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Collagen—A Biomaterial for Delivery of Growth Factors and Tissue Regeneration

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Abstract—Research on collagen as a carrier for growth factors and cells is an actual field in modern tissue engineering. The progress in this field of research will make possible approaching the problem of regeneration of injured organs and large tissue fragments.

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INTRODUCTION

Growth factors—protein molecules stimulating cell growth and differentiation—are finding growing application in the modern regenerative medicine [1–3]. Different types of such molecules can considerably accelerate regeneration and repair of bone tissue, skin, cornea, cartilage, vessels, and other tissues. Protein growth factors ensure efficient interaction of cells with the extracellular matrix, as well as trigger a complex cascade of biochemical processes responsible for regeneration of damaged tissues [4]. For growth factors to be used in clinical practice, systems for their controlled delivery to target body compartments and improving pharmacokinetic and pharmacodynamics characteristics of these agents are required. To enhance the efficiency of tissue repair, techniques for tissue engineering based on biocomposite materials comprising a polymer carrier, cells, and growth factors have been developed [5, 6]. These materials are injected directly into an injured tissue and function simultaneously as a source of growth factors, mechanical support, and cell scaffold.

It is desirable that the action of growth factors in the regeneration site be local and limited by the application area of the composite. The concentration of growth factors maintained for a long time in the regeneration site should be high enough to ensure cell activation. At the same time, the total quantity of the protein released from the composite should be as small as possible to avoid triggering systemic immune response, since injection of growth factors poses a risk of induction of immune response in patients, especially in the case of repeated injections. The immunogenicity of therapeutic protein preparations may give rise to clinically significant side effects. First, a humoral immune response to endogenous intrinsic growth factors can be induced, which may entail systemic suppression of reparation processes [7]. Second, the circulating autoantibodies may suppress the therapeutic activity of the injected proteins. The most dangerous side effect is the development of hypersensitivity of the body to a specific protein formulation. Thus fairly rare type of response is associated primarily with biopharmaceutical formulations containing recombinant proteins of bacterial origin.

The same is true of polymers used for composite fabrication. Among known polymer materials, collagen holds the greatest promise for tissue engineering in view of its biopermeability, biocompaticibility, and weak antigenicity. It was found that collagen implants

favor proliferation of fibroblasts and vascularization of surrounding tissues [8]. In the body collagen molecules break into smaller molecules, and the latter are either excreted or actively involved in biosynthesis at the cellular level and stimulate reparative processes, including endogenous collagen formation [9]. In terms of drug commercialization, collagen is an accessible biological material, because it is contained in great amounts in connective tissues of mammals. Due to the structural characteristics and compatibility with synthetic polymers, collagen holds promise for a great diversity of applications.

Polymeric delivery systems for drugs, including growth factors, make possible controlled release of therapeutic proteins and thus provide a required concentration of therapeutic proteins in a local site.

The present review focuses on the research on the properties of collagen materials, methods of retaining proteins inside collagen matrices (including encapsulation), and application of collagen delivery systems for growth factors in tissue repair.

General Information about Collagens

Collagen is a fibrillar protein, it is the main structural of the connective tissue, forms the scaffold of the extracellular matrix, and is a component of the interstitial tissues of virtually all parenchymal organs [10].

Drug delivery systems are most commonly fabricated using type I and III collagens. Collagens of both types feature similar amino acid sequences and low contents of aromatic amino acids [11], which makes animal collagens feasible for clinical applications.

There is some concern about the introduction of collagen in clinical practice: Massive immune response is possible, entailing secondary side effects, such as organ injury by immune complexes or cross-coupling of antibodies to animal collagen derivatives with human collagen, which may induce autoimmune diseases. Whether immune response on collagen will arise or not depends on the source of collagen, fraction of native protein (no less than 80%), and procedure of its purification [12].

