REVIEW ARTICLE

Review of the Benefits of Eggshell Content in Body Tissue Structure Repair

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ABSTRACT

Eggshell (ES) is a waste material that cannot be consumed with low economic value. ES mainly contains calcium carbonate (CaCO3) and an organic matrix in the form of proteins, glycoproteins, and proteoglycans. Meanwhile, the eggshell membrane (ESM) contains osteopontin collagen, fibronectin, keratin, histones, avian beta defensins, ovocalyxin-36, apolipoproteins, protocadherin, chondroitin sulfate, ovotransferrin, hyaluronic acid, and sialic acid as well as various amino acids. Recently, ES has been widely used in industry, agriculture, food, and medical fields. The potential of ES in the medical field is interesting to discuss, especially in relation to tissue repair. Efforts to reduce the prevalence of wounds that generally originate from acute wounds but become chronic due to various factors that are neglected in their management. In particular, this review will describe the benefits of ES content in repairing body tissues. ES-derived active ingredients such as CaCO3, brushite, and hydroxyapatite exhibit osteoconductive properties that promote bone regeneration. Calcium ions can increase insulin and leptin sensitivity in the liver and can induce repair of acute kidney injury. Meanwhile, ESM contributes positively to neural tissue repair and plays an important role in wound healing, response to external stimuli, defense response, inflammatory response, cell-substrate adhesion, promoting cell growth, migration, differentiation, and tissue remodeling.
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INTRODUCTION

Eggshell (ES) is a solid waste produced by industry and households. Besides not being consumed, this product also has a low economic value and it is usually dumped in landfills with high management costs (1). According to the UN's Food and Agriculture Organization, Indonesia produced roughly 3% of the world's eggs in 2017, or 33,940,000 eggs per year, and ranked 8th (2). The existence of ES as an abundant waste material has the potential to be an environmental pollutant if it continues to be left unchecked.

Chicken eggs have three main components: shell, egg white, and yolk (3). In particular, the chicken ES consists of a cuticle, a crystalline and a spongy calcareous layer, a core, and a mammillary layer. The membrane on the inner surface seems to be a single layer, but it is separated into two layers of fibers. One layer surrounds albumin, while the other is attached to the calcified ES in the inner and outer shell membranes, respectively. ES is formed by controlled deposition of calcium carbonate on the outer membrane fibers in the extracellular space between the shell membrane that covers the albumen and the uterine mucosa (4-6).

ES is a calcareous structure consisting mostly of $CaCO_3$ which is around 90%, Other research indicates that eggshell $CaCO_3$ can be around 95% and the organic matrix contains around 3.5% proteins, glycoproteins, and proteoglycans (4,7). Eggshell membrane (ESM) is primarily composed of collagen proteins (I, III, V, and X), osteopontin, fibronectin, keratin, histones, avian beta defensins, ovocalyxin-36, apolipoproteins, protocadherin, chon droitin sulfate, ovotransferrin,

hyaluronic acid, and sialic acid, and various amino acids such as glycine, proline, and serine (8-15).

The complete form of chicken ES can be used as fertilizer, while the powder form is used as a filter for polypropylene composites, lime, and calcium supplements. They are also synthetic supplements containing calcium carbonate, calcium glutamate, monocalcium and dicalcium phosphate, organic calcium, and hydroxyapatite. Furthermore, they are also used as a purification material for water and soil from heavy metals. In the medical field, the membrane polypeptide extraction helps stimulate the growth of osteoblast cells, used as a hyaluronic acid extraction material for treating osteoarthritis, bioactive anti-inflammatory components, and components for treating gastrointestinal disorders, hypertension treatment, and cosmetic ingredients (16). In the food sector, it is an alternative source of calcium (17-19). ES is also widely used as food additives in bread (20), biscuits (21), chocolate cake (22), and yogurt (23).

Various studies on ES continue to be developed, one of which is interesting to discuss that ES have benefits in the medical field that have the potential as alternative materials to encourage tissue repair. Tissue repair is a natural body process that aims to restore the structure and function of damaged tissues. Tissue repair is seen as a form of wound healing. Tissue repair includes two separate processes, including regeneration and replacement, depending on the part and type of tissue injured (24). Wounds are divided into two types based on the length of the healing process, namely acute and chronic. This can be influenced by the structure of the tissue under the wound, the depth of the wound, and the primary wound care. Acute wounds will develop into chronic if left untreated or with conventional therapy, which takes time. Efforts to reduce the prevalence of chronic wounds and seek alternative treatment materials are still a concern. A faster wound healing process will also reduce the costs incurred for treatment (25).

