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The Effectiveness of Milkfish (Chanos Chanos) Scales Chitosan on Soft and Hard Tissue Regeneration Intooth Extraction Socket: A Literature Review

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ABSTRACT

Background: Milkfish (Chanoschanos) is a superior fishery product of South Sulawesi, but fish scales wastehas not been used optimally. Chitosan from chitin extracted from milkfish scales is a non-toxic, biocompatible, and biodegradable polymer material. Tooth socket healing is a pathophysiological process that involves proliferation, cell migration and tissue remodeling. It is hoped that the effectiveness of chitosan in tissue regeneration can be used as a regeneration material for soft tissue and scaffold to increase bone regeneration. **Objective:** To discuss the potential of chitosan in milkfish scales waste for soft and hard tissue regeneration in post extraction socket. **Literature Review:** Milkfish scales can be used as a source of chitosan. Chitosan (poly-β- 1,4-glucosamine) is a natural polysaccharide that provides anti-inflammatory effects by inhibiting prostaglandin E2 and cyclooxygenase-2 protein expression and weakening pro- inflammatory cytokines, as well as increasing anti-inflammatory cytokines. Chitosan can accelerate wound healing, stimulate the production of growth factors, antibacterial, increase angiogenesis and the function of inflammatory cells. Chitosan also has potential as a scaffold because it is biocompatible, degrades with tissue formation, without inflammatory and allergic reactions, adequate porosity, and low degradation products. **Discussion:** Milkfish scales chitosan shows biocompatible, anti-inflammatory, and osteoconductive properties. However, chitosan itself has low water solubility at neutral or high pH and low mechanical properties therefore requires modification of the surface of chitosan or combining chitosan with other biomaterials. **Conclusion:** The use of milkfish scales chitosan has potential as an anti-inflammatory agent, stimulates the activity of fibroblasts, osteoblasts and differentiation of mesenchymal stem cells, as well as osteoconductive bone regeneration scaffold, but in use it is better combined with polymers / biomaterials, and / or other bioactive molecules to improve mechanical properties, absorption of protein and biomineralization of chitosan.

Keyword: ***chitosan, bone regeneration, socket preservation***

INTRODUCTION

The use of natural ingredients is currently growing rapidly in the field of dentistry, one of which is the use of chitosan. Chitosan is a diacetylated, non-toxic and biocompatible chitin-derived polymer material, derived from crustacean shells, fungal and algae cell walls, insect exoskeletons, and radula mollusks.[1]However, several studies have used milkfish scales as the basic ingredient of chitosan because they contain chitin.[2–5]Milkfish (*Chanoschanos*) is a product of brackish water fishery which is abundant in South Sulawesi, which is consumed

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by the public and is known as a fish that has many bones. Milkfish processing often only uses meat without utilizing the bones and scales, so the percentage of fish waste is quite high.[5,6]In the field of dentistry, the application of chitosan has been widely used, among others, as an antimicrobial, anti-inflammatory, bioactivity with dental materials, hemostasis and wound healing, and bone repair.[7]

Tooth extraction is a common practice in dentistry. This procedure can cause minimal trauma by leaving a wound in the tooth socket. The process of wound healing after tooth extraction has the same principles as wound healing in general. Tooth socket healing is a complex pathophysiological process involving cell proliferation, cell migration, extracellular matrix protein synthesis, and deposit and tissue remodeling.[8]The inflammatory phase will occur immediately after tooth extraction, where the wound will be dominated by neutrophil cells, then a phagocytosis process occurs followed by macrophage cells. Macrophages will increase osteoclast activity and release pro-inflammatory cytokines, anti-inflammatory and growth factors, which will decrease the extracellular matrix, stimulate cell differentiation and proliferation for the recovery of damaged tissue and continue with the bone formation process.[9,10]A study conducted by Gupta et al reported that chitosan was effective in accelerating the wound healing process and the occurrence of osteogenesis in the tooth extraction socket by increasing the function of inflammatory cells, such as PMN leukocytes, macrophage cells, fibroblasts, and osteoclasts.[11]

Milkfish chitosan has never been studied for bone and soft tissue regeneration. Therefore, this review will discuss the potential of milkfish scale waste chitosan for soft and hard tissue regeneration after extraction through a general review of research on chitosan.

