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Advances in collagen, chitosan and silica biomaterials in oral tissue regeneration: from basics to clinical trials

Inés Alvarez Echazú\textsuperscript{1,2}, Maria Victoria Tuttolomondo\textsuperscript{1,2}, Maria Lucia Foglia\textsuperscript{1,2}, Andrea Mathilde Mebert\textsuperscript{1,2}, Gisela Solange Alvarez\textsuperscript{1,2}, Martin Federico Desimone\textsuperscript{1,2*}

\textsuperscript{1}Universidad de Buenos Aires.\textsuperscript{2} CONICET. Instituto de Química y Metabolismo del Fármaco (QUIMEFA). Facultad de Farmacia y Bioquímica. Junín 956 Piso 3. (1113), Ciudad Autónoma de Buenos Aires, Argentina.

ABSTRACT

Different materials have distinct surface and bulk characteristics; each of them potentially useful for the treatment of a particular wound or disease. By reviewing those materials that have reached a clinical stage the reader will have a broad panorama of the possibilities a particular material can offer, regarding its ability to support fast tissue regeneration. This review covers the most recent advances made towards the development of biomaterials aimed to support regenerative process. Indeed, we highlight key examples, from basic research to clinical trials, of biomaterials for a specific biomedical application. In this context, the focus is made on collagen, chitosan and silica which are key representatives of a protein, a polysaccharide and an inorganic material usually employed as biomaterials. Particularly, this review article presents an overview of their potential therapeutics in the treatment of disorders within the oral mucosa and tooth supporting tissues. Finally, it is highlighted the importance of \textit{in vivo} and \textit{in vitro} studies, clinical evidence studies, systematic reviews and meta-analyses as an adequate guidance for biomaterial design and development.
1. Introduction

Biomaterials have been developed over the last decades in order to stimulate wound healing without any side effects. A broad diversity of polymers, including natural based materials and synthetic ones were successfully applied in the design of biomaterials. Several ingenious design strategies from biological models have been appropriated into novel synthetic materials and structures for regenerative and material-based tissue engineering. The important physico-mechanical and chemical properties of various polymeric scaffold materials for corneal, skin, cartilage, cardiac and bone tissue engineering were recently discussed by Vashist and Ahmad. The simultaneous variation of several composition-dependent properties announces an unparalleled technological world of multifunctional devices, where it is possible to produce 2-dimensional as well as 3-dimensional supports and also address the response of cells from different origins. The present review focuses on materials for application within the oral mucosa and tooth supporting tissues. For this purpose, the particularities and components of these tissues are presented along with the characteristics of the employed materials and results obtained in vivo/in vitro together with recent clinical trials. Particular emphasis is placed on those systems developed in recent years (>70% of references have been reported since 2012).

The oral mucosa has not received enough attention due to the low morbidity associated to the pathological processes that affect it. Yet, the psychological impact of an underlying illness on the patient’s life quality should not be left aside. As an example, periodontal disease and its consequences, which include tooth mobility, gingival recession and bad breath, has been related to a diminished life quality. Subsequently, a study undertaken by Fisher and collaborators showed an improvement in the life quality of 61 patients with periodontal disease who were subjected to implant procedures, which in some cases included soft tissue augmentation and guided bone regeneration.
Additionally, soft tissue disease of the oral cavity is very often related to bone loss, which ultimately leads to tooth loss, a process which cannot be reversed. Vaccination strategies to engender protective mucosal immune responses had been identified as an alternative strategy.\(^{13}\) Moreover, dental implants could potentially improve the aesthetical and functional aspects of tooth loss, though the procedure is not always linked to success and in many cases implant rejection occurs. Until now, a single model has not been able to completely recreate anatomic, physiologic, biomechanic and functional environment of the human mouth and jaw.\(^{14}\) As a consequence, a complete understanding of the structure and function of the oral mucosa as well as tooth supporting tissues, the underlying pathologies that affect them and the available treatments are necessary for the design of biomaterials aimed to sort out the problems associated to the existing ones.

### 2. Oral mucosa and tooth supporting tissues

Much is known about the skin structure and physiology. The oral mucosa is not very different from it, though there are some particularities that make it unique. To start with, one noteworthy characteristic is its capability to heal without a scar and at a faster speed than injured skin. The difference in the expression of some extracellular matrix components such as procollagen I and tenasin-C, as well as the reduced presence of immune cells and profibrotic mediators induced by oral tolerance, may be responsible for it.\(^ {15}\)

Histologically, the oral mucosa is composed by a stratified squamous epithelial layer, a basal lamina, a lamina propria and a submucosal layer, the latter in contact with bone or with muscle depending on the area. Keratinization of the epithelial layer provides extra protection against mechanical forces associated with mastication as in the case of the hard palate and gingiva. On the other hand, when more flexibility is required the epithelium remains non keratinized.\(^ {16}\) Additionally, it is interesting to note that the mucosal epithelium is thicker than
that of the skin and its thickness has been positively related to a higher blood flow, probably
due to a higher metabolic demand.\textsuperscript{17}

The oral cavity mucosa can be divided in gingival mucosa, found in hard palate and gum,
lining mucosa, observed in lips, cheeks, soft palate and finally, specialized mucosa,
constituted by lingual papillae and gustatory corpuscles.

The gingival mucosa, along with the junctional epithelium constitutes the gingival tissue,
which is firmly attached to the alveolar bone and teeth. This gingival tissue, together with
periodontal ligaments and alveolar bone provides the necessary support for teeth. The
periodontal ligament, a fibrous connective tissue, has regions of loose and dense connective
tissue where fibroblasts and collagen fibers can be found.

The periodontium, which in latin means “around the tooth” not only refers to gingival
mucosa but also to the cementum, periodontal ligament and alveolar bone.

The presence of salivary glands accounts for another characteristic within the oral cavity: the
presence of saliva, lots of it all over the mucosal surface. Saliva is a complex secretion, even
though it is composed of a 99% of water and a 1% of a variety of organic and inorganic
molecules whose function is to maintain the overall oral health through antimicrobial
protection, lubrication, dilution and buffering.\textsuperscript{18, 19} Among the organic compounds we can
find glycoproteins, enzymes, immunoglobulins and some peptides with antimicrobial activity.