The immunogenicity of collagen can be reduced, for example, by selectively removing non-helical terminal regions (telopeptides) from the collagen under the action of enzymes (tripsin, chemotripsin). Additional chemical cross-linking, for example, by glutaraldehyde, too, reduces the antigenicity of collagen but not eliminates it completely [13, 14]. Moreover, chemical cross-linking (cross-linking induced by gamma γ-irradiation) imparts additional mechanical strength to the material. However, excessive chemical treatment may cause a loss of low immunogenicity and denaturation, as well as reduce the number of cell–scaffold interaction sites, thus leading to a loss of the main advantages of collagen [15].

This is just the reason why preference should be given to collagen structures reduced from an extracted and purified native collagen. The process of preparation of collagen biomaterials includes preparation of gels, fibril suspensions, or solutions, which are then subjected to chemical cross-linking, drying, lyophilization, mineralization, or a combination of these treatments. To retain the triple-helix collagen structure and prevent denaturation, one should avoid long-term heating at temperatures higher than 41°C. Thus, different forms of collagen carriers (hydrogels, sponges, microparticles, films, etc.) can be produced

Collagen Structures

Fibrils. Collagen endows body's connective tissues by the ability to withstand substantial mechanical loads. This is made possible by the fact that collagen molecules are packed in fibrils which form 3D structures. Depending on environment conditions, the diameter of fibrils *in vitro* varies from 10 to 500 nm [16], whereas in the connective tissue collagen fibrils form bunches up to 10 μm in thickness.

Collagen fibrils are formed by ordered packing of collagen molecules in parallel to each other with a shift by 67 nm and a gap of 40 nm. Collagen molecules in a fibril are held together by covalent cross-linking between the telopeptide region of one molecule and the helical region of the neighboring molecules, in a staggered order [17]. Fibril formation *in vitro* is only possible if the collagen structure is not disturbed (i.e. in the case of preserved nativity; gelatin which is a denatured collagen cannot form fibrils). Fibril formation is affected by partial or complete removal of collagen telopeptides, because the latter are responsible not only for collagen self-aggregation into fibrils, but also for the subsequent stabilization of intermolecular bonds in fibrils via covalent cross-linking involving oxidized lysine residues [18].

Collagen gel. Collagen gel *in vitro* represents a fibril network containing 0.5–3% protein and 97–99.5% aqueous salt solution. The transfer of *in vitro*

Table 1. Example applications of growth factors in tissue engineering [103, 104]

Growth factor, abbreviation	Function	Regenerated organ
Vascular endothelial growth factor, VEGF	Migration and proliferation of endothelial cells	Lymphatic and blood vessels
Insulin-like growth factor 1, IGF-1	Proliferation and inhibition of apoptosis	Cartilages, skin, nerves, kidney, bones, muscles
Hepatocyte growth factor, HGF	Proliferation, migration, and differentiation of mesenchymal stem cells	Liver, muscles, bones
Epidermal growth factor, EGF	Proliferation and differentiation of fibroblasts and epithelial and mesenchymal cells	Skin
Bone morphogenetic proteins, BMP-2/3/7	Differentiation and migration of osteoblasts	Bones, cartilages, kidney
Platelet-derived growth factor, PDGF-AA/BB/AB	Ebryonic development, migration and proliferation of endothelial cells	Bones, skin, muscles, blood vessels
Transforming growth factor, TGF-α/β	Proliferation and differentiation of basal, nerve, and bone-forming cells	Brain, skin, cartilages, bones

solution of native collagen into a gel form is necessary for forming stable gel-like structures capable of prolonged maintaining integrity of the collagen gel in physiological conditions. The functioning of the biomaterial is modified by drying or freeze-drying to increase the gel concentration to 10–20%, as well as by treatment with stabilizing or cross-linking agents.

Release of Growth Factors from Collagen Carriers

Table 1 presents examples of growth factors and their applications in tissue engineering. It is important that a local release of macromolecular growth factors prevents side effects associated with their effect on healthy tissues or with their high concentration in the injection site. Another important advantage the release of growth factors from collagen carriers offers over injections consists in prolonged action, which is provided by a slow release of protein molecules of the matrix.

