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A Review on Some Natural Biopolymers and Their Applications in Angiogenesis and Tissue Engineering

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Abstract

One of the concerning challenges for engineering and regenerating tissues is providing a suitable condition for development of a utilitarian vascular matrix. Natural polymers such as collagen, gelatin, chitosan, silk fibroin and fibrin are used as bio-compatible scaffolds to prepare appropriate biological and mechanical conditions for regenerative medicine and tissue engineering approaches. A wide range of studies demonstrated that using these biomaterials as scaffolds or engineered constructions such as hydrogels can provide a microenvironment to improve regeneration and repair of target tissues and organs through enhancing angiogenesis. They can be used single or in composition with each other. This review focused on some different natural polymeric constructs that have been incorporated in tissue engineering.

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Introduction

Tissue engineering is a reparative means which merges biomaterials, cells and functional environmental factors to induce growth, proliferation and differentiation signals for promotion of tissue repair or functional regeneration.[1,2] This interdisciplinary field faces many limitations which some of them are listed here[3-5]:

- Proper selection of bio-compatible and bio-active materials for the purpose of repairing or regenerating the target tissue;
- Optimizing mechanical properties to produce a well-developed scaffold suitable for soft or under load tissues;
- The technique used to construct the appropriate engineered scaffold or structure;
- Identification of angiogenic factors in each tissue;
- Utilizing factors that can stimulate the production and secretion of angiogenic factors in target tissue; and
- How to transfer these factors to the tissue in absence of the native factors.

Natural polymers are biologically suitable materials to use as structures in tissue engineering applications (Figure 1).[6] In this study, we focused on some polymers that used in research works in angiogenesis and other tissue engineering applications in different tissues and organs. The studied polymers are collagen, gelatin, chitosan, silk fibroin and fibrin. The aforementioned polymers have the ability of mimicking many properties of the extracellular matrix (ECM). Therefore, they can potentially induce the attachment, growth, migration, organization and differentiation of cells during regeneration of tissue or wound healing.[7,8]

The biological process that pre-existing vessels form new ones is called angiogenesis. As mentioned, angiogenesis is one of the most important issues in regeneration of a tissue.[9] Vessels deliver nutrients and take away wastes and their lack is one of the problems for scaffolds or engineered constructions implanted in body.[10] One of the current purposes in tissue engineering is to solve this concern by utilizing biomaterials that can act as a suitable environment to provide a condition for enhancing angiogenesis during tissue regeneration. These materials have also the potential of homing and releasing other angiogenic factors or effective elements while used as engineered constructs. Induction of endothelial cell homing, stimulation and activation of pro-angiogenic factors such as osteopontin, Forkhead box protein C2 (FOXC2), basic

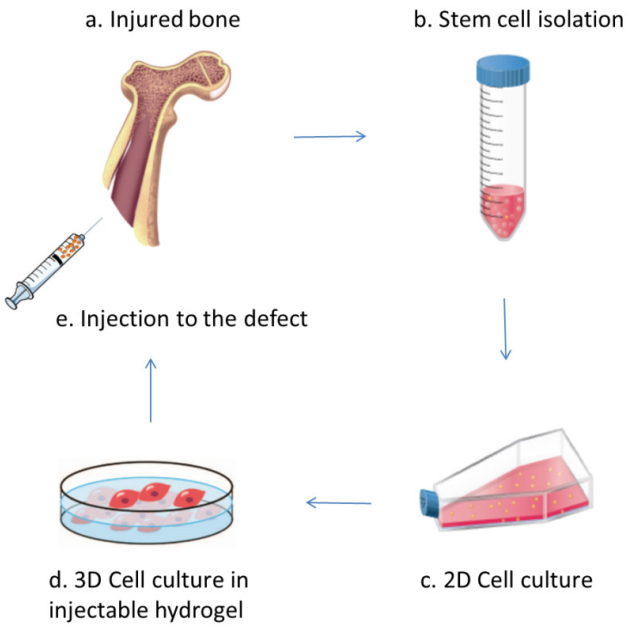

Figure 1. Using Natural Polymers as Hydrogel in Bone Tissue Engineering.

fibroblast growth factor (bFGF), Tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and vascular endothelial growth factor (VEGF), inducing proliferation and migration of endothelial cells, improving the mitogenic response to angiogenic factors and overexpression of angiogenic genes are the effects of elements such as silicon, phosphorus, copper, magnesium and europium used in natural-based constructs to improve angiogenesis in tissue engineering approaches.[1] In the following part, some natural polymers and their applications will be reviewed.