On the other hand, the inorganic fraction contains sodium, potassium, chloride and
bicarbonate in concentrations that render saliva hypotonic when compared to other body
fluids.\textsuperscript{20} Saliva itself as well as gingival secretions provide the nutrient foundations for the
microbiota colonizing the oral mucosa, the members of which may change due to dietary
habits and underlying diseases.\textsuperscript{21}

3. Immune and inflammatory response in oral tissues
The oral mucosa is a physical barrier for pathogens and other harmful environmental agents. Innate immune cells like macrophages, polymorphonuclear leukocytes and immunoinflammatory mediators have an important role in the oral immune response. Nevertheless, oral keratinocyte-derived biologic mediators, salivary secretory immunoglobulin A and gingival crevicular fluid components are also relevant in the understanding of the oral cavity mucosa immunity. Oral cavity mucosa immunity intends to limit pathogenic microorganisms colonization, generate immunoinflammatory responses against pathogens and mediate tolerance of commensal microorganisms.22

In case of mucosa injury, three distinct but overlapping stages can be observed: i) inflammation, ii) proliferation and iii) remodeling. Inflammation takes place in a matter of minutes and involves the activation of the coagulation cascade and the immune system in an attempt to stop the bleeding, prevent infection and remove cell debris. Platelet activation results when the endothelial layer is disrupted and the collagen from the underlying matrix is exposed. Collagen interacts with platelets and von Willebrand factor to induce the activation of the coagulation cascade, leading to the formation of a fibrin mesh constituting the platelet plug, which serves as a scaffold for the migrating cells and prevents further bleeding, resulting in haemostasis.23 Neutrophils are the first cells to arrive to the injured site as their recruitment is initiated by changes in the endothelium in response to inflammation mediators. Although considered proinflammatory in nature, neutrophils themselves play a major role in healing by removing dead cells and bacteria, which could perpetuate the inflammation response in time. Furthermore, neutrophils secrete various proteases such as MMP9, an enzyme which in turn activates VEGF to promote revascularization of the injured site, as well as several signals which lead to the recruitment of monocytes and their extravasation to the wounded area. The recruited monocytes are then activated and responsible for the phagocytosis of apoptotic neutrophils and foreign material.24 When biomaterials are tested in
in vivo the presence of macrophages is often seen as a menace. The truth is that macrophages are necessary during the healing process and two distinct phenotypes have been recently associated with it, namely M1 and M2 phenotypes. Hence, macrophages have been shown to transition in vitro from a proinflammatory or M1 to a reparative phenotype or M2, the latter characterized by the expression of anti-inflammatory mediators. Such transition is though not completely understood, and both types might coexist or even hybrid macrophages may be found in vivo, therefore suggesting that it is the ability to transition and the balance between both populations what drives proper wound healing. Indeed, macrophages are one of the most important cells present in the last stages of the inflammatory process (48-72 hours), responsible for the progression into the proliferative phase. During the proliferation stage, which lasts up to 2 weeks, fibroblasts are recruited and stimulated to proliferate along with the sprouting of new vessels, leading to the formation of the highly vascularized granulation tissue. Additionally, fibroblasts differentiate into myofibroblasts and promote wound contraction. Finally, the last stage, the remodeling phase, is reached, during which new collagen is deposited and remodeled in order to restore the normal tissue architecture.

Another interesting feature of the healing process within the oral mucosa is the final result, as wounds tend to heal in a scarless manner, similarly to what occurs in fetal skin, a process that has been associated to the differential expression of certain extracellular matrix components. As an example, fibronectin, fibronectin ED-A, and chondroitin sulphate were more expressed in the oral mucosa when compared to the skin, a pattern similar to that found in a previous study when human fetal and adult skin were compared. Additionally, oral wounds display a lower number of immune cells such as neutrophils and macrophages. Fibroblasts, on the other hand, are thought to be important players determining the fate of oral wounds, as they are able to proliferate at a higher rate and contract more efficiently than their dermal counterparts.
In short, periodontal healing and regeneration need different sequential processes. First, there is a clot/vascular phase; secondly, an inflammatory and granulation phase is set. Afterwards, angiogenesis and bone mineralization occurs, typical of periodontal regeneration phase. Finally, periodontal remodeling and stability phase is established.  

4. Periodontal disease

Periodontal disease is a leading cause of tooth loss, very commonly associated to risk factors such as diabetes mellitus, obesity, metabolic syndrome and osteoporosis, among others.\textsuperscript{31} The interaction between pathogenic microorganisms and the host immune system triggers, under certain circumstances, an inflammatory response causing the gums to become swollen and bleed easily. This early stage is also known as gingivitis, the mildest form of periodontal disease. When left untreated, plaque biofilm spreads and continues to invade the gingival tissue, causing further irritation of the gums leading to the chronic stage known as periodontitis. At cellular level, dental plaque generates a local inflammatory response which involves the secretion of proinflammatory factors, such as cytokines and reactive oxygen species, as well as the deregulation of matrix metalloproteinases (MMPs).\textsuperscript{32} Among these, MMPs are one of the most important host factors responsible for collagen and extracellular matrix degradation involved in periodontal disease.\textsuperscript{33} As it progresses, cytokines, such as IL-1, and RANK-L promote bone osteoclast resorption.\textsuperscript{34} Gradually, damage in supporting tissues leads to gingival recession and dental pockets formation. Subsequently, alveolar bone absorption and destruction of periodontal ligament and cementum take place, constituting the main cause of tooth loss in adults.\textsuperscript{35}

Oral cavity molecular and cellular events from inflammatory and immune response mechanisms initially respond “normally” to subgingival biofilm. Nevertheless, subgingival
biofilm bacterial products and patient’s risk factors encourage periodontal disease development.

Periodontal disease treatment differs according to the stage of the disease. Systemic antibiotics, antimicrobial oral delivery devices, scaling and root planning represent examples of different clinical strategies. In the case of chronic periodontitis, a clinical approach consists in guided tissue regeneration.

Despite all available treatments, periodontium regeneration is a challenge in odontology. Early diagnosis of gingivitis along with the implementation of an adequate oral health still constitute the best prognosis for patients at risk of developing periodontal disease. However, current knowledge of the underlying causes of gum disease provides the basis for the rational design of new materials aimed to stop its progression and aid in the regeneration of the affected tissue.

5. Materials in oral health

A biomaterial is any matter, surface, or construct that interacts with living systems. Biomaterials can be obtained from nature or synthesized in the laboratory using a variety of different approaches (metallic components, polymers, ceramics or composite materials). In addition, biomaterials are widely used every day in dental applications, surgery, and drug delivery.

Biomaterials for tissue regeneration possess some basic characteristics such as: i) suitable microstructures and mechanical properties, ii) proper surface topography and chemistry, iii) biodegradability and non-cytotoxic degradation products, iv) simple and cost effective manufacturing technology. Moreover, these materials must provide a specific
microenvironment including unique physical structures, special chemical composition, surface properties, and biosignals to direct cell behavior.

Natural biomaterials such as collagen, gelatin, fibrin, chitosan and hyaluronic acid have been widely used for in situ tissue regeneration. In general, natural biomaterials have excellent biocompatibility and biodegradability properties. Natural biomaterials can mimic many features of the extra cellular matrix and the recognition sites they carry are beneficial for cell behavior.