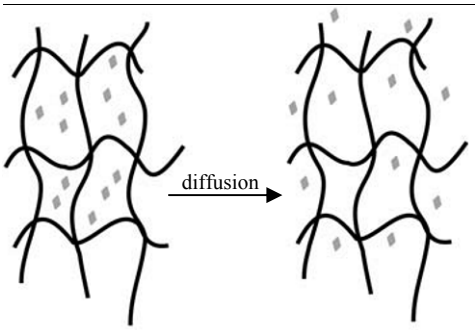
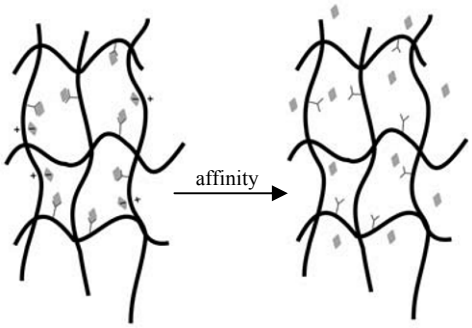
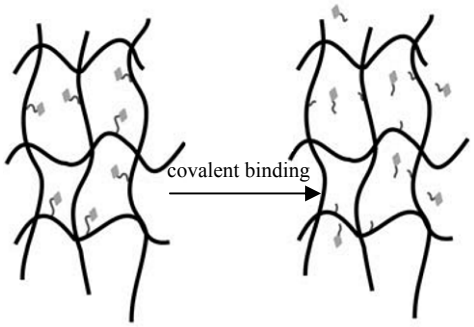
Controlled release of proteins occurs due to their retention in the matrix. There two groups of retention mechanisms: (1) diffusion restrictions: the collagen scaffold of the gel prevents free diffusion of protein molecules because of the small size of pores between fibrils and (2) binding of protein molecules with the collagen scaffold: proteins forms either covalent or noncovalent bonds with collagen molecules, and the dissociation rate of these bonds depends on the composition and properties of the solvent (pH, ionic strength, protein concentration, etc.), and, therefore, cannot be controlled from the outside.

Protein release from a collagen carrier *in vivo* is usually more rapid than *in vitro*. This effect is associated by the fact that an *in vivo* drug delivery system has to function in a complicated environment [19]. Cells produce enzymes destructive for the collagen scaffold; components of the tissue fluid react with growth factors or compete for their binding sites with collagen; mechanical loads deform the scaffold. To understand the mechanisms underlying release of growth factors from collagen carriers and assess the principal parameters responsible for the release rate are necessary for explaining and predicting the release profiles of growth factors from implants *in vivo*. Nor infrequently several different mechanisms are effected to reach the required release rate of a growth factor. Below we consider the mechanisms of controlled protein release from collagen materials (see also Table 2).

Diffusion-Controlled Release

Collagen carriers for drug delivery, such as gels, sponges, and microparticles, are biphasic materials. The solid phase or scaffold consists of collagen molecules and fibrils, forming a network. The communicating pores of the network are filled by the liquid phase which is a continuous medium, where solute molecules can move. Protein molecules incorporated in the collagen scaffold and not reacting with fibrils, move with respect to scaffold molecules due to diffusion [20]. Moreover, in the case of a directed flow of the liquid phase with respect to the scaffold, protein molecules are transported by the flow. Such a convective transport may arise *in vivo* as a result of pressure gradient, gel contraction or swelling, and mechanical loads which cause gel contraction or expansion. The same mechanisms is operative in the transport of signaling biomolecules inside native

Table 2. Mechanisms of release of growth factors from collagen materials

Mechanism	References
 Fick's law Concentration gradient Geometry-dependent release rate Protein size < pore size	[105]
 Protein adsorption due to weak interactions (electrostatic, hydrophobic, hydrogen-bond) Affinity to sites in additionally introduced molecules (heparin, antibodies, hyaluronic acid)	[35] [32] [33]
 Covalent binding with scaffold Possible bond cleavage Hydrolytic or enzymatic cleavage	[64] [63]

tissues by extracellular fluid flows. Thus, the transport of macromolecules in the collagen gel is similar to that occurring in biological tissues.