METHOD

Data Source

Data was collected through the PubMed and Cochrane searches published from 2016 to 2021. The data search was performed using keywords; (((Socket preservation) OR (Bone regeneration)) OR (Soft tissue healing)) AND (Chitosan).

Research criteria

A. Inclusion Criteria
   - Published articles from 2016-2021
   - Articles in English and Indonesian
   - Articles assessing soft and hard tissue regeneration in tooth socket or bone defects or on cell activity

B. Exclusion Criteria
   - Articles in the form of systematic reviews, literature reviews, books, meta-analysis, and case reports
   - Full text is not available for free

Data collection

The data used in this review literature are secondary. The data is obtained from articles which are then reviewed based on the criteria made by the author

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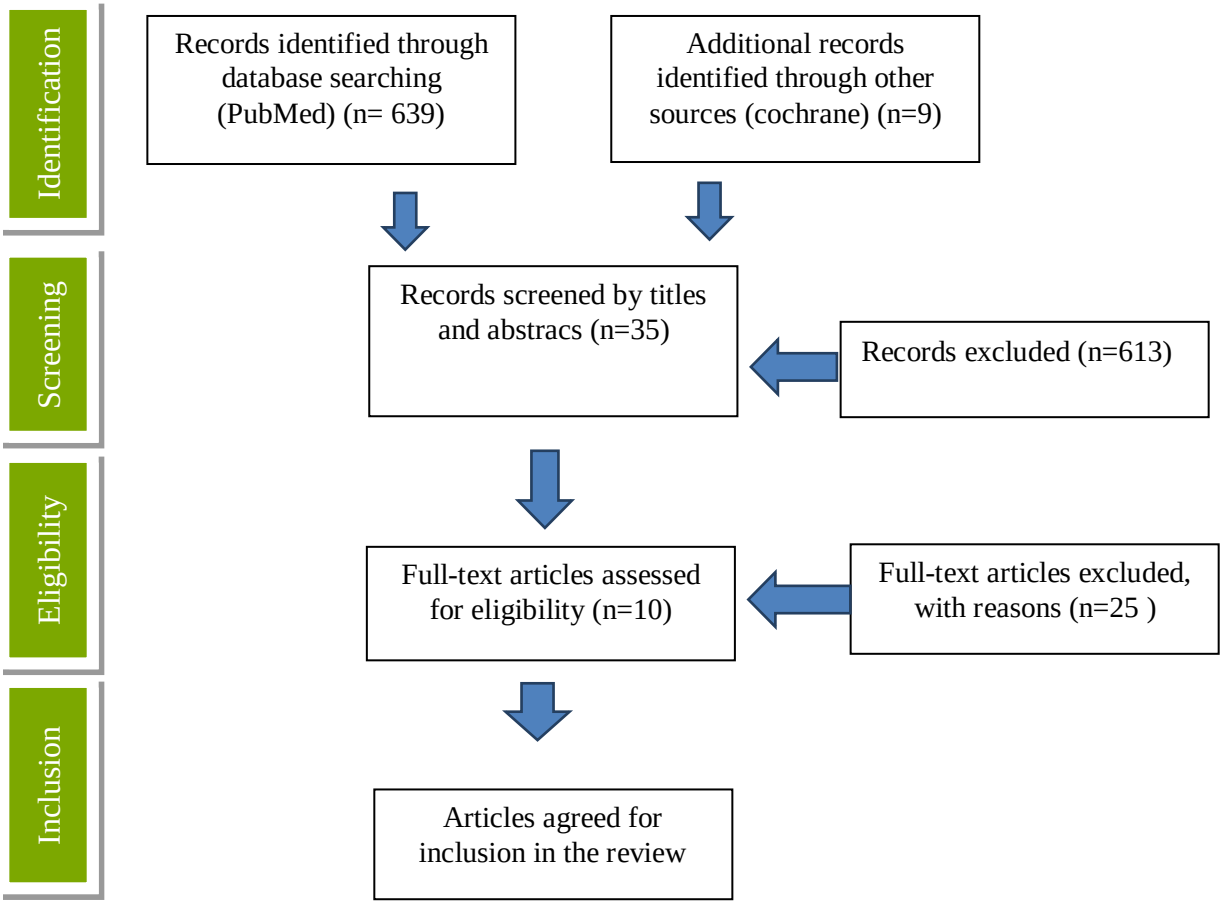