Collagen

Collagen is an essential protein of ECM which supports tendons, skin, cartilage, bones, ligaments and cornea tissue.[11-13] Therefore, it is considered as a proper matrix or scaffold. Collagen interaction with connective tissue cells transduces crucial signals for the regulation of cell adhesion, survival, proliferation, migration and differentiation.[14] Collagen has many types but type I collagen is the most studied for tissue engineering and biomedical uses.[15] Its properties including high mechanical strength, low antigenicity, good biocompatibility and ability of being cross-linked, make collagen an ideal biomaterials for tissue engineering applications.[16,17] Laiva et al developed a scaffold with pro-angiogenic gene-activated properties to influence angiogenesis by producing paracrine angiogenic factors in wound healing. They combined nanoparticles of polyethyleneimine containing stromal derived factor-1 alpha (SDF-1α) gene with a collagen-chondroitin sulfate scaffold. They evaluated the effect of this structure on mesenchymal stem cells (MSCs) angiogenic potential. The MSCs on scaffold showed SDF-1α mRNA over-expression associated with VEGF and CXCR4 activation which are angiogenic factors. They conclude that combination of SDF-1α gene with collagen-based scaffolds can provide a suitable condition for angiogenic response during wound closure.[18] Some of these applications are summarized in Table 1.

Gelatin

Gelatin is a natural polymer derived from collagen which is widely used for medical applications and pharmaceutical due to its biocompatibility and biodegradability in physiological environments.[49,50] In addition, gelatin has low antigenicity in comparison to collagen which is shown antigenicity because of its animal origin. Gelatin is a denatured protein received by collagen processing using alkaline and acid.[51,52] The alkaline process affects asparagine and glutamine amide groups and hydrolyses these group into carboxyl ones. Acidic treatment has little influence on the amide groups. Therefore, acidic treatment is electrically different from alkaline-processed gelatin. The different conditions of gelatin processing allow polyion complexation flexibility of a gelatin scaffold, carrier or hydrogel with either negatively or positively charged biomolecules and cells.[49,53] Because of mentioned properties of gelatin, it is been used in engineering of various tissues and drug delivery applications. We summarize many of these applications in table. Nemati et al used alginate-gelatin microcapsules to provide an appropriate microenvironment for human umbilical vein endothelial cells (HUVECs) during a 5-day period. In vitro studies revealed promotion of HUVECs proliferation and cell survival. The expression of VEGFR-1, VEGFR-2, Tie-1 and Tie-2 were enhanced however it was not significant. They investigate the potential of the encapsulated cells in angiogenesis by implanting it in immune-compromised mouse model for 7 days. The result showed an increase of encapsulated cells angiogenic response in comparison to non-capsulated ones. They claimed that angiogenic response could be promoted by alginate-gelatin encapsulation.[54] In Table 2, some of gelatin applications in tissue engineering are summarized.

Chitosan

Chitosan is a chitin-obtained cationic polymer consists of copolymers of N-acetyl-D-glucosamine and β (1→4)-glucosamine. Chitin as a natural polysaccharide found in the cuticles of insects, shell of crustacean and fungi cell walls. Chitosan is the partially or fully deacetylated form of chitin [71]. The chitosan deacetylation degree is usually a range between 70% and 95%, and the molecular weight is also between 10 to 1000 kDa.[72] Its application in tissue engineering and drug delivery fields is wide ranging from cartilage, bone, vascular grafts and skin to substrates for cell culture. Biologically renewable, biocompatible, biodegradable, non-toxic and non-antigenic properties of chitosan make it a bio-functional useful biomaterial.[52,73,74] In addition, hydroxyl and amino groups of chitosan can be modified chemically to provide a high chemical diversity. It also has bio-adhesive properties.[72] Chitosan exhibits different behaviors at various pH levels. It doesn't dissolve at high pH while it is soluble at lower pH ranges.[75] This property makes chitosan a suitable tool for delivery applications. The summarized applications of chitosan in tissue engineering exhibited in Table 3. Cheng et

