KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS HASANUDDIN
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RSGMP

REKOMENDASI PERSETUJUAN ETIK

Nomor: 0055/PL.09/KEPK FKG-RSGM UNHAS/2022

Tanggal: 18 April 2022

Dengan ini menyatakan bahwa protokol dan dokumen yang berhubungan dengan protokol berikut ini telah mendapatkan persetujuan etik:

No. Protokol	UH 17120639	No Protokol Sponsor	
Peneliti Utama	Dr. drg. Arni Irawaty Djais, Sp.Perio(K)	Sponsor	Pribadi
Judul Peneliti	Efektivitas Kitosan Limbah Sisik Ikan Bandeng (Chanos chanos) terhadap Regenerasi Jaringan Periodontal Melalui Analisis Ekspresi TNF-α, IL-1, RANKL dan OPG pada Tindakan Socket Preservation (In Vivo)		
No. Versi Protokol	1	Tanggal Versi	07 April 2022
No. Versi Protokol		Tanggal Versi	
Tempat Penelitian	1. Laboratorion Biokimia TPHP Politani Poltek Pangkep, 2. Laboratorium Terpadu Kimia, Fak.MIPA Unhas, 3. Klinis Hewan La Costae , 4. Laboratorium Patalogi Anatomi RSP Unhas, 5. Laboratorium Biokimia-Biomolekuler Fakultas Kedokteran Universitas Brawijaya		
Dokumen Lain			
Jenis Review	☐ Exempted ☒ Expedited ☐ Fullboard	Masa Berlaku 18 April 2022-18 April 2023	Frekuensi Review Lanjutan
Ketua Komisi Etik Penelitian	Nama: Dr. drg. Marhamah, M.Kes	Tanda Tangan	Tanggal
Sekretaris Komisi Etik Penelitian	Nama: drg. Muhammad Ikbal, Sp.Pros	Tanda Tangan	Tanggal

Kewajiban peneliti utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum diimplementasi
- Menyerahkan laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 dan lapor SUSAR dalam 72 jam setelah peneliti utama menerima laporan.
- Menyerahkan laporan kemajuan (*progress report)* setiap 6 bulan untuk pene resiko tinggi dan setiap setahun untuk penelitian resiko rendah.
- Menyerahkan laporan akhir setelah penelitian berakhir.
- Melaporkan penyimpangan dari protokol yang disetujui (*pr deviation/violation)*
- Mematuhi semua aturan yang berlaku.

Original Article

The Utilization Of Milk Fish Scales Chitosan For Bone And Soft Tissue Regeneration After Tooth Extraction : A Literature Review

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DOI: 10.47750/pnr.2023.14.S02.121

Abstract

Introduction : Chitosan from milkfish scale chitin is a polymer material that is biocompatible, biodegradable, and non-toxic. Chitosan has been used as an antimicrobial, anti-inflammatory, bioactivity with dental materials, hemostasis, wound healing, and bone repair. Wound healing after tooth extraction is a pathophysiological process involves proliferation, cell migration, and tissue remodeling. The ability of chitosan to regenerate tissue is use as a soft tissue regeneration material and scaffold for bone regeneration. This literature review aims to discuss the ability of chitosan contained in milkfish scales to regenerate bone tissue and soft tissue after tooth extraction.

Methods: Data were collected through article searches on PubMed and Cochrane published from 2016 to 2022. Data search was conducted using keywords; ((Tooth Extraction) OR (Bone Regeneration)) OR (Soft Tissue Healing)) AND (Chitosan).

Result: An initial search of Pubmed and Cochrane found 392 articles. Furthermore, 357 articles were excluded because they did not meet the inclusion criteria desired by the author in the title and abstract, or there were duplications, so that 35 articles were obtained. Then from these 35 articles, 23 articles were excluded, and the final results were 12 articles that would be reviewed and included in the synthesis table.

Conclusion: Milkfish scale chitosan has the potential as an anti-inflammatory agent, stimulates the activity of fibroblasts, osteoblasts, and mesenchymal stem cell differentiation, and osteoconductive bone regeneration scaffold. However, the utilization will be better when combined with polymers/biomaterials, and/or other bioactive molecules to improve mechanical properties, protein absorption, and biomineralization of chitosan.