Figure 1. Review journal search flowchart

RESULT

After obtaining 648 articles in Pubmed and Cochrane searches, 613 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 35 articles. Of the 35 articles, 25 were excluded. Ten articles will be reviewed and included in the synthesis table.

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Table 1. The effect of the combination of chitosan with polymers/biomaterials and/or other bioactive molecules on soft and hard tissue regeneration

No.	Reference	Preparation	Polymer / Biomaterial Combinations	Combination of Bioactive Molecules	Method	Result
1.	Hendrijantini et al (2018) *The effect of combination spirulina–chitosan on angiogenesis, osteoclast, and osteoblast cells in socket models of hyperglycemic Rattus norvegicus*[12]	gel	combination of 20% chitosan and 12% spirulina		A laboratory study involved 36 Rattus norvegicus, divided into three groups (nondiabetic Mellitus (DM), uncontrolled DM, and controlled DM) and further divided into six subgroups. The control group (K1, K2, and K3) was induced with 3% carboxymethyl cellulose Na, while the treatment group was induced with 12% spirulina and 20% chitosan. On day 14, the lower jaws of the rats were removed. Capillary	The combination of 12% spirulina and 20% chitosan increases angiogenesis and the number of osteoblast cells and decreases the number of osteoclasts in diabetic conditions. This combination can inhibit the accumulation of reactive oxygen species (ROS) so that it also inhibits the expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) which play a role in bone resorption. A decrease of IL-6 will increase the amount of osteoprotegerin that

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				lumen, osteoblasts, and osteoclasts were calculated by examining the hypothalamus-pituitary-adrenal examination and the results were analyzed using Shapiro-Wilk, Levene's, one-way ANOVA, and Tukey's post hoc difference test.	inhibits RANK and RANKL interactions. This condition causes a decrease in the number of osteoclasts. The osteoinductive properties and osteointegratability of chitosan stimulate bone regeneration.
2.	Maryani et al (2018) *Effect of Gel Combination Platelet Rich Plasma and ChitosantoIncreaseOsteoblastas Bone Regeneration in WoundHealing Post Extraction Teeth in Mice Wistar*[13]	gel	Platelet-rich plasma	The true experimental post-test only control group design was carried out on 28 Wistar rats. The samples were divided into 4 groups, namely PRP gel, combination PRP and chitosan gel, chitosan gel, and Povidone Iodine control group. The	There was a significant increase in the number of osteoblasts in the combination PRP and chitosan gel groups compared to the PRP, chitosan gel, and povidone-iodine groups 14 days after tooth extraction.

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				mandibular right incisor was extracted and treated according to the group. The number of osteoblasts in the post-extraction socket was observed microscopically after 14 days using IHC staining. Data were analyzed using the One-Way ANOVA parametric test followed by the Post Hoc LSD test.	
3.	Xu et al (2019) *Influence of in vitro differentiation status on the in vivo bone regeneration of cell/chitosan microspheres using a rat cranial defect model*[14]	microsphere	bone-marrow-derived mesenchymal stromal cells (BMSCs)	Stromal mesenchymal cells (BMSCs) derived from mouse bone marrow were inserted into the apatite-coated chitosan microsphere. The constructs were osteogenically	There was the closure of the bone defect in the chitosan scaffold group cultured with osteogenic media for 14 days, after eight weeks. The new bone is thick and almost covers the entire defect.

