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Review Article

Marine based biomaterials: A Marvel in Periodontal Regeneration – A Review

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Abstract

This article is intended to reach out to the readers about the potential of marine based bio materials in the field of periodontal regeneration. Periodontal Regeneration is the reproduction or reconstruction of a lost or injured tissue so that the architecture and function of lost or injured tissues are completely restored. Regeneration done using guided tissue regeneration procedures helps in guiding the regeneration of periodontal structures through differential tissue responses. Various types of barrier membranes or bone grafts used for regenerative procedures are autograft, allograft, xenograft and alloplast. Xenografts such as bovine, porcine had been used commonly in the regenerative procedures. Since the bovine derived collagen have higher risk of encephalopathy other sources of collagen have been researched upon. Besides mammalian collagen, it has been suggested that fish collagen have the potential that can be used in the development of GTR/GBR. In order to overcome the shortcomings in collagen and to improve the regenerative potential, researches are being made with the addition of various natural bioactive substances like chitosan and hydroxyapatite.

Keywords: Guided tissue Regeneration, Guided bone Regeneration, Collagen, Chitosan, Hydroxyapatite

Introduction

The periodontal ligament and alveolar bone are gradually destroyed by periodontitis, which is defined as "an inflammatory disease of the supporting tissues of the teeth caused by individual microbe or groups of specific bacteria with pocket formation, recession, or both."[1] According to Glossary of Periodontal Terms (1992), Regeneration is the reproduction or reconstruction of a lost or injured tissue in such a way that the architecture and function of the lost or injured tissues are completely restored. In order to access the sick root surface and remove local elements such plaque, calculus, and infected cementum, traditional periodontal procedures are frequently carried out. Gingivectomy, open flap debridement, and osseous surgery are surgical procedures that may reduce probing depth and improve clinical attachment.[2,3] However, fresh connection obtained by these treatments typically follows healing, long junctional epithelium, and cementum development, with little to no new connective tissue attachment.[4] Through the use of diverse tissue reactions, guided tissue regeneration (GTR) aims to restore damaged periodontal structures. The GTR concept promotes periodontal regeneration and the exclusion of undesirable cells from the wound area (Figure 1).

The membranes allow cells from the periodontal ligament and bone to

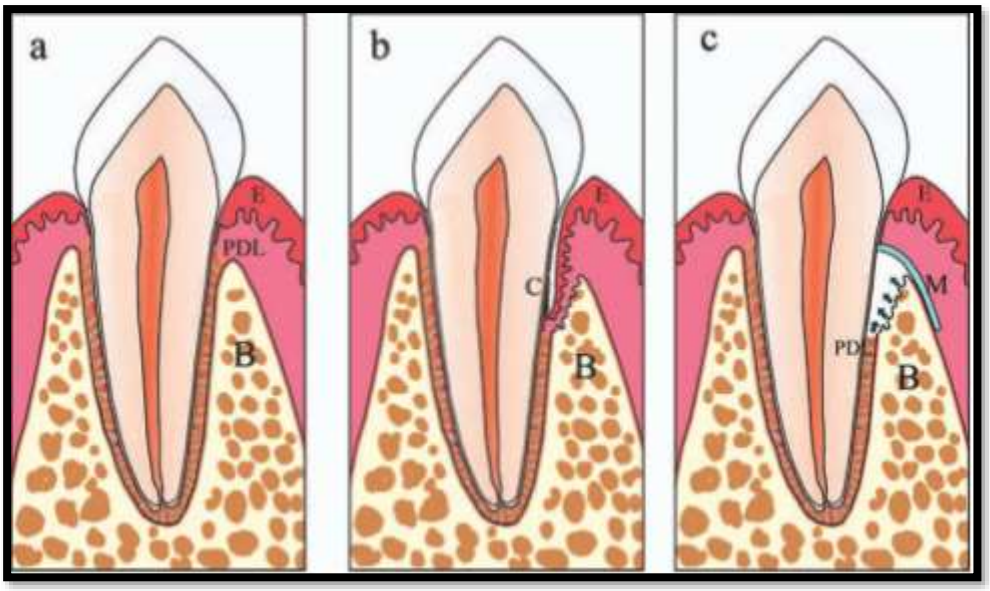

Figure 1: Drawing illustrating: (a) the normal periodontal tissue, (b) in periodontitis and (c) principle of GTR.