The need for a wound management approach as well as choosing therapy for wounds is the basis of this review. Later, it is hoped that ES that are abundant as unused material in the environment will become a source of raw materials that are useful in the health sector. This study explains the benefits of ES content in body tissue repair. Then it can be used as a reference to develop the potential and utilization of ES waste.

METHOD

Search criteria

The articles that will be included in this review are experimental research articles, which are conceptually research in the health sector. Research articles published from 2002–2021, written in English, involve research subjects in the form of experimental animals, using ES interventions or derivative materials derived from ES, focusing on the benefits of ES content on body tissue repair. Review manuscripts, thesis manuscripts, incomplete articles and articles received but not published, and incomplete data information are declared ineligible for acceptance.

The keywords used in the article search are Eggshell, eggshell membrane, and tissue repair. All three keywords are searched simultaneously in Harzing Publish or Perish software, which will automatically collect various related articles in each database.

Information Sources

Relevant articles published from 2002 to 2021 were searched in four databases, namely PubMed, Crossref, Google scholar, and Semantic scholar. These four databases were chosen because they are readily available and accessible on Harzing Publish or Perish software.

Data management

The collected articles are identified as whether there are duplicate articles, whether they are written in English, and whether they meet the criteria set for an article to be included in the data collection.

Selection Process

The article identification in four databases provides 2395 articles. 121 duplicate articles were found after further identification. There are eight articles written in addition to using English. 81 articles were published as review articles. Each article was adjusted to the inclusion criteria that had been set, including the research design, intervention, research subjects, and reported results, so that 20 complete articles were obtained that would test the eligibility criteria. An article manuscript that has been received but has not been published is removed because it is considered that the manuscript has not been fully processed by the relevant journal (figure 1).

Data collection

Articles that met the criteria were then extracted to collect the main author's name, year, article title, country, study design, intervention, subject, results, and conclusions (table 1).

EGGSHELL APPLICATION AGAINST BODY TISSUE REPAIR

Benefits of eggshell on nervous tissue

Research on neural tissue by Farjah et al. (2013) evaluated the process of peripheral nerve regeneration in vivo using an ESM as a guide. It showed that the sciatic functional index (SFI) in the ESM-treated group increased significantly at 49 and 60 days compared to the autograft group. On day 90, the ESM group had a higher average nerve conduction velocity (NCV) than the autograft group, however, this was not statistically

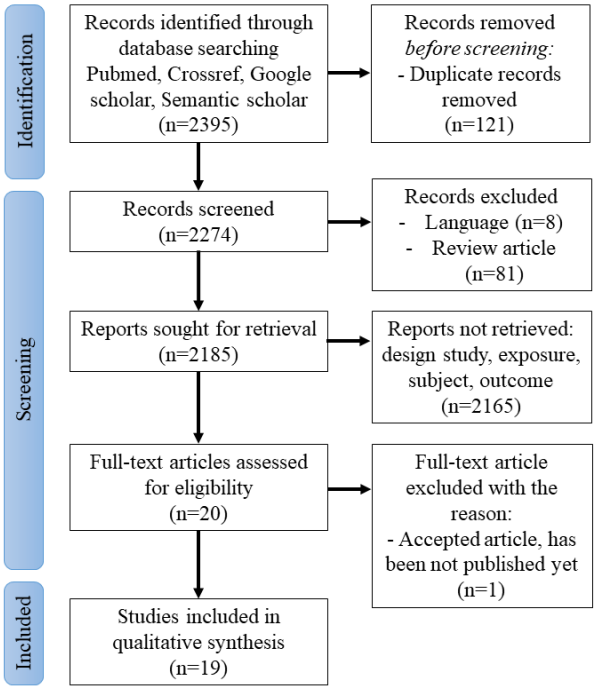

Figure 1: Flowchart article selection procedure

significant (26). In the following years, Farjah et al. (2016) reported the results of the evaluation and comparison of histology of nerve cell regeneration given a similar treatment. It was known that nerve fibers appear to contain axon fascicles. Blood vessels and epineurium were observed in the regenerated tissue, consisting of several layers of fibroblasts and collagen fibers arranged in a circle. Therefore, ESM effectively promotes nerve regeneration and functional recovery in the sciatic nerve (27).

