Review

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Hydrogels based on collagen and fibrin – frontiers and applications

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Abstract: Hydrogels are a versatile tool for a multitude of applications in biomedical research and clinical practice. Especially collagen and fibrin hydrogels are distinguished by their excellent biocompatibility, natural capacity for cell adhesion and low immunogenicity. In many ways, collagen and fibrin represent an ideal biomaterial, as they can serve as a scaffold for tissue regeneration and promote the migration of cells, as well as the ingrowth of tissues. On the other hand, pure collagen and fibrin materials are marked by poor mechanical properties and rapid degradation, which limits their use in practice. This paper will review methods of modification of natural collagen and fibrin materials to next-generation materials with enhanced stability. A special focus is placed on biomedical products from fibrin and collagen already on the market. In addition, recent research on the in vivo applications of collagen and fibrin-based materials will be showcased.

Keywords: biohybrid polymer; clinical application; structural proteins; tissue engineering.

Introduction

The first hydrogels used in the biomedical context appeared in the 1970s, when the need for a new material for cell cultivation was high. Complex reactions in a tissue-like system could not be properly investigated at the time. Materials like collagen, alginate and carrageenan were used as an immobilization matrix for fibroblasts [1] and microbial cells [2, 3]. Langer and Vacanti gave an overview of the first hydrogels in tissue engineering [4]. The application of hydrogel scaffolds in tissue engineering and biomedicine has taken great steps since then [5].

Classical hydrogels with excellent biocompatibility and bioactivity are natural, protein-based polymers like collagen [6], gelatine [7], silk fibroin and fibrin [8]. Especially proteins from the extracellular matrix (ECM) like collagen or fibrin are promising candidates in the field of tissue implants due to their ability to mimic key biochemical factors vital for tissue regeneration. Fibrin plays an important role in wound healing, hemostasis and angiogenesis, and serves as a provisional matrix. Collagen, as a major constituent of the ECM, adds to the mechanical strength and flexibility of different tissues, and contains important RGD binding sites. The ECM serves as the mechanical framework for cells and tissues by providing adhesion molecules and growth factors. The fibrin matrix during wound healing provides attachment sites for cells, which in turn lay out a novel extracellular matrix, composed predominantly of collagen. Both proteins serve as a scaffold for tissue regeneration and can promote the migration and ingrowth of cells. Fibrin and collagen are an ideal biomaterial for hydrogel scaffolds, because of their distinguished biocompatibility and cell adhesion capability [9, 10].

The formation of a hydrogel from fibrin is based on its natural polymerization process with thrombin following vascular injury. Thrombin cleaves two small amino acid sequences in the amino-termini of the Aα and Bβ chain of the fibrin precursor fibrinogen. As a consequence, polymerization sites within the molecule are exposed, which leads to the polymerization of single fibrinogen molecules to a 3D fibrin network. Further stabilization of this network is achieved by covalent crosslinking which is catalyzed by factor XIIIa (as reviewed in [11]). Polymerization can

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be influenced by temperature, as well as thrombin and fibrinogen concentrations [12, 13]. Increased mechanical stability can be achieved by casting techniques leading to a compacted fibrin matrix [14]. It should be noted that the fibrinogen material for biomedical research is typically sourced from blood, which always carries a residual risk of pathogen transmission.

Native collagen polymerizes into fibrils on its own [15]. When using extracted collagen, a fibrillar scaffold similar to the ECM can be formed by increasing the pH and temperature of the collagen solution. Collagen biomaterial can be obtained from a variety of natural sources. The most common materials are from a bovine or porcine origin [16] but also discarded human tissue (e.g. placenta) provides a good source of collagen [17]. As the extraction of biomedical material from mammalian tissue is always accompanied by concerns about the safety of the material, new sources have received attention in recent years. These include poultry as well as marine sources of collagen like fish, shark, jellyfish and marine sponges [18]. However, the production of collagen from these sources is still limited.

Despite the fact that collagen and fibrin-based hydrogels are already widespread in clinical applications, in practice they display some disadvantages like mechanical instability [14, 19] or in the case of fibrin gels, rapid degradation [20]. To overcome these drawbacks, more stable materials can be achieved by blending with natural and synthetic polymers or by influencing the crosslink density. The resulting materials combine the inherent bioactivity of collagen and fibrin with tailor-made mechanical properties. More detailed information about the classification of hydrogel materials and the preparation of hydrogels can be found in recent reviews [21, 22]. In this paper, we will give an overview of modifications of collagen and fibrin hydrogels with natural and synthetic polymers leading to augmentation of mechanical properties. In addition, we review current commercial biomedical applications of fibrin and collagen gels, as well as recent research of in vivo applications.

