REVIEW ARTICLE

Collagen and Chitosan as Scaffold in Tissue Engineering

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ABSTRACT

Objective: **To highlight collagen and chitosan as natural material, and their modifications to be used as scaffold in tissue engineering.**

Materials and methods: **PubMed and Google scholar searches were performed using keywords: 'scaffold', 'biomaterial', 'natural polymer', 'collagen modification biomaterial' AND 'tissue engineering', and 'chitosan modification biomaterial' AND 'tissue engineering'. The information was tabulated and presented as text and Tables.**

Results and discussion: **Tissue engineering has the intention to restore the structures and functions of impaired or damaged tissues. Scaffolds, which are supporting biomaterials mimic the structure and function of the natural extracellular matrix (ECM), play an important role in tissue engineering. Collagen and chitosan are natural polymers that have suitable properties to be used in tissue engineering. Both are the most abundant biopolymer and can be extracted in large quantities from a wide range of sources. However, collagen scaffolds have some drawbacks due to their poor mechanical properties. To eliminate the drawbacks of collagen and enhance the capacity of chitosan as natural materials, combinations to form a composite with other materials are needed.**

Conclusion: **Combination of collagen or chitosan with other materials, such as synthetic polymer, other bioactive materials, such as glass or ceramics or other natural products are conducted in several studies and showed that the modified product could be applied in various tissue engineering.**

KEY WORDS

collagen, chitosan, bioactive, biopolymer, scaffold, tissue engineering

INTRODUCTION

Tissue engineering has the intention to restore the structures and functions of impaired or damaged tissues. The aim is to develop tissues to replace the damaged tissues that mimic tissues in nature. The main factors in tissue engineering are cells, scaffolds and growth factors. Scaffolds are supporting materials, either natural or synthetic materials that provide a mimicking structure and function of the damaged tissue natural extracellular matrix (ECM)[1)]. A good scaffold should have suitable biological properties (biomaterial). Based on the consensus conference of the European Society for Biomaterial (ESB), biomaterials are defined as interfacing materials in biological systems for the evaluation, treatment, augmentation, repair or replacement of tissues or organs of the body[2)].

Scaffolds provide a three-dimensional support for initial cell attachment, proliferation, migration, and subsequent tissue formation. Three-dimensional scaffold characteristics for tissue engineering should have a good biocompatibility and bioactivity, and have a three-dimensional architecture, which have a highly porous microstructure. Moreover, a scaffold needs to be biodegradable, has a suitable surface chemistry, desired mechanical properties and flexible[3)]. The design of scaffolds need to be adjusted to perform the functions of promoting cell-biomaterial interactions, cell adhesion, and extra cellular matrix deposition, and allowing sufficient transport of gases, nutrients, and regulatory factors to promote cell survival, proliferation, and differentiation. In addition, the design should ensure biodegradation at a controllable rate that approximates the rate of tissue regeneration under natural conditions, and ensure a minimal degree of exposure to inflammation or toxicity in vivo[4)].

The development of tissue engineering is on-going worldwide, and includes tissue engineering of tissues such as bone, cartilage, tendon, ligament, and heart muscles and also organs like liver, kidney, pancreas, nerve, and intestine, or parts of an organ, such as heart valves. Moreover, achievements of tissue engineering in the areas of bone, skin, bladder and airway have been translated in patients[2)]. Biomaterials can be used in biomedical and clinical applications, especially in bone tissue engineering to fill in small bone defects[5)].

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Table 1: Modified collagen scaffold

Composite scaffold	Method	Application	Reference
Coll 1 - PCL	Electro-spinning	Diaphragmatic muscle tissue engineering	18
Coll ─ highly porous 45S5 bioactive glass	Coating	Bone tissue engineering	17
PLGA - Coll	Coating	Skin tissue engineering	6
Coll-CPC	Composite	Bone tissue engineering	19

Coll 1 = Collagen type 1, PCL = poly (ε-caprolactone), Coll = Collagen, PLGA = poly(lactic-co-glycolic acid), CPC = self-setting calcium phosphate cement

Table 2: Modified chitosan scaffold

Composite scaffold	Method	Application	Reference
Chitosan-carbon nanofiber	Composite	Cardiac tissue engineering	23
fMWCNT-chitosan-HAp	Composite	Bone tissue engineering	24
PEG-chitosan	Composite loaded with ciprofloxacin hydro chloride	Wound dressing	27
Keratin-chitosan	Composite	Human adipose stem cell culture	28

fMWCNT = functional multi-walled carbon nanotube, HAp = hydroxyapatite [Ca10 (PO4)6 (OH)2], PEG = polyethylene glycol

Scaffold materials can be natural or synthetic, which might be degradable or non-degradable. Both natural and synthetic material have been used in tissue engineering[4]. Natural materials can be organic or inorganic. Organic natural materials have bioactive properties that could interact with cells and enhance the cell activities in tissue engineering. Inorganic natural materials, such as bioactive ceramics, which are composed of rigid bonds, might be used in hard or soft tissue engineering. They may interact with physiological fluids and cellular activity, but their biodegradability and biocompatibility are often insufficient, and limit their potential use in clinical practice[4].