Dental materials have always been a useful tool in dentistry. Their use is related with orthodontics, restorative purposes, endodontics, even esthetics. The improvement in clinical outcomes has shown their advantages in dental practice.

Dental materials involve several types of materials such as metals, ceramics and organics. Each type of material with its inherent characteristics reflects different mechanical, optical and biological properties. These differences among others are considered in the selection by the dentist for oral diseases treatment.

Dental materials have evolved due to the patient's needs and the growing knowledge in biomaterials and oral pathology (Fig. 1). They are in continuous development in order to increase their quality and biocompatibility as well as decrease toxicity. (Fig. 2).

As mentioned above in this review, the focus is made on collagen, chitosan and silica which are key representatives of a protein, a polysaccharide and an inorganic material usually employed as biomaterials.

Abundant naturally occurring polymers such as starch, collagen, gelatin, alginate, cellulose and chitin have been reported and used in tissue regeneration. Nowadays silica, collagen and chitosan have gained great interest in oral health because of their several clinical applications. In odontology, organic materials reinforced with silica, have improved their mechanical properties. Moreover, in the last decade the use of polymers and mesoporous
silica materials as efficient drug delivery carriers has attracted great attention.\textsuperscript{45, 46} Chitosan and collagen have been considered as potential scaffolds for tissue regeneration as well.

### 5.1. Collagen

Collagen is the most abundant protein of the extracellular matrix (ECM) naturally present in human tissues (e.g. skin, bone, cartilage, tendon and ligaments). It represents 25\% of the total protein body content, providing strength and integrity to tissue matrices. In addition, collagen can also interact with cells and help essential cell signaling that will regulate cell anchorage, migration, proliferation, differentiation and survival.\textsuperscript{47-50}

Twenty-seven types of collagens have already been identified, with types I–IV being the most common. Type I collagen is the most abundant protein present in mammals and the most studied protein for biomedical applications. Collagen is enzymatically degraded within the body, mostly via collagenases, gelatinases and metalloproteinases. In general terms, collagens are triple helix proteins that present high mechanical strength and good biocompatibility. Collagen can form stable fibers and its mechanical, degradation and water-uptake properties can be further enhanced by chemical cross-linking (i.e.: using glutaraldehyde, genipin, carboiimide along other)\textsuperscript{51-53} or by physical cross-linking (i.e.: using freeze-drying) or by the formation of hybrids and composite materials (Fig. 3).\textsuperscript{54, 55} Low inflammatory and cytotoxic responses and biodegradability are other attractive properties of collagen. As a result, and since collagen is one of the major components of human ECMs, it is usually considered as an ideal biomaterial for tissue engineering and for wound dressing applications (Fig. 4).\textsuperscript{56, 57} Collagen is usually isolated from animal tissues. However, enzymatic purification techniques (to eliminate those immunogenic telopeptides that induce foreign body response) may be employed.\textsuperscript{58}
Naturally and synthetically produced collagen matrices as well as more sophisticated three-dimensional collagen scaffolds provide cues at nano-, micro- and meso-scale for molecules, cells, proteins and bulk fluids.\textsuperscript{59} Collagen mimics periodontal soft tissue. For this reason, collagen can enhance biological sealing and osseointegration in dental implants and promote tissue reconstruction in severe periodontitis, characterized for gingival recession and dental pockets formation. Sometimes collagen scaffolds are combined with hydroxyapatite, fibrin, among others, for many purposes.\textsuperscript{60, 61}

5.2. Chitosan

Chitosan is a natural, cationic amino polysaccharide linear copolymer of D-glucosamine and N-acetyl-D-glucosamine, which is obtained by alkaline deacetylation of chitin. The term chitosan is also usually employed to describe a series of chitin derivatives having different degrees of deacetylation. The chemical, physical and biological properties of chitosan are directly related to its deacetylation degree and to its molecular weight\textsuperscript{62, 63} and chitosan is generally regarded to be biodegradable, biocompatible, non-antigenic, non-toxic, bioadhesive, anti-microbial, bioactive and to have hemostatic effects.\textsuperscript{64-66} Indeed, lysozyme, a saliva enzyme can degrade chitosan. This biodegradation, influenced by chitosan degree of acetylation value is relevant for the design of biodegradable oral delivery devices.\textsuperscript{62} Biodegradable oral delivery devices have demonstrated better patient compliance and acceptance. In addition, chitosan amino and hydroxyl groups can be chemically modified, thus allowing a high chemical versatility.\textsuperscript{58, 67}

Chitosan can be easily processed into hydrogels (Fig. 5)\textsuperscript{68}, membranes\textsuperscript{69}, nanofibers\textsuperscript{70}, beads\textsuperscript{71}, micro/nanoparticles\textsuperscript{72}, scaffolds\textsuperscript{73} and sponges\textsuperscript{74} for various types of biomedical applications such as drug delivery, wound healing and tissue engineering\textsuperscript{42, 75}. Chitosan provides a non-protein matrix for 3D tissue growth and activates macrophages for
tumorcidal activity. It stimulates cell proliferation and histoarchitectural tissue organization. Muzzarelli et al., reported that chitosan and ascorbic acid can be used together in the form of a gel suitable for application in the oral cavity presenting a number of biological advantages useful for the stimulation of an ordered regeneration of wounded tissues. Indeed, few days after the chitosan treatment, it was still possible to notice the presence of this macromolecule showing a tridimensional honeycomb organization. This appearance is a functional condition required for the stimulation of the tissue reconstruction (Fig. 6). Chitosan is a hemostat, which helps in natural blood clotting and blocks nerve endings reducing pain. Chitosan will gradually depolymerize to release N-acetyl-β-D-glucosamine, which initiates fibroblast proliferation, helps to promote ordered collagen deposition and stimulates increased levels of natural hyaluronic acid synthesis at the wound site. It helps in faster wound healing and scar prevention. It is also easily degraded by chemical hydrolysis as well as by certain human enzymes. Biomaterials based on chitosan, are natural and biodegradable. They present good features for guided tissue regeneration and local drug delivery. Local drug delivery, permits lower systemic toxicity and sustained drug concentrations. For instance, antioxidants, growth factors and antimicrobial agents are chosen for oral local delivery.

