GENERAL REVIEW

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The use of collagen membranes in guided tissue regeneration

Primena kolagenih membrana u vođenoj regeneraciji tkiva

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Introduction

Dental implants' rehabilitation success is defined by sufficient quantity of the jaw bone. Filling of bone defects with bone substitutes is a procedure of choice in order to maintain bone, but ingrowth of the connective tissue from mucoperiosteal part can compromise the coalescence process of the bone substitute with bone defects walls. Usage of membrane as a barrier is indicated as a solution for this problem [1, 2].

Guided bone regeneration (GBR), a method which originates from guided tissue regeneration (GTR), is based on a concept of dividing bone from soft tissue, i.e. preventing apical migration of the gingival epithelial and connective tissue inside the defect with a membrane as a barrier which favorites proliferation of regeneration-potent cells and their differentiation in the desired tissue type [3].

Five surgical objectives should be reached in order to achieve the goal of guided bone regeneration. This implies the following: using the appropriate membrane; reaching primary soft tissue healing; creating and maintaining a location protected by the membrane; adapting and stabilizing membrane with the surrounding bone; and enabling a sufficiently long healing period [4, 5].

According to Hardwick et al. [6], the main purpose of the membrane's barrier function is creating suitable surroundings in which the natural biological potential for functional regeneration is pushed to the maximum. Creating and preserving the location where a blood coagulum is placed, preventing inflammation which can occur as a result of bacteria penetration, isolating regeneration space from unwanted tissue, and ensuring mechanical stability and compactness of the organized coagulum are just a few of the most important factors for creation of a suitable place for regeneration. Their role in preventing permeation of epithelial cells in solid bone substitute applied, has to be mentioned, and also better fixation of the applied bone substitute [6, 7].

Membrane's features for GBR have been described by several authors [7–9].

They include: biocompatibility; appropriate barrier potency (mechanical prevention of soft tissue proliferation); tissue integration; immunological inertness; preservation of the location for new alveolar bone; and application simplicity.

Membrane must resist chewing force and cut tissue tension, and prevent collapsing of the soft tissue and narrowing the wound space. The ability to integrate into the tissue secures wound stabilization and epithelial migration inhibition [10].

Based on clinical and histological researches of different barrier materials so far, neither of them showed as an ideal one for every clinical situation, because each has its specific characteristics, advantages and limitations.

Depending on the reaction to their biological surroundings, membranes can be grouped as non-resorbable and resorbable.

Non-resorbable membranes keep structure and shape in tissues, and it is necessary to have another surgery in order to remove it; this increases trauma to the patient, the wound healing process, costs and duration of the whole treatment.

Resorbable membranes are not needed to be removed after placement, which reduces the inconvenience and cost of the treatment, and also the risk of surgical complications. Due to the resorbable membrane's nature, it is not possible to precisely determine duration of their degradation. The process of degradation begins immediately after the placement. Data from the literature regarding the desirable duration of membrane persistence *in vivo* show that it varies from 4 weeks

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to a few months [11]. Due to biological degradation, resorbable membranes induce tissue response, which may negatively impact wound healing and disturb regeneration.

The ideal bioresorbable membrane for GBR has the following characteristics: biocompatibility, the absence of inflammatory reaction, total resorption, degradation and elimination. It should be easy to handle, cut, contour and adapt, maintain desired shape and configuration, be easily secured in place, reliably exclude non-osseous tissues from defect, be resistant to bacterial attachment and colonization, have a predictable resorption time, compatible with bone formation [12, 13].

Two materials are mainly used to manufacture resorbable membranes: synthetic aliphatic polyester and collagen, derived from different animal sources, including bovine tendon, bovine dermis, calf skin, or porcine dermis [14–16].

Collagen membranes

The ability of collagen to stimulate adhesion, chemotaxis and physiological degradation of progenitor cells, together with the possibility of its own degradation, makes it an ideal material for building the membrane. Collagen is an insoluble fibrous protein that is an essential component of the connective tissue stroma. There are at least 16 types of collagen found in interstitial tissues, matrix of bone, cartilage, epithelial and blood vessel basement membrane and the vitreous of the eye, among others. Types I, II and III collagen constitute 80– 90% of the body`s collagen. Commercially available collagen products are composed mainly of types I and III collagen [17, 18].