repopulate the previously periodontitis-involved root surface and promote the regeneration of periodontal ligament and alveolar bone. They also prevent the epithelium and gingival connective tissue from making contact with the root surface during healing.[5]

Review of Current Literature

For Guided tissue regeneration procedure, various types of membranes have been researched. Both resorbable and non-resorbable membranes fall within this category. With non-resorbable membranes, there is a need for secondary surgery. Thus, it is not frequently used in the present day. Resorbable membranes take only less time for the treatment. They are obtained from various sources such as connective tissue transplants, collagen and synthetic materials etc. Disadvantages of resorbable membranes include:

1. Lack of space making ability.
2. Unpredictable degradation rate.
3. Risk of disease transmission. [6,7,8]

Researchers investigated the usage of membranes with functionally graded, multi-layered architectures that preserve adequate mechanical characteristics, degradation rate, and bioactive qualities in order to overcome the limitations.

Collagen material has been used for its biocompatibility and improved healing in the field of medicine and dentistry.[6] Due to these qualities, it excelled in many applications and was the best option for a bioresorbable GTR membrane.

Over time, several xenografts have been applied in the medical profession as bandages for wounds and to speed up healing. Burns had previously been treated with wound dressings made of porcine skin.[9] For periodontal regeneration in dentistry, xenografts such of those from pig, bovine, equines, and more recently, fish, have been applied. Placing a barrier stops the growth of lengthy junctional epithelium and directs the proliferation of certain cells to regenerate into its matching periodontal tissues using directed tissue regeneration techniques.[10] Research must be done to find more biocompatible

replacements for bovine derived collagen since it is linked to an increased risk of encephalopathy in barrier membrane materials.[11] The growth rates and differentiated functions of the periodontal ligament fibers are higher in fish collagen compared to mammalian collagen.[12]

Fish collagen

Various studies using fish collagen has proved its excellent biocompatibility[13]. However, it doesn't have enough mechanical or antibacterial qualities to be employed as a solo membrane in GTR/GBR procedures1.[14] So further studies are required for the same.

Sources of Marine collagen:

Other than fish collagen, different types of bioactive substances are being studied for improving the biocompatibility and regeneration potential to be used as a barrier membrane. They include chitosan, hydroxyapatite and various other substances. Among these the former two are readily available and in abundance from marine sources (Table 1)

Chitosan

Chitin, which is generated from the exoskeleton of shellfish, primarily crab shells and shrimp, can be hydrolyzed to produce chitosan. Chitosan has the greatest potential of all the natural polymers and can be employed as a barrier membrane because of its biocompatibility, biodegradability, antibacterial activity, bacteriostasis, and tissue healing capabilities.[5] Pure chitosan scaffolds, on the other hand, typically exhibit inferior strength and a rapid rate of breakdown. To perform at its best as a barrier membrane in directed tissue regeneration, it is crucial to optimise the mechanical strength and rate of disintegration. combining polymer and bioceramics, which could enhance the membrane's mechanical performance and bioactivity.