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				differentiated for 0, 7, 14, and 21 days followed by implantation of the in vivo calvarial defect for up to 8 weeks.	
4.	Gaihre et al (2018) *Comparative investigation of porous nano-hydroxyapatite/chitosan, nano-zirconia/chitosan and novel nano-calcium zirconate/chitosan composite scaffolds for their potential applications in bone regeneration*[15]	composite	Nano hydroxyapatite/Nano ZrO_2 /Nano $CaZrO_3$	The porous composite scaffold was developed using the freeze-drying technique. Cell culture studies were carried out using mouse pre-osteoblast cells (OB-6). The sterilized samples were incubated with culture media for 5 hours at 37^oC and 5% CO_2. The scaffold was then removed and 200 µl of cell suspension containing 50,000 to 105 cells was added to the top of the	The addition of bioceramicnanopowder to the chitosan scaffold increased mechanical strength, cell proliferation, and cell spread on the scaffold.

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				scaffold slowly so that the cells could spread over the entire surface, then incubated at 37°C and 5% CO_2 for 3 hours. The proliferation of pre-osteoblasts along the surface and into the scaffold was observed using a confocal laser scanning microscope (CLSM) (Leica, USA) after staining cells with calcein AM	
5.	Saravanan et al (2018) *Chitosan-based thermoresponsive hydrogel containing graphene oxide for bone tissue repair*[16]	hydrogel	Glycerophosphate + graphene oxide	The hydrogel pore architecture was examined using scanning electron microscopy (SEM), swelling properties, protein adsorption ability, degradation rate, and exogenous	The hydrogel is biocompatible with mesenchymal stem cells and is metabolically active. Hydrogels increase osteogenic differentiation of mouse mesenchymal

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			biomineralization. In in vitro biological studies, mesenchymal stem cells derived from mouse bone marrow (rBMSCs) were isolated. rBMSCs were used for in vitro biocompatibility analysis and mouse mesenchymal stem cells (C3H10T1 / 2) were used for differentiation studies. rBMSCs were homogeneously suspended in HEC solution (2 × 105 cells / ml; 20 mg / ml HEC dissolved in DMEM), and mixed with CS / GP gel solution. To qualitatively assess the viability of the encapsulated cells, FDA and DAPI	stem cells by upregulating Runt-related transcription factor 2 (Runx2), Alkaline phosphatase (ALP), collagen type 1 (COL-1), and osteocalcin (OC) in osteogenic conditions.

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					stains were performed to assess their distribution in the hydrogel. After 4 days, cell hydrogels were washed and fixed with 2.5% glutaraldehyde for 1 hour, washed with ethanol series (gradient elution), dried, gold-sputtered, and analyzed by SEM to assess cell distribution in the hydrogel.	
6.	Keller et al (2019) *Preclinical safety study of a combined therapeutic bone wound dressing for osteoarticular regeneration*[17]	composite	poly-ε-caprolactone	BMP-2	A 1.5 mm osteochondral round defect was induced with a short drill in the femur to subchondral bone bleeding (approx. 2 mm). A NanoM1-BMP2 membrane was placed at the bottom of the break,	Bone and cartilage regeneration was seen in both experimental animals without cytotoxicity. BMP-2 is released gradually, thereby reducing the side effects of inflammation.

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which was then filled with the hMSCs / hydrogel mixture and gelled by adding 102 mM calcium chloride (SigmaAldrich), for 5 minutes. Rats in experimental group 1 (ARTiCAR; n = 20 rats; 10 males and 10 females; 3.5 µl hydrogel containing 105,000 ± 10% cells) and group 2 (n = 20 mice; 10 males and 10 females; 3.5 µl control hydrogel) underwent the same procedure with different ingredients. After gelation, the articulation capsule is closed, the muscles and skin are sutured and the wound is disinfected. Rats were observed daily for wound

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					healing, leg mobility, morbidity, mortality, and signs of toxicity, and twice a week for each weight loss for up to 90 days. Hematocrit, hemoglobin concentration, erythrocyte count, leukocyte count, mean cell volume, and platelet count is determined in a blood sample. Histopathological analysis was performed on animals used for blood tests.	
7.	Ansarizadeh et al (2019) *Fabrication, modeling and optimization of lyophilized advanced platelet-rich fibrin in combination with collagen-chitosan as a guided bone regeneration*	membrane	collagen	Advanced platelet-rich fibrin (A-PRF)	Response surface methodology (RSM) was used to design experimental conditions and to correlate effect parameters,	Analysis of alkaline phosphatase (ALP) showed an increase in osteoblast differentiation due to the addition of A-PRF