Table 1. Collagen Applications in Tissue Engineering

Polymer	Condition	Cell Type	Tissue Engineering Application	In Vitro/In Vivo (Animal)	Reference
Collagen	As sponge	Chondrocytes	Cartilage	In vivo (implantation in nude mice)	19
Collagen	As gel	Stromal cells of bone marrow	Bone/cartilage	In vivo (mice)	20
Collagen	As gel	-	Skin	In vivo (rabbit)	21
Collagen	As gel	-	Angiogenesis	In vitro	22
Collagen	As a gel in heparan sulfate matrix	-	Angiogenesis	In vivo (rat)	23
Collagen	As a gel with gelatin microspheres	-	Adipose	In vivo (mice)	24
Collagen	As sponge	Osteoblasts	Bone	In vivo (defect in mice skull)	25
Collagen	Electrospun nanofibers	Bone marrow-derived MSCs	Bone	In vitro	26
Collagen	As sponge and hydrogel	Human intervertebral disc cells	Intervertebral disc	In vitro	27
Collagen	As sponge	Porcine third molar cells	Tooth	In vivo (rat)	28
Collagen	As sponge	Autologous chondrocytes	Cartilage	In vivo (sheep)	29
Collagen	As membrane	Chondrocytes	Cartilage	In vivo (rabbit)	30
Collagen	As sponge	Human preadipocytes	Adipose	In vivo (nude mice)	31
Collagen	Associated with GAG	Bone marrow-derived MSCs	Cardiovascular	In vivo (rat)	32
Collagen	Scaffold	Human smooth muscle cells	Urogenital	In vivo (nude mice)	33
Collagen	As gel	Glomerular mesangial and epithelial cells	Glomerular tissue of kidney	In vitro	34
Collagen	Fibrous scaffold	Meniscus cells	Meniscus tissue generation	In vitro	35
Collagen	Electrospun fibers composed by poly(L-lactide-co-caprolactone)	Autologous tracheal epithelial cells and chondrocytes	Trachea	In vivo (rat)	36
Collagen	As hydrogel	MSCs and MCs	Meniscal tissue	In vitro	37
Collagen	Freeze dried porous scaffold composed by β-tricalcium phosphate	MSCs	Bone	In vitro	38
Collagen	As films composed by silk fibroin	Adipose-derived stem cells and human osteoblasts	Bone	In vitro	39
Collagen	Associated with alginate as bioink for three-dimensional (3D) cell printing	Chondrocytes	Cartilage	In vitro	40
Collagen	Composed with hydroxyapatite and PLGA and alginate incorporated microparticles	Osteoblasts	Bone	In vitro/In vivo (rat)	41
Collagen	As hydrogel composed with hydroxyapatite and alginate	Chondrocytes	Osteochondral	In vitro	42
Collagen	Composed with bioglass and phosphatidylserine	rMSCs	Bone	In vitro/In vivo (rat)	43
Collagen	Membrane scaffold Composed with GAG	Tendon cells	Tendon	In vitro	44
Collagen	Electrospun scaffold composed with human tropoelastin	-	Skin	In vitro	45
Collagen	Composed with bacterial cellulose	Osteogenic cells	Bone	In vitro	46
Collagen	Composed with elastin-like polypeptide	MC3T3-E1 pre-osteoblast cells	Bone	In vitro	47
Collagen	Scaffold with a cement of calcium phosphate	Umbilical cord stem cells	Bone	In vitro	48

Abbreviations: MSCs, mesenchymal stem cells; MCs, meniscus cells; rMSCs, Rat MSCs

al carried out a study to construct a thermo-sensitive hydrogel of chitosan/gelatin composition that is suitable for angiogenic applications by sustained release of adipose-derived stem cells which is a result of gelatin gradual degradation. In vitro studies revealed a significant concentration of VEGF in the supernatant of chitosan/gelatin hydrogels containing adipose-derived stem cells. Tube-like structures were formed in co-culture of the encapsulated adipose-derived stem cells and SVEC4-10 endothelial cells. This result demonstrates the potential of chitosan/gelatin hydrogel in inducing angiogenesis.[76] Recent applications of chitosan are summarized in Table 3.