On the other hand, synthetic materials including synthetic polymers are widely used in biomedical and medical field because of their properties that could be designed for specific applications. Synthetic polymers have better mechanical and physical properties over natural materials, but they are usually inert, non-bioactive, and might cause adverse tissue reactions due to their acidic degradation product. Moreover, the lack of hydrophilic functional group on a synthetic polymer may cause hydrophobicity of the synthetic polymer surface that may reduce cell adhesion and growth[6]. These disadvantages of the synthetic material can be reduced by combining a synthetic with a natural material to overcome its shortage[1].

Collagen and chitosan are natural biomaterials that can be used as scaffold in tissue engineering, and both are the most abundant biopolymers that can be extracted in large quantities from a wide range of tissue sources. Collagen and chitosan have low immunogenicity, regulated biodegradation and might be resorbed after a certain time of implantation. Moreover, they are biocompatible, lack of toxicity, and have good plasticity. Therefore, they are attractive to be used as scaffold in tissue engineering[7,8], and their combination with other material is interesting to be discussed. Accordingly, this review aimed to highlight collagen and chitosan as natural material, and their modifications to be used as scaffold in tissue engineering, by addressing natural scaffold, various types of collagens, modified collagen scaffold, chitosan, modified chitosan scaffold, and future direction.

MATERIALS AND METHODS

PubMed and Google scholar searches were performed to look for articles that were published in English on March 29, 2021 and June 24, 2021. The used keywords were 'scaffold', 'biomaterial', 'natural polymer', 'collagen modification biomaterial' AND 'tissue engineering', and 'chitosan modification biomaterial' AND 'tissue engineering'. All studies concerning the description of collagen and chitosan, and studies on their modification and usage in tissue engineering were used. The information was tabulated and presented as text and Tables.

RESULTS AND DISCUSSION

Natural Scaffolds

Natural scaffolds are drawing a great attention due to their beneficial properties such as high surface-to-volume ratio, high porosity with very small pore size, biodegradability, and suitable mechanical property. Natural scaffolds are natural polymers and can be classified as proteins, polysaccharides, and polynucleotides. Proteins are available as silk, collagen, elastin, fibrinogen, gelatin, keratin, actin, and myosin. Polysaccharides are glycosaminoglycans, chitin, dextran, amylose, and cellulose, while polynucleotides are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)[4].

Natural polymers have been used in tissue engineering due to their non-toxicity, biocompatibility, and biodegradability without releasing toxic substances. Collagen and chitosan are natural polymers that have suitable properties to be used in tissue engineering. Both are the most abundant biopolymer and can be extracted in large quantities from a wide range of sources. Collagen and chitosan scaffolds also can be designed according desired morphological, mechanical, physical, and chemical properties to achieve specific functions, by varying manufacturing conditions and changing composition, concentration and cross-linking conditions[9].

Natural polymers may serve as three-dimensional scaffolds and get more attention due to their biocompatibility, lower cost and processing readiness[5]. Natural polymers, as their name implies, are derived from natural sources. They are biocompatible, typically composed of a polymeric network, which may contain high water content, and are suitable for tissue engineering[3].

Combination of various natural polymers can create new materials to be used as scaffolds that have improved physical and chemical properties compared to a single-component scaffold[5]. Moreover, the new material can be made either from various natural or synthetic polymers or a combination of both. The resulting new composite materials can be modified using physical and biochemical new technologies to yield biomimetic scaffold materials and nanomaterials. The main purpose of those methods are to modify the morphology, spatial structure, mechanical properties and surface of the scaffolds to improve the bioactivity of composite materials that are suitable for seeded cell adhesion and distribution[10].

In addition, an ideal scaffold for tissue engineering should show the characteristics of a homogenous microstructure, with suitable pore size, and high porosity to allow cells to grow in, migrate, and form vascularization[11]. Furthermore, interconnecting pores are required to allow diffusion of nutrients into the scaffold, and waste and degradation products out of the scaffold to prevent interference with the tissue or organ to be engineered[2].