5.3. Inorganic materials: silica

Silica, an example of a ceramic material, is widely used in dentistry. Bioactive ceramic materials promote bone formation, a key point in diseases like periodontitis, in which the inflammation and immune response causes bone and periodontal tissue destruction. Additionally, silica composites are usually employed as dental composite fillers. However, all these bioceramics are brittle, and for this reason their main application for years has been as a grafting material for the filling of small bone defects and periodontal anomalies.
Silicate-based bioceramics, as a new family of biomaterials, have proven to be excellent materials for bone tissue regeneration, and their preparation, mechanical strength, apatite mineralization and biological properties have been comprehensively reviewed.\textsuperscript{80-85} Briefly, in the early 1970s, Hench and his colleagues developed a new class of biomaterials, $\text{SiO}_2$–$\text{CaO}$–$\text{Na}_2\text{O}$–$\text{P}_2\text{O}_5$ glasses.\textsuperscript{86} One of the significant characteristics of Ca-Si-based bioactive glasses is that they can induce the formation of a hydroxyapatite layer or hydroxyl carbonated apatite on their surface similar to the mineral phase of bone in simulated body fluids. This class of Ca–Si-based glasses is able to osseointegrate with host bone. Further studies have also shown that the Ca- and Si-containing ionic products released from the 45S5® contribute to its bioactivity, as both Ca and Si are found to stimulate osteoblast proliferation and differentiation \textit{in vitro}.\textsuperscript{87} Results indicated that Ca and Si ionic products from bioglass might increase IGF-II availability in osteoblasts by inducing the transcription of the growth factor and its carrier protein and also by regulating the dissociation of this factor from the binding protein. The unbound IGF-II was likely to be responsible for the increase in cell proliferation observed in cell culture experiments. Similar bioactive induction of the transcription of extracellular matrix components and their secretion and self-organization into a mineralized matrix might be responsible for the rapid formation and growth of bone nodules and differentiation of the mature osteocyte phenotype in the presence of bioglass.\textsuperscript{88} $\text{SiO}_2$ is a documented differentiation promoter and its incorporation improved adhesion, proliferation, differentiation, and mineralization of MG63 osteoblasts (\textbf{Fig 7})\textsuperscript{89, 90}.

To date, ceramic scaffolds present important advantages compared to polymeric scaffolds, such as suitable porous structures and chemical textures that promote mesenchymal stem cells differentiation and mineralization of the extracellular matrix, as well as the lack of toxic by-products found in several polymeric materials.\textsuperscript{91} The possibility to synthesize bioceramic materials with a variety of methods, such as sol–gel, gives the opportunity of developing
materials with controlled porous structure, morphology and in many cases biocompatible.\textsuperscript{92-96} The coating of bioceramic scaffolds with biodegradable polymers has proved to impart functional properties to the scaffolds surfaces, as well as to improve scaffolds’ mechanical properties. Some examples include gelatin,\textsuperscript{91} where the results obtained by Gouduri et al., show that the pores of the scaffolds were completely interconnected, while the SEM microphotographs presented a pore size of about 200–400 mm and strut thickness of about 150–200 mm, considering that a scaffold should have an interconnected porous structure with pore sizes between 300 and 500 mm for cell penetration, vascularization, and nutrient and metabolic waste transportation, the strategy proved to be suitable.\textsuperscript{91} Silica can not only be used as scaffold, but also in the form of nanoparticles to create fillers.\textsuperscript{97, 98} The properties of nanoparticles clearly differ from those of their corresponding bulk materials.\textsuperscript{99, 100} The solubility and reactivity of nanoparticles are significantly increased because of their high surface energy and large surface area.\textsuperscript{101} This large surface area provides a high affinity and allows them to easily deposit on irregular spaces. Inspired by the properties of calcium phosphate precipitation (CPP), silica, and nanomaterials, Tian et al., used mesoporous silica nanoparticles with the addition of Ca\textsuperscript{2+} and PO\textsubscript{4}\textsuperscript{3-} for dentinal tubule occlusion.\textsuperscript{102} Their results showed good mechanical plugging and remineralization.

Diatomite particles, basically porous silica nanoparticles, have also been used to improve the mechanical properties of dental fillers. In 2011 Wang et al., studied the benefits of combining mesoporous and nonporous silica particles as fillers. Their results show that the advantage of using co-filling particles in hybrid-type composites is that nanoparticles can “fill into” the regions between the microparticles. Therefore, the packing density of the inorganic skeleton is increased, leading to a larger mass fraction of fillers and reduced polymerization shrinkage. As the authors point out, porous diatomite fillers can be considered as an important factor for enhancing mechanical properties and filler content of dental resin composites. On the other
hand, the authors noted that diatomite particles have an abundance of impurities, which may block the pores and affect the esthetics and mechanical properties. Therefore, these samples should be purified before use.\textsuperscript{103}

One thing to take into account when dealing with oral implantations is the potential bacterial colonization of the mentioned implants that in the end leads to the loosening of the implant and, in severe cases, to its loss. In order to overcome this drawback, Massa $et\, al.$, prepared an antibacterial coating on the titanium implant surface using silver nanoparticles embedded on a silica coating using a sol-gel approach. The coating produces a strong antibacterial effect on the titanium surface by killing the adherent bacteria and inhibiting biofilm formation. The long-term antibacterial activity was tested against \textit{Aggregatibacter actinomycetemcomitans} and the long term results indicate that this type of nanoscale surface modification is a promising strategy to control infections associated with dental implants.\textsuperscript{104}

6. \textit{In vitro} and \textit{in vivo} periodontal tissue regeneration

6.1. Collagen based biomaterials

Collagen, an example of biodegradable material, presents several advantages due to its hemostatic and fibroblast chemotactic properties. Collagen easy manipulation and alveolar topography adaptation are also benefits.\textsuperscript{105}

Marin-Pareja $et\, al.$, investigated collagen-functionalised titanium surfaces. Collagen was immobilised on the titanium surface by two different methods: (1) physical adsorption on the plasma orpiranha-treated surfaces (the samples were coded as PL-col or PH-col, respectively); and (2) covalent bonding through a silanisation process of the previously plasma or piranha-treated surfaces with 3-chloropropyl-(triethoxy)silane (samples coded as PL-CP-col and PH-CP-col, respectively). A significant over expression of fibroblast activation genes and extracellular matrix remodeling in collagen-coated surfaces was
observed. Their results also showed that collagen favors primary human dermal fibroblast proliferation and adhesion (Fig. 8).\textsuperscript{106}

In parallel, the gingival connective tissue response to 3-dimensional collagen type I nanofiber-coated titanium dental implants was studied. Dental implants were coated with collagen nanofibers by an electrospray deposition technique. Posterior scanning electron microscopy images revealed uniform collagen fibers deposition. Implantation procedure was performed, and histological evaluation revealed that gingival connective tissue attachment ratio was significantly higher in the case of collagen-coated nanofiber implants compared to titanium implants.\textsuperscript{107}

Moreover, intrafibrillar-silicified collagen scaffolds have shown that human dental pulp stem cells proliferation, osteogenic differentiation and mineralization rates were higher when compared to nonsilicified collagen scaffolds \textit{in vitro} and \textit{in vivo}. Li-na Niu et al., \textit{in vitro} studies evaluated cell viability, osteogenic related genes, alkaline phosphatase activity and mineralization. \textit{In vivo} results in 5 week-old male nude mice demonstrated calcium deposition through alizarin red staining and osteocalcin expression after 8 week scaffold ectopic subcutaneous implantation (Fig. 9)\textsuperscript{108}.

