to include and to assess the use of this material in different types of studies *in vitro*, animal, and human. Also, we tried to find if there were certain documented ways of use to enhance its properties.

Methods

We conducted a review during in September 2021 on PubMed to find the uses and properties of this material. The search strategies included the following keywords ”eggshell”, ”bone” and ”regeneration”. We included all published articles until the date of search.

The summary of the inclusion and the exclusion criteria can be seen in table I.

Table I. The summary of the inclusion and the exclusion criteria of the included studies.

Inclusion Criteria	Exclusion Criteria
Published in Pubmed	Abstracts, technical notes, position papers, letters to the editors and articles with insufficient information.
Date of publish: until September 2021	Incomplete data
Keywords: eggshell, bone, regeneration	Impossibility of accessing the full text of the article
Study type: randomized clinical studies, nonrandomized clinical trials, prospective clinical trials, animal studies, *in vitro* studies	
Interventions included were bone regeneration procedures	
Examinations of the material were included, such as histology, electronic microscopy	

The studies included in the review met the following criteria: randomized clinical studies, nonrandomized clinical trials, prospective clinical trials, animal studies, *in vitro* studies. The interventions included were bone regeneration procedures. Also, examinations of the material were included, such as histology, electronic microscopy.

The exclusion criteria were the following: abstracts only, technical notes, position papers, letters to the editors and articles with insufficient information.

The following parameters were noted and summarized: experiment type, experimental animal, type of material used, processing method of the material, results of the procedure. For the *in vitro* studies, the properties of the material that was examined were noted, as well as the methods used to process and the techniques of examination.

Results

The conducted search used the following keywords on PubMed: “eggshell”, “bone” and “regeneration”. Forty-one studies were found and were included in the present review, the first being in published in 1966. Out of these studies, 5 were clinical studies, 14 *in vivo* studies, and 20 *in vitro* studies. More extensive research begun after 1994.

A summary of studies is shown in table II.

Table II. Summary of the included articles.

Human studies	*In vivo* studies	*In vitro* studies
[11–15]	[16–37]	[10,17,21–23,29,30,38–55]

All the studies were analyzed, and we have found that eggshell is a potential biomaterial, it has high biocompatibility, it can be processed in various ways (fine powder, blocks). Its characteristics can be enhanced using different additive techniques. There was no evidence of bone inducing capabilities. Significant evidence has been found for its use as a supplement for calcium deficiencies.

The research includes some clinical studies but with few patients enrolled and short follow-up. There is documented use of the eggshell in cystectomy defects, apicectomy defects and after third molar extractions. A high risk of bias has been found for these studies as shown in a recent review [56].

Animal research was performed on *in vivo* models using small animals: Sprague-Dawley rats, Wistar rats and New Zealand rabbits. The defects and the type of model ranges from a very small 3 mm circular defect to a rectangular defect of 3 x 4 cm. The most widely used model was the calvaria defect. The animal studies have revealed a wide range of models that can be used with such materials. Also, the results show good biocompatibility and good integration with the host bone.

The clinical studies employed imaging and clinical assessment in monitoring the outcome of the procedures. Histology and micro-CT evaluation was mostly used for assessing *in vivo* studies.

Discussion

Continuing the research that our team begun years ago, we stopped to look at eggshell, a biomaterial with potential of bio-regeneration. Eggshell is mostly comprised of calcium carbonate, and it has a lot of important properties to allow the embryo to form and evolve.

In the attempt to summarize all these results and knowledge, our team has recently undertaken extensive research on clinical studies and *in vivo* studies [56,57].

The eggshell is composed of the hard-shell and the eggshell membrane. The eggshell membrane has a multitude of well-known components which include collagen, osteopontin, fibronectin and many other which are documented to induce bone formation [58]. The authors agree that by processing, the eggshell membrane is degraded, and its properties and components are degraded. Furthermore, the sterilization protocols deteriorate all the active components [59].

Multipotent cells migration, proliferation, and differentiation sustain bone repair through a complex interaction of molecular mechanisms [60]. Various studies were recently directed at identifying these molecular processes, and progress was made regarding the molecular underpinnings of bone regeneration. Many researchers have been successful in identifying key signaling molecules and transcriptional regulators of bone regeneration.

Bone morphogenetic proteins (BMPs) are pleiotropic members of the transforming growth factor beta (TGF-β) superfamily [61]. They are important for brain and bone formation in utero and have been implicated in human disease. BMPs contain a signal peptide, a prodomain, and a mature peptide [62]. It has been hypothesized that BMP-2, -6, -7, and -9, various BMP isoforms, have the greatest osteogenic capacity [63].

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is often used in spinal fusion, as well as orthopedic trauma and dental procedures [64]. There are relatively scarce *in vivo* data comparing the two. Some studies have considered it more osteoinductive than BMP-7 based on *in vitro* analyses [65].

TGF-β has been implicated in cell cycle regulation, angiogenesis, wound healing, and skeletogenesis [66]. TGF-initiated signaling, like BMP-mediated signaling, uses protein intermediates to activate specific target gene transcription [67]. It may also be important for the coupling of bone resorption and formation [68].

