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Effectiveness Nacre Pearl Shell (*Pinctada Maxima*) as Bone Graft for Periodontal Bone *Remodeling*

Sri Oktawati[1], Surijana Mappangara[1], Hendra Chandra[2], Harun Achmad[3]*, Sitti Raoda Juanita Ramadhan[4], Gustivanny Dwipa A[4], Muhammad Yudin[4]

[1]Lecturer of Periodontology Department, Faculty of Dentistry, Hasanuddin University, Indonesia [2]Lecturer of Maxillofacial Surgery Department, Faculty of Dentistry, Hasanuddin University, Indonesia

[3]LecturerofPediatricDentistryDepartment,FacultyofDentistry,HasanuddinUniversity,Indonesia

[4]PeriodontologySpecialistEducationalProgramofDentistryFaculty,HasanuddinUniversity,Indonesia

Corresponding Author: harunachmader@gmail.com

ABSTRACT

Background: Infection of periodontal tissue is characterized by the occurrence of tissue resorption from both alveolar bone to cementum which can result in tooth loss. Spontaneous regeneration does not always occur, so the need for regenerative therapy that can accelerate healing and formation of new bone. Tissue engineering is a biomedical technology developed to help regenerate limbs that cannot be repaired by tissues. One type of tissue engineering to regenerate periodontal is the application of bone graft. Some of the marine biota structures contained in it can be used to build tooth structures (bone, dentin, pulp, and periodontal ligaments) that are damaged or lost due to disease.**Aim :**to discuss the potential of nacre pearl shells as bone graft material. **Methods:**Data was collected through Medline search (via PubMed) and Wiley Online Library published from 2016 to 2021. Data search was performed using the keywords Bone Graft, Nacre, Bone Remodeling**. Literature Review :**Nacre is the inside of the shell, is composed of calcium carbonate. The shells and bones have some similarities, the acellular structure of the shells is formed by the outer skeleton of the mollusks, while the acellular structure of the bones is formed by the internal skeleton of the invertebrates. These two structures share an organic matrix deposited by specialized cells, the organic form of which forms a scaffold for direct crystallization and mineralization. Bone grafting is a surgical procedure that replaces lost bone with material from the patient's body, artificial substitutes, synthetic, or natural. As bone naturally grows, this bone generally replaces the graft material completely, resulting in a new, fully integrated area of bone.**Results:** After obtaining 32 articles in the Medline search (via PubMed) and the Wiley Online Library, 20 articles were excluded because they did not meet the inclusion criteria desired by the author on the title and abstract, or there were duplications, resulting in 12 articles. From 12 articles, 5 articles were excluded. Obtained 7 articles to be reviewed. Literature review: **Discussion:** Several studies have been conducted to prove the potential content of nacre pearl shells as bone graft which has osteoconductive, osteoinductive, and osteogenetic properties. **Conclusion :** Nacre pearl shells are a material that shares some similarities with bone. Nacre pearl shells has the potential to be used as a bone substitute material in periodontal bone remodeling.

Keywords: bone graft, nacre, pinctadamaxima,bone remodeling.

Introduction

Currently medical research in the biomolecular field has developed rapidly, the use of

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the Earth's sea as a source of basic medical ingredients is believed to provide better results than chemicals. Some marine structures ingredient can be used in building the structure of the teeth (bone, dentin, pulp, and periodontal ligament) are damaged or lost due to illness.[1]

Infection of periodontal tissue is characterized by the occurrence of tissue resorption from both alveolar bone to cementum which can result in tooth loss. This infection can cause defects in the bones, resulting in irregular and complex bone damage. When the resorption exceeded then either the high bone formation or bone density is reduced. Spontaneous regeneration does not always occur, so the need for regenerative therapy that can accelerate healing and formation of new bone. Tissue engineering is a biomedical technology developed to help regenerate limbs that cannot be repaired by tissues. [3-8]

One type of tissue engineering to periodontal regenerate is the application of bone graft. Bone grafts are used to reconstruct intraosseous defects that form as a result of periodontal disease. Bone graft can help bone regeneration through three methods, namely osteoinductive, osteoconductive, and osteogenesis. The broadly classified, there are four types of bone graft, namely autograft, allograft, xenograft and alloplastic synthetic materials. Autograft is still the main choice in restoring bone defects, but it is still very limited, so a replacement bone graft material is needed that can help bone regeneration. [9,10,11]