Kim et al., observed various differentiation properties in human dental pulp cells according to the kind of natural scaffold material. Chitosan, gelatin and collagen type I and III was evaluated. Cell growth within a period of 36 hours on chitosan was lower than on collagen and gelatin. Human dental pulp cells alkaline phosphatase activity (ALP) was measured in differentiation-inducing and control medium. On collagen scaffolds, higher ALP activity was observed when compared to gelatin and chitosan. Cells grown on collagen showed a mineralized extracellular matrix stained with alizarin red S staining after 30 days.\textsuperscript{109}

In an experimental animal study, following the extraction of the distal roots of the mandibular second and fourth premolars of four dogs, the sockets were preserved using a combination of
a collagen membrane intimately covering the socket plus a collagen matrix or a collagen membrane alone. After 5 months of healing, the histological analysis revealed a similar picture of bone formation in both groups. However, the mucosa covering the alveolar ridges was significantly more abundant in post-extraction sockets preserved with the double-layered approach. Thus, the collagen matrix associated with a collagen membrane could be a clinical option to preserve post-extraction ridges. Similarly, the quality of early healing processes developed in the former sockets preserved with a collagen matrix alone or associated with a bone substitute in comparison with naturally-healed sockets, using an animal model was also reported. In this work, the bovine bone substitute seemed to delay hard tissue development.

Vignoletti et al., employed 12 mini pigs to histologically evaluate the healing of a xenogeneic collagen matrix used to augment the width of keratinized tissue around teeth. The tested collagen matrix demonstrated uneventful healing, being resorbed within the surrounding tissues in absence of significant inflammation. Additionally, growth factors from plateletrich plasma (PRP) and platelet-rich fibrin (PRF) enhance the multiplication and recruitment of osteocompetent cellular strains, promoting alveolar bone restoration. These glycoproteins are often included in bone grafts. Growth factors local concentration promote tissue regeneration and wound healing. In this sense, the addition of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) to a chemically cross-linked collagen matrix and a non-cross-linked collagen matrix influenced tissue integration, angiogenesis, and matrix degradation. Indeed, it was observed that the compact layer (in non-cross-linked collagen matrix) delayed angiogenesis and connective tissue formation, while the spongy cross-linked collagen matrix facilitated early vascularization and demonstrated network presence over a longer time.

6.2. Chitosan based biomaterials
Chitosan biomaterial has gradually been considered for oral diseases treatment. In addition, because of its antibacterial properties, it has been introduced in dental area. Indeed, scaffolds, nanoparticles and composites are being taken into account as alternatives.

Mota et al., described a chitosan/bioactive glass nanoparticles composite synthesized by solvent casting. Authors analyzed metabolic activity and proliferation in periodontal ligament cells and in adult human bone marrow cells. Metabolic activity was higher in chitosan combined with bioactive glass membrane for both type of cells. Composite membranes showed a higher content of calcium in comparison to chitosan membranes.

Hernández et al., performed an in vitro study in order to investigate mesenchymal stem cells proliferation obtained from human gingival tissue on a chitosan scaffold. Proliferation was confirmed by cristal violet staining and light microscopy. However, it was observed that chitosan scaffolds lost their physical properties in cell media. Nevertheless, chitosan evidenced good proliferation signs for tissue regeneration.

Zang et al., synthesized a thermosensitive chitosan hydrogel for tissue regeneration. DAPI staining revealed human periodontal ligament cells infiltration and proliferation on chitosan hydrogel. Alternatively, scaffolds were implanted in male mixed breed dogs with class III furcation defects. After twelve weeks, histological analysis evidenced the enhancement of periodontal tissue regeneration.

In addition, a carbon fiber-reinforced polyetheretherketone/nanohydroxyapatite ternary biocomposite functionalized by covalently grafting carboxymethyl chitosan, followed by the decoration of a bone-forming peptide assisted via the polydopamine tag strategy was recently reported. Antibacterial test with Staphylococcus aureus indicated that the substrates significantly suppressed bacterial adhesion. In vitro assays demonstrated that it disclosed greatly accelerated adhesion, proliferation and osteo-differentiation of human mesenchymal stem cells (hMSCs) (Fig. 10). Chitosan development is not as advanced as collagen in dentistry. Nevertheless, many
accomplishments have been achieved in the area, leading to the possibility of its implementation in dental practice in a future.

6.3. Inorganic materials

Osseointegration is a vital issue for dental implants. Tooth loss, a consequence of gingivoperiodontal diseases and caries includes dental implants for its treatment. They intend to preserve patient masticatory and phonetic functions. Osseointegration is dismissed in severe periodontitis, where alveolar bone and other periodontal tissues are damaged because of inflammatory and immunological mechanisms. In these patients, bone regeneration before dental implants placement is necessary. In the case of osteoporosis and trauma, bone regeneration is also part of their treatment.

Autologous, synthetic grafts and allografts are widely used in dentistry for this procedure. Examples of synthetic grafts are silica, hydroxyapatite and calcium phosphate. Although autologous bone is considered as the "gold standard", bone substitutes are suggested in order to avoid a second surgery. Synthetic grafts present the advantage to decrease the possibilities of infection and immunological rejection.

Moreover, nano-bone graft materials are emerging in the bone regeneration field. They should be osteoconductive, highly porous, synthetic and nanostructured.

Hydroxyapatite structure and composition is similar to natural bone mineral. Bone is an organic-ceramic composite. Bioactive glass, a SiO$_2$-based glass, shows fairly mechanical strength and bioactivity. These ceramic-based materials have extensively improved the regenerative periodontal strategies. Ceramics provide stiffness and osteoconductivity to regenerative periodontal scaffolds.