The fibroblast growth factor (FGF) family of cytokines mediate processes including cellular proliferation, migration, and differentiation; mitogenesis; angiogenesis; embryonic development; and wound healing [69]. Mutations in FGFs or FGFRs are involved in the development of various skeletal dysplasias, including achondroplasia and craniosynostosis [70,71]. FGF, a subtype of growth factor, has been connected to several osteoinductive pathways. FGF-2 treatment decreases levels of differentiation markers and augments osteoclast formation, thus resulting in net bone resorption [72]. By contrast, intermittent FGF-2 treatment enhances bone formation [73].

Platelet-derived growth factor (PDGF) plays an important role in a number of biological processes, including embryological development and inflammatory reactions [74]. PDGF is a potent mitogen and chemotactic agent for cells and tissues of mesenchymal origin and crucial in bone homeostasis and repair [75]. PDGF, in addition to its direct mitogenic effect on osteoblasts, indirectly enhances bone regeneration by stimulating angiogenic cytokines and promoting bony healing via interactions with other growth factors [76]. The effects of PDGF on osseous repair in the clinical setting have been investigated [77].

Surgeons select grafting solutions based on information retrieved from the literature as well as information offered by suppliers. There are few randomized statistically significant correlations evaluating materials in similar patient models. Serial measurements using a cone-beam computer tomograph scanner can be used to objectively review a grafting material and method [78].

The ability of bone to form on a material and intermingle with the graft can be termed osteoconduction [79]. It might be important for the surface morphology of a graft material to be similar to that of native bone [80]. Investigators propose that when the grain size of the processed xenograft is similar to native bone, then bone formation is increased [81].

When looking at the properties of the components of the eggshell, it has been found to have excellent biocompatibility, with a low potential of osteoinduction and osteoconduction [82]. It seems to resemble the behavior of hydroxyapatite in other studies when compared in bone regeneration models [37].

Physiological characteristics can play an important role in the osteogenesis features of a processed xenograft [83]. These include the graft's resorption properties, porosity, crystallinity, and mechanical strength. Because of vascularization and cell recruitment, larger pores encourage osteogenesis [84]. The clinician should choose a xenograft whose characteristics match the clinical need [85,86].

In the anterior maxilla, the remaining thin buccal bone will most likely resorb, resulting in loss of ridge contour [87]. This is a normal consequence of tooth loss or extraction. If the socket is not grafted, in case of a large defect, then clinicians are concerned about incomplete bone formation [88]. In the maxilla, in the presence of thin or lost buccal bone, a graft material with fast resorption can be useful, such as an allograft within the socket, with a contour graft of a slower resorbing material to maintain the esthetics [89]. Scar development and delayed healing occurs when using non-resorbable materials, such as ceramics, e.g., sintered hydroxylapatite, for coverage [84].

Many graft materials operate well in extraction sockets, with no difference between allografts and xenografts handled at moderate or high temperatures. The major difference is the time it takes to form bone by the graft material within the socket [90]. These evidences indicate that using a material in the proper position can support preservation of the ridge contour, but it is still unclear which material gives the optimal ridge width and contour maintenance [5].

Prior to the extraction of a tooth, the buccal bone might be already lost due to the pathologic process that led to the extraction of the tooth. One solution is to use a graft that will resorb along with the bone formation process within the "socket" and another that has a very slow resorption rate overlying the socket graft to maintain ridge contour [91]. A layering technique, employing different graft materials to augment dehisced or deficient alveolar bone is also used around dental implants.

Xenografts with different processing methods have been used for increasing bone height in the posterior maxilla [92]. The goal of the augmentation is to provide bone that is capable of integrating next to the implant and is maintained over time without substantial resorption [93]. Xenografts were equivalent to autogenous bone grafts when evaluating implant survival and the reaction of the peri-implant hard and soft tissues. Merely limited amounts of new bone have formed within the biomaterial. Despite these discrepancies, the clinical outcomes of the various grafting procedures were comparable.

Autogenous bone grafting can create sufficient bone volume for implant placement, but individual variations in resorption pattern make the grafting procedure unpredictable for long-term prognosis [94]. Onlay augmentation in the mandible and maxilla may use iliac crest or chin grafts [90]. Ridge augmentation with autogenous block grafts and bovine particulate filler, covered with a collagen membrane exhibited great predictability and effectiveness in horizontal ridge augmentation [91].

There is a great deal of research potential regarding the use of eggshell. The current use regards it as a bone substitute filler material like hydroxyapatite. Ongoing research attempts to improve the uses by altering the components and properties. The quest, in our opinion, is to emulate nature and develop new processing methods to maintain the elements of the eggshell membrane to be able to preserve the bone regeneration potential.

Conclusions

Procedures for bone regeneration are predictable and can be used safely in current dental implantology and oral surgery. Eggshell has been proven to be a reliable material to support bone regeneration. Several methods for processing eggshell into finite products have been described. There is a lack of strong clinical trials available to be able to draw a conclusion regarding human use. The summary of the *in vivo* and *in vitro* animal studies can confirm that the material is highly biocompatible and can be used in different sizes and shapes. There is a need for more studies regarding the clinical use. Further developments need to address the use of the eggshell in combination with different elements to enhance its properties.

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