Diffusive transport. The first use of collagen gel for local delivery carrier and prolongation of drug action dates back to 1960s, when research on the diffusion characteristics of collagen gels was initiated. Rosenblatt et al. [21] studies the diffusion coefficient of proteins as a function of fibril and pore size. Further research concerning diffusive and convective transport in collagen gels focused on transport of macromolecules in tumors [22].

The degree to which macromolecular diffusion is slowed down in gel depends on the scaffold microstructure, specifically, on the size and volume fraction of pores and on the size of fibrils. Because of hindrances, macromolecules should make a longer way than in the absence of the scaffold, and, therefore, the apparent diffusion coefficient in gel is lower than in solution [20]. There are theoretical models for calculation of diffusion coefficients depending on gel characteristics [23].

It is noteworthy that the diffusion coefficient is almost independent on whether gel formation has already occurred in the collagen solution or no and depends on the concentration of collagen and the size of its fibrils. Table 3 lists the diffusion coefficients in solution and in gels for selected proteins with different sizes and shapes. The gel prepared from a dilute collagen solution and compressed by centrifugation (a fibrillar gel) contains thick (up to 500 nm) and long (10–100 μm) fibrils. A fibrillar collagen gen with the

Table 3. Diffusion coefficients of proteins in collagen gels and in solution[a]

Type of collagen gel, collagen content, average pore diameter	Protein, molecular weight, maximum size of protein molecule	Diffusion coefficient	
		in solution, 10^{-8} cm^2/s	in gel, 10^{-8} cm^2/s
Fibrillar, 3.5%, 58 nm	Fibrinogen, 330 kDa, 70 nm	20.2	4.6
	Chymotrypsinogen, 23 kDa, 1.7 nm	95	95
Nonfibrillar, succinylated, 3.8 %, 5.6 nm	Chymotrypsinogen, 23 kDa, 1.7 nm	95	20
Nonfibrillar, 3%	Bovine serum albumin (BSA), 66 kDa, 4 nm	90	36
	Liposomes, 20 nm	25	2.5
Nonfibrillar, 0.24%	BSA, 66 kDa, 4 nm	90	85
	Liposomes, 20 nm	25	20
Fibrillar, 0.24%	BSA-rhodamine (conjugate), 66 kDa, 4 nm	45.1	43.9
Fibrillar, 0.24% + hyaluronic acid, 0.5%	BSA-rhodamine (conjugate), 66 kDa, 4 nm	45.1	21.4

[a] The data were obtained by the FRAP technique [22] fluorescent correlation spectroscopy [25], and fitting of the experimental dependence of protein release from gel by a theoretical curve [24].

collagen concentration of up to 4 wt %, pores are fairly large, and, therefore, sustained release is only possible for fibrillar (fibrinogen) or large globular proteins [24]. In a nonfibrillar gel (contains smaller fibrils or individual collagen molecules not associated in fibrils) or a gel with pores comparing in size with proteins, the diffusion coefficient decreases in proportion to protein diameter ([22, 24], see Table 3).

Diffusion-controlled release occurs with a concentration gradient, it is proportional to the diffusion coefficient and obeys the Fick's law.