Silicate glasses favor angiogenesis and osteogenesis. For this reason, they constitute a remarkable option for bone tissue engineering. Bioactive glass ion dissolution products are
involved in the activation of genes which are associated with vascularization and bone
formation.\textsuperscript{129} Moreover, sol-gel nanosilica represents an alternative to be applied for bone
substitutes or cements in restorative dentistry.\textsuperscript{130}

Considering bioactive glass advantages, El-Fiqi \textit{et al.}, synthesized osteoinductive scaffolds of
biopolymer and mesoporous bioactive glass nanocarriers. The electrospun fibrous scaffolds
of polycaprolactone-gelatin incorporating mesoporous bioactive glass nanoparticles (mBGn)
were proposed to be excellent matrix platforms for bone tissue engineering. After 10 days
incubation with rat periodontal ligament stem cells (rPDLSCs), these scaffolds with different
concentrations of mesoporous bioactive glass nanospheres (0-2.5-5-10\% mBGn) showed
good cell compatibility by SEM and confocal laser scanning microscopy techniques (\textbf{Fig.
11}). These results are particularly interesting because rPDLSCs play a major role in bone and
ligament formation. So, they could be a good alternative for guided bone regeneration and
dental pockets reconstruction.\textsuperscript{131}

Moreover, it was observed that nano-hydroxyapatite increased the expression of bone
morphogenic protein-2 (BMP-2), a protein with osteoinductive properties in human
periodontal ligament cells for 72hs. Measurements of calcium and phosphate extracellular
concentration remained constant. These results indicated that probably nano-hydroxyapatite
effect on BMP-2 expression was not influenced by Ca\textsuperscript{2+} and PO\textsubscript{4}\textsuperscript{2-} concentrations.
Consequently, nano-hydroxiapatite may play a role in human periodontal ligament cells
differentiation.\textsuperscript{132}

\textit{Wu et al.}, fabricated electrospun fibrous scaffolds combined with hydroxyapatite. They
analyzed periodontal ligament cells morphology, proliferation and differentiation on
collagen-poly (\varepsilon\text{-}caprolactone) with and without hydroxyapatite scaffolds immersed in
simulated body fluid solution. Scaffolds combined with hydroxyapatite showed good
biocompatibility and osteoinductive ability.\textsuperscript{133}
As mentioned before, an equally important ceramic material used in odontology besides hydroxyapatite and silica is calcium phosphate. It has great tissue biocompatibility. For example, tricalcium phosphate (TCP) is sometimes used as a carrier because of its bioabsorbability.\textsuperscript{134}

Calcium phosphate cements (CPC) have many craniofacial and periodontal applications. Neira \textit{et al.}, have successfully reinforced CPC with different hydroxyapatite crystals. Besides material characterization, they carried out mechanical and culture cell tests. Cytocompatibility in murine preosteoblastic cells (MC3T3-E1) was performed by MTT assay and cell differentiation was determined by ALP enzyme assay (\textbf{Fig. 12}). CPC and CPC-15% hexagonal prism like HA (one of the strongest composites) showed good cytocompatibility. In the case of this kind of composites, HA promoted early pre osteoblast cell differentiation.\textsuperscript{135}

Provided calcium phosphate cements present good features for periodontal bone regeneration, Y. Xiao \textit{et al.}, hypothesized that a macroporous calcium phosphate scaffold with growth factors and collagen would present better periodontal regenerative performance. For this purpose, different composites were implanted in beagle dogs. A histological analysis was carried out along with the determination of degradation ability and osteogenesis by micro computed tomography (micro-CT) scan. Results illustrated that macroporous calcium phosphate scaffold with growth factors and collagen degradation ability was largely increased and osteogenesis was encouraged.\textsuperscript{136}

As has been noted, silica, calcium phosphate and hydroxyapatite have good perspectives for oral tissue regeneration. Considering this, Y. Zhou \textit{et al.}, synthesized a $\text{Ca}_7\text{Si}_2\text{P}_2\text{O}_{16}$ ceramic powder, with a single phase-crystal structure which contains Ca-, Si-, P-. They studied \textit{in vitro} apatite-mineralization ability and its effect in PDLC (dental pulp ligament cells). This bioceramic was soaked in simulated body fluid for 7 days. Scanning electron microscopy
showed apatite formation after 1, 3 and 7 days (Fig. 13). Moreover, ionic products from Ca$_7$Si$_2$P$_2$O$_{16}$ significantly stimulated the expression of genes related to osteogenesis and cementogenesis: ALP, Runx2, CEMP1 and Col I.$^{137,138}$

7. Clinical applications

*In vitro* and *in vivo* results demonstrate good perspectives for patients who are in need of periodontal tissue regeneration. (Table 1) Several articles report good signs of cell proliferation and differentiation in many types of periodontal cells in collagen scaffolds and synthetic bone grafts. $^{139}$ Collagen revealed good features for periodontal regeneration, as a resorbable barrier membrane or as a scaffold. This biomaterial enhances tissue reconstruction. It was observed improvement of gingival tissue attachment and extracellular matrix remodeling in collagen-coated surfaces. The point is, after extensively studies, which are the true clinical applications of collagen biomaterial and synthetic bone grafts?

In this sense, a case report, which describes the placement of particulate hydroxyapatite and a resorbable collagen membrane in a 27-year old female patient and her posterior radiographic results, suggested this synthetic bone graft combined with a collagen membrane as a suitable treatment approach. Six-month radiograph showed a significant vertical bone fill.$^{140}$ Singh *et al.*, carried out a parallel-group, randomized, controlled clinical trial, in order to evaluate nanocrystalline hydroxyapatite efficacy combined with a bioresorbable collagen membrane compared to open flap debridement alone in patients with intrabony defects. In both groups, it was recorded clinical parameters such as plaque index, gingival index, probing pocket depth, gingival recession and clinical attachment level at baseline and after six months (Fig. 14). It was also performed intra-oral periapical radiographs. There were significant differences between test and control group in probing pocket depth reduction, clinical attachment level gain and radiological results. Consequently, it was observed an
improvement in clinical parameters in the test group, which was treated with nanocrystalline hydroxyapatite and a bioresorbable collagen membrane.\textsuperscript{141}

Debasish Mishra \textit{et al.}, reported two cases of gingival recession. The chosen treatment for these patients with Miller’s class I gingival recession consisted in a synthetic collagen membrane or platelet rich fibrin. To evaluate the outcome of both cases, it was recorded sulcus depth, gingival thickness, keratinized gingiva and recession depth before the required surgery and after six months. Results indicated that synthetic collagen membranes and platelet rich fibrin could be a good alternative for gingival recession treatment.\textsuperscript{142}

A comparative, randomized, 6-month controlled clinical trial was performed basically to evaluate the clinical outcomes of two different treatment strategies for Miller’s class gingival recession: subepithelial connective tissue graft or a bioabsorbable bilayer collagen membrane. Both treatments showed a significant improvement in clinical parameters. Subjects from bioabsorbable bilayer collagen membrane group expressed greater overall satisfaction according to collected data from patient response forms.\textsuperscript{143}