The combined effect of ESM plus other active ingredients has also been investigated. One of published research was conducted by Farjah et al. (2020) to assess the effect of ESM supplemented with lycopene for peripheral nerve regeneration. Lycopene has a high anti-oxidant ability as a free radical scavenger (28–30). The research reported that SFI in the ESM+lycopene group increased over time, indicating a progressive increase in axon regeneration for microfascicles with little connective tissue. These results show the positive effect of ESM-containing soluble protein supplemented by lycopene in nerve cell regeneration. The benefits of soluble protein content in ESM in the form of collagen, fibronectin, and hyaluronic acid are also thought to contribute positively

Table I: Data collections

Author, Year	Country	Study design	Exposure	Subject	Outcome	Conclusion
Farjah, 2013 (26)	Iran	Experimental	Eggshell membrane (ESM)	Sprague Dawley rats	Sciatic functional index (SFI) values improved from the first to the last evaluation, nerve conduction velocity (NCV) was great	ESM significantly enhances peripheral nerve regeneration and promotes functional recovery in injured sciatic nerve
Farjah, 2016 (27)	Iran	Experimental	Eggshell membrane (ES)	Sprague Dawley rats	The nerve cables contained fascicles of axons. Blood vessels were observed throughout the regenerated tissue. The neural tissue was surrounded by an epineurium composed of several layers of circumferentially arranged fibroblast and collagen fiber layers	ESM effectively enhances nerve regeneration in injured rat sciatic nerve
Farjah, 2020 (28)	Iran	Experimental	ESM + Lycopene	Sprague Dawley rats	The nerve tissue was successfully connected to two terminal ends nerve. Progressively, the myelinated axons were more organized in microfascicles with a small connective tissue	The ESM + Lycopene may be beneficial for treating peripheral nerve damages
Li, 2016 (37)	China	Experimental	Copper-containings bioactive glass/eggshell membrane (Cu-BG/ESM)	C57/BL6 mice	Epithelial in the wound edge and the new epidermis was more continuous and uniform	Cu-BG/ESM enhances the wound-healing quality indicated by the improved angiogenesis rate and they formed a new epidermis
Liu, 2017 (38)	China	Experimental	Silver nanoparticles of the eggshell membrane (EM/AgNPs)	BALB/c mice	EM/AgNPs accelerated the wound healing process and promote granulation tissue formation	EM/AgNPs not only exhibited anti-inflammatory properties but also could efficiently accelerate wound healing.
Vuong, 2018 (36)	Canada	Experimental	Processed eggshell membrane powder (PEP)	C57BL6/J mice	PEP stimulates activity and protein expression of matrix metalloproteinases (MMPs) in the epithelial layer and the granulation tissue, which declined in the remodeled tissue, enhances fibroblast and keratinocyte proliferation, myofibroblast differentiation, and regulate the activity of various MMPs	PEP is seen as an accelerator in the first phase of wound healing which enables cells to restructure new tissue
Ohto-Fujita, 2019 (9)	Japan	Experimental	Solubilized eggshell membrane (S-ESM)	Hairless mice (HR-1)	S-ESM upregulated extracellular matrix (ECM) molecules, such as type III collagen, decorin, v2, and hyaluronan synthase 2	ESM has anti-aging effects by improving the ESM environment in the papillary layer
Furukawa, 2021 (34)	Japan	Experimental	Powdered-eggshell membrane (ESM)	IL-10 KO mice	Based on microarray data, genes related to calcium signaling and collagen synthesis might be partly involved in ESM's effect. Based on the Telomerase reverse transcriptase (Tert) result, pESM might improve telomere shortening, a hallmark of aging. Based on growth factors results, pESM might affect or inflammatory-related gene.	ESM plays an important role in improving skin health and aging

Table I: Data collections (continued)

