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Review Article

Alginate And Collagen Composite For Hard Tissue Generation: A Mini Review

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ABSTRACT

The bone regeneration process involves the formation of the regenerative cells, the cell proliferation to produce new tissue and subsequently will repair the fractured area. During regeneration process, the osteoblast cells will secrete several proteins, organic and inorganic components as the base components to form the bone tissues or known as matrix. However, some conditions such as bone tumor removal, massive bone loss injury, extensive infections at the bone, minerals deficiency resulting the failure of bone regeneration and lead to prolonged bone fracture. To overcome this, bone scaffold is implanted or encapsulated bioactive cells are applied at the damaged area to support the bone regeneration. The materials should be osteogenic, osteoconductive and have osteoinductive properties. Natural polymer such as alginate and collagen are often used as biomaterials due to their high biocompatibility, degradability, low toxicity and easy to form a crosslinker with other materials through various method process. Therefore, this review will highlight the properties of alginate and collagen, the fabrication method to form composite and its application in biomedical area.

INTRODUCTION

Hard tissue regeneration has become more important as the world's population has grown to address conditions including bone fractures, bone tumour removal, severe bone loss injuries, extensive infections at the bone, mineral deficiencies, a lack of regenerative cells, and other bone illnesses. Due to the inability of bone to regenerate regularly, there will be insufficient regeneration, which will cause a prolonged bone fracture. To meet this urgent requirement, several advanced techniques for regenerative medicine and smart biomaterial-based have been developed. The responsiveness of the material can be manipulated, and the effects on cells and tissues can be triggered by either an instructional or an inductive response to internal or external stimuli (Zhang et al., 2018). The properties and functions to encourage tissue repair and regeneration can be tailored. The development of scaffold will assist the bone healing by stimulating the differentiation of mesenchymal stem cells, migration of resident cells, guidance of bone growth and formation of new bone by osteoblast cells at the injury site. The osteoblast cells will also secrete several proteins, organic and inorganic components as the base components to form the bone tissues or matrix (Filippi et al., 2020).

The materials used to produce the bone scaffold will need to fulfill several requirements such as biocompatible, low toxicity, adequate mechanical support at the injury area and porous to provide the space for bone cells and blood vessels growth (Filippi et al., 2020). Besides, the material will be able to induce cell growth via deposition of the extracellular matrix on its surface via apatite layer, enhance the cell differentiation and stimulate the fusion of materials with the native bone tissue (osseointegration). In some cases, the scaffold will need to be able to deliver the drugs that can speed up the healing process (Filippi et al., 2020). Polymer matrix and its composite is suitable to be employed as drug delivery carrier as they possess appropriate mechanical properties, bioresorbable and have

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specific drug release profile (Calori et al., 2020). Polymer composites, whether natural or synthetic, will act as both matrix and reinforcement in a composite, degradable or non-degradable have significant applications. Both alginate and collagen have been identified to be natural composite that can be used for the hard tissues regeneration process due to its unique property such as low toxicity, high availability, able to perform the in-situ gelation, water soluble, mucoadhesive nature and able form protective barrier for the cells and particle release systems. Therefore, this review will discuss the composite of alginate and collagen, its application and recent technology to fabricate it.

Alginate And Its Composites

Alginate is a natural polymer that can be extracted from algae or seaweed, and certain bacteria such as Azotobacter spp. and Pseudomonas spp. Alginate is a carbohydrate composed of homopolymeric units of -D-mannuronic acid (M block) and -L-guluronic acid (G block) at position 1 and 4 of the molecule backbone (Hernández-González et al., 2020). Since alginate exhibit certain properties such as low toxicity, high availability, able to perform the in-situ gelation, water soluble, mucoadhesive nature and able to form protective barrier for the cells and particle release systems, thus alginate in form of hydrogel is one of the commonly used materials for bone regeneration/scaffold, providing the site or space to load the bone cells (Hernández-González et al., 2020). Alginate has another special property which is not exhibited by most of the bone regeneration biomaterial, that is, it is injectable due to the presence of the inherent ionic crosslinking (Hernández-González et al., 2020). However, the pure alginate has its disadvantages when used as the scaffold for bone regeneration, it has a slow and incomplete degradation as only alginate with high molecular weight will be used to fulfill the mechanical properties similar to the bone as required for the bone regeneration (Hernández-González et al., 2020). Sodium alginate (SA) is the main form of alginate. The weakness of pure alginate can be countered by forming the composite with other biomaterials to enhance its physical and chemical properties.