Various types of collagens

Collagens are a family of molecules, which have a triple helix configuration of three polypeptide subunits that are known as α-chains. These helices are molecules of tropocollagen, which form the basic building block of collagen fibres[9]. There are various types of collagens, and the four most common are: type I collagen that is found in skin, tendons, and bone; type II collagen that is found in cartilage; type III collagen that is found in blood vessels; and type IV collagen that is found in basement membranes. These various types of collagens allow for enhanced biomimetics due to the possibility to choose the suitable type of collagen that is specific to the tissue to be engineered[12]. Type I bovine collagen is a suitable material for fabrication a porous collagen scaffold. It was used in a study to promote angiogenesis[13], which is very important to keep an engineered tissue or organ viable. The porous structure of the collagen scaffold allowed diffusion of nutrients and waste product in and out, as well as migration of cells that were important features of vascularization[13]. Another study used porous collagen scaffold to produce a model of human endometrium. In the study, the porous collagen scaffold was seeded with either decidual tissue derived stromal cells or endometrial organoid cells. The resulting human endometrium models were both functionally responsive to hormone stimulation[14]. Type I collagen is the most abundant collagen type in the cornea. A study synthesized a corneal tissue substitute using rat tail collagen I as the scaffold. Pure type I collagen has strong mechanical strength, high water content, high clarity, and relatively slow degradation that can be prolonged by UV cross-linking. The scaffold supported epithelial cell and keratocyte growth, which was needed to produce the corneal tissue substitute[15]. In the future, the corneal tissue substitute might be used in human transplantation.

Modified collagen scaffold

Collagens are the most abundant natural protein in the human body. Collagens, which are produced by fibroblasts, are found in ECM and provide tensile strength for tissue growth[16]. Collagen is a fibrous protein, which is hydrophilic in nature, and contains special sequences of amino acids i.e. Arg-Gly-Asp (RGD) that are important to promote cell adhesion[17], proliferation, and differentiation[6]. However its poor mechanical properties may interfere with its function as a scaffold[2]. Therefore, combination with synthetic polymer is used to overcome the drawbacks of collagen. Synthetic polymer can be designed with a desired architecture, and their degradation characteristics can be controlled by varying the composition of the polymer. Various synthetic polymers have been used to develop modified collagen scaffold (Table 1), including polystyrene, poly-I-lactic acid, polyglycolic acid[2], poly(-lactic-co-glycolic acid) (PLGA)[6], and poly(ε-caprolactone) (PCL)[18]. Numerous studies reported various modifications of collagen with other materials to make collagen composites to be used in tissue engineering of bone, cartilage, skin, lung, urethra, and endometrium.

In a study, collagen type I and PCL were used to develop an aligned fibrous scaffold by electro-spinning that seemed to be analogous to collagen fibers in ECM of diaphragmatic muscle tissue. The electro-spun collagen-PCL hybrid scaffold could guide cell alignment and enhance myotube formation in vitro, and might be used in diaphragmatic muscle tissue engineering[18].

In several studies, collagen was use as coating material of relatively non bioactive materials, as collagen might improve cell attachment and proliferation, and promote the incorporation of biomolecules and signaling cues. The use of collagen as coating material was applied to highly porous 45S5 bioactive glass that was suitable for bone tissue engineering[17], and PLGA-collagen hybrid scaffold that was used in skin tissue engineering[6]. The study on highly porous 45S5 bioactive glass showed that application of thick cross-linked collagen coating had no detrimental effect to the scaffold microporosity and compressive strength of the hybrid scaffold was increased by a factor of five[17]. The study on PLGA-collagen hybrid scaffolds showed that collagen coating helped adhesion and spreading of the cells. However, collagen coating caused faster and more water absorption that promoted hydrolysis and degradation of the PLGA polymer. The negative effects of collagen coating due to oligomers that resulted from hydrolysis were balanced by increase in hydrophilic property[6]. In a study, type I collagen was used in combination with self-setting calcium phosphate cement (CPC) that showed suitable scaffold strength with improvement in actin stress fiber density that enhanced cell attachment and differentiation of umbilical cord derived stem cells. Type I collagen RGD sequence is important for attachment of osteoblasts via their membrane receptors, i.e. integrins. In the study, human umbilical cord derived mesenchymal stem cells (hUCMSCs) were seeded on collagen-CPC, which showed superior load-bearing capability, and hUCMSC differentiation improved bone regeneration that might be promising in a wide range of orthopedic and craniofacial applications[19].

Other studies used biological materials to modify collagen to construct composite collagen scaffolds. A study developed collagen scaffolds that were covalently conjugated with stem cell specific antibodies for cardiomyocyte regeneration. The stem cell specific antibodies were used to enrich autologous Sca-1 positive cells. The composite collagen scaffold played a role in specific stem cell enrichment at target site of tissue regeneration, where it provided a 3-D porous scaffold for stem cells adhesion, proliferation and differentiation[20]. Collagen binding domain of vascular endothelial growth factor (CBD-VEGF) can specifically bind to collagen I and maintain VEGF biological activity in vitro and in vivo. A study used CBD-VEGF in liver fibrosis mouse model and got promising results[21]. Another study developed a composite collagen scaffold using CBD-VEGF, and used the scaffold for wound healing in a diabetic rat model. The scaffold attracted VEGF and provided an effective VEGF local concentration in the wound, and showed a promising result[22].