Mechanical Compression and Contraction

In a living body, soft tissues are subject to constant mechanical deformations, and this also relates biopolymer materials introduced into the injury site. The deformation of gels makes the fluid phase to move with respect to the scaffold, thereby transporting substances. Hsu et al. [26] measured the amount of the growth factor rhBMP-2 released from a collagen sponge compressed after being implanted into an injured bone. The volume of fluid released from a sponge 4.4 mL in volume reached up to 250 μL, which resulted in a release of about 3% rhBMP-2 during sponge implantation. In another experiment, the collagen sponge compressed by 40% in the implantation site, and this led to a release of a half of the encapsulated protein within 30 min after implantation [19].

Abundant evidence has been accumulated showing that a cyclic mechanical loading induces a cyclic fluid flow inside hydrogels, thus much accelerating release of growth factors encapsulated in them [19, 27]. Even if the amplitude of cyclic deformation is as little as 5%, the rate of protein release may increase by 10–20% compared to that from a non-deformed gel [28]. Increased contraction–expansion amplitudes accelerate the transport of growth factors form the carrier to surrounding tissues or fluid reservoirs.

In vivo mechanical loads may lead to an irreversible contraction of collagen gel and release the encapsulated drug together with the fluid phase [29]. Gel contraction can also be caused by cells (fibroblasts) migrating into the implant and affecting the scaffold due to adhesion to collagen molecules.

Protein Binding with Collagen Scaffold Molecules

Noncovalent and covalent binding of proteins/growth factors with the hydrogel scaffold makes it possible to reach the goal: prolonged delivery of a drug injected in a single high dose. Therewith, the drug concentration does not exceed an acceptable dose, because protein molecules are bound, while cell receptors react exclusively in a free drug in solution. Protein release may be modulated by chemical factors (enzymes, pH, salt concentration, etc.). External mechanical loads have a weaker effect of protein release.

Noncovalent Binding

The simplest approach to binding proteins to the scaffold makes use of the natural properties of collagen, due to which collagen molecules can adsorb proteins. Such properties include the positive and negative charges distributed over the collagen mole-

cule [30] and the presence in the collagen molecule of binding sites affine to different proteins [31]. In cases where the natural properties of collagen cannot provide efficient retention of protein macromolecules, the collagen biomaterial is ether modified to enhance its binding ability by introducing into it additional agents able to bind both with collagen and with growth factors (heparin [32], hyaluronic acid [33], fibronectin, and chondroitin sulfate [34]) or by binding to collagen of antibodies to growth factors [35]; another approach involves modification of a protein to be delivered by fusing cross-linking to it a collagen-binding domain to endow the protein with additional affinity [36, 37]. Since noncovalent binding is reversible, i.e. no proteolytic enzymes are required for adsorbed proteins to release from the scaffold, the proteins start to release, when their concentration in the surrounding fluid decreases.

Let us consider in more detail the above-mentioned approaches.

(1) Electrostatic interactions. *Heparin*. In a living body, heparin and heparin-containing molecules (heparin sulfate) bind signaling molecules, and, therefore, they are important components of the extracellular matrix, which control cell adhesion, migration, proliferation, and differentiation. A great number of protein growth factors contain a site specific to heparin (proteins bind with the negatively charged heparin molecule by electrostatic forces).

One of the first uses of heparin for drug delivery purposes was reported by Wissink et al. [38] who cross-linked heparin to collagen by an *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide/*N*-hydroxysuccinimide bridge to provide a better retention of the basic fibroblast growth factor (bFGF). Thus modified collagen scaffolds were used in many works as vehicles for delivery of such growth factors as VEGF, FGF-2 [32, 39], stromal cell-derived factor 1α (SDF-1α), transforming growth factor b2 (TGF-b2), and epidermal growth factor (EGF) [34, 40]. Heparin is an anticoagulant, and its immobilization on collagen materials attenuates the thrombogenic activity of collagen, which prevents thrombocyte adhesion and blood clotting [41]. Conjugation with heparin stabilizes the growth factors which are rapidly inactivated in the free form, for example, TGF-b2. In this connection, a positive effect can be obtained even if the carrier is fabricated by simply mixing collagen with heparin [42].