In parallel, a 9-month preliminary study was carried out to compare fish origin collagen barrier membrane (test group) versus open flap debridement (control group) clinical and radiological parameters in patients with periodontal intrabony defects. Results showed no significant differences between the two groups, but each group showed clinical improvement in comparison to baseline parameters.\textsuperscript{144}

Alternatively, Yadav \textit{et al.}, conducted a clinical evaluation to compare intrabony defects treatment clinical outcomes with collagen membranes and combined with autogenous bone graft or a mixture of bioactive glass-autogenous bone. Clinical findings have shown differences in clinical and radiological parameters. They were not significant between collagen-autogenous bone group and collagen-autogenous bone-bioactive glass group. However, in these three groups an improvement was observed in probing depth reduction,
defect resolution and clinical attachment level gained after six months. These results implied that bioactive glass could be combined with autogenous bone for guided tissue regeneration in intrabony defects treatment.\textsuperscript{145}

Moreover, Kher \textit{et al.}, undertook a controlled, randomized, clinical study to evaluate the effectiveness of bioabsorbable collagen membrane with or without decalcified freeze-dried bone allograft for guided tissue regeneration. Clinical measurements were recorded and a radiological analysis was performed throughout the study. It was found a significant improvement in clinical parameters like probing pocket depth, clinical attachment level and also in radiographic defect depth in collagen-bone allograft group.\textsuperscript{146}

A systematic review, which analyzes the clinical efficacy between the use of a bioabsorbable membrane and non-bioabsorbable membrane for guided tissue regeneration concluded that any barrier type or enamel matrix derivative improved the parameter clinical attachment level in comparison to open flap configuration.\textsuperscript{147}

It is worth to mention that Almazrooa, Noonan and Woo reported six cases in which collagen membranes couldn’t be absorbed. After corresponding biopsy, histological analysis was performed after hematoxylin-eosin and Masson trichrome staining techniques. Results revealed that in 5 cases resorbable collagen membranes could have delayed healing process.\textsuperscript{148}

Interestingly, in 15 volunteers, 6-mm punch biopsies were harvested at both palatal sites. A collagen matrix was sutured in one site; the other one was left untreated (control). The results of this clinical study indicate that the use of a collagen matrix enhances oral soft tissue healing compared with control sites at selected early time-points. This was documented by a faster re-epithelization at 4 and 8 days and re-epithelization was completed in all subjects by day 15. It was suggested that the collagen matrix improved the healing by an early
stabilization of the coagulum (matrix function) and an enhancement of the epithelial proliferation from the surrounding soft tissue.\textsuperscript{149}

In a multicentre single-blinded, randomized, controlled, split-mouth trial, 90 recessions (Miller I, II) in 45 patients were employed to evaluate the clinical outcome of the use of xenogeneic collagen matrix (CM) in combination with the coronally advanced flap (CAF) in the treatment of localized recession defects. CAF + CM was not superior regarding root coverage, but enhanced gingival thickness and width of keratinized tissue when compared with CAF alone. On the other hand, for the coverage of larger defects, CAF + CM was more effective.\textsuperscript{150}

Singh and Suresh evaluated clinical efficacy of rhPDGF-BB plus beta tricalcium phosphate for root coverage procedures. During this 6-month study, several clinical parameters were performed in order to assess its efficacy. It was not found a statistically difference between collagen group and rhPDGF-BB plus beta tricalcium phosphate with collagen. For this reason, both strategies could be applied for root coverage procedures.\textsuperscript{151}

8. Commercialized products

There are many products that have successfully reached the market. In order to mention few examples of success the list can begin with Mucografterm (Geistlich Pharma AG [Wolhusen, Switzerland]). This is a xenogeneic collagen matrix of porcine origin; which belongs to the class III medical device, according to the Medical Device Directive 93/42 (Medical Device Directive REF 30773.1, approved by the FDA for use in the US). This three-dimensional matrix consists in two functional structures: a thin compact layer consisting of collagen fibers in a wide dense porous layer where the collagen fibers are loosely arranged. This scaffold, mostly spongy, provides a space that favors the formation of a blood clot and the in-growth of tissue from adjacent sites. Healguide\textsuperscript{TM} (Advanced Biotech) is a sterile bioresorbable
collagen membrane for guided tissue regeneration. This commercialized product is a thin sheet made of high purity Type I collagen derived from animal tissues. BIO-GIDE®, a bioabsorbable collagen (type I and III) membrane of porcine origin (Geistlich Biomaterials, Geistlich Pharma AG, Wolhusen, Switzerland), and Dembone®, (Pacific Coast Tissue Bank, California, USA) a demineralized freeze-dried bone allograft are other examples. Geistlich Bio-Oss Collagen® consists of 90% Geistlich Bio-Oss® granules with the addition of 10% porcine collagen which makes it a biomaterial with improved handling characteristics employed in regenerative dentistry. Similarly, nanocrystalline hydroxyapatite (NcHA) bonegraft (Sybograf®) in combination with collagen membrane (PerioCol®) demonstrated successful clinical outcomes in the treatment of periodontal intrabony defects\(^{141}\).

GEM 21S® (Osteohealth, Luitpold Pharmaceuticals, Inc) is a growth factor enhanced matrix, which combines an osteoconductive matrix, beta tricalcium phosphate, with a bioactive highly-purified recombinant human platelet-derived growth factor (rh-PDGF-BB) which showed good clinical results\(^{151}\).

8. CONCLUSIONS

Although clinical findings demonstrate good perspectives in dental practice, there are many issues to be improved and further critical assessments are required for evaluating proof-of-principle concepts, safety and possible unwanted reactions of candidate biomaterials. Indeed, sometimes, clinical relevance is not that easy to predict from \textit{in vitro} and \textit{in vivo} assays outcomes. Silicate cements are an example: \textit{in vitro} assays showed good results, however, in usage tests they turned out to be irritating.\(^{152}\) Anyway, clinical studies and case reports indicate that collagen and ceramic materials seem to be a promising clinical approach in restorative dentistry. Nevertheless, most clinical studies enroll a low number of patients and last no more than six months. It would be more convenient to design clinical trials with a
larger number of subjects and whose duration exceed a few months. Periodontal tissue regeneration involves several mechanisms and requires a prolonged period of time. Additionally, more high clinical evidence studies, like systematic reviews and meta-analyses in the field could be an adequate guidance for biomaterial design and development.