Author, Year	Country	Study design	Exposure	Subject	Outcome	Conclusion
Choi, 2021 (35)	Korea	Experimental	Modified eggshell membrane (ESM)	Sprague Dawley rats	Markedly higher granulation construction developed a full-thickness epidermal layer and exhibited a healed epithelial layer, with a tightly regenerated dermis filled with sufficient appendants beneath	The modified ESM significantly accelerated skin wound healing to support re-epithelialization and granulation tissue formation in the early stages of wound healing
Arias, 2008 (43)	Chile	Experimental	Eggshell membrane (ES)	rats, rabbits	The angiogenic process was increased, continuity of the cortical bone with an abundant bone matrix presence of dense infiltrative collagen tissue	The interposition of eggshell membranes between the two ends of a sectioned bone would be interesting not only for the treatment of premature epiphyseal closure, but also because it could be an experimental procedure for developing a non-union model in rabbits
Uraz, 2013 (42)	Turkey	Experimental	Eggshell-derived calcium carbonate	Sprague Dawley rats	Bone formation was increased and progressively inflammation was absent	Eggshell gel or powder in intrabony defects are potential candidate graft material
Sah, 2017 (48)	India	Experimental	Soluble eggshell protein (SEP) modified silk fibroin (SF)-polyvinyl alcohol (PVA) scaffold	mice	The scaffolds were completely degraded without causing any tissue necrotic, encapsulated by thin fibrovascular tissues layer of the recipient, showed fibroblast grown with minor inflammation around, and showed the fibroblasts were infiltrated into the implant	The scaffolds are non-immunogenic, support tissue growth, and highly promising for tissue engineering application
Salama, 2019 (10)	Egypt	Experimental	Nano-sized eggshell powder "Membrell's® BONEhealth™ Plus D3 & K2"	New Zealand rabbits	Histological section of defect revealed deposition of osteoid newly-formed bone trabecula which regularly arranged, separated by narrow bone marrow space, and surrounded y an osteoblastic layer	Eggshell powder is a material that accelerates bone healing in surgical defects and may serve as a graft in maxillofacial reconstruction.
Jayasree, 2019 (44)	India	Experimental	Eggshell derived brushite cement (EB)	Wistar rats	The formation of new blood vessels was increased. Formation of woven bone can be seen from the edge of the bone defect with attached by active osteoblast. fibroblast and inflammatory cells were found around partially degraded cement material	A brushite forming cement was fabricated using an eggshell with a low inflammatory response and high osteoconductive
Zaker, 2020 (45)	Iraq	Experimental	Biphasic Hydroxyapatite/Tricalcium Phosphate (HA/TCP)	Rabbits	There was an increased granulation tissue formation with well-defined blood vessels, infiltration of inflammatory cells and progressively, filled the defect with few compact bones	HA/TCP showed a good bone regeneration capacity and excellent biocompatibility with very little inflammatory response
Alhussary, 2020 (46)	Iraq	Experimental	Nanohydroxyapatite (nHA) of eggshell in gold 5 %	Rabbits	Eggshell nHA in gold 5 % increased the healing and osteogenesis, which is highly dense in bone and bigger osteoclast size. Showed more newly formed bone trabecular and a high number of lacunae	Gold increases bone healing and increases more when it is added to nHA from eggshell
Jia, 2017 (52)	Japan	Experimental	Eggshell membrane powder (ESM)	C57BL6/J mice	ESM have functional effects such as repairing the epithelium, regulating energy requirements, and eventually alleviating mucosal inflammation	ESM promoted candidates for the prevention and treatment of inflammatory bowel disease
El-Zeftawy, 2020 (47)	Egypt	Experimental	Ca^{+2} from eggshell	Sprague Dawley rats	Liver of rat with 7.2 g Ca^{+2} ES/Kg rat chow treatment showed normal liver anatomical structure with a normal central vein. Meanwhile, in 18 g Ca^{+2} ES/Kg rat chow treatment showed ameliorated hepatocytes with the distribution of small aggregations of inflammatory cells	The reduction in adiposity resulted from Ca^{+2} in ES treatment that enhanced insulin and leptin sensitivity leading to lower adiposity and protection from poetry metabolic disorders.

to the repair of nervous tissue (31-33).

Benefits of eggshell on skin tissue

ESM supplementation plays a vital role in improving skin health and delaying aging. This is based on the research of Furukawa et al. (2021), which reported microarray examination data that allow involvement of ESM in influencing genes associated with calcium signaling and collagen synthesis. Furthermore, telomerase transcriptase (Tert) expression shows that ESM can improve telomere shortening, which is a sign of aging. The expression of growth factors indicates the possibility of ESM influencing genes associated with inflammation (34).

In vivo assays on hairless mice showed a protective effect against moisture after exposure to ultraviolet B-light, reduced radiation-induced wrinkles, and a significant increase in type III collagen in the palilla dermis after treatment with ESM. As a result, it can be inferred that ES may be used for skin maintenance to reduce tissue elasticity loss and promote type III collagen formation (9).

Modified natural ESM treated with carboxylic acid showed positive potential in accelerating skin

regeneration and enhancing wound healing early in vivo testing. This ingredient promotes skin regeneration, cytokine secretion, epidermal cell proliferation, and inflammatory response control (35). In line with the investigation results of Vuong et al. (2018), ESM can stimulate the activity and protein expression of matrix metalloproteinases (MMPs) in the epithelial layer and granulation tissue. It can also increase fibroblast and keratinocyte proliferation, myofibroblast differentiation, and regulation of various MMPs. As a result, ESM is appreciated as a wound healing accelerator in the early stages, allowing cells to reconstruct new tissues (36).