Keywords: Bone Regeneration, Chitosan, Tooth extraction, Wound healing

INTRODUCTION

The development of the use of natural materials in the field of dentistry is currently growing rapidly, one of which is chitosan. Chitosan is an acetylated, non-toxic, and biocompatible chitin-derived polymer material. Chitosan can be derived from crustacean shells, cell walls of fungi and algae, insect exoskeletons, and radula molluscs.[1] In addition to these sources, several studies have stated that milkfish scales can be used as the basic material for chitosan because they contain chitin.[2–5] Milkfish (Chanos chanos) is a brackish water fishery product that is abundant in Indonesia and is widely consumed by the public and is known as a fish that has many bones. During this time, milkfish processing mostly only uses the meat, so fish scales are only considered as waste.[6,7] In dentistry, chitosan has been widely used as antimicrobial, anti-inflammatory, bioactivity with dental materials, hemostasis, wound healing, and bone repair.[8]

Tooth extraction is an action that can cause minimal trauma by leaving a wound in the tooth socket. The process of wound healing after tooth extraction has the same principle as wound healing in general. Physiologically, the socket healing process can be divided into four successive overlapping phases, namely the hemostasis phase, the inflammatory phase, the proliferative phase, and the maturation/remodeling phase.[9,10] 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 proinflammatory cytokines, anti-inflammatory and growth factors, which will degrade the extracellular matrix, stimulate cell differentiation and proliferation for the restoration of damaged tissue, and continue the process of bone formation. [11,12] Gupta et al reported that chitosan was effective in accelerating the process of wound healing and osteogenesis in tooth extraction sockets by enhancing the function of inflammatory cells, such as PMN leukocytes, macrophages, fibroblasts, and osteoclasts.[13] Research on milkfish scale chitosan on bone and bone tissue regeneration has not been done much. Thus, this literature review will discuss the potential of milkfish scale waste chitosan for soft and hard tissue regeneration after tooth extraction through an overview of health research on chitosan.

MATRIAL AND METHOD

Data Source

Data were collected through article searches on PubMed and Cochrane published from 2016 to 2022. Data search was conducted using keywords; ((Tooth Extraction) OR (Bone Regeneration)) OR (Soft Tissue Healing)) AND (Chitosan).

Research criteria

A. Inclusion Criteria

- Published articles from 2016-2022
- 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.

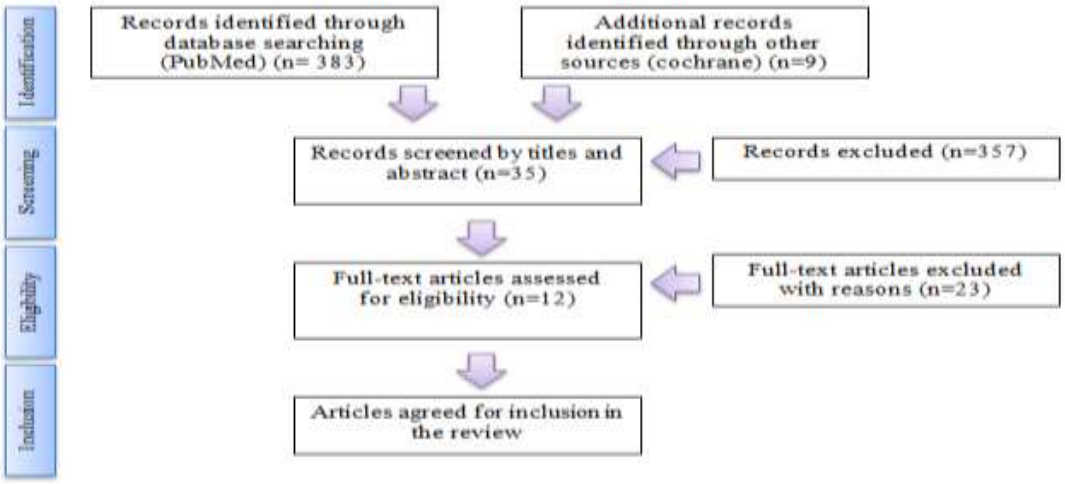

Figure 1. Review journal search flowchart

RESULT

An initial search of Pubmed and Cochrane found 392 articles. Furthermore, 357 articles were excluded because they did not meet the inclusion criteria desired by the author in the title and abstract, or there were duplications, so that 35 articles were obtained. Then from these 35 articles, 23 articles were excluded, and the final results were 12 articles that would be reviewed and included in the synthesis table.