The discovery of dental implants in the skull of the Mayan tribe was the beginning of a number of studies on clam shells. "Nacre", commonly called "mother of pearl", is part of the shell which has the main content of Ca (CO3) 2. Nacre is able to facilitate osteoblast proliferation, accelerate extracellular matrix production, and mineralization. Nacre containing inorganic and organic materials that have a similar basic structure of the bone. Therefore, the purpose of writing this literature is to discuss the potential of nacre pearl shellsas bone graft material. [12,13]

METHODS

Data Source

Data collection was carried out by searching the literature on article search sites, namelyMedline (via PubMed) and the Wiley Online Library published from 2016 to 2021. The data search was performed using keywords; (((Bone Graft) AND (Nacre)) AND (Bone Remodeling)).

Research Criteria

A. Inclusion criteria
   - Published articles from 2016-2021
   - Articles in English and Indonesian
   - Article looking at the effectiveness of shell nacre as bone graft in bone remodeling

B. Exclusion criteria
   - Articles included in systematic reviews, literature reviews, and case reports.
   - Articles that cannot be accessed for free

Data Collection

The data that will be used in this research are secondary data. The data is obtained from

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articles that are searched for in the article database which will then be reviewed according to the research criteria set by the researcher. The literature search was carried out on an online database, namely Pubmed using the keywords (((Bone Graft) AND (Nacre)) AND (Bone Remodeling)) found 32 articles.