Modifications for enhanced stability

Overcoming the mechanical instability yet preserving the excellent biocompatibility of protein-based hydrogels is the main focus of the modification process. Their natural origin leaves the proteins susceptible to protease degradation, which can be advantageous in the case of a biodegradable matrix, but also a limitation, if the scaffold structure is being degraded too fast for tissue regeneration. Some instability issues can be counteracted with a higher concentration of polymer in the gel, but attention has to be paid to the resulting pore size. Higher concentrated gels form a dense network which impedes cell migration and ingrowth into the matrix [23, 24]. Lower concentrated gels have poor mechanical properties and may tear easily during implantation. The general challenge for designing hydrogels is the balancing act between providing a cell-promoting environment and having good mechanical properties.

There are several possibilities of modifying natural protein hydrogels. Stabilization can be achieved by blending with natural or synthetic polymers, as well as chemical crosslinking. Alternative strategies include using composites like interpenetrating polymer networks (IPNs) or semi-IPNs. IPNs are formed when polymer monomers are polymerized within a pre-polymerized network. Semi-IPNs are formed when linear polymers are entrapped within a pre-polymerized hydrogel [25]. In IPNs or semi-IPNs only physical interactions and no chemical interactions occur [26].

Here, we focus on modifications for enhanced stability through the addition of different natural and synthetic polymers or some form of crosslinking manipulation. We do not review modifications aimed primarily at bioactivity enhancement, such as those with adhesion peptides or heparin.

Modification with natural components

Collagen and fibrin can be modulated into a stronger hydrogel by combining them [27]. It has been shown that composites of different concentrations of collagen and fibrin display increased compaction, which leads to mechanically robust matrices. Of all composites tested, the construct with 1:1 collagen to fibrin ratio corresponding to a total protein concentration of 1 mg/mL, resulted in the highest cell concentration (cell number combined with gel compaction) and best mechanical properties [material modulus of 52.2 kPa in contrast to 11.2 kPa (pure collagen) and 21.9 kPa (pure fibrin)] [27]. This result is supported by other studies, which also proved that a composite of collagen and fibrin possesses enhanced mechanical features compared to pure collagen and pure fibrin alone [28, 29]. Other natural components can be used to modify fibrin, as well as collagen in order to stabilize the protein gels. These include chitosan, hyaluronic acid and genipin. Chitosan-modified protein gels form stable scaffolds with enhanced mechanical features and

are a promising biomaterial to be used as a wound dressing. Collagen sponges crosslinked with chitosan and embedded with human acidic fibroblast growth factor led to improved diabetic wound healing [30]. Membranes fabricated from collagen and chitosan were functionalized with silver. The result was a film with a diffusion coefficient similar to human cornea. Additionally, the silver ions led to bactericidal activation of the scaffold, but also caused a small degree of hemolysis [31]. Another modification with chitosan is a composite of fibrin, chitosan and sodium alginate used for veterinary wound healing [32]. It showed enhanced mechanical features, because of the ionic bond between the amine groups of the chitosan and the carboxyl groups of the alginate.

Using hyaluronic acid with fibrin and collagen is another successful strategy for improving the mechanical stability of protein-based hydrogels. Fibrin and hyaluronic acid (HA) with methacrylic anhydride (MA) form stable hydrogels with enhanced mechanical strength, which could be correlated to increasing HA-MA concentrations [33]. Furthermore, gene expression studies proved that proliferation of bone marrow-derived mesenchymal stem cells and even differentiation into chondrocytes was promoted by this composite. Hyaluronic acid can be modified with tyramine (tyr) to produce hyaluronic acid-tyramine polymers, which in turn can be used as a modification for fibrin networks. The formation of a hydrogel consisting of fibrin and HA-tyr led to improved structural stability due to the mechanical support provided by the modified hyaluronic acid [34].

Modification of the structural proteins fibrin and collagen with genipin, a natural aglycone, also resulted in mechanically stronger hydrogels. Two approaches were taken to crosslinking fibrin with genipin which showed slightly different results. First, crosslinking was performed after the gelation process was finished. The resulting gel had a high crosslinking density and was stable for a long time. The second method used crosslinking and gelation at the same time and resulted in a gel with enhanced mechanical features [35]. The second approach is more suitable for tissue engineering as the mechanical properties can be modulated at the same time the cell encapsulation takes place. Furthermore, genipin decreased the proteolytic degradation of the fibrin network [36].