Hyaluronic acid. It was shown that heparin-binding proteins exhibit affinity to sulfonated hyaluronic acid [40]. Bone morphogenetic proteins (BMP) are affine both to native collagen and hyaluronic acid. Kim et al. [33] compared the release of rhBMP-2 (recombinant human bone morphogenetic protein) from a 0.3% collagen gel and 10% hyaluronic acid sponge. After 28 days of soaking, 88% of protein released from the collagen gel and against 31.8% from the hyaluronic acid sponge. These findings allowed the researchers to conclude that the BMP-2 protein is stronger bound with hyaluronic acid than with collagen.

Succinylation. Singh et al. [43, 44] studied the electrostatic interactions between collagen, modified collagen, and model proteins: lysocyme and polylysine. Lysocyme slower released from the native collagen scaffold than expected by theoretical calculations by the Fick's second law of diffusion. Succinylation of collagen which imparts a negative charge to the scaffold; as a result, the release of the positively charged lysocyme and polylysine gets even slower due to electrostatic interactions [43]. Lysocyme is retained longer than polylysine, on account of hydrophobic interactions with collagen.

It is important to note that the binding capacity of collagen is much dependent on the source of collagen, since collagens from different sources have different amino acid sequences [45, 46].

(2) Hydrophobic interactions. Hydrophobic interactions are responsible for retention of the rhBMP-2 growth factor in collagen gels (collagen in itself is a hydrophilic substance). Note that rhBMP-2 belong to the group of bone morphogenetic proteins used in clinic for bone tissue regeneration [19].

One more approach to introduce a charged agent in collagen was described by Wang et al. [47]. The authors made use of the ability of native collagen for association into a triple helix and found that a synthetic collagen mimetic peptide (proline–hydroxyproline–glycine) is adsorbed on type I collagen fibrils. They synthesized a peptide with the *N*-terminus bearing a negative charge which can retain growth factors. This peptide was used to immobilize the VEGF growth factor in a highly concentrated collagen gel.

Affine Binding

(1) Use of the natural properties of collagen. Collagen molecules have a number of binding sites for extracellular matrix proteins (fibronectin, von Wille-

brand factor) and cell integrins. The growth factors BMP-2 [48] and BMP-7 and FGF-2 [49, 50], too, are able to affinity-based binding with collagen [51, 52]. The fibronectin binding site is between the amino acids 643 and 819 [53]. There are two bonding sites for the von Willebrand factor (VWFC): between the amino acids 32 and 90 (a non-helical region) in the alpha 1 chain and the domain responsible for fibril formation between the amino acids 1253 and 1487.

Probably, the binding sites in collagen for growth factors are the same as for other proteins of the extracellular matrix and blood plasma. Release of growth factors from tissue implants is faster than *in vitro*, which can be explained by a competitive expulsion of growth factors by proteins dissolved in the surrounding medium.

Censi et al. [54] reported successful clinical trials of growth factors affinity bound to a collagen carrier. Thus, the BMP-2 protein encapsulated in a collagen sponge is used to treat bone fractures and fissure fractures [55]. Another example [56]: the BMP-7 protein in a collagen scaffold is used to treat bone defects.

(2) Modification of proteins by collagen-binding domains. The most used collagen-binding domain is a *Clostridium histolyticum* collagenase domain. This domain is well characterized [57], it was used for modification of the EGF [58, 59] and bFGF growth factors [59]. According to [34], recombinant growth factors containing and not containing this domain compare in biological activity.

Ishikawa et al. [60] studied the EGF growth factor conjugated with the fibronectin collagen-binding domain by fusing cross-linking. This domain made it possible to increase the concentration of the biologically active protein EGF administered in a wound together with a collagen material. It was also found that the domain–EGF conjugate is retained in the wound and damaged vessel walls due to binding with the tissue collagen.