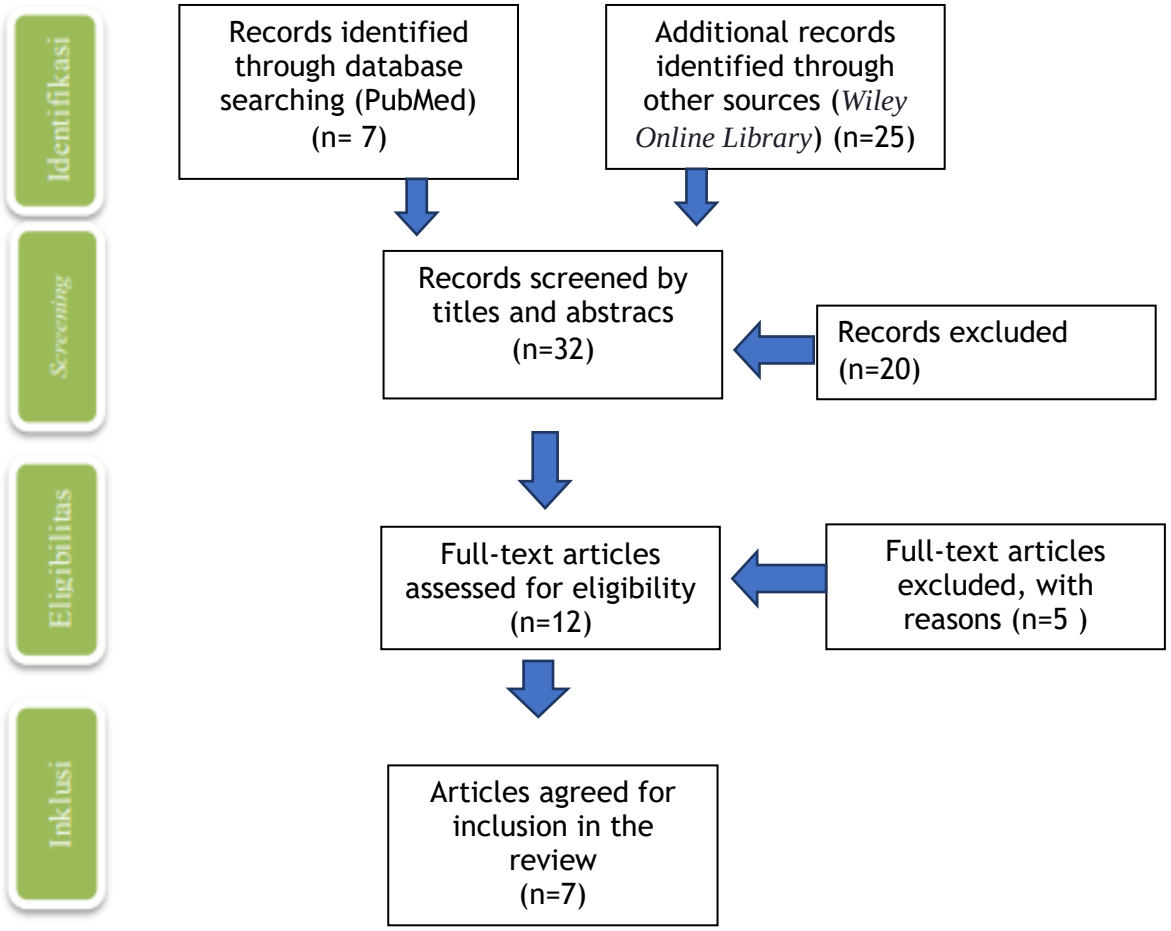

***Figure 1**. The flow chart of the journal search to be synthesized*

Result

After eliminating duplicate articles, the titles and abstracts of each article were analyzed across 32 articles resulting in an exclusion of 20 articles. The full-text articles in the remaining 12 articles were re-analyzed and excluded 5 articles, resulting in 7 articles which were then entered into the analysis.

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Table 1. Use of Nacre in Bone Remodeling

NO	Author	Year	Title	Method	Result
1	Zhang et al [29]	2016	A New Method For The Separation And Purification Of The Osteogenic Compounds Of Nacre Ethanol Soluble Matrix.	This research was conducted using the P. margaritifera species in vitro ESM extracted from nacre was placed on MC3T3-E1 and human osteoblasts for 21 days	ESM can improve the mineralization capacity of osteoarthritis osteoblasts
2	Brundavanam et al [30]	2017	Synthesis of bone like composite material derived from waste pearl oyster shells for potential bone tissue bioengineering application	This study utilized pearl oyster shells as a source of hydroxyapatite in periodontal tissue regeneration	The results showed that hydroxyapatite is calcium phosphate which can be considered as a bone substitute
3	Alakpa E V [13]	2017	Nacre topography	This study was conducted in	From the results of this

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			Produces Higher Crystallinity in Bone than Chemically Induced Osteogenesis	vitro where the researchers isolated the topography of the nacre from its inherent chemical properties	study it was found that nacre has osteoinductive properties to bone
4	Coringa R et al [31]	2018	Bone substitu made from a Brazilian oyster shell functions as a fast stimulator for bone forming cells in an animal model	This study used bone substitutes obtained from oyster shell (CrassostreaRhizophora). 72 rats were divided into three parts and randomly assigned to submandibular defects. The first part was a negative control (-C), the second part was a positive control (+ C; Bio-Oss) and EBS	From the results of this study, it was found that EBS (Experimental Bone Subtitute) provides good biocompatibility and stimulates the formation of bone cells in experimental animals.
5	Rahayu S et.al[26]	2018	Utilization Of Nacre Pearl Shell (Pinctada Maxima) As A Source Of Hydroxyapatite	This research was conducted in Mataram Indonesia. By using nacre pearl shells (Pinctada maxima). In this study,	The results of this study obtained HAp and TCp in pearl shell powder. This research can be used as the

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				researchers chose to use a precipitation technique based on previous studies	basis for the synthesis method of nacre Pearl shells to be used as bone graft material which is osteoinductive, osteoconductive and osteogenesis.
6	Williem AS et al[32]	2019	The Effect Of Nacre Extract On Cord Blood-Derived Endothelial Progenitor Cells: A Natural Stimulus To Promote Angiogenesis?	The levels of gene and protein expression of endothelial cell specific markers (EC) were determined in EPC cultured in the presence of nacre extract (ethanol soluble matrix [ESM] at two concentrations: 100 μg / mL and 200 μg / mL (abbreviated as ESM100, respectively) and ESM200)). Cell functionality was explored by proangiogenic factor production and in vitro tube formation assays. The ESM200	It was found that stimulation with 200 μg / mL ESM increased trace angiogenesis. This in vitro study focuses on the proangiogenic effects of ESM. Due to its osteogenic properties, which have been demonstrated previously, ESM can be a key element for developing the ideal bone replacement.