Examples for polysaccharides combined with collagen to form stable hydrogels are alginate, glycosaminoglycans, cellulose and dextran. The modification of collagen with alginate-dialdehyde led to a stabilized collagen matrix and also to a denser network structure. The resulting hydrogel promoted cell attachment and proliferation [37]. Copolymerization with glycosaminoglycans resulted in a stiffer and tougher hydrogel with a decreased degradation rate of collagen [38]. Glycosaminoglycan-modified collagen type I hydrogels are already used in a variety of in vivo and in vitro applications [39]. Oxidized glycosaminoglycans have been investigated by Zhao et al. who proved that they have no negative effect on cell behavior [40]. Dialdehyde carboxymethyl cellulose (DCMC) as a crosslinker of collagen improved the thermal stability of collagen and did not interfere with the triple helical structure of the molecules [41]. Thermal stability enhancement was also the result of collagen gels modified with aldehyde-functionalized dextran (DAD). The maximum compressive strength of the collagen-DAD gels was about 20 times higher than that of a pure collagen hydrogel [42].

Proteins can also be utilized to modify collagen gels, e.g. it was shown that soy protein had a strengthening effect on the gel and was well distributed in the network [43]. Modifications at the amino acid level of collagen can alternate the structure of the protein and lead to favorable changes. The use of D-alanine in the amino acid sequence of collagen led to conformational changes and thus inhibited collagenase activity at the cleavage site [44].

Biohybrid fibrin and collagen hydrogels

Modification with natural materials does not always lead to the desired result of a mechanically stable and biologically active hydrogel. On the other hand, synthetic hydrogels do not carry natural binding sites for cells which hinders their usage in tissue engineering. As a result, combinations of synthetic polymers and natural proteins are an attractive option for certain applications. The resulting hydrogels display great mechanical strength and it is possible to modify their physicochemical characteristics in a controllable way.

Polyvinyl alcohol (PVA) is a biocompatible molecule which shows good mechanical properties [45] and the ability to simulate natural tissues [46]. Pure PVA gels display no degradation [47] and cell membrane protein binding occurs to a low extent only [21]. Bidault et al. showed that the storage modulus of a (methylacrylated)-PVA-fibrin-IPN was 3–50-fold higher in comparison to pure fibrin gels [45]. Furthermore, they have proven that the biohybrid material is non-cytotoxic. However, no cell proliferation of human fibroblasts was observed. In 2015 the same research group improved cell growth and biodegradability by introducing (methylacrylated)-serum albumin to the PVA-fibrin-IPN [47]. A combination of collagen and PVA improved the compressive strength of the pure hydrogels. By crosslinking collagen and PVA

with glutaraldehyde, the compressive strength could be increased even further [48].

Another attractive modification is the usage of polyethylene glycol (PEG): a biocompatible, biodegradable polymer which is inert to cell or protein adsorption [49]. PEG can be modified with fibrin(ogen) or collagen in various ways leading to increased mechanical strength in all cases. For example, one approach described in the literature is the encapsulation of fibrin ribbons into a PEG hydrogel [50]. Another group, Jiang et al., loaded fibrin into a porous PEG hydrogel resulting in a decreased degradation rate of fibrin, which in turn induced vascularized tissue ingrowth due to the prolonged fibrin lifetime [51]. Collagen can be modified with PEG in a semi-IPN leading to higher viscoelasticity and elongation. Furthermore, cell adhesion and cell proliferation was promoted in this composite, which makes it a good scaffold for viscoelastic tissues [52].

Instead of using pure components, one could use PEGylated hydrogel-precursors for modification. PEGylated precursors are made by crosslinking denatured fibrinogen and collagen [the latter being modified with succinimidylacetyl-thioacetate (SATA)] to PEG. Photopolymerization of these PEGylated precursors leads to scaffolds which enable migration, cell spreading and controllable proteolytic degradation [53]. Stabenfeldt et al. examined the influence of synthetic PEGylated fibrin knob peptides on the polymerization process and the resulting mechanical properties of the fibrin gels. An increase of the complex moduli, porosity and a decrease in the degradation rate could be observed when a fibrin knob 'B' peptide was used [54].