Silk

Silk is a protein biopolymer produced by spiders, silkworms, mites, scorpions and flies.[98] Spider silk is an interesting biomaterial that is weightless, elastic and strong that is comparable to the best fibers synthetized by new technology

Table 2. Gelatin Applications in Tissue Engineering

Polymer	Condition	Cell Type	Tissue Engineering Application	In Vitro/In Vivo (Animal)	Ref.
Gelatin	As hydrogel	-	Bone	In vivo (nude mice)	49
Gelatin	Freeze dried scaffold composed with siloxane	Osteoblast-like cells (MC3T3-E1 line)	Bone	In vitro	55
Gelatin	Microspheres encapsulated in hydrogel	-	Cartilage	In vitro	56
Gelatin	Microspheres encapsulated in hydrogel	Bovine chondrocytes	Cartilage	In vitro	57
Gelatin	As sponge	-	Bone/cartilage	In vivo (canine)	58
Gelatin	Porous disks	Adipose-derived adult stem cells of human	Cartilage		59
Gelatin	Photocured styrenated microspheres	-	Adipose	In vivo (nude mice)	60
Gelatin	microspheres encapsulated in hydrogel matrix	Bone marrow stromal osteoblasts of rat	Bone	In vitro	61
Gelatin	Associated with modified hyaluronic acid hydrogel	Bone marrow-derived MSCs of rabbit	Osteochondral	In vivo (rabbit)	62
Gelatin	Microcarrier beads	Nasal chondrocytes of human	Cartilage	In vivo (nude mice)	63
Gelatin	Porous sponge	Adult human MSCs	Cartilage	In vivo (rabbit)	64
Gelatin	As VEGF-containing hydrogel	HUVECs and Schwann cell line (RT4-D6P2T)	Peripheral nerve	In vitro/vivo	65
Gelatin	Photocrosslinkable hydrogel	Keratinocyte	Skin (epidermal tissue)	In vitro	66
Gelatin	Composed with polycaprolactone, photocrosslinkable scaffold	Adipose stem cells	Tendon	In vitro	67
Gelatin	Photocrosslinkable methacrylated scaffold	Mature adipocytes	Adipose tissue	In vitro	68
Gelatin	Composed with collagen and bioactive glass	Osteoblast	Bone	In vitro	69
Gelatin	Electrospun bilayered nanofibers scaffold, composed with poly(lactic acid-co-glycolic acid)	meniscal cells derived from New Zealand white rabbits menisci	Meniscal tissue	In vitro	70

Abbreviations: HUVECs, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells.