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				enhances the expression of several EC-specific genes. The in vitro tube formation test showed that the ESM200 stimulates tubulogenesis which affects angiogenic parameters	
7	Darbois et al [33]	2020	In Vivo Osseointegration And Erosion Of Nacre Screws In An Animal Model	This study compared the in vivo osseointegration and erosion of nacre screws in animal models with titanium screws. Rough examination reveals clinical osseointegration to treat titanium and nacre.	Nacre is a resorbable and osteoconductive material that supports bone apposition without triggering an inflammatory reaction

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Literature Review

Bone Graft

Periodontal disease is an infectious disease which in its advanced development can cause bone loss and tooth loss.Periodontal disease can lead to the formation of various defects in the alveolar bone, which affects loss of the supporting bone around the tooth and changes in tooth placement.Periodontal regeneration is the restoration of tooth supporting tissues such as bone, cementum, periodontal ligaments back to their condition before disease.Tissue engineering is a biomedical technology developed to help regenerate limbs that cannot be repaired by tissues.Bone grafts are used to reconstruct intraosseous defects that form as a result of periodontal disease. [4,14,15]

Bone grafts should be sterile, non-toxic, non-antigenic, biocompatible and easy to use.Graft is a material used to replace or repair damaged tissue. The biological mechanisms of bone graft, among others, are osteoconduction, which means that the bone graft material functions as a scaffold for new bone growth.Osteoconduction in its function acts as a medium for stem cells and osteoblasts to adhere, live and develop properly in a bone defect or as a form of bone graft that gives scaffold dimensions or a scaffold for osteoblasts, facilitates vascularization, and prepares migration of new host cells by osteogenic activity.[16-19]

Osteoinduction involves stimulating osteoprogenitor cells to differentiate into osteoblasts which then form new bone.Cell mediators known to be osteoinductive are Bone Morphogenetic Proteins (BMPs). Osteoinduction is a process that stimulates osteogenesis, the release of substances that stimulate bone formation. [16-19]

Bone graft material which is osteoconductive and osteoinductive not only provides a framework for existing osteoblasts but will also trigger the formation of new osteoblasts, which promotes faster graft integration.Osteogenesis when osteoblasts derived from bone graft materials contribute to new bone growth. Osteogenic grafts originate or consist of tissue that is involved in the natural growth or repair of bone. Osteogenic cells can promote bone formation in soft tissue or activate faster bone growth in the area ofbone.[17,20,21]

The ideal characteristic of bone graft is to have good biocompatibility. Resorption is slow enough and is high osteoconductive to stimulate the formation of new bone from the bone defect area, has high porosity so that it is totally integrated with the bone, is able to regenerate the formation of new bone, cementum, and periodontal ligament fibers, is stable, has an adequate modulus of elasticity, is easy adapt to pressure and strain.[22]

Bone grafting is a surgical procedure that replaces missing bone with material from a patient's body, substitutes artificial, synthetic, or natural.Bone grafting is possible because

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bone tissue has the ability to fully regenerate when there is space where it can grow. As bone naturally grows, this bone generally replaces the graft material completely, resulting in a new, fully integrated area of bone.[20]

There are several things that must be fulfilled in order to obtain a bone graft that is satisfactory in treating all clinical defects, that is:[23]

Shape : the scaffold must be able to fill the defect cavity

Function: has the ability to withstand pressure

Fixation: the scaffold can be well attached to the bone at the edge of the defect to eliminate movement between the bone host and the scaffold.

Formation: scaffold can stimulate bone formation

Nacre and Bone

Nacre is part of the shell, composed of acellular calcium carbonate which is produced by bivalves, gastropods, and cephalopods.Nacre consists of aragonite crystal tablets coated with an organic matrix. Aragonite and calcite are two polymorphs of calcium carbonate which is a constituent shells of mollusks and give strength and resistance to the overall architecture of the shell.Most small invertebrates shell consists of an organic matrix that is responsible for the process of nucleation, growth, and inhibition of calcium carbonate. [13, 23,25,26]

The special structure of the nacre pearl shell is composed of a brick-shaped wall containing pseudohexagonal aragonite tablets about 0.5 mm in thickness and 5-15 mm in diameter.The tablets are arranged in parallel laminae and separated by interlamellar sheets of organic matrix. P. Margaritifera and P. Maxima are the largest nacre species, with a maximum size of up to 30 cm in P. Margaritifera species and 20-25 cm in P. Maxima species.The micro-particles and layered components of the nacre provide excellent and better pressure to the bone.[23,25]

The discovery of dental implants in the skull of the Mayan tribe was the beginning of a number of studies on clam shells. "Nacre", commonly called "mother of pearl", have shown promising results as a resorbable biomimetic graft material. Nacre often exhibits natural osteoconductive substitution with strong effects in osteoprogenitor, osteoblast, and osteoclast during bone tissue formation and morphogenesis. In addition, nacre also exhibits biocompatible and biodegradable properties in bone tissue.[25]