Fibrin and collagen can also be modified with polylactic acids. For example, porous polylactic acid can be filled with a fibrin gel. Zhao et al. decelerated the weight loss rate of fibrin with such a polylactic acid construct [55]. A combination of poly lactic-co-glycolic acid, polylactic acid and fibrin achieved mechanical stability and a stable construct size during cultivation [56]. Porous collagen was also modified with surface activated polylactic acid fibers [57] or nanoparticles [58] leading in both cases to an increase in the compression modulus.

Another promising approach in research are thermosensitive hydrogels based on collagen and fibrin in combination with the Pluronic® F127 copolymer. Pluronic® F127 copolymer undergoes reverse thermal gelation in response to temperature changes. The conjugation of fibrinogen to Pluronic® F127 enables the control over physical properties (variable G′ and G″ values) while retaining full cell compatibility of the hybrid hydrogel [59]. The combination of collagen with Pluronic® F127 led to a composite gel which showed improved viscous characteristics compared to pure Pluronic® F127 gels, but with a small decrease in elastic behavior [60]. This thermosensitive gel may be used as an injectable hydrogel in tissue regeneration, for example [59, 60].

Carbon nanotubes (CNTs) can be used for modification as well. They are rolled-up graphite sheets building single-walled (SWCNT) or multi-walled (MWCNT) hollow cylinders [61]. CNTs are interesting materials because of their unique mechanical and electrical properties [62]. They may be used as scaffolds for cardiac or neural tissue [63]. MacDonald et al. showed that incorporation of SWCNT into a collagen hydrogel embedded with human dermal fibroblast cells led to a hydrogel with decreased gel compaction. The gel also exhibited electrical conductivity like native soft tissues [64]. Kim et al. demonstrated that the addition of SWCNT to collagen led to thicker fibers resulting in a less flexible matrix. Cultivation of human decidua parietalis placenta stem cells revealed no negative effects on cell proliferation and supported neural differentiation [65].

The tensile strength of collagen can be further improved with functionalized silver nanoparticles [66] or curcumin-caged silver nanoparticles [67]. Both approaches resulted in a biocompatible network which enabled cells to proliferate and even inhibited microbial growth, making nanoparticle-functionalized collagen hydrogels promising candidates in wound dressing applications [66, 67].

The group of Tranquillo increased the stability of fibrin-based engineered tissue through a ruthenium-catalyzed crosslinking system. This system leads to crosslinks between tyrosine residues of the fibrin and cell-deposited proteins, and resulted in a 3-fold increase in mechanical strength and a 10-fold increase in stiffness. These positive effects could be transferred to collagen-based engineered tissues [68].

Collagen and fibrin-based materials in vivo

Common medical applications of fibrin and collagen hydrogels are in the context of nerve regeneration, skin repair and cartilage tissue engineering. Even though some of these applications are typical for fibrin, as well as for collagen, the two proteins do not necessarily fulfill the same function in the preparation. Many fibrin dressings employ fibrin's natural capacity for hemostasis and angiogenesis. Collagen is mostly used as a bioactive scaffold

for cells or as a dressing for wound healing, which corresponds to its role in the ECM.

Various applications in biomedical research require higher mechanical strength than the one provided by the natural hydrogels themselves. Therefore, in the applications listed in Tables 1 and 2 the hydrogels have been compressed, crosslinked or blended with natural or synthetic polymers to enhance their mechanical properties. The resulting hydrogels are used in different in vivo environments.

Commercial products

Various products of fibrin and collagen are commercially available for clinical use. These include treatments for skin, nerves, vascular tissue and soft tissue. In contrast to the collagen and fibrin-based biomaterials which are the focus of current research, the commercial products of fibrin and collagen constitute mainly of isolates of the individual proteins. In current commercial applications, fibrin and collagen are used in their natural role as a hemostatic agent and an ECM protein, respectively.

Tisseel® was the first fibrinogen product approved by the US Food and Drug Administration (FDA). It has been marketed as a sealant for colonic anastomoses and as a hemostat when sutures or ligatures are ineffective to control bleeding since 1998 [79]. Since then, additional FDA-approved fibrinogen products have appeared on the market acting as hemostats, sealants and tissue adhesives in different clinical settings. Most of these commercial applications mimic the last step of the coagulation cascade, leading to the formation of a fibrin clot. The major differences between these medical devices are to be found in the source of fibrinogen and their application forms. Fibrinogen can be applied either in liquid or dried form. Liquid fibrin sealants are sprayed directly onto the wound, whereas dried fibrinogen is embedded into a solid matrix which is then placed onto the wound.