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	membrane[18]			including the weight ratio of chitosan/collagen (chit/col) and A-PRF concentration to Young's modulus, the viability of mesenchymal stem cells (MSCs), and degree of membrane degradation.	
8.	Elango et al (2019) *Chitosan- Collagen 3D Matrix Mimics Trabecular Bone and Regulates RANKL-Mediated Paracrine Cues of Differentiated Osteoblast and Mesenchymal Stem Cells for Bone Marrow Macrophage-Derived Osteoclastogenesis*[19]	composite	Chondroitin sulfate + hydroxyapatite	Collagen is cross-linked with chitosan, hydroxyapatite (H), and chondroitin sulfate (Cs), to produce a natural, bone-like 3D structure and to evaluate its effect on bone homeostasis using bone marrow mesenchymal stem cells, osteoblasts, and bone marrow macrophages. XRD and micro-CT data	there is increased osteoblast differentiation through increased cellular ALP and bone mineral. The matrix regulates RANKL secretion which can promote osteoclastogenesis so that bone resorption is limited and bone regeneration is enhanced.

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			confirm the arrangement of H crystals in the three-dimensional (3D) chitosan-collagen-H-Cs (CCHCs) matrix and the three-dimensional structure of the matrix. The stimulatory osteoblastogenic and exploitative osteoclastogenic activities of the 3D matrix were identified using differentiated osteoblasts and osteoclasts.	
9.	Sularsih et al (2016) *Influenced of UsingChitosanwith Weight Molecul Different to TumourExpression of Necrosis Factor Alpha (Tnf A) in Wound HealingPost ExtractionTeethRattus*	gel	Rattusnorwegicus strain Wistar was divided into 3 treatment groups, namely group I with chitosan gel treatment which has	Chitosan gel with high molecular weight showed an increase in the amount of TNF-α expression that was greater than that of the treatment group with

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	Norvegicus[20]		a high molecular weight, group II with chitosan gel treatment which has a low molecular weight, and group III without chitosan gel. Chitosan is applied to the tooth socket. The mandibular jaw was decapitated 3 and 4 days after treatment, then an anatomical histopathological examination was carried out to observe the angiogenesis process.	chitosan which had low molecular weight both at 3 and 4 days of observation. Chitosan with high molecular weight contains more N-acetyl so that it will stimulate macrophage cells to release more TNF-α cytokines. Also, the polymer chains are long so that the molecular bonds are getting stronger. The more N-acetyl monomer, the higher the effect of accelerating wound healing.
10.	Gupta et al (2019) *Efficacy of Chitosan in promoting wound healing in extraction socket: A prospective study*[11]	wound dressing	Symmetrical mandibular third molars were extracted simultaneously in 27 patients and Chitosan dressing was placed	Chitosan is effective in accelerating wound healing and early osteogenesis of the post-extraction socket.

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into the extraction socket. Pain scores were recorded on the Visual Analog Scale (VAS) using a pain score of 0-10. Wound healing compared between right and left side. The radiographic findings were evaluated by observing the lamina dura and extraction socket density.

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LITERATURE REVIEW

Wound healing is a specific biological process related to tissue growth and regeneration. Wound healing takes place through various stages of matrix and cellular components that work together to rebuild the integrity of damaged tissue and replace lost tissue. The wound healing process involves complex biochemical and cellular processes, which consist of 3 phases, namely the inflammation, proliferation, and remodeling phases.[21]