Nacre is a material that has several advantages, there are cheap, modern design, hierarchical structure, and architecture, intrinsic biological function, low immogenity, low toxicity, safe and easy storage. The shells and bones have some similarities, the acellular

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structure of the shells is formed by the outer skeleton of the mollusks, while the acellular structure of the bones is formed by the internal skeleton of the invertebrates.These two structures share an organic matrix deposited by specialized cells (bone cells in vertebrates and epithelial matle cells in mollusks), their organic form forming scaffolds for direct crystallization and mineralization.[18] The chemical composition of Pinctada maxima shells is 97% inorganic and 3% organic, which consists of proteins, peptides, glucoproteins, chitin, lipids, and pigments. The composition of Pinctada maxima is Ca, Mg, Na, P, Fe, Cu, Ni, B, Zn, and Si. The main content of this nacre is calcium carbonate (Ca_2CO_3).Hydroxyapatite is a compound containing calcium ions ($Ca^{2+)}$ which converts toxic metal ions and absorbs organic chemical elements in the body. This structure is similar to bone, the inorganic structure has extraordinary strength, while the organic matrix is able to increase osteoconductivity when compared to other synthetic materials. In the organic matrix of the shells, a biological molecule is found capable of activating the chemical signals of osteoblasts. .[25,26]

Bone tissue consists of osteogenic cells, extracellular matrix, protein, growth factors, calcium phosphate minerals to form hydroxyapatite and a complex vascular system.Ossification occurs via intramembranous or endochondral pathways. The building cells of bone include osteoprogenitor cells, osteoblasts, and osteocytes. While the organic matrix in bone consists of 95% type I collagen and the remaining 6% is composed of proteoglycans such as chondroitin sulfate and hyaluronic acid as well as a number of non-collagen proteins such as osteonectin, osteocalcin (bone GLA-protein), osteopontin (bone sialoprotein I), bone sialoprotein II, growth factors (IGF-I and II), transforming growth factor-β (TGF-β), and bone morphogenic protein (BMP). Other than that, the main inorganic material is crystal salt which is deposited in the bone matrix consisting mainly of calcium and phosphate.[23]

Bone Remodeling

Bone remodeling is a lifelong process, in which old bone is resorbed from the skeletal, and new bone is added through a process called ossification. Remodeling includes continuous bone resorption and is replaced by matrix synthesis and mineralization to form new bone.This process also regulates bone formation or replacement during growth and following injuries such as fractures and microdamages, it prevents the accumulation of minor bone damage through replacement of old bone with new bone that occurs through normal activities.Remodeling also responds to mechanical loading. As a result, new bone is added where it is needed and removed where it is not needed. This process is important in

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maintaining bone strength and mineral homeostasis.Skeletal is an organ that is metabolically active and undergoes continuous remodeling throughout life. Remodeling is important for maintaining the structural integrity of bone and also for its metabolic function as a storage area for calcium and phosphorus.[27,28]

The normal bone remodeling cycle requires bone resorption and bone formation in a coordinated pattern, which ultimately depends on the development and activation of osteoclasts and osteoblasts.The ability of bone, which constantly absorbs old bone and forms new bone, makes bone a very dynamic tissue that allows the maintenance of bone tissue, repair of damaged tissue, and homeostasis of phosphocalcic metabolism.The bone remodeling cycle includes well-regulated steps that depend on the interaction of two cell strains, there are mesenchymal osteoblastic derivative and the hematopoietic osteoclastic derivative. The balance between bone resorption and deposition is determined by the activity of two types of cells, namely osteoclasts and osteoblasts. Osteoblasts and osteoclasts, which are combined via a paracrine signaling process, are referred to as bone remodeling units.[27,28]

Known functions of bone remodeling include maintaining mechanical strength of bone by replacing bone with minor damage with new healthy bone, and through calcium and phosphate homeostasis. Bone remodeling can be divided into six phases, namely quiescent, activation, resorption, reversal, formation, and mineralization. Mineralization is the last stage. This process occurs in the remodeling area, which is distributed randomly, but is more focused on areas that need improvement.[27,28]

The inorganic material and organic matrix of the nacre have good mechanical properties and biological effects similar to those of bone so that they are well integrated with the bone and can be specially designed to suit each clinical need.