Collagen has weak immunogenicity, induces hemostasis, and can augment tissue thickness; during healing of the wound occurs, an interaction between collagen and different types of cells [19, 20]. Collagen is made from animal skin, tendons or offal. First, it is isolated and purified with enzymes and chemicals, then processed in different forms. Most common chemical modification of collagen creates transverse connections, usually with exposure to aldehyde, which decreases absorption of water, influences ability to melt, degradation rate and increases firmness [21].

Collagen membranes are degraded by macrophages and polymorphonuclear neutrophils, and the absorption rate is different, based on the collagen source and modifications. Collagen membrane resorption begins with the activity of collagenase enzyme (matrix-metalloproteinase), which divides collagen molecule on specific position. Created parts are denatured and transformed into gelatin, which is then degraded to amino acids by gelatinase and other proteinase [22].

During enzymatic degradation, it will incorporate with in the flap to support new connective tissue attachment [20]. This may result in augmenting tissue/flap thickness to protect further bone formation.

Cross-linking of collagen: pro and contra

Structure stability increased by cross-linking slows down the degradation process. Cross-linking of collagen is achieved by ultraviolet and gamma rays, hexamethylene glutaraldehyde, diphenyl phosphorylase and ribose. Cross-linking is controlled and reduces *in vivo* the rate of collagen material resorption and increases mechanical characteristics. The essence of the process is building different mutual connections between specific amino-acids, and between aminoacids and carboxylate groups, under chemical and physical agents' influence [23].

There is a controversy arising from whether to apply cross-linked or non-cross-linked membranes in GBR. Although many studies have proved that with cross-linking the biodegradation of the collagen membrane is being expanded, and that they have shown positive, but limited effect on GBR in different types of experimental defect models [24 25], other studies have shown that their application associated with the initial reaction of foreign body, reduces tissue integration and with compromised trans membrane vascularization [26, 27]. Despite all the disagreements, it has been shown that membrane vascularization is being improved in 2 weeks after its submucosal implantation in rats by using certain procedures of cross linking. [28]. This is probably because the initial hyperemia is being caused in the neighboring tissue, which directs angiogenesis toward experimental membrane. In 2006 Schwarz et al. [28] examined the model of angiogenesis in natural and crossed-linked collagen membranes, because previous tests have shown that vascularization is weaker in cross-linked membranes. The conclusion was that angiogenesis in different types of membranes is without statistical significance.

In two studies done in the Military Medical Academy, defects covered with cross-linked collagen membranes showed a better level of vascularization in comparison with defects with non-cross-linked membrane or with empty defects [29, 30].

In 2012, Thoma et al. [31] studied the differences in cross-linked collagen, but instead of collagen membranes they used high and low degree collagen patterns, which have been chemically cross-linked and they have put them in the soft tissue of mice. Histopathologic and histomorphometric researches were performed 3 and 6 months after surgical intervention, and referred to the presence of tissue integration, collagen biodegradation and formation of new blood vessels. The results have shown that the level of crosslinking was in negative correlation with observed parameters, because collagen with lower level of crosslinking has shown better tissue integration, stability and angiogenesis.

All of these studies showed the importance of crosslinking. Despite few negative characteristics, many authors suggested that the use of cross-linked collagen membranes brought many benefits to GBR.

Exposure of collagen membranes

Several periodontal pathogens are capable to produce collagenase, an enzyme which can lead to premature membrane degradation. These are *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* [32]. Bacterial colonization may lead to early degradation of the collagen membranes, which can compromise the procedure. Both cross-linked and non-cross-linked membranes are being equally exposed to lysis

under the influence of bacterial proteases, although some studies have shown that cross-linked membranes are more resistant to proteolysis [32, 33]. Therapeutic concentrations of antibacterial and antibiotic agent, such as chlorhexidine, minocycline and doxycycline, partially inhibit the enzymatic membrane degradation.

Collagen membranes differ in their microarchitecture (space between collagen molecules, fibers, beams and layers within the membrane) and crosslinking.

Microarchitecture and cross-linking define membrane characteristics, such as tension power, easy manipulation, flexibility, tissue integration, biodegradation.