Chitosan

Chitosan is derived from chitin, and chitin is the most abundant biopolymer that is generally found in crustaceans or insects. Chitosan is a promising biomaterial for tissue engineering due to its biocompatibility, hydrophilic properties, biodegradability, nontoxic, and minimally immunogenic nature[23]. Moreover, it can be developed into porous structure, which is suitable for cell ingrowth, and osteo-conduction. In addition, it has an intrinsic antibacterial property[24]. Chitosan has protonable amino groups in its D-glucosamine residues that generate muco-adhesive properties. The positive charges on chitosan backbone provide haemostatic properties, as they interact with negatively-charged membrane of red blood cells[25].

Modified Chitosan scaffold

Chitosan structure has similarity to glycosaminoglycans in tissue ECM, thus it may be a good candidate to be used as a scaffold in tissue engineering. It contains carbohydrate biopolymer that is suitable in promoting the healing process of connective tissues proper and specialized connective tissues such as bone and cartilage[26], either chitosan alone or its modification. Several studies described the combination of chitosan with other biopolymers, carbon nanofibres or nanotubes, bioactive ceramics, or growth factors to enhance tissues regeneration that showed better results compared to chitosan alone (Table 2).

A study on cardiac tissue engineering used modified chitosan-carbon nanofibre scaffold that showed combined properties of chitosan, which was biodegradable, with carbon nanofibre properties that showed excellent mechanical strength and electrical conduction[23]. The study showed that the use of composite porous chitosan-carbon nanofibre scaffolds improved the resulting cardiac tissue constructs as the composite scaffold supported attachment and proliferation of cardiac cells and enhanced cardiogenic properties. The resulting cardiac construct showed endogenous electrical conduction without exogenous electrical stimulation[23].

A study developed combination of chitosan with bioactive ceramics to improve chitosan bioactivity. The bioactive ceramics, i.e. hydroxyapatite [Ca10 (PO4)6 (OH)2] (HAp) was used to increase the interconnected porosity, which was important for cell attachment, and mechanical strength of chitosan. Natural Hap was derived from Thunnus Obesus bone, and chitosan matrix was grafted with functional multi-walled carbon nanotube (fMWCNT) to develop fMWCNT-chitosan-HAp composite scaffold for bone tissue engineering. The scaffold showed interconnected porosity, which is suitable for cell attachment and proliferation, good thermal stability, mechanical strength, and controlled in vitro degradation[24].

Synthetic polymer-chitosan composite was intended to overcome low biodegradability and immune stimulation properties of the polymer. Polyethylene glycol (PEG) is a polymer that has less mechanical strength, but biocompatible and non-toxic. PEG-chitosan composite can be easily fabricated into porous three dimensional scaffolds. In a study, PEG-chitosan composite scaffold, which was loaded with ciprofloxacin hydro chloride, was applied for wound dressing and showed inhibition of bacterial growth. PEG-chitosan composite improved drug loading and cumulative release compared to pure chitosan scaffold[27].

Modified chitosan was also fabricated using natural polymer. Keratin-chitosan composite can be produced into films that induce cell

adhesion and proliferation of human adipose stem cells. Lyophilized keratin-chitosan composite produced highly interconnected pores that lead to porous scaffolds that might provide an ideal environment for cell attachment and growth. Moreover, their cross-linkable properties might produce 3D natural polymer scaffolds that are suitable for tissue engineering purposes[28].

Future directions

As various modified collagen and chitosan scaffolds showed promises in various tissue engineering studies, translational studies in regenerative medicine are warranted to be conducted. For critical size bone defect or bone fracture with delayed union, the use of scaffold to stimulate bone healing is highly needed. Therefore, further translational studies using collagen coated highly porous 45S5 bioactive glass, Collagen/CPC, or fMWCNT-chitosan-HAp composite, which are seeded by mesenchymal stem cells, either natural or engineered to secrete bone morphogenetic protein 2, are recommended. In addition, diabetic chronic ulcers that are difficult to heal may need skin tissue engineering to cover large denuded areas, or appropriate wound dressing to accelerate healing. Therefore, further translational studies using PEG-chitosan composite loaded with antibiotics to serve as a wound dressing to prevent infection and to stimulate granulation tissue formation, which is followed by the use of collagen coated PLGA to engineer new skin to cover the granulation tissue, are highly recommended.

CONCLUSION

The drawbacks of collagen and chitosan as natural materials can be overcome by combination to form a composite with other materials, such as synthetic polymer, other bioactive materials, such as glass or ceramics or other natural products. Several studies showed that the modified product could be applied in various tissue engineering.

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