Graphene oxide (GO) which acts as the filler to the alginate hydrogel, was produced to have stronger mechanical properties. The SA/GO composite showed enhanced young modulus (from 1.9 to 4.3 GPa) and maximum tensile strength (from 0.32 to 0.62 GPa), nearly double of the original strength (Raus et al., 2021). The research by Choe et al. in 2020 also found that SA/GO composite has a high MSC proliferation rate and survival rate even within an oxidative stress environment, while possessing an enhanced osteogenic differentiation on composite (Choe et al., 2019). SA/GO composite also exhibits a high structural stability due to low swelling rate as uncontrolled expansion will reduce the mechanical toughness, thus can maintain the original shape and mechanical properties (Li et al., 2018). Strong hydrogen bonding between GO and SA chains lead to thermal stability of composite (Li et al., 2018).

Gelatin is the same as alginate, it is a natural polymer. It can form a composite with alginate to overcome the mechanical weakness of both alginate and gelatin. However, the bioactivity of alginate/gelatin composite is not sufficient, thus nano apatite layer is coated on the composite via in situ mineralization. With nano apatite coating, the composite possesses high structural integrity and rigidity due to enhanced young modulus (Luo et al., 2018). The composite also has a high swelling ratio, thus resulting in larger mesh size that can ease the nutrient and growth factor diffusion at the application site to aid the bone recovery (Luo et al., 2018). The coated composite also showed a higher bone cell proliferation and differentiation rate due to upregulation of certain marker genes that play important roles in mesenchymal cell differentiation (Luo et al., 2018). High protein absorption of the coated composite as result of surface roughness and porosity of the coating also leads to a better cell adhesion as the absorbed protein on material will bind its receptor to the cell membrane of the surrounding cells (Luo et al., 2018).

In the alginate-peptide composite, the protein is added to the alginate hydrogel to form a composite, the main purpose is to increase the bioactivity and biocompatibility of the composite. If fluorenylmethoxycarbonyl-diphenylalanine (FmocFF) dipeptide is added to the alginate hydrogel, the alginate/FmocFF composite with high bioactivity is formed. Under scanning electron microscope, the long-entangled fibrils with mm in length are observed and will form nanofibrous texture on the composite, resembling the fibrillary nature of the extracellular matrix (ECM) (Ghosh et al., 2019). The composite has a high population of cells in an in vitro cell culture experiment, and the fibrillary nature of the composite directs the elongation of cells during the cell culture (Ghosh et al., 2019). The alginate/FmocFF also showed an ability to induce the osteoblast differentiation and mineralization of preosteoblast cells (Ghosh et al., 2019). The alginate/FmocFF also has an improved mechanical property due to supramolecular organization of the FmocFF within the alginate matrix (Ghosh et al., 2019). Bone formation peptide-1 (BFP-1) derived from bone morphogenetic protein-7 (BMP-7) can also be added to the alginate (Heo et al., 2017). The composite produced showed a uniform cell spread during the cell culture experiment and has an improved osteogenesis capability as a result of its ability to increase the alkaline phosphatase and calcium deposition, thus resulting in an accelerated bone regeneration (Heo et al., 2017) (Yang et al., 2018).