Examples for liquid fibrin include Tisseel®, Evicel® or Artiss®. Tisseel® and Evicel® are both assisting in hemostasis but differ in their formulation [79, 80]. Both contain human fibrinogen and thrombin from pooled human

Table 1: In vivo applications of fibrin hydrogels.

Form	Material	Application	References
Gel	Fibrin	Autologous blood vessel implant	[14]
	Fibrin-PEG	Vascular network formation	[51]
	Fibrin-hyaluronic acid	Scaffold for cartilage repair	[69]
	Fibrin-PVDF	Vascular graft	[70]
	Fibrin-PLA	Vascular graft	[71]
	Long-term stable fibrin-polycaprolactone-based polyurethane scaffold	Cartilage engineering	[12]
Sponge	Fibrin-PLLA-PLGA	Regulation of 3D vessel formation and neovascularization	[56]

PLLA, Poly(L-lactic acid); PLGA, polylactic-co-glycolic acid; PVDF, polyvinylidenfluorid.

Table 2: Examples of in vivo applications of collagen hydrogels.

Form	Material	Application	References
Disk	EDC-crosslinked collagen	Corneal substitute	[72]
Film	Collagen-poly(glutamic acid)	Surgical adhesive for damaged lungs	[73]
Gel	Collagen I (dehydro-thermally crosslinked)	Spinal cord repair	[74]
Matrix	Collagen-elastin	Skin wound coverage	[75]
Scaffold	Collagen I compressed	Artificial corneas	[76]
	Collagen II-glycosaminoglycan	Cartilage tissue engineering	[77]
	Collagen-chitosan	Peripheral nerve regeneration	[78]
	Collagen crosslinked with aminosilane functionalized silver nanoparticles	Wound dressing	[66]
Sponge	Collagen-chitosan	Diabetic wound healing	[30]

EDC, 1-ethyl-3(-3-dimethylaminopropyl)carbodiimide.

plasma. Tisseel® contains synthetic aprotinin as an adjuvant to inhibit fibrinolysis [79]. Artiss®, however, differs from Tisseel® and Evicel® in its utilization, although it also contains homologous human fibrinogen and human thrombin. It is used as a tissue adhesive in the treatment of burned skin for the binding of autologous skin grafts. Furthermore, it can be applied in face-lifting procedures to adhere tissue flaps [81]. Most of these liquid formulations carry the disadvantage that they have to be stored cooled, whereas dried formulations are ready-to-use which makes them ideal for emergency cases.

In 2010, TachoSil® received approval of the FDA as the first fibrinogen patch. This patch contains an equine collagen sponge as a carrier matrix providing mechanical stability and flexibility. It is indicated in hepatic and cardiovascular surgery to control bleeding [82]. Another patch (Evarrest®, Ethicon/J&J) is composed of layers of oxidized regenerated cellulose and polyglactin G910 which contain embedded fibrinogen and thrombin. It is used in liver surgeries or to stop soft tissue bleeding [83].

All previously mentioned products contain homologous fibrinogen and thrombin from pooled human plasma. These materials always carry the residual risk of containing blood-borne pathogens like viruses or prions and are highly dependent on blood donations. A different approach is the isolation of autologous fibrinogen from the patient's own blood during or before surgery.

One possibility to get autologous fibrinogen is offered by the Vivostat® System. After blood withdrawal, a non-physiological cleavage of the fibrinopeptide A is achieved by using the snake venom batroxobin. The resulting fibrin I polymer can be separated from blood plasma through centrifugation. This fibrin I network polymerizes into a fibrin clot by pH-adjustment which activates residual endogenous prothrombin, resulting in a cleavage of fibrinopeptide B and activation of endogenous Factor XIII [84]. The CryoSeal® FS System automates the cryoprecipitation step. The resulting fibrin sealant is indicated as a hemostat in liver resection [85].

Dyna-Stat® and formerly CoStasis®, now Vitagel™, is an application system which contains a solution of bovine collagen and thrombin. A mixture of this solution with autologous blood plasma leads to a collagen/fibrin matrix [86].