1. Milkfish Scales Chitosan

Chitosan (poly-β-1,4-glucosamine) was first discovered by Rouget in 1985, is a natural biopolymer that has a linear chain with the structural formula ($C_6H_{11}NO_4$). Chitosan is a natural polysaccharide that can be obtained from the deacetylation of chitin, which comes from fungal cell walls, crustaceans, and insect exoskeletons. The process of obtaining chitosan is carried out by chemical extraction methods including deproteinization, demineralization, and deacetylation of chitin.[22,23]Chitosan has a composition of carbon (40.30%), nitrogen (6.35%), and hydrogen (5.83%), and is non-toxic, biodegradable, biocompatible, antibacterial, bioactivity and can be used in various forms of solutions, gels, pastes, mixtures, sponges, membranes, tablets, and micro-granules depending on the application.[18,24]

Fish scales are waste that has not been used optimally. Fish scales contain chitin, calcium, proximate, alkaloids, steroids, saponins, phenol hydroquinone, molisch, benedict, biuret, and ninhydrin. Fish scales can be used as raw material for chitin extraction and further modified into chitosan. Extraction and modification of chitin into chitosan begins with the preparation, demineralization, deproteinized, and deacetylation steps. Fish scales with content similar to bone contain potential antimicrobial ingredients that can be used as dental materials.[25] Chitosan from milkfish scales (Chanoschanos) has antimicrobial activity against Candida albicans. The higher the chitosan concentration given, the larger the diameter of the formed inhibition zone. Also, milk scales chitosan gel can also inhibit the growth of Aggregatibacteria actinomycetemcomitans and Porphyromonas gingivalis which are the bacteria that cause periodontitis, and the higher the concentration of milk scales chitosan gel, the higher the inhibition zone produced.[2,4]

2. Effect of Chitosan on Soft Tissue Regeneration

Chitosan has been shown to promote tissue healing, as well as to stimulate the production of platelet growth factors and exhibit antibacterial activity which can play a role in reducing

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postoperative discomfort.[26]Chitosan is a cationic polymer that has an antimicrobial effect due to destabilization of the outer membrane of gram-negative bacteria and permeabilization of the microbial plasma membrane. Besides, chitosan can also increase granulation tissue and angiogenesis, and accelerate wound healing by increasing the function of inflammatory cells, such as macrophages, polymorphonuclear leukocytes (PMN), and fibroblasts or osteoclasts.[11]Chitosan is metabolized by certain enzymes, such as lysozyme so that it can be broken down and can act as a tissue engineering scaffold because it has structural similarities to glycosaminoglycans and is hydrophilic.[27]

Stimulation of fibroblasts produces interleukin-8 which plays an important role in chemotaxis and angiogenesis processes. Complement activation occurs by increasing the production of C5a which triggers the migration of neutrophils and monocytes to the walls of blood vessels. Chitosan also activates macrophages, cytokine production, giant cell migration, and stimulation of type IV collagen synthesis. Chitosan also can promote the formation of adequate granulation tissue accompanied by angiogenesis and deposition of collagen fibers.[1]

3. Effect of Chitosan on Hard Tissue Regeneration

The process of bone remodeling depends on the interaction of RANK-RANKL and OPG. Osteoclastogenesis is inhibited by OPG produced by osteoblasts whose function is to inhibit RANK-RANKL interactions.[28]If the OPG expression is higher then bone formation is increased. However, if the RANKL expression is higher then bone resorption occurs. In this case, OPG is needed to prevent bone resorption and to achieve bone metabolism.[29]

One of the current treatments for bone regeneration includes the development of a scaffold that resembles the composition and structure of the extracellular matrix of bone. The structure with high porosity and suitable topography and surface chemical properties can facilitate cell adhesion, cell growth and proliferation, diffusion of oxygen and nutrients, and facilitate the removal of metabolic waste that occurs during the regeneration process. Materials with porosity>75% and pore size>300 µm have the most optimal osteoconductive effect.[30]

Chitosan from milkfish scales has never been used to regenerate soft tissue and bones. Chitosan has a positive effect on soft tissue and bone healing, both in stimulating anti-inflammatory cells and osteoblast activity, as well as by triggering differentiation of mesenchymal stem cells into osteoblasts. However, its use is limited, so it is necessary to combine it with other materials to increase its utilization in both physical, chemical, and biological properties.