Chitosan is a natural degradable polycation polysaccharide and its degradation product is nontoxic and noncarcinogenic (Abedini et al., 2018). Alginate within the composite acts as a linker to promote the interaction between the chitosan and hydroxyapatite (Liao et al., 2017). The sodium alginate/hydroxyapatite/chitosan composite still has a low compressive strength that will change with the citric acid concentration that is used to produce the bone cement (Liao et al., 2017). When immersed in Simulated Body Fluid (SBF), the HA on the composite will first dissolve during the first week but will grow again due to calcium phosphate (CaP) salt deposition during the third and fourth week with an optimal rate, and the newly formed HA is similar with the bone HA (Liao et al., 2017). This scenario indicates that this composite is a good material for bone replacement. Besides, the composite also showed an enhanced cell viability for the first 24 hours of cell culture, thus promoting cell proliferation at the applied area (Liao et al., 2017). The composite also possesses a good cell affinity to improve the cell adhesion on the composite for proliferation (Bi et al., 2019). The cell adhesion can be further improved via film coating or fabricating the composite in suitable geometry (Bi et al., 2019).

The wet spinning method is one of the processes to prepare the alginate fiber such as calcium alginate fiber. The wet spinning device used to prepare the alginate fiber is small since the common device is large, so the device is not versatile for the fabrication of alginate fiber with special shape and function (Zhang et al., 2022). To stimulate ion cross-linking and subsequently create the primary fibre, the homogenous alginate spinning solution extruded from the spinneret is added into a coagulation bath that consists of calcium salt solution. The numerous draught rollers are set up in the coagulation bath to provide the primary fibre with a suitable drawing ratio. This is helpful in enhancing the mechanical performance of fibrous tissue. Calcium ion, Ca^{2+} is combined with other metal ions such as Zn^{2+}, Ba^{2+}, Cu^{2+}, and Al^{3+} to form the multi-metal ions coagulation bath that helps to enhance the alginate fiber's application properties (Zhang et al., 2022). The primary fibre is obtained during manufacture which is by extruding the alginate spinning dope in the syringe into the $CaCl_2$ solution to complete the ion exchange and prepare alginate fibre. To improve the mechanical performance and application features of alginate fibre, several additional functional polymers or cross-linkers were added to the spinning dosage to create hybrid fibre.

Collagen and its composites

Collagen is another natural polymer that is commonly used in bone regeneration due to its high biocompatibility and bioactivity compared to the synthetic polymers as natural polymers may possess certain similarities to the extracellular matrix within the body (Filippi et al., 2020). Collagen is a fibrous glycoprotein derived from the animal and it is the most abundant protein in the human body, contributing about 25% to 35% of the whole protein content as it is an essential component in building the bones, skin, tendon, and cartilage (Filippi et al., 2020). The bones are composed of type I collagen that is mineralized (Filippi et al., 2020). Collagen is observed as elongated fibrils in the form of triple helix. The collagen will be fabricated in the form of nanofibers via electrospinning technique, or in the form of hydrogel via polymerization (Filippi et al., 2020). The fabricated collagen will then be used to produce the collagen scaffolds, with suitable properties such as hydraulic permeability for the mechanical properties, oxygen and nutrient exchange, pore size, orientation of material and so on (Filippi et al., 2020). The scaffold will then be used for the cell holding to ensure the sustainable growth of cells or bones, then gradually degrading along the healing process (Filippi et al., 2020). However, even if the natural collagen has high biocompatibility and bioactivity, the bone scaffold that is only made of collagen is fragile, not producing adequate mechanical performance and a stable geometrical property to provide the support along the bone regeneration process (Filippi et al., 2020). And even if collagen exhibits a high bioactivity, it is insufficient to stimulate the formation of the bone cells at the applied site (Filippi et al., 2020). Thus, collagen is added with the fillers to form the composites that can overcome the weakness of pure collagen scaffold.