Chitosan can suppress both gram-negative and gram-positive bacteria, according to earlier research. Actinobacillus actinomycetemcomitans is inhibited at very low concentrations, according to in vitro research with Streptococcus mutans and Actinobacillus actinomycetemcomitans.[17] Chitosan can destroy the fungal cells by penetrating the biofilms created by the pathogenic fungus Cryptococcus neoformans, according to earlier investigations. As a result, it lessens the biofilms' metabolic activity.[18] The periodontal tissues' biocompatibility for guided tissue regeneration revealed that chitosan was not poisonous and had a good level of biocompatibility.[19] The mesh type chitosan membrane facilitates effective adhesion, hemostasis, healing, and re-epithelialization of the wound in clinical applications of chitosan as a wound healing dressing.[20] Chitosan showed no significant clinical symptoms of any allergic reactions up to 12 weeks in human trails.[21]

Chitosan can be formed into micro/nanoparticles, fibres, film sponges, gels, and injectable devices to provide local medication delivery systems for the treatment of periodontitis, dental cavities, or root canal procedures. It keeps therapeutic medication concentrations and drug release in periodontal pockets for long periods of time.[22] Scale of milkfish to improve mechanical properties, protein absorption, and biomineralization of chitosan, it is better combined with polymers, biomaterials, and/or other bioactive molecules. Chitosan has demonstrated promising results as an anti-inflammatory agent, enhancing the activity of fibroblasts, osteoblasts, and differentiation of mesenchymal stem cells, as well as an osteoconductive bone regeneration scaffold.

Table 1: Different sources and types of marine collagen that have been isolated and used[8]
(ASC- Acid Soluble Collagen , PSC-Pepsin Soluble Collagen)

v	Source of Collagen	Source Tissue	Yield
Type I	Bigeye snapper Large fin	Bone	ASC: 1.59%
	Long barbel catfish		ASC: 10.94%
	Seaweed pipefish	Skin	ASC: 16.8%; PSC: 28.0%
	Brown backed toadfish		ASC: 5.5%; PSC: 33.2%
	Ocellate pufferfish		PSC: 54.3%
	Lizard fish		ASC: 10.7%; PSC: 44.7%
	Horse mackerel	Scales	ASC: 0.79%
	Grey mullet		ASC: 1.51%
	Flying fish		ASC: 0.43%
	Yellowback seabream		ASC: 0.72%
	Bigeye tuna	Bone	ASC: 0.90%
	Squid	Skin	53%
	Cuttlefish	Skin	ASC: 0.58%; PSC: 16.23%
	Edible Jellyfish	Umbrella	46.4%
Type II	Brown banded bamboo shark	Cartilage	ASC: 1.27%; PSC: 9.59%
	Blacktip shark Ribbon jellyfish	Cartilage Umbrella	ASC: 1.04%; PSC: 10.30% PSC: 9%–19%
Type IV	Marine Sponge		30%

Hydroxyapatite ceramics

In contrast to synthetic raw materials, hydroxyapatite ceramics made from organic materials are preferred because they have similar chemical and physical properties, don't cause cellular toxicity, don't cause inflammatory or pyrogenetic reactions, and don't cause the formation of fibrous tissue around the healing area.

Based on their crystal phase, hydroxyapatite comes in a variety of forms. For instance, there are several different types of calcium phosphate ceramics, including hydroxyapatite, precipitated hydroxyapatite, calcium deficient hydroxyapatite, tricalcium phosphate, -tricalcium phosphate, amorphous calcium phosphate, biphasic calcium phosphate, dicalcium phosphate anhydrous, monetite, carbonated apatite, brushite, monocalcium phosphate monohydrate These forms of bone substitute materials include calcium phosphate ceramics, hydroxyapatite, and its combination with tricalcium phosphate.

Since trace ions are present in natural sources, the HA produced from them is not stoichiometric.[24] The cations Na+, K+, Mg2+, Sr2+, Zn2+, and Al3+ as well as the anions F-, Cl-, SO4 2-, and CO3 2- are beneficial for bone regeneration.[25] Bovine spongiform encephalopathy and foot and mouth disease have raised concerns about the safety of hydroxyapatite made from pigs and cows.[26] Because there have been no unfavourable reports on the safety of products produced from fish, it appears that hydroxyapatite made from fish wastes is safer.[27]

Fish bones can be processed in a number of ways to yield hydroxyapatite and -tricalcium phosphate. Thermal calcination, alkaline hydrolysis, hydrothermal, and laser ablation are a few of them. The traditional method is "Thermal calcination". The calcination method is used to make hydroxyapatite from natural sources or various biowastes such as fishbone, coral, bovine bone, egg shell, and sea shells[28]. Cuttlefish bones can be used as a natural resource for hydroxyapatite[29,30].