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DISCUSSION

Milkfish scales are abundant waste in Indonesia, but their use is not optimal. Milkfish scales are rich in chitin, calcium, proximate, alkaloids, steroids, saponins, phenol hydroquinone, molisch, benedict, biuret, and ninhydrin. The content of chitosan in milkfish, which is obtained from chitin, can reach 37.4% after dehydration.[23]Chitosan consists of glucosamine β-1,4-linked N-acetyl-D and glucosamine-D units which are natural cationic polysaccharides. N-acetyl glucosamine functions as an anti-inflammatory that is synthesized in the human body from glucose. Chitosan provides anti-inflammatory effects by inhibiting prostaglandin E2 and cyclooxygenase-2 protein expression and weakens the pro-inflammatory tumor necrosis factor-α and interleukin-1β cytokines, as well as increasing the expression of the anti-inflammatory cytokine interleukin-10.[27]Research conducted by Sularsih et al (2016) has reported that chitosan gel can stimulate macrophage cells to release TNF-α cytokines on the 3rd and 4th days so that it has the effect of accelerating wound healing.[33]The same thing with the research of Gupta et al (2019) reported that chitosan is effective in accelerating wound healing and early osteogenesis in the post-extraction socket.[11]

Chitosan also has high potential as a scaffold material because of its biocompatible properties, degrades with tissue formation, without inflammatory and allergic reactions, adequate porosity, and low degradation products.[31–34]Chitosan is hydrophilic so it supports cell adhesion and proliferation. In several in vitro studies, chitosan has been shown to increase the adhesion and proliferation of osteogenic cells and mesenchymal stem cells.[32,35,36]Osteogenic cells cultured on chitosan produce an extracellular matrix that mineralizes into bone tissue. Chitosan also increases osteogenic differentiation of mesenchymal stem cells.[37]This is consistent with research by Maryani et al [13], Sularsihet al[20,38]and Gupta et al[20,38]which showed that the use of chitosan in the tooth socket showed a significant increase in osteoblasts and collagen, and decreased the number of osteoclasts so that it could support bone formation in the socket.

However, chitosan itself has several disadvantages, such as low water solubility at neutral or high pH and low mechanical properties.[34,36,39]For this reason, the latest research is to modify the surface of chitosan or combine chitosan with other synthetic or natural polymer materials (poly (vinyl alcohol), poly-ε-caprolactone, alginate, collagen, etc.], biomaterials (hydroxyapatite, β-tricalcium phosphate, SiO2, etc.), or bioactive molecules (bone morphogenetic protein 2 (BMP-2), vascular endothelial growth factor (VEGF),

bisphosphonates, etc.) to increase mechanical resistance, protein absorption, and biomineralization. According to Xu et al[20,38]bioceramicnanopowder added to chitosan increased mechanical strength, cell proliferation, and cell spread resulting in a new bone that was thick and almost completely covered the defect 14 days after defect creation.Besides, Danilchenko et al [40]also showed the formation of bone tissue due to the increased osteoconductive properties of the combination of hydroxyapatite and chitosan. Combinations with bioactive molecules, for example, PRP, BMP-2, BMSCs, and A-PRF carried out by each Maryaniet al[13], Keller et al[13], Xu et al[14] andAnsarizadehet al[14], showed that there was a significant increase in activation and osteogenic differentiation compared to only using chitosan.In gel form, chitosan protects the wound area and has a cooling effect that can reduce pain. Several studies have shown that chitosan gel with a degree of acetylation of 80% -84% and a molecular weight of 150-252 kDa is the most optimal in increasing bone regeneration.[41,42,43,44,45,46]

CONCLUSION

The use of chitosan from milkfish scales has the potential as an anti-inflammatory agent as well as stimulating the activity of fibroblasts, osteoblasts, and differentiation of mesenchymal stem cells. Also, chitosan can be used as a scaffold for osteoconductive bone regeneration, but in use, it is better combined with a polymer/biomaterial and/or bioactive molecule to improve mechanical properties, protein absorption, and biomineralization of chitosan.

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