Hydroxyapatite (HA) can be added to the collagen matrix to produce Col/HA composite. Col/HA has enhanced compression modulus and stiffness that enable the composite to withstand a higher stress at the application site (Filippi et al., 2020). Besides, adherence surface area and roughness of the composite also showed improvement, thus providing a better cell adhesion, resulting in a higher bioactivity and cell proliferation rate (Filippi et al., 2020). The Col/HA composite also possesses a higher transcription factor and osteogenic genes expression, which indicates differentiation of mesenchymal stem cells (MSC) into osteoblasts, compared to the pure collagen (Sun et al., 2018). This can be proved with denser cell spreading morphology observed in research conducted by Sun et al. in 2018. Another special characteristic of Col/HA is that certain composite properties can be modified by substituting the anions or cations within the HA. For example, the HA substituted with magnesium ions will exhibit regulatory effect on bone regeneration process (Filippi et al., 2020). On the other hand, if the HA cations are substituted with zinc ions, the composite will have a higher ability to stimulate bone cells proliferation (Filippi et al., 2020). After years and months of development and research, the Col/HA composite can now have controllable interconnectivity, micropore organization and isotopic equiaxed structures, that will affect the performance of the scaffold produced (Filippi et al., 2020).

Calcium phosphate (CaP) can also be added to the collagen matrix as a filler. This type of composite is able to simulate the intrinsic inorganic compartment of the bone as the CaP mainly consists of calcium ions and phosphate ions as in bones (Filippi et al., 2020). The addition of CaP as filler into collagen strengthens the compression modulus of composite to a value 3 times higher than that of pure collagen (Zhang et al., 2018). CaP filler also works as a stabilizer that stabilizes the composite structure with smaller swelling and pore size variation (Zhang et al., 2018). A controlled swelling of the collagen will provide better contact of the material with the surrounding cells (Zhang et al., 2018). Beta or alpha tricalcium phosphate (TCP) is chosen as the filler, Col/β-TCP has a higher degradation rate and fusion rate due to lower Ca/P ratio, thus enabling complete replacement in the newly formed bone tissue (Filippi et al., 2020) (Lu et al., 2019). The percentage of β-TCP (wt 5% to 10%) must be controlled so that the composite will exhibit appropriate mechanical properties and release of the calcium ions, to enhance cell proliferation, bone formation and neovascularization (Filippi et al., 2020). $\boldsymbol{\alpha}$-TCP is more soluble than β-TCP and can have cement reaction, which means that $\boldsymbol{\alpha}$-TCP will harden forming a calcium-deficient hydroxyapatite (CDHA) within aqueous condition, speeding up the bone formation (Kim et al., 2017). However, Col/$\boldsymbol{\alpha}$-TCP has a low mechanical property compared to the real trabeculae bone due to porosity, even the composite already has enhanced compression modulus (Kim et al., 2017).

One of the calcium silicates is bioglass composed of sodium oxide, calcium oxide and silicon oxide. Bioglass can also be added to the collagen matrix to increase the compression modulus and elastic modulus, as well as enhancing stability of the composite in terms of physical and mechanical (Dhinasekaran et al., 2021; Sergi et al., 2020). Col/BG composite also showed reduced infections and improved osteogenic when applied to the damage area (Zhang et al., 2018) (Sergi et al., 2020). Bioglass as compared to the CaP, has a higher surface reactivity, and due to that, has a higher bioactivity (Zhang et al., 2018). The bioglass of Col/BG composite will release the sodium ions, calcium ions and soluble silica, which will stimulate osteogenesis by triggering cellular proliferation and differentiation (Zhang et al., 2018). The formation of the silanol functional group enables the BG to directly bind with the tissues and provides the active site for apatite layer deposition (Zhang et al., 2018). For the bioglass, the nanosized particles have a better performance than micro sized filler, in terms of mechanical properties and osteoconductive ability (Zhang et al., 2018).