The benefits of ESM combined with bioactive have also been reported, for instance, the combination of ESM with copper (Cu-BG/ESM) by Li et al. (2016) provides information that Cu-BG/ESM can improve the quality of wound healing by increasing the rate of angiogenesis and neoepidermis formation (37). Liu et al. (2017) also reported the potential for nano-silver ESM particles (EM/AgNPs) to exhibit anti-inflammatory properties and promote the formation of granulation tissue to accelerate healing efficiently. ESM can be used as a supplementation material with a positive effect in maintaining skin elasticity because it contains type III collagen. Its use is also quite flexible because it can be used without or with modifications and active ingredients combination (38).

In addition, collagen content in ESM has been reported to play an important role related to wound healing, including response to external stimuli, defense response, inflammatory response, and cell substrate adhesion (39). Meanwhile, fibronectin, in addition to its role in wound healing, is also known to increase cell growth, migration, and differentiation (40,41).

Benefits of eggshell on bone tissue

The effectiveness of the calcium carbonate formulation in ES has the potential for bone healing in the form of candidate material. Clinical data in rat samples revealed a reduction in bone degeneration, no inflammation, and vigorous new bone growth with an osteoid production rate of 12.31% after the 45th day after transplantation (42). This is consistent with Salama et al. (2019), where supplementation containing nano-sized ES particles as alloplasts indicated a significantly higher rate of osteoid formation accompanied by bone trabeculae regularly arranged in the center of the bone defect (10).

Arias et al. (2008) reported that ESM applied to bone defects can enhance angiogenic processes, showing continuity of cortical bone with massive matrix and dense collagenous tissue infiltration. The excised bone also exhibits ESM interposition to treat premature physis closure and experimental procedures for developing models in rabbits (43).

Moreover, using brushite graft preparations from ES positively affected the bone regeneration process through compaction of the calcified bone zone after 6 weeks of treatment using calvariate samples of white mice. In this case, active osteoblasts were attached to the newly formed bone anastomosis. The new bone formation will continue to form and reach its peak at week 12, characterized by dense histological integration of minerals and decreased inflammatory reactions (44). This is supported by Zaker et al. (2020), which explained that bone grafts in the form of Biphasic Hydroxyapatite/Tricalcium Phosphate (HA/TCP) increased the formation of granulation tissue with well-defined blood vessels. This indicates good bone regeneration capacity with a slight inflammatory response (45).

The addition of nanohydroxyapatite ES in 5% gold can promote healing and osteogenesis, with high bone density and larger osteoclast size (46). The use of ES is increasingly recommended in tissue engineering because the resulting graft can be thoroughly degraded and encapsulated by the recipient's fibrovascular tissue layer, which is non-immunogenic (47). Therefore, ES functions as a potential bone graft because the calcium carbonate content is derived as hydroxyapatite which can be integrally compounded to regenerate and maintain bone. In addition, osteopontin in ESM has been reported to modulate osteoclast differentiation, regulate apatite size and growth, and tissue remodeling (48-50).

Benefits of eggshell on gastroenterohepatology tissue

Jia et al. (2017) reported that in a dextran sodium sulfate-induced colitis rat model, ESM supplementation significantly suppressed the expression of inflammatory mediator genes. This indicates re-epithelialization of ulcerated areas, energy demand regulation that may ultimately reduce intestinal mucosal inflammation (52). Calcium ions (Ca^{2+}) from ES administered to an obesity rat model showed normal hepatic and central venous anatomic structures upon administration of 7.2 g Ca^{2+} ES/Kgw rat. Meanwhile, the 18 g Ca^{2+} ES/Kgw rat administration reported hepatocyte repair with an inflammatory cell aggregation distribution. Due to Ca^{2+} from ES treatment, decreased adiposity increases insulin and leptin sensitivity and protects against metabolic disorders (47).

CONCLUSION

Active ingredients derived from ES such as $CaCO_3$, brushite, and hydroxyapatite exhibit osteoconductive properties that promote bone regeneration. Calcium ions can increase insulin and leptin sensitivity in the liver and can induce repair of acute kidney injury. Meanwhile, ESM, which contains the proteins collagen, fibronectin, and hyaluronic acid, contributes positively to the repair of nerve tissue by increasing regeneration and promoting functional recovery in nerves. Collagen and fibronectin also play important roles related to wound healing,

promoting cell growth, migration, and differentiation in skin tissue. The content of osteopontin in ESM is also known to modulate osteoclast differentiation, regulate apatite size and growth, and remodel bone tissue.

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