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 Combination**	**Combination of Bioactive Molecules**	**Method**	**Results**
1	Hendrijantini et al[14] (2018) The effect of combination spirulina–chitosan on angiogenesis, osteoclast, and osteoblast cells in socket models of hyperglycemic Rattus norvegicus	Gel	Combination of 20% chitosan and 12% spirulina		A laboratory study involved 36 Rattus norvegicus, divided into three groups (non diabetic 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 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.	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 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[15] (2018) Effect of Gel Combination Platelet Rich Plasma and Chitosan to Increase Osteoblastas Bone Regeneration in	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	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

	Wound Healing Post Extraction Teeth in Mice Wistar				groups, namely PRP gel, combination PRP and chitosan gel, chitosan gel, and Povidone Iodine control group. The 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.	povidone-iodine groups 14 days after tooth extraction.
3	Xu et al[16] (2019) Influence of in vitro differentiation status on the in vivo bone regeneration of cell/chitosan microspheres using a rat cranial defect model	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 differentiated for 0, 7, 14, and 21 days followed by implantation of the in vivo calvarial defect for up to 8 weeks.	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.
4	Gaihre et al[17] (2018) Comparative Investigation of porous nano-hydroxyapatite/chitosan, nano-zirconia/chitosan and novel nano-calcium	Composite	Nano hydroxyapatite/Nano ZrO2 /Nano CaZrO3		The porous composite scaffold was developed using the freeze-drying technique. Cell culture studies were carried out using mouse	The addition of bioceramicnanopowder to the chitosan scaffold increased mechanical strength, cell proliferation, and cell

	zirconate/chitosan composite scaffolds for their potential applications in bone regeneration				pre-osteoblast cells (OB-6). The sterilized samples were incubated with culture media for 5 hours at 37ºC and 5% CO2. The scaffold was then removed and 200 µl of cell suspension containing 50,000 to 105 cells was added to the top of the scaffold slowly so that the cells could spread over the entire surface, then incubated at 37oC and 5% CO2 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	spread on the scaffold.
5	Saravanan et al[18] (2018) Chitosan-based thermoresponsive hydrogel containing graphene oxide for bone tissue repair	Hydrogel	Glycerophosphate + graphene oxide		The hydrogel pore architecture was examined using scanning electron microscopy (SEM), swelling properties, protein adsorption ability, degradation	The hydrogel is biocompatible with mesenchymal stem cells and is metabolically active. Hydrogels increase osteogenic differentiation of mouse mesenchymal stem cells by

					rate, and 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 exogenous 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),	upregulating Runt-related transcription factor 2 (Runx2), Alkaline phosphatase (ALP), collagen type 1 (COL-1), and osteocalcin (OC) in osteogenic conditions.

					dried, gold- sputtered, and analyzed by SEM to assess cell distribution in the hydrogel.	
6	Keller et al[19] (2019) Preclinical safety study of a combined therapeutic bone wound dressing for osteoarticular regeneration	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, 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	Bone and cartilage regeneration was seen in both experimental animals without cytotoxicity. BMP-2 is released gradually, thereby reducing the side effects of inflammation.

					wound is disinfected. Rats were observed daily for wound 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[20] (2019) Fabrication, modeling and optimization of lyophilized advanced platelet-rich fibrin in combination with collagen-chitosan as a guided bone regeneration membrane	Membrane	Collagen	Advanced platelet-rich fibrin (A-PRF)	Response surface methodology (RSM) was used to design experimental conditions and to correlate effect parameters, 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.	Analysis of alkaline phosphatase (ALP) showed an increase in osteoblast differentiation due to the addition of A-PRF

8	Elango et al[21] (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	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 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.	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.
9	Sularsih et al[22] (2016) Influenced of Using Chitosan with Weight Molecul Different to Tumour	Gel			Rattus norwegicus strain Wistar was divided into three treatment groups,	Chitosan gel with high molecular weight showed an increase in the amount of TNF-α

	Expression of Necrosis Factor Alpha (TNF-α) in Wound HealingPost ExtractionTeethRattus Norwegicus				namely group I with chitosan gel treatment which has 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	expression that was greater than that of the treatment group with 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[13] (2019) Efficacy of Chitosan in promoting wound healing in extraction socket: A prospective study	Wound dressing			Symmetrical mandibular third molars were extracted simultaneously in 27 patients and Chitosan dressing was placed 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	Chitosan is effective in accelerating wound healing and early osteogenesis of the post-extraction socket.