(3) Modification of collagen by protein-binding agents: antibodies and biotin. Zhao et al. [35] immobilized polyhystidine antibodies on a collagen scaffold demineralized bone matrix (DBM) to initiate specific binding between BMP2 containing a six-histidine tag (His-BMP2) and DBM. The heterobifunctional agent sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate and the Traut's reagent were used to conjugate monoclonal antibodies with DBM, and, the resulting conjugated mutrix can bind more His-BMP-2 molecules than the starting bone matrix. Boyce et al. [61] biotinylated bovine skin collagen. The model protein was avidin-conjugated horseradish peroxidase. Cross-linking a modified collagen with a biotin/avidine bridge was also used to enhance binding of peptide growth factors in collagen coatings for wound healing [46, 62].

Covalent Binding

First attempts to form covalent bonds between growth factors and collagen molecules were based on the use of bifunctional polyethylene glycol (3.4 kDa) cross-linking agents containing succinimidyl groups on both ends [34, 63, 64]. This method was developed before the technology of concurrent cross-linking of a collagen scaffold and cross-linking of growth factors to the latter. Thus incorporated growth factors TGF-β2 [63] and VEGF [64] much slower released *in vivo* than their non-cross-linked analogs.

The second most popular method of covalent binding of collagen is similar to that for heparin cross-linking (vide supra). The covalent immobilization of the rhBMP-2 bone protein in collagen sponges using the hydrophobic synthetic copolymer of *N*-isopropylacrylamide and N-acryloyl succinimide favored a much longer retention of rhBMP-2 in the implantation site [19, 65].

Covalent immobilization ensures the longest retention times of drugs in carriers *in vivo*. However, this methods, too, is not free of drawbacks. Collagen has quite a complicated biochemical and spacial structure, and, therefore, covalent immobilization of growth factors cannot be controlled to a sufficient degree [47, 64], low reaction yields are observed, and the biochemical characteristics of collagen get even worse. The yield of a covalently bound protein depends both on the site of injection and on the activity of cells such as fibroblasts which produce poteinases capable of cleaving the protein or degrading the scaffold to let the protein in [19].

Application of Collagen Biomaterials

Collagen Sponges

The first developed collagen sponges were designed to coat wounds and stop bleeding. They are prepared by lyophilization of aqueous solutions of an acid or an alkaline collagen with 0.1–5% dry-substance contents. The porosity of lyophilized sponges can be

varied by varying the concentration of collagen and the rate of freezing before lyophilization [66–69]. Collagen can be combined with other materials, like elastin [70, 71], fibronectin [72], or glucosamine glycans [66–69]. Collagen sponges are actively used in treatment of burn wounds, decubituses, ulcers [74], as well as *in vitro* test systems.

The main advantages of collagen sponges consist in their ability to absorb much tissue exudate, tightly stick to wet wound surfaces and therewith preserve the wet microclimate in the wound, protect the wound from mechanical exposures, and prevent secondary bacterial infections [69, 73]. Furthermore, collagen favors infiltration and growth of cells responsible for tissue regeneration, as well as infiltration of inflamematory cells [74]. This allows collagen sponges to be used for tissue regeneration, and their combination with various growth factors makes such systems more efficient [75]. Thus, the use of the recombinant growth factor PDGF combined with collagen sponge caused fibroblast infiltration into sponges 3 days and capillary formation 7 days post-implantation [75]. Early dermal and epidermal healing was observed in wounds coated with collagen sponges combined with the FGF growth factor [76].

One more illustrative example is a combination of collagen sponges with bone morphogenetic proteins (BMP). These proteins are used in clinic for regeneration of bone tissue injuries. They can induce the differentiation of mesenchymal cells into chondogenic and osteogenic cells, as well as favor osteoblast proliferation [77]. Preclinical and clinical trials of the rhBMP-2 proteins incorporated into collagen sponges gave evidence for a high efficiency of this combination in the repair of critical-size bone defects [78], spinal fusions [79–81], and bone fractures [82], as well as in dental practice [83].