Excellent osteoconduction, bioactivity, thermal stability, and biocompatibility are all characteristics of hydroxyapatite. They therefore have a tremendous potential to enhance porous scaffolds used in tissue engineering and bone regeneration.[31]

Fish scale hydroxyapatite from tilapia scales can be isolated by enzymatic hydrolysis method.[32] . Fish scale hydroxyapatite promotes cell proliferation and differentiation and has potential for medical applications providing cost effectivity and environmental compactability.[33] The scaffolds made from cuttlefish bone demonstrated good bioactivity in simulated body fluid and biocompatibility with osteoblasts, indicating that they can be used in tissue-engineering and bone grafting procedures in the clinic.[33-35] In a recent study using rat subcutaneous tissue test to evaluate the in vivo biocompatibility of hydroxyapatite (8 m pore width) obtained from Whitemouth croaker (Micropogonias furnieri) by calcination at 800°C for 5h, it was discovered that after implantation of hydroxyapatite for a month there is no cytotoxicity and genotoxicity in the blood, liver, lung, and kidney. we can deduce that the biocompatibility of hydroxyapatite is satisfactory.[36]

Another investigation using white barramundi (Lates calcarifer) fish scales to make hydroxyapatite powder as a bone graft material on rats with mandibular lesions demonstrated significant bone development radiographically and improved body weight, showing hydroxyapatite biocompatibility.[37]

Composite membranes

Fish collagen composite membrane

In comparison to chitin/chitosan and synthetic polymers, fish collagen exhibits superior biocompatibility, a high level of direct cell adhesion, minimal antigenicity, and a high degree of biodegradability.[13]

Without any further inducing factors, it has been discovered that hydrolyzed fish collagen increases the cell survival and osteogenic-related genes and proteins of human periodontal ligament cells. Fish collagen can be utilised alone as a barrier membrane, but it doesn't have enough mechanical or antibacterial qualities to be used in GTR/GBR operations, necessitating further study.[14] The membrane showed a fibrous surface with a decreased mechanical strength and an optimal porosity size, according to an invitro examination of its manufacture and characterisation for guided tissue regeneration. This implies the requirement for additional studies aimed at enhancing mechanical strength.[38]

The tensile strength of the GTR/GBR membrane, which is made of fish collagen, bioactive glass, and chitosan nanofibers, is 13.1 0.43 Mpa, which is higher than the tensile strength of pure fish collagen (6.72 0.44 Mpa).[38] Additionally, the membrane increased the expression of genes related to osteogenesis, such as RUNX-2, ALP, and OPN, demonstrating their potential for osteogenic differentiation of human periodontal ligament cells. Despite the addition of small amounts of bioactive glass and chitosan, the composite membrane still inhibits S. mutans adhesion

and growth when compared to membranes made entirely of fish collagen. In an animal trial, the composite membrane was able to encourage bone healing in beagle pups with furcation abnormalities.[39]

Chitosan composite membrane

Chitosan scaffold has been shown in a number of in vivo experiments to promote the production of alkaline phosphatase and osteopontin in proliferating human periodontal ligament cells as well as recruit vascular tissue formation. A chitosan-collagen membrane was discovered to be able to boost the number of fibroblasts and new blood vessels in the wound healing process in a study utilising rat mandibular lesions.[38] Chitosan preserved the morphology and space for bone repair in subcutaneous tissue in rats for six weeks. The appropriate degradation rate for chitosan to function as a barrier membrane depends on its molecular weight and processing techniques. In vitro experiments using the right concentrations can promote the growth of human periodontal ligament cells.[40]