					observing the lamina dura and extraction socket density.	
11	Gani et all[23] (2022) Effectiveness of Combination of Chitosan Gel and Hydroxyapatite from Crabs Shells (Portunus pelagicus) Waste as Bone graft on Periodontal Tissue Regeneration through IL-1 and BMP-2 Analysis	Gel		Hydroxyapatite	Experimental laboratory research and clinical trials with posttest only control group design. Twenty-seven Wistar rats were divided into three groups. The femoral bone defect was made, the positive control group was given placebo gel,the positive control group was given BATAN hydroxyapatite, and the test group was given a combination of chitosan gel and hydroxyapatite crab shells.Wistar rats were sacrificed on days 7, 14, and 21, and the femur bone was then taken for immuno histochemical analysis to determinethe levels of IL-1 and BMP-2. Kolmogorov–mirnov test, Levene test,and one-way ANOVA analyzed the data.	On days 7, 14, and 21, the expression levels of IL-1 and BMP2 were significantly different between the three groups. The group added with chitosan gel and crab shell HA showed a faster decrease in IL-1 expression than the control group.BMP-2 expression increased in the test group compared to the control group.
12	Djais et al[24] (2022) South Sulawesi Milkfish (Chanos Chanos) Scale Waste as a New Anti-inflammatory Material in Socket Preservation	Gel		Hydroxyapatite	This is a post-test-only control group design study. Thirty-two Cavia cobaya were divided into four groups: (1) Socket preservation using milkfish scales chitosan, (2) milkfish scales chitosan + bovine	On days 3, 7, 14, and 28, groups with chitosan added showed lower levels of TNF-α and a faster decrease in IL-6 expressions compared to those without chitosan.

					xenograft, (3) bovine xenograft as a positive control, and (4) placebo as a negative control, then were sacrificed on 3rd, 7th , 14th , and 28^{th} days. The mandible jaw specimen was taken for immunohistochemical analysis to determine the levels of TNF-α and IL-6. The data were analyzed using the Kolmogorov–Smirnov test, Levene's test, and one-way analysis of variance.	

LITERATURE REVIEW

Wound healing is a biological process of repairing or replacing functional tissue damaged by injury. This process involves inflammation and bone healing which sequentially goes through several stages, characterized by the presence of blood clots, migration and proliferation of mesenchymal stem cells, formation of granulation tissue, inflammatory infiltrates, angiogenesis, fibroblast proliferation and collagen synthesis which ends with bone remodeling.[9,25]

1. Milkfish Scales Chitosan

Chitosan is a natural polysaccharide derived from N-deacetylated chitin and a semicrystalline polysaccharide consisting of glucosamine and N-acetyl glucosamine units in -(1-4)2-acetamide-2-deoxy-D-glucopyranol bonds. The main source of chitosan is chitin which can come from the cell walls of fungi, crustacean exoskeletons, and insects.[26] The process of obtaining chitosan is carried out by chemical extraction methods including deproteinization, demineralization, and deacetylation of chitin.[4] 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.[20,27] 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 stages of preparation, demineralization, deproteinization, and deacetylation. Fish scales with a content similar to bone contain potential antimicrobial materials that can be used as dental materials.[28] Chitosan from milkfish scales (Chanos chanos) 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.[2,29]

2. Effect of Chitosan on Hard Tissue Regeneration

One of the treatments for bone regeneration includes the development of a scaffold that resembles the composition and structure of the extracellular matrix of bone. Structures with high porosity and topography and suitable surface chemical properties can facilitate cell adhesion, cell growth, proliferation, diffusion of oxygen and nutrients, as well as facilitate the disposal of metabolic wastes that occur during the regeneration process.[24] Chitosan can be used as a drug, as a conductor of growth factors, and as a scaffold material for bone regeneration.[26,30,31] Currently, chitosan has been combined with polymer materials or other natural materials, biomaterials, or bioactive molecules with the aim of increasing mechanical resistance, protein absorption, and protein biomineralization.[1,17,21,32,33] The combination of bioceramic nanopowder and chitosan can increase the mechanical strength, cell proliferation, and cell dispersion on the chitosan scaffold.[17] In addition,the formation of bone tissue due to the increased osteoconductive properties of the combination of hydroxyapatite and chitosan materials.[34] Recent research using natural chitosan sources from crab shells with a combination of hydroxyapatite showed an increase in the expression of BMP-2 which plays a major role in the differentiation of osteoblasts for bone tissue regeneration.[23]