Glycosaminoglycan (GAG) is a natural polymer, the examples are chondroitin sulfate, hyaluronan, heparin sulfate, dermatan sulfate, keratan sulfate and so on (Zhang et al., 2018). GAGs can bind with the growth factors and cytokines, thus improving the binding of osteoblast and osteoclast to the material as well as attracting the growth factors to the material (Zhang et al., 2018). Collagen added with GAGs showed improved cell migration and proliferation, as well as enhanced structural stability (Zhang et al., 2018). At the same time, the degradation rate of the composite is reduced. Col/GAG composite when first discovered, the researchers think that the GAGs will inhibit the hydroxyapatite crystal growth that will affect bone regeneration. But the further study of the composite showed that the Col/GAG composite does not inhibit the growth of HA crystal (Zhang et al., 2018). This type of composite provides a suitable 3D environment for the differentiation of MSCs to bone cells, and scaffolds made of Col/GAG showed an effective bone formation result, even without addition of progenitor stem cells or exogenous growth factors (Zhang et al., 2018). Most used GAGs in composite are hyaluronan and chondroitin sulfate (CS). Hyaluronan will recruit the osteoblast precursor cells to the materials, while the CS binds the osteoblast and osteoclasts to the material and capture the soluble mediators which are essential for bone formation (Förster et al., 2017).

3D printing refers to establishing the particular trace in advance in accordance with the required model, and then printing the scaffold in the required shape (Ma et al., 2019). Mineralized collagen scaffolds (MCSs) have been increasingly exploited as bone substitutes via tissue engineering procedures due to their high biocompatibility, appropriate mechanical strength, and similar structure and composition to genuine bone. MCSs are usually fabricated by extrusion printing, which provides advantages such as customized shapes, precise control of pore size, and rapid production (Charbonnier et al., 2020) (Fahimipour et al., 2018).

Application of alginate- and collagen- based composite

Alginate-based composite

The excellent property of alginate causes it to extensively be used in various biomedical fields such as tissue engineering, wound healing and drug delivery applications as the alginate gel retains similarities in its structures with the extracellular matrices in body tissues. Alginate exhibits its special characteristic that can be processed and used in 3D scaffolding material which includes microcapsules, foams, hydrogels, fibers and sponges. Hence, bio-polymeric alginates have often been used in tissue and bone injuries healing, repair and treatment in wound cartilage, new bone regeneration and scaffolds for the cell growth particularly (Sahoo et al., 2021). Porous scaffolds with highly functional properties are applied as delivery vehicles and used extensively in the field of tissue engineering for the application of temporary skeletons to accommodate and stimulate new tissue growth. The alginate-based composite can easily be formulated into porous scaffolding matrices in various forms in response for the cell culture and response, which makes it practically acceptable for regenerative medicine. Various critical diseases such as osteoarthritis, osteoporosis, bone cancer, major bone fracture and bone infections needed extra treatment for complete tissue recovery (Sahoo et al., 2021). After blood, bone is the second most transplanted tissue and alginate is often used as a good therapeutic tissue for the solution of pain relief together with anti-inflammatory and antibacterial agents (Sahoo et al., 2021).

The application of alginate-based composite materials was extensively studied and applied in the regeneration of endodontics. Alginate is categorized as a polysaccharide class with complete special properties such as biodegradable, biocompatible and non-toxic biopolymer, thus, it is considered as an injectable bio-polymeric material suitable for dentin regeneration (Sun & Tan, 2013). However, due to its lack of mechanical properties in strength, the application of the alginate-based composite in hard tissue engineering is limited. Therefore, Calcium Chloride (CaCl2) is mixed with alginate which serves as a cross-linking agent to increase the covalent bonding cross-linking and resulting in high mechanical strength (Sun & Tan, 2013). Besides that, a combination of alginate scaffold with nano-bioglass ceramic is used as a resource material for successful artificial dental implantation (Sun & Tan, 2013). Alginate is also used in a variety of wound dressings, such as hydrogels, sponges, and electrospun mats, which encourage wound healing substrates with various benefits, such as ability of gel forming and hemostatic capability against wound exudate absorption. Furthermore, the desirable properties of alginate used for wound dressing include good water absorptivity, flexibility, optimal transmission rate of water vapour, mild antiseptic properties, and non-toxicity. The cytokines which produce pro-inflammatory factors at wound sites help in aid in wound healing and the presence of endotoxin in alginate causing the high dressing bioactivity in wound healing. Alginate-based dressings may also prevent secondary injury peeling off and promote healing when compared to gauze (Sun & Tan, 2013).