Discussion

The use of nacre in the medical world has a long history. In ancient Chinese medicine, nacre were used as an ingredient to treat hepatopathy for more than 100 years (in the classic medical textbook, BencaoTujing 1061). A nacre-based tooth implant was found in the skull of the Mayan tribe, which fused well with the surrounding bone. [22-24]

Various studies have been conducted that show the potential content of nacre as a new ingredient in bone regeneration.Several previous studies have found that Pinctada maxima is simultaneously biocompatible and osteoconductive to bone. Research using the nacre pinctada maxima chip on human osteoblasts in vitro showed the formation of a thick osteoid matrix consisting of foci with mineralized structures and bone.Nacre pinctada maxima has osteogenic activity in vivo. In addition, a study using nacre powder and blood was carried out

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on the bone defects of 8 patients suffering from bone loss and the results showed that nacre had good biocompatibility to the visible bone 6 months after treatment. The bone formed fuses well with the nacre without soft or fibrous tissue intervention. [22-24]

Green DW et al[1] also conducted research. This research used nacre pearl shells which were made in the form of chips and carried out on human marrow bone stromal cells and carried out for 21 days. The results of the study found that pearl clam shells affect the early stages of differentiation of human bone cells. Several studies have conducted tests using nacre as a substitute for bone graft to treat bone defects in animals and humans which can be seen in Table 1.

Zhang et al's study in 2016 found that ESM on nacre can improve the mineralization capacity of osteoblasts in osteoarthritis. In 2017 Brundavanam also conducted research on pearl shell as a source of hydroxyapatite in periodontal tissue regeneration and from the results of his research it was found that hydroxyapatite is calcium phosphate which can be considered as a bone substitute. In the same year (2017) Alakpa EV conducted research on nacre in vitro by isolating the topography of nacre from the chemical properties inherent in nacre, it was found that nacre has osteoinductive properties to bone.[13,29,30]

In the following year (2018) Coringa R et al conducted a research using bone substitutes obtained from shellfish (CrassostreaRhizophora). 72 rats were divided into three parts and randomly assigned to submandibular defects. The first part was a negative control (-C), the second part was a positive control (+ C; Bio-Oss) and EBS it was found that EBS (Experimental Bone Subtitute) provided good biocompatibility and stimulated the formation of bone cells in experimental animals.[31]

This research was also conducted in Indonesia in 2018 by Rahayu S et al, using nacre pearl shells (Pinctada maxima). In this study, researchers chose to use a precipitation technique based on previous studies. In this study, HAp and TCp were obtained in pearl shell powder. So that it can be the basis for the synthesis method of Pearl clam shells to be used as bone graft material which is osteoinductive, osteoconductive and osteogenesis.[26]

In 2019, Willien AS et al conducted a study on nacre with the method of gene and protein expression levels from specific markers of endothelial cells (EC) determined in EPC cultivated in the presence of nacre extract (ethanol soluble matrix [ESM] at two concentrations: 100 μg / mL and 200 μg / mL (abbreviated ESM100 and ESM200), respectively). Cell functionality was explored by proangiogenic factor production and in vitro tube formation assays. The ESM200 enhances the expression of several EC-specific genes. The in vitro tube formation test showed that the ESM200 stimulates tubulogenesis which

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affects angiogenic parameters. It was found that stimulation with 200 μg / mL ESM increased trace angiogenesis. This in vitro study focuses on the proangiogenic effects of ESM. Because of its osteogenic properties, which have been demonstrated previously, ESM can be a key element for developing the ideal bone replacement.[32]

The most recent study of Nacre was carried out by Darbois et al in 2020 comparing in vivo osseointegration and erosion of nacre screws in animal models with titanium screws. Rough examination reveals clinical osseointegration for titanium and nacre screws. And the results of his research showed that Nacre is a resorbable and osteoconductive material that supports bone apposition without triggering an inflammatory reaction.[33,34,35,36,37]

Based on the research that has been done, it shows that nacre has the potential as an alternative to bone-substituting composite materials, because of the hydroxyapatite and calcium phosphate content which can be used in tissue engineering. Nacre Pearl (Pinctada maxima) exhibits osteoinductive, osteoconductive, and osteogenetic properties, is good biocompatible and biodegradable as a bone substitute.

Conclusion

Nacre is a material that shares some similarities with bone. The inorganic material and the organic matrix in the nacre have good mechanical properties and biological effects similar to the bone so that they are well integrated with the bone.Nacre has several advantages, namely cheap, modern design, hierarchical structure and architecture, intrinsic biological function, low immogenity, low cytotoxicity, safe storage, ease, and can be planned to suit each clinical need.

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