Collagen Hydrogels

In 1962 Grillo and Gross [84] prepared collagen gel from a neutral solution of acidic collagen and proposed it for medical application. It was shown collagen gel forms *in vivo* upon injection of a neutral collagen solution in animals.

Collagen hydrogels possess viscoelastic properties: They are semisolids in a quiescent state, and fluids can be induced to flow through them by pressure (for example, if the fluid is forced out of a syringe) [85]. They exhibit a good cell and tissue compatibility [86]. The most popular are two forms of injection collagen hydrogel: suspensions of collagen fibrils [85] and nonfibrillar viscous aqueous solutions [87]. The main advantage of collagen hydrogels over other forms of collagen biomaterials is that they can serve as substrates for cells [88]; they also show impressive results when combined with growth factors [89].

At the same time, collagen hydrogels has a number of disadvantages. The most important of them is that collagen gels are mechanically too weak for surgical manipulations, and they cannot resist to tensile loads *in vivo* [90]. Furthermore, collagen gels are susceptible for cell-mediated contraction [91]. The problem of enhancing the mechanical strength of collagen hydrogels is approached by several ways: (1) combining gels with harder collagen biomaterials, such as sponges [90], (2) increasing the concentration of collagen in gels, and (3) forcing fluid out of hydrogel before its use [92]. The mechanical strength of collagen gels increases with increasing collagen concentration. The strongest are gels with the maximum collagen concentration of 20 mg/mL [93]. Increased collagen concentration decreases porosity, which restricts cell migration inside gels [93] and can cause another negative impact on cells.

Collagen Microparticles

Collagen microparticles are generated on formation of water-in-oil emulsions [94]. To fix the spherical shape of particles, collagen microspheres are crosslinked, which can affect the immunogenicity of such carriers. In view of the small size of particles, protein molecules diffuse rapidly, and, therefore, there is a need to slow down protein release. To this end, collagen microparticles are generally combined with other polymer materials, such as poly(lactic-co-glycolic) acid [95], polylactide [96], and polycaprolactone [97]. Note that such carriers have never been used in clinic.

Collagen Films

Dry collagen films 0.5–3 mm in thickness are prepared by drying collagen hydrogels. They are primarily applied in ophthalmology to treat the cornea [98]. Before use they are commonly soaked (rehydrated), and, therewith, the film thickness increases only slightly. The rehydrated collagen film is a thin collagen hydrogel. When applied on eyes, collagen film completely reproduces the corneal shape, it is permeable for oxygen required for metabolism and

fulfills the function of a temporary patch [99]. Dissolving film leaves on the cornea a collagen solution layer which attenuates friction of eyelids on the cornea and favors its epithelization [98].

As a rule, collagen films are used in combination with water-soluble antibiotics, for example, gentamicin [100], or with growth factors, in particular, EGF [101]. If collagen films are used as carriers for protein growth factors, collagen–protein should be provided, because free diffusion of proteins from the thin (1 mm) collagen film occurs within a few hours. Another approach to the problem consists in forming a multilayer collagen film by fastening the layers together under pressure [102]. From this system, the PDGF growth factor released *in vivo* at a constant rate over the course of 100 h.

CONCLUSIONS

Even though there is a broad range of potential applications of collagen as a carrier for growth factors, only a few collagen drug delivery systems have found clinical application. At the same time, synthetic polymer carriers, such as poly(lactic-co-glycolic), are widely promoted in the literature.

The reason for such a poor introduction of collagen in clinic is its high cost and complicated fabrication of a pure native collagen, as well as variable parameters of collagen products (density, fibril and pore size).

The most successful applications of collagen carriers for drugs and growth factors are associated with ophthalmology, bone surgery, and burn wound healing. This is explained by low immunogenicity of a pure collagen product and its biocompatibility, degradation to nontoxic physiological compounds, and ability to ensure cell ingrowth into the carrier and regeneration of injured tissues.

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