Table 3. Chitosan Application in Tissue Engineering

Polymer	Condition	Cell Type	Tissue Engineering Application	In Vitro/In Vivo (Animal)	Ref.
Chitosan	Photopolymerizable hydrogels associated with collagen	Mouse BMSCs	Bone	In vitro	77
Chitosan	A TCP/ chitosan hydrogel	-	Bone	In vivo (rat)	78
Chitosan	Freeze-dried scaffolds	Rat calvarial osteoblasts	Bone	In vitro	79
Chitosan	Composed with collagen scaffolds periodontal TGF-β1 plasmid Human	Periodontal ligament cells of human	Periodontal bone	In vivo (nude mice)	80
Chitosan	Freeze-dried sponge	Fetal rat calvarial osteoblastic cells	Periodontal bone	In vivo (calvarial defect of rat)	81
Chitosan	Composed with gelatin, freeze-dried scaffolds	Rabbit articular chondrocytes	Cartilage	In vitro	82
Chitosan	microspheres in chitosan freeze-dried scaffolds	Porcine articular chondrocytes	Cartilage	In vitro	83
Chitosan	microspheres in chondroitin sulfate–collagen–chitosan freeze-dried scaffolds	Rabbit articular chondrocytes	Cartilage	In vitro	84
Chitosan	As hydrogel, composed with glycerol phosphate	-	Osteochondral	In vivo (rabbit)	85
Chitosan	Injectable hydrogels composed with heparinoid	HUVEC	Angiogenesis	In vivo (mice)	86
Chitosan	As hydrogels	-	Vascularization	In vivo (rabbit)	87
Chitosan	As hydrogel	-	Skin	In vivo (rat burn wound)	88
Chitosan	As photo cross-linked hydrogel	-	Skin	In vivo (mice full-thickness skin incisions)	89
Chitosan/ chitin	Tubular device with PLGA microspheres	Mice neural stem cells	Peripheral nerve regeneration	In vitro	90
Chitosan	As injectable hydrogel, composed with chondroitin sulfate	Articular chondrocytes	Cartilage	In vitro	91
Chitosan	As scaffold, containing calcium polyphosphate and pigeonite	MSCs	Bone	In vivo (rat)	92
Chitosan	Composed with gelatin as macroporous scaffold	Human dermal fibroblasts	Blood vessel	In vitro	93
Hydroxyethyl chitosan	Hydrogel scaffolds with bubble-like porous structure composed with cellulose	Osteoblastic MC3T3-E1 cells	Bone	In vitro	94
Chitosan	Composed with nano-hydroxyapatite	Human bone MSCs	Bone	In vitro	95
Chitosan	Composed with poly(lactic acid) as a nano-fibrous scaffolds	Cardiomyocyte	Cardiac tissue	In vitro	96
Chitosan	As silicone-modified membrane	-	Corneal epithelium	In vitro	97

Abbreviations: HUVECs, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells; BMSCs, bone marrow stromal cells.

in terms of mechanical properties.[99] It is also a bio-degradable material and environmentally safe. Because of the limited amount of spider silk, silk fibroin as a natural polymer which produced by silkworms is a good alternative.[100,101] Sericin and fibroin are the major components of it. Fibroin, a fibrous protein creating the silk core, is composed of composed of fibroin Light chain, fibroin heavy chain and fibrohexamerin.[102] Excellent mechanical properties, biocompatibility and slow degradability make this material interesting [103,104]. Recently, silk is used as a biomaterial in corneal tissue engineering due to its transparency potential. Some of applications are mentioned in Table 4. Liu et al fabricated a Porous scaffold composed of basic FGF-immobilized silk fibroin. Proliferation and growth of L929 cells were improved on bFGF-immobilized silk fibroin scaffolds. In vivo studies contained implantation of the scaffold into the skin defect of rat that displayed significant re-epithelialization and skin regeneration. Formation of new vessels and deposition of collagen after 4-week treatment, showed the potential of these scaffold in angiogenesis and tissue regeneration.[105] Table 4 shows silk fibroin applications in regenerative medicine studies.

Fibrin

The applications of this natural polymer are well developed because of its innate ability to cellular interaction induction and scaffold remodeling in comparison to synthetic scaffolds.[135] Fibrin-based materials biochemical specifications make them ideal for drug and cell delivery. It can also be harvested autologous that provides an immuno-compatible carrier for drug, cell and active biomolecules delivery.[136,137] Keratinocytes, tracheal epithelial cells, murine embryonic stem cells, urothelial cells and mesenchymal progenitor cells are examples that could be carried by this biomaterial.[138-140] It is also very used in chondrocytes encapsulation for tissue