Membranes with a higher level of crosslinking remain intact for a longer period [34]. The studies have shown that premature membrane resorption or its removal can lead to incomplete bone healing, so it is advised that the membranes applied in GBR should have degradation period between 3 to 9 months, the time needed to achieve bone formation [4].

Biodegradation of collagen membranes

Rothamel et al. [26] studied biodegradation over time, the reaction to tissue, tissue integration and the vascularization of commercially available collagen membranes as well as those experimental, after being placed subcutaneously in 40 rats. Histological and histometric researches were performed 2, 4, 8, 16 and 24 weeks after placing the membrane. The conclusion was that the cross-linked collagens types I and III of bovine and porcine origin extend biodegradation, but reduce tissue integration and vascularization, and foreign body reaction appears which is characteristic for cross-linked membranes. This study shows the abovementioned differences between cross-linked and non-cross-linked membranes, proving that the non-cross-linked membranes have better vascularization and tissue integration, longer-lasting barrier role, slower degradation, but also that the cross-linked membranes have weaker tissue integration. The absorption rate directly correlated to the crosslinking degree – higher level of connection, longer resorption rate [27].

In 2006 and 2008, Tal et al. [15, 35] studied clinically and histologically the barrier function duration and biointegrity in places that have been treated by cross-linked and non-cross-linked collagen membranes. Special attention was given to the spontaneous mucosal perforations through the barrier membranes. It was shown that cross-linked membranes were more resistant to tissue degradation and that they maintained integrity in the longer period. Neither type of membranes was resistant to tissue degradation when being exposed. Exposure occurred more frequently in cross-linked membranes. However, a complete primary closure is essential to prevent early exposure.

The impact of membrane thickness on bone regeneration

So far, there has not been a lot of published researches on the impact of the resorbable membrane thickness on bone regeneration. The attempt of applying a thicker membrane was published in 2005 by Busenlechner and et al. [36]. The purpose of their study was to question the possibility of slowly resorbable prototype 3-layer membrane in bone regeneration during augmentation of the alveolar ridge after the extraction of the first and second molars in the lower jaw of a monkey, and after making the cavity three months after extraction. Experimental animals were sacrificed after 9 months. The study supports implementation of the slowly resorbable three-layer membrane, because the best achieved bone regeneration was made using this membrane and bone graft. The membrane was made by adding a polylactide layer between two layers of collagen in order to increase the degradation time and also the barrier's function. Polylactide fragments were found in histological examinations even after 9 months. The 3-layer membranes' design can be the important step in improving membrane stability with a specific exposure rate. In this study, it amounted 8.33%, which is extremely low compared to 43.75% recorded in the study done by Sculean et al. [37].

The same 3-layer membrane prototype was examined by von Arx et al. [38]. The aim of their study was to examine the three-layer membrane prototype in combination with a variety of materials for augmentation. Patterns were analyzed histopathologically and histomorphometrically after four and a half months. The 3-layer membrane prototype in combination with autograft showed the best bone regeneration.

In 2009, Kozlovsky et al. [39] made histological comparison of Bio-Gide® membrane biodegradation (non cross-linked collagen membrane) placed in one and two layers in mechanical defects created on rat's calvarias. Application of the second layer of Bio-Gide® membrane (double layer technique) resulted in a significantly greater residual amount of collagen, at least up to 9 weeks following surgery in rats. Also there was much more barrier material left in the bilayer membrane tissue, which indicates a longer-term barrier role of membranes, but also that monolayer membrane could not achieve barrier function in the long period of time. Therefore the bilayer membrane made better bone regeneration and defects ossification. It should be noted that the second layer achieves a reduction of micro movements and improves its stability. Transmembranous vascularization of the membrane was manifested histologically already 4 weeks following implantation and become well-defined through all layers of membrane 9 weeks following implantation. In spite of the difference in the thickness of 2-membrane preparations, similar degradation rate of 80% for both membranes was measured at 9 weeks. Since the transmembranous formation of blood vessels is essential for collagen resorption [28], it seems that vascularization of the double layer membrane was not impaired by its increased thickness. It has been claimed that increasing the density of cross links between collagen molecules has a negative effect on membrane biocompatibility [29, 40], membrane to tissue integration and vascularization, and inhibits attachment and proliferation of PDL fibroblasts and osteoblasts [5, 40]. Using a second layer of resorbable cross-linked membrane avoids these disadvantages, while extending membrane longevity. In the double layer 9 weeks membrane specimens, central intramembrane neo-ossification was clearly identified with collagen fibers embedded in the osteoid [41, 42].