3. Effect of Chitosan on Soft Tissue Regeneration

Chitosan has been shown to promote tissue healing, stimulate platelet growth factor production, and exhibit antibacterial activity that can reduce postoperative discomfort.[35] Chitosan has an antimicrobial effect because it can cause destabilization of the outer membrane of gram-negative bacteria and permeabilization of the microbial plasma membrane. Chitosan can also increase granulation tissue and angiogenesis, and accelerate wound healing by enhancing the function of inflammatory cells, such as macrophages, polymorphonuclear leukocytes (PMN), and fibroblasts or osteoclasts.[13] Chitosan is metabolized by lysozyme so that it can be broken down and can act as a tissue engineering scaffold because it has a similar structure to glycosaminoglycans and is hydrophilic.[36]

The production of interleukin-8 (IL-8) due to fibroblast stimulation plays an important role in the process of chemotaxis and angiogenesis. Complement activation occurs by increasing the production of C5a which triggers the migration of neutrophils and monocytes to the vessel wall. Chitosan can activate macrophages, cytokine production, giant cell migration, and stimulate type IV collagen synthesis. In addition, chitosan can also promote the formation of adequate granulation tissue accompanied by angiogenesis and collagen fiber deposition.[1]

DISCUSSION

Milkfish scale waste is very abundant in Indonesia, but its utilization is not optimal. Fish scales contain chitin, calcium, proximate, alkaloids, steroids, saponins, phenol hydroquinone, molisch, benedict, biuret, and ninhydrin. The content of chitosan in milkfish comes from chitin which can reach 37.4% after dehydration.[37] Chitosan consists of -1,4-linked N-acetyl-D and glucosamine-D units, which are natural cationic polysaccharides. N-acetyl glucosamine functions as an anti-inflammatory which is synthesized in the human body from glucose. Chitosan exerts an anti-inflammatory effect by inhibiting the expression of prostaglandin E2 and cyclooxygenase-2 proteins and attenuating the pro-

inflammatory cytokines tumor necrosis factor-α and interleukin-1β, as well as increasing the expression of the anti-inflammatory cytokine interleukin-10.[36]

Sularsih et al (2016) reported that chitosan gel can stimulate macrophage cells to release TNF- cytokines on days 3 and 4 so that it has the effect of accelerating the wound healing process.[22] Similar results were found in the study of Gupta et al (2019) who reported that chitosan was effective in accelerating wound healing and early osteogenesis in post-extraction sockets.[13] Chitosan has potential as a scaffold material because it is biocompatible, degraded by tissue formation, does not cause inflammatory and allergic reactions, adequate porosity, and low degradation products.[38–40] The hydrophilic nature of chitosan can support cell adhesion and proliferation. Several in vitro studies have proven that chitosan can increase the adhesion and proliferation of osteogenic cells and mesenchymal stem cells.[41,42] Osteogenic cell culture in chitosan produces extracellular matrix which is mineralized into bone tissue. Chitosan also enhances the osteogenic differentiation of mesenchymal stem cells.[43] This is shown in the study of Maryani et al[15], Sularsih et al[22], and Gupta et al[13] which stated that the use of chitosan in dental sockets showed a significant increase in osteoblasts and collagen, as well as a decrease in the number of osteoclasts so that they could support bone formation in the sockets.

Chitosan has several disadvantages, such as low water solubility at neutral or high pH and low mechanical properties.[39,42] Therefore, currently many studies are conducting surface modification of chitosan or combining chitosan with bioactive inorganic materials (hydroxyapatite, tricalcium phosphate) or synthetic polymers (poly (vinyl alcohol) and poly (ethylene glycol)) and natural polymers (collagen) with the aim of improve mechanical resistance, protein absorption, and biomineralization.[32,42] Xu et al[22] bioceramic nanopowder 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. Danilchenko et al[34] and Asdar Gani et al[23] 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[15], Keller et al[19], Xu et al[16] showed 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.[26]

CONCLUSION

Chitosan from milkfish scales can be used as an anti-inflammatory agent and stimulates the activity of fibroblasts, osteoblasts, and mesenchymal stem cell differentiation. In addition, chitosan can also be used as a scaffold for osteoconductive bone regeneration, but optimization of its utilization can be done by combining it with polymers/biomaterials and/or bioactive molecules to improve mechanical properties, protein absorption, and biomineralization of chitosan.

ACKNOWLEDGEMENT

We are very thankful to all the staff members of the Department of Periodontics, Hasanuddin University for their valuable suggestions and encouragement during making this journal

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