Pure collagen gels can also be applied as hemostats. Several companies are producing collagen-based hemostats as an alternative to manual compression [87, 88]. These are available as dry materials like powder, sheet, sponge, or as a sprayable liquid. Examples for dry material products include Helistat® and Helitene® sponges, D-Stat® bandages and Avitene® powder or sheets.

Dermal wound dressings consisting of collagen are commonly used and present a different example of commercial translation. Apligraf® is one example of an artificial skin matrix used for venous leg ulcers and diabetic foot ulcers [89]. It consists of bovine type I collagen seeded with human fibroblasts in the lower dermal layer and human keratinocytes in the upper epidermal layer forming a skin-like structure. Alloderm® of LifeCell™ also started as a dermal dressing, but its main use has shifted towards reconstruction of soft tissues like breasts and abdominal organs. The biological matrix used in Alloderm® is decellularized human skin tissue which promotes a regenerative process after implantation. A similar effect is provided by the INTEGRA™ Matrix Wound Dressing which is composed of bovine tendon collagen type I and glycosaminoglycans. It can be combined with INTEGRA™ Bilayer Matrix Wound Dressing for a flexible covering with water vapor loss control. Special treatment is necessary for the care of burned skin. Biobrane® of Smith & Nephew consists of porcine collagen and allows faster healing time than conventional burn care [90]. It can also be used for the healing of transplanted skin.

Apart from dermal applications, collagen is also used for peripheral nerve regeneration, as for example, NeuraGen® from INTEGRA™. This collagen tube consists of crosslinked collagen which supports axonal growth across a nerve gap [91]. Another example of collagen application in nerve regeneration is DuraGen® from INTEGRA™. It consists of bovine Achilles tendon collagen type I and is used in cranial and spinal operations as a substitute of injured dura mater to prevent leakage of cerebrospinal fluid [92]. TissuDura of Baxter is a very similar but transparent product made of equine collagen. It has the same application field and has shown comparable results in clinical application [93, 94].

Another application field of collagen is in the regenerative therapy of bone and cartilage. Matricel produces membranes consisting of porcine collagen which promote bone regeneration (Remaix) or cartilage regeneration (Cartimaix). The membrane functions as a barrier for the recovery area and as an adhesion site for cells. Thus, it can support the regeneration of the damaged tissue [95].

Conclusion and future perspectives

The challenge of designing a novel biomaterial is to provide an environment which closely resembles the

native tissue which has to be replaced. As the understanding of the basic underlying mechanisms of ECM-cell interactions becomes increasingly elucidated, we can expect that additional knowledge will flow into material design. Hydrogels with an ECM-like composition offer the best possibility to mimic native tissue and are the first step to accomplish structure and organization similar to native ECM.

Structural proteins like fibrin and collagen are a good starting point for creating ECM-based materials as they are already approved for clinical application. As pure collagen and fibrin hydrogels show poor mechanical stability, major advances have been made in the development of modified and functionalized biomaterials. Modification with additional proteins, polysaccharides or synthetic polymers has led to hydrogels with improved stability for various tissue regeneration procedures.

Due to the fact that collagen and fibrinogen are usually extracted from biological sources, batch-to-batch variations will invariably appear which could hinder the GMP requirements for a particular product. A more secure way to obtain pure proteins of consistent quality and free of the risk of pathogenic transmission is recombinant production. Most heterologous expression systems for fibrinogen production are based on mammalian cells (e.g. baby hamster kidney cells [96] or PER.C6 cells [97]) due to the complex structure and post-translational modifications involved. Furthermore, fibrinogen could be isolated from the milk of transgenic dairy cows [98]. In contrast to fibrin, recombinant collagen production has been successfully demonstrated in yeast systems [99]. Stein et al. could prove that production of collagen type I is also possible in tobacco plants [100].

Another strategy for obtaining novel ECM-based materials involves the production of tailor-made proteins with characteristic sequences of interest to create fibrin and collagen-mimetic recombinant materials. These protein fragments could be produced with simple and cost-effective expression systems like *Escherichia coli* or *Pichia pastoris*.

The next level of design of collagen and fibrin-based biomaterials includes considerations of the fabrication technique which allows the manufacture of complex 3D structures with defined geometry and architecture. Techniques like stereolithography, micro-molding, bioprinting, and laser structuring require bioactive materials which can be quickly polymerized using changes in temperature, enzymatic or light reactions. In this way, it is possible to structure the physiological environment provided by the hydrogel into precise micro-scale structures, which is a major step towards the engineering of artificial 3D tissues and organs.

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