Experiments using hydroxyapatite were undertaken to enhance the chitosan membrane's physical characteristics, and it was shown that a 4/11 weight ratio of hydroxyapatite to chitosan sol is ideal for the creation of composite membranes useful for guided tissue regeneration.[41] Electrospun poly(lactide-co-glycoside) and chitosan/poly(vinyl alcohol) nanofibrous composite membranes have been shown to enhance fibroblast growth.[42] The fish collagen-chitosan barrier for GTR therapy was reported to improve clinical outcomes with a low incidence of gingival recession and device exposure when a layer of chitosan was impregnated to a thin film of fish collagen for a GTR membrane in the infra bony defects.[43]

Chitosan scaffolds were created using the freeze-drying method, which allows for precise control over the scaffolds' pore diameters, and then examined for their capacity to regulate drug release. To efficiently manage the wound healing process through the modulation of particular cell proliferation, a dual drug-releasing biocompatible chitosan scaffold with growth factors, bFGF and TGF-1, was developed.[44]

By adding bio glass- nanoparticles into chitosan, the stiffness of the membrane increased along with extensibility in wet conditions and osteoconductive potential while immersing in the simulated body fluid. In contrast with pure chitosan membrane, human periodontal ligament cells have also increased cell proliferation and facilitated greater cell matrix mineralization both in human periodontal ligament cells and human bone marrow stromal cells[45]. More recently, a flexible composite foam containing chitosan and bioactive glass was prepared and with the addition of bioactive glass to the samples, the degradation rate was found to decrease. This is most likely explain by the hydroxyapatite mineralization, bioglass, and chitosan intermolecular interactions that were seen on the composite foams. Thermal examination showed that the degradation temperature of the composite samples had increased; this finding was supported by the results of XRD, FTIR, EDS, water uptake, and weight loss. Both materials had substantial porosity before and after degradation, according to SEM microscopy and micro-CT analyses. The fact that the chitosan/bioactive glass-based foams can combine regulated bioactivity and degrading behaviour is supported by this result, which is crucial for cell movement. The potential of matrices in tissue regeneration is thus demonstrated.[46]

Hydroxyapatite ceramics

The hydroxyapatite is brittle in nature. To overcome the shortcomings, various studies are being carried out. The osteogenic effects of nHA/Chitosan composite scaffolds have been studied in the past[47]. Few research recently in the realm of periodontal regeneration, have focused on mHA/Chitosan[48,49]. Salmon bone-based hydroxyapatite with fucoidan and chitosan was used to create a three-dimensional scaffold. The composite's pores ranged from 23 to 354 m, making it easy to add growth agents and nutrients.[50] When nanofibrous fish scales were introduced to a poly(3-hydroxybutyrate-co-3-hydroxyvalerate) composite scaffold, the mechanical properties, biomineralization propensity, cell survival, alkaline phosphatase activity, and type I collagen formation were all improved.[51] Without the use of exogenous inductive agents, the integration of hydroxyapatite nanoparticles (nHA) in the chitosan/gelatin/nanohydroxyapatite scaffolds can improve the deposition of nanocrystalline mineralized tissue within the cell-seeded scaffolds.[52]

Conclusion

Since sea is a huge reservoir of natural bio polymers, these raw marine sources which are cost effective, indigenous and easily available, have shown to be promising tool for periodontal regeneration and reconstruction. It has been discovered that fish collagen works just as well as animal collagen as a substance for periodontal regeneration. Bioactive compounds like chitosan and hydroxyapatite can be introduced into the collagen membrane to promote its biocompatibility, bacteriostasis, and angiogenesis qualities as well as its mechanical properties.Further, future studies are needed before commercialisation of such regenerative materials.

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Conflicts of Interest:

No conflicts of interest are disclosed by the authors.

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