Table 4. Silk fibroin applications in tissue engineering

Polymer	Condition	Cell Type	Tissue Engineering Application	In Vitro/In Vivo (Animal)	Ref.
Silk fibroin	Fibre scaffolds	Bone marrow-derived mesenchymal	Bone	In vitro	106
Silk fibroin	As hydrogel	Osteoblasts	Bone	In vivo (rabbit)	107
Silk fibroin	Electrospun align net	Endothelial cells	Angiogenesis	In vitro	108
Silk fibroin	Porous scaffolds	MSCs	Cartilage	In vitro	109
Silk fibroin	Electrospun fiber scaffolds	Fibroblasts and keratinocytes	Wound dressing	In vitro	110
Silk fibroin	Composed with collagen	Hepatocytes	Liver	In vitro	111
Silk fibroin	Multi fiber matrix	Bone marrow-derived MSCs	Anterior crucial ligament	In vitro	112
Silk fibroin	Electrospun scaffold	Human MSCs (hMSCs)	-	In vitro	113
Silk	Biohybrid scaffold, composed with PLGA	Mesenchymal progenitor cells	Ligament/tendon	In vitro	114
Silk fibroin	Macro/microporous scaffolds prepared byobtained by combining the salt-leaching and freeze-drying methods	-	Articular cartilage and meniscus	In vitro	115
Silk fibroin	Scaffold	Human adipose-derived stem cells	Bone	In vitro	116
Silk fibroin	Electrospun sulfated nanofibrous scaffolds	Endothelial cells and smooth muscle cells	Vascular tissue	In vitro	117
Silk fibroin	Lactose conjugated	Rat hepatocytes, FLC-4, and HepG2 cell lines	Hepatic tissue	In vitro	101,118,119
Silk fibroin	Composed with alginate, chitin or collagen	Human oral/epidermal keratinocytes and fi broblasts	Skin	In vivo (rat)	120-123
Silk fibroin	Scaffold	Rat olfactory ensheathing cells	Spinal cord tissue	In vitro	124,125
Silk fibroin	Scaffold	Fibroblasts and chondrocytes	Tracheal tissue	In vivo (rabbit)	126
Silk fibroin	Scaffold composed with chitosan	Fibroblasts and chondrocytes	Tracheal tissue	In vivo (rat)	127
Silk fibroin	Scaffold	Human smooth muscle cells and myoblasts	Muscle tissue	In vitro	101
Silk fibroin	Scaffold	Human tympanic membrane keratinocytes	Eardrum tissue	In vitro	128,129
Silk fibroin	Containing cardiac tissue-derived ECM	HL-1 atrial cardiomyocytes and human embryonic stem cell-derived cardiomyocytes	Cardiac tissue	In vitro/vivo (rat)	130
Silk fibroin	As nano-hydroxyapatite composite hydrogels	Osteoblastic cells	Bone	In vitro	131
Silk fibroin	bFGF-incorporated porous scaffolds produced by freeze-drying	Dental pulp stem cells	Dental pulp	In vitro	132
Silk fibroin	Composed with poly (glycerol sebacate)	Dorsal fibroblasts of newborn littermates of C57 BL/6 mice	Skin	In vitro	133
Silk fibroin	Electrospun scaffold	Bone marrow MSCs	Sciatic nerve	In vivo (dog)	134

Abbreviations: HUVECs, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells; BMSCs, bone marrow stromal cells; hMSCs, human MSCs; ECM, extracellular matrix.

engineering of cartilage. Rapid degradation of fibrin can be considered as a disadvantage in tissue engineering. Therefore, improving composition of fibrin is an important issue to produce a scaffold system with appropriate mechanical properties.[141-143] An investigation was performed by Dohle et al to establish co-culture system of primary osteoblasts and outgrowth endothelial cells within injectable platelet-rich fibrin matrices. They were in an effort to determine the effect of platelet-rich fibrin on angiogenesis and wound healing by activating endothelial cells in this system. Histological studies indicated vessel-like structures formation after 7 days culturing. Expression of the VEGF, as a pro-angiogenic factor, was increased on the mRNA and protein levels. Therefore, platelet-rich fibrin might be ideal for wound healing through promoting angiogenesis.[144] Applications of fibrin-based constructs in tissue engineering are summarized in Table 5.