The efficiency of bilayer membranes in bone grafts application, in terms of bone resorption, has been analyzed in a study on rabbits [43]. Bone blocks of parietal bones were taken from one side and placed on the other, and covered with membranes. Histological and histomorphometrical analysis were performed 2, 4 and 6 months after surgery. The results of the study show that the double membrane application decreases bone resorption of the graft significantly more in respect to the single layer one [41].

A study done in the Military Medical Academy examined the impact of collagen membranes of different origin and thickness on post-extraction ridge preservation. The results show that the best outcome was reached with application of thicker membranes [30].

The results of these few studies regarding the thickness of the membranes, show that membranes of greater thickness, whether they are arranged in several layers or are thicker, show greater barrier ability and remain for longer time in tissue, because they decompose slowly and enable better bone defect ossification. While this finding has never been fully understood, it may be speculated that the significant increase in membrane thickness and longevity results in increasing angiogenesis and cellular population of collagen matrix, leading to cell proliferation, differentiation and ossification.

Collagen membranes of human origin

Special attention should be given to resorbable collagen membrane of human origin. The role of the resorbable human demineralized membrane in GBR and GTRhas been insufficiently studied. There are a few experimental studies done in the Military Medical Academy, Belgrade. The authors examined the impact of thickness and origin of human resorbable membranes on bone regeneration. The resorbable human demineralized membrane (RHDM) was prepared by the combination of physical and chemical methods (demineralization of cortical bone with successive removal of lipoproteins) from human cadaver in calvarium region. These studies showed that RHDM proved a greater degree of bone regeneration compared to other membranes, especially the thicker one [43–46].

Disadvantages of collagen membranes

Compared to non-resorbable membranes, collagen membranes lack space-making ability. The use of bone graft material to preserve space tends to improve the outcome of GBR. Alveolar ridge augmentation can be expected only if the space under the collagen membrane is created and preserved in an appropriate period while the new bone is being formed. It is therefore advisable to use materials which will provide support as to prevent collapse of the barrier due to pressure of overlay issue or due to chewing forces [47].

These membranes are often used with tenting or supporting materials (different bone grafts or bone fillers) to prevent space collapse. When grafting materials are used with bioresorbable membranes, the results of GBR procedures are generally favorable and even comparable to the results achieved with non-resorbable barriers, especially in management of localized alveolar horizontal ridge defects [48–52]. Grafting materials alone seems to be less effective than the combination of a supporting material and a barrier. Combination of bioresorbable membranes and non-resorbable membranes with grafting material can achieve good results in treating vertical alveolar ridge defects because one of the main disadvantages of collagen membrane is disability to achieve vertical height of bone. In order to solve this problem, the mentioned combination was used. Membranes, in these cases, needed an extra-stabilization with mini screws and tacks [52–54].

Combination of membranes and growth factors

Lately, the incorporation of growth factors and differentiation in the membrane has also been explored. There is sufficient evidence that certain growth factors and similar mediators can influence regeneration of many tissues, among others, regeneration of bones. An example is the development of combined membranes, which would control release of transforming growth factor (TGF-β). The local delivery of a wide variety of growth factors, such as platelet-derived growth factors (PDGF) and bone morphogenetic proteins that are both osteoinductive growth factors, have been utilized in dentistry possessing capability to further stimulate cell recruitment, proliferation and differentiation. Numerous *in vitro*, animal and clinical trials have demonstrated the advantages of these growth factors in combination with membranes [55–62]. Such combinations could lead to major changes in the outcome of GBR.

Conclusion

This paper reviews the basic principles in membranes utilized in guided bone regeneration. Much advancement has been made since the original non-resorbable polytetraflouroethylene (e-PTFE) membrane was used. Synthetic and natural biomaterials have now been utilized in dentistry with great clinical success for over 20 years, and improvements are continuously being made regarding their mechanical properties and degradation rates.

The next generation of membranes is expected to combine more functional biomolecules projected to increase the success of GBR therapy.

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