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Legends to figures

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**Figure 9.** Schematic representation of cells employed in periodontal tissue regeneration *in vitro* assays

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**Figure 12.** Bar graphs of measured ALP (A) and MTT (B) enzyme activities of MC3T3 E1 osteoblast cells cultured on pure CPC and CPC-15%HX biocomposite specimens as a function of time. The insets in A are representative light microscopy images from the Giemsa stained biocomposite surface after 8 (left) and 21 (right) days of cell culture experiments. "Reprinted with permission from 135. Copyright (2010) American Chemical Society."
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**Figure 14.** Schematic representation of parameters employed to evaluate clinical performance
Table 1. Recent examples of collagen epidemiological studies

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<th>Article</th>
<th>Study design</th>
<th>Published</th>
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<th>Material</th>
<th>Objective</th>
<th>Conclusions</th>
<th>References</th>
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<tr>
<td>A comparative evaluation of the effectiveness of guided tissue regeneration by using a collagen membrane with or without decalcified freeze-dried bone allograft in the treatment of intrabony defects: a clinical and radiographic study</td>
<td>randomized, controlled trial</td>
<td>2013</td>
<td>16</td>
<td>collagen</td>
<td>compare GTR efficacy using a collagen membrane barrier with or without calcified freeze-dried bone allograft (DFDBA) for the treatment of periodontal intrabony defects</td>
<td>GTR with DFDBA showed statistically significant improvements in CAL gain, PPD reduction and radiographic defect resolution for infrabony defects</td>
<td>146</td>
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<td>Clinical and radiographical evaluation of a bioresorbable collagen membrane of fish origin in the treatment of periodontal intrabony defects: A preliminary study</td>
<td>randomized, controlled trial</td>
<td>2013</td>
<td>10</td>
<td>collagen</td>
<td>determine clinical parameters, alveolar crestal bone level and percentage of defect fill with and without a bioresorbable collagen barrier membrane in intrabony defects treatment</td>
<td>no significant differences were found either by using a bioabsorbable membrane or OFD for treatment of intrabony defects clinically and radiographically.</td>
<td>144</td>
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<tr>
<td>Clinical evaluation of subepithelial connective tissue graft and guided tissue regeneration for treatment of Miller’s class I gingival recession (comparative, split mouth, six months study)</td>
<td>randomized, controlled trial</td>
<td>2014</td>
<td>30</td>
<td>collagen</td>
<td>compare and evaluate GTR based root coverage using bioabsorbable collagen membrane and SCTG based root coverage procedure for shallow Miller’s Class I recession defects treatment</td>
<td>SCTG and GTR can be successfully used to treat recession defects. A GTR technique may offer many advantages over SCTG, patient acceptance, reduced surgical time and post surgical discomfort</td>
<td>143</td>
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<tr>
<td>Clinical evaluation of GEM 21S® and a collagen membrane with a coronally advanced flap as a root coverage procedure in the treatment of gingival recession defects: A comparative study</td>
<td>human case series</td>
<td>2012</td>
<td>7</td>
<td>collagen-ß-TCP</td>
<td>evaluate clinical efficacy of rhPDGF-BB plus ß-TCP with a collagen membrane in root coverage using a coronally advanced flap and compare rhPDGF-BB plus ß-TCP with a collagen membrane (group A) versus collagen membrane (group B) in root coverage using a coronally advanced flap, on clinical parameters.</td>
<td>better clinical results were achieved in rhPDGF-BB plus ß-TCP with collagen group and only collagen group with a statistically significant difference. But there was no statistically significant difference between them, suggesting that rhPDGF-BB plus ß-TCP with collagen and only collagen can be used effectively in root coverage procedures.</td>
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<tr>
<td>Clinical evaluation of guided tissue regeneration combined with autogenous bone or autogenous bone mixed with bioactive glass</td>
<td>randomized, controlled trial</td>
<td>2011</td>
<td>22</td>
<td>collagen-bioactive glass</td>
<td>compare GTR with collagen membrane (CM) (control group) or CM combined with autogenous bone graft (test group 1) or clinical attachment gain in both test groups was not significantly different, suggesting that autogenous bone can be mixed with bioactive glass if harvested bone is not</td>
<td>clinical attachment gain in both test groups was not significantly different, suggesting that autogenous bone can be mixed with bioactive glass if harvested bone is not used.</td>
<td>145</td>
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bioactive glass in intrabony defects

Improving Gingival Aesthetics Using Platelet Rich Fibrin and Synthetic Collagen Membrane: A Report of Two Cases

<table>
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<tr>
<th>Case Report</th>
<th>Year</th>
<th>Patients</th>
<th>Treatment</th>
<th>Outcomes</th>
</tr>
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<tbody>
<tr>
<td>Case 1</td>
<td>2015</td>
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<td>Collagen-platelet rich fibrin</td>
<td>Present two cases of young patients with gingival recession treatment</td>
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<tr>
<td>Case 2</td>
<td>2015</td>
<td>2</td>
<td>Bioactive glass (test group 2) in intrabony defects clinical outcomes</td>
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Nano-crystalline hydroxyapatite bone graft combined with bioresorbable collagen membrane in the treatment of periodontal intrabony defects: A randomized controlled clinical trial

<table>
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<th>Study</th>
<th>Year</th>
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<th>Treatment</th>
<th>Outcomes</th>
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<tr>
<td>Case 1</td>
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<td>Collagen-HA</td>
<td>Evaluate NeHA bone replacement graft with a bioresorbable collagen membrane efficacy compared to OFD</td>
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<td>Case 2</td>
<td>2012</td>
<td>16</td>
<td>Bioactive glass (test group 2) in intrabony defects clinical outcomes</td>
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</table>

Two dimensional alveolar ridge augmentation using particulate hydroxyapatite and collagen membrane: A case report

<table>
<thead>
<tr>
<th>Case Report</th>
<th>Year</th>
<th>Patients</th>
<th>Treatment</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>2014</td>
<td>1</td>
<td>Collagen-HA</td>
<td>Describe particulate HA and collagen membrane potential to correct alveolar ridge defect with resin-bonded prosthesis to achieve esthetics and health</td>
</tr>
<tr>
<td>Case 2</td>
<td>2014</td>
<td>1</td>
<td>Bioactive glass (test group 2) in intrabony defects clinical outcomes</td>
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</tbody>
</table>

Platelet rich fibrin and collagen membrane can be an efficient and less invasive approach to treat gingival recession. In both cases good aesthetic results were achieved regarding to colour, contour and consistency of the gingiva.

Nano-crystalline hydroxyapatite bone graft in combination with collagen membrane demonstrated better clinical outcomes compared with OFD.

HA: hydroxyapatite OFD: open flap debridement GTR: guided tissue regeneration CAL: clinical attachment level PPD: probing pocket depth SCTG: subepithelial connective tissue graft ß-TCP: beta tricalcium phosphate
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26009x19507mm (1 x 1 DPI)
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258x323mm (150 x 150 DPI)
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144x108mm (120 x 120 DPI)
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254x190mm (96 x 96 DPI)
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168x125mm (113 x 113 DPI)
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254x190mm (96 x 96 DPI)
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