Conclusion

We reviewed different usages of some natural polymers in tissue engineering based on previous researches. Among the materials, polymers have been widely applied and have excellent potential to regenerate tissues due to their flexible features.[166] As mentioned one of the issues in the tissue regeneration is to provide blood supplying.[1] For this purpose, the quality of the regenerated vascular network is more important than the quantity. In other words, amount of perfused blood through a vascular network is the criterion, not just the number of vessels. Therefore, the importance of the vascular structure organization and maturation is clear. On the other hand, over-stimulating of angiogenesis leads to the creation of many unorganized vessels which are poorly perfused and have inefficient performance.[167] Organization of vascular structures is not the only factor that determines the success chances of engineered tissues, but it seems to be a basic principle.[168] Tissue engineering is in an effort to focus on vascular cells patterning in the target tissue to control the organization and maturation of vascular structures.

Incorporation of active bio-molecules like growth factors, angiogenic factors and elements is a novel strategy that is highly useful to improve tissue regeneration by improving angiogenesis,[169] but achieving a tissue with normal and functional vascular structures is still a challenge.

Table 5. Silk Fibroin Applications in Tissue Engineering

Polymer	Condition	Cell Type	Tissue Engineering Application	In Vitro/In Vivo (Animal)	Ref.
Fibrin	As a combined gel with PCL/TCP	Human osteoblasts	Bone	In vitro	145
Fibrin	As gel	-	Bone	In vivo (rat)	146
Fibrin	As a heparin containing gel	HUVECs	Vascularization	In vivo (mice)	147
Fibrin	As gel	Human fibroblasts cell line	Skin and Cardiovascular	In vitro	137
Fibrin	As gel and beads	Chick dorsal root	Spinal cord injury	In vivo (rat)	148
Fibrin	As gel	Chick dorsal root	Peripheral nerve regeneration	In vivo (rat)	149
Fibrin	As collagen containing gel	Embryonic chondrogenic cells	Cartilage	In vitro	143
Fibrin	As gel	Bovine articular chondrocytes	Cartilage	In vitro	142
Fibrin	As a gel in a PGA non-woven mesh	Pig chondrocytes	Cartilage	In vitro	150
Fibrin	Porous gel	Articular chondrocytes of human	Cartilage	In vitro	151
Fibrin	Scaffold	Murine embryonic stem cells	Spinal cord injury	In vitro	139
Fibrin	As gel	Rat aortic smooth muscle cells	Vascularization	In vitro	152
Fibrin	As a gel in a fiber-based scaffold	Human venous myofibroblasts	Cardiovascular	In vitro	153
Fibrin	Injectable beads composed with alginate	hUCMSCs	Bone	In vitro	154
Fibrin	Injectable	Endothelial cells	Cardiac tissue	In vivo (ischemic myocardium of sheep)	155
Fibrin	As a glue	Skeletal myoblasts	Cardiac tissue	In vivo (rat)	156
Fibrin	Fibrin gel in silicone tube	Cardiac myocytes	Cardiac tissue	In vivo (rat)	157
Fibrin	Injectable	Bone marrow mononuclear cells	Cardiac tissue	In vivo (rat)	158
Fibrin	As fibrin heart valves seeded with autologous cells	Carotid artery-derived cells	Cardiac tissue	In vivo (Implantation in sheep)	159
Fibrin	Injectable to the ischemic region	Cardiac-derived stem cells	Cardiac tissue	In vivo (rat)	160
Fibrin	As a glue	Adipose-derived stem cells	Cardiac tissue	In vivo (rat)	161
Fibrin	Injectable	Human mesenchymal progenitor cells	Cardiac tissue	In vivo (nude rat)	162
Fibrin	Implantable scaffold	-	Nervous tissue	In vivo (rat spinal cord injury)	163
Fibrin	Injectable hydrogels functionalized with cartilage ECM	infrapatellar fat pad-derived stem cells	Cartilage	In vitro/vivo (nude mouse)	164
Fibrin	As a keratin-fibrin-gelatin composite scaffold fabricated by freeze drying	NIH 3T3 fibroblast and human keratinocytes (HaCaT) cell lines	Wound dressing	In vitro	165

Abbreviations: hUCMSCs; human umbilical cord MSCs; HUVECs, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells; ECM, extracellular matrix.

Authors' Contributions

All authors contributed equally to this research.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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