Collagen-based composite

Collagen is primarily used in burn and wound cover dressings, bone filling, osteogenic, anti-thrombogenic surfaces and the immobilization of therapeutic enzymes. The very first application of a biomaterial is as a barrier membrane. Collagen film is used to treat tissue infections such as infected corneal tissue, as well as as usage for drug carriers for antibiotics and gene delivery carriers to promote bone formation (Khan et al., 2013).

Collagen has unique properties such as controlling fluid evaporation resulting in wound become pliable, promoting the development of granulation tissue, reducing pain, and providing mechanical protection against physical and bacterial infection. As a result, it has been used continually in dentistry because collagen powders have excellent wound adhesion and enable sufficient cell reactivity simulation with the formation of a highly vascularized granulation bed. Collagen-based membranes are prevalently used as barriers in implant treatment and periodontal to prevent epithelial cell migration and promote wound repopulation by regenerative cells (Khan et al., 2013).

Collagen has numerous sources, including bone, cartilage, tendon, ligament, blood vessel, nerve and skin as the main strcutural protein with most soft and hard tissues. Based on the excellent performance exhibited in collagen properties, this natural polymer has a good potential in biomaterial application for scaffolding in tissue engineering. However, the lack of mechanical strength and stability due to hydration condition which limits its use in specific tissues. Thus, intermolecularly cross-linking of collagent scaffolds with natural/synthetic polymers or inorganic materials is a straight-forward method to improve the mechanical and biological properties by enhancing the physical and chemical interactions of the polymers (Dong et al., 2016). Thus, the modification of collagen scaffold can be applied to other organ parts such as nerve, ligament, tendon, cartilage, and bone in tissue engineering.

In bone regeneration, the application of collagen composites as scaffold is to support the fractured bone, and at the same time provide the spot for the MSCs to undergo proliferation and differentiation to form osteoblast (Sun et al., 2018). The scaffold will also increase the osteogenic capability at the applied area by increasing the expression of marker genes that are essential to induce osteogenesis (Sun et al., 2018). Certain collagen composites, such as collagen/B-TCP can be used to replace the autologous bone treatment as it has a comparable deposition rate and fusion rate with autogenous bone group, to fuse with the bone of the patient, normally at the spine (Lu et al., 2019). In some cases, the cells can be seeded on the scaffold or printed together with the composite hydrogels to form a cell-laden scaffold; such scaffolds can provide a homogenous cell distribution (Kim et al., 2017). Sometimes, collagen composite such as Col/GAG is not used to build the scaffold, instead, it is used to form the coating on the bone implant for enhanced bone healing (Förster et al., 2017).

CONCLUSION

In conclusion, both alginate and collagen composite are a vital part in the hard tissue regeneration treatment process. Due to their unique properties, both composites help to advance research and development for this field of studies. Alginate is a material that can be employed in a variety of biomedical applications, including those involving tissue engineering, medication administration, and wound healing. because it is easily moldable in a variety of geometries, biocompatible, degradable, capable of creating gels, and may enclose substances. Alginates based on biopolymer alginates have been utilised mostly in tissue healing, bone wounds, scars, and wounded cartilage. Alginates have unique features that can be easily processed as a 3D framework material incorporating hydrogels, foam, sponges, fibers, and alginates based on these. Collagen has special abilities to regulate fluid evaporation, keep wounds elastic and flexible, encourage the growth of granulation tissue, lessen pain, and offer mechanical defense against bacterial and physical harm. Collagen is found in many different tissues, including bone, cartilage, tendons, ligaments, blood vessels, nerves, and skin. It is the primary structural protein of most hard and soft tissues. Collagen is a natural polymer that is a viable biomaterial for tissue engineering because of its great characteristics. Thus, tissue engineering techniques for collagen backbone alteration can be used on other organ components such as neurons, bone/cartilage, and tendon.

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