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Current Research Status of Biomimetic Scaffolds and Mechanically Stimulating the Efficacy of Combined Growth Factors in Promoting Stem Cells in Ligament Repair

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Abstract

Ligaments are dense, fibrous connective tissue that maintains the stability of the joint through interosseous connections. Ligament tears due to sports injuries or tissue aging often require surgical intervention, and reconstitution using autologous, allogeneic or artificial ligaments as grafts is currently the gold standard for treating such diseases, but these grafts have many drawbacks. With the development of material science and manufacturing technology, artificial ligament tissue based on biological scaffold is expected to become a new type of tissue donor, by simulating the structure, composition and biomechanical characteristics of natural tissues, the purpose of tissue regeneration can be achieved, in addition to growth factors, mechanical stimulation and other factors also have positive significance for stem cell repair ligament, this paper summarizes the *in vitro* and animal experimental research of ligament tissue engineering, with biomimicry as the design principle, all aspects of ligament tissue scaffolding are evaluated. In addition, the performance and effect of various factors on stem cells to promote ligament repair and reconstruction are summarized, and the future development direction of bionic scaffolds and mechanical stimulation combined with growth factors on stem cells in ligament tissue engineering research is summarized and prospected, in order to provide new ideas for clinical repair of ligament damage.

Keywords: Ligament tissue engineering; Scaffold; Bionic; Biological materials; Biological factors

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Introduction

Ligaments are dense connective tissue that connects the bone and maintain joint stability during exercise and rest [1]. The mechanical properties of the ligament make it prone to laceration after sports injury or tissue aging. The repaired ligament is mainly disordered fibrous scar tissue, and its biomechanical properties are far inferior to those of normal tissues [2]. About 33 million musculoskeletal injuries are reported in the United States each year, with 50% of tendon/ligament injuries, and on average over 300,000 patients undergo surgery to repair a damaged tendon or ligaments annually [3]. In China, the incidence of rotator cuff injury is 20% at 60 years old and 31% at 70 years old, but there is no accurate statistics on the incidence of ligament injury [4-8]. Although transplantation can restore some mechanical function in a short period of time, the use of autologous, allograft or xenograft brings some problems, such as artificial ligament/tendon products represented by Leeds Keio and LARS show good initial mechanical strength and postoperative joint stability in clinical use, lack of viscoelasticity, wear and aging, limited autologous tissue regeneration caused synovitis, medium and long-term injury mechanical strength and even graft failure, making the clinical application of artificial ligament/tendon graft controversial [9]. The purpose of ligament tissue engineering is to combine the scaffold, cells, growth factors, and mechanical stimuli *in vitro* to reconstruct the structure and function of the injured ligaments. Seed cells, growth factors, and mechanodynamic stimulation can greatly increase the application effect of fiber scaffolds in ligament tissue engineering, and the review of scaffold manufacturing technology is conducive to the development of ligament tissue engineering. As a structural basis, the scaffold provides mechanical support and a cellular microenvironment, it functioning similar to the Extracellular Ligament Matrix (ECM). Composition and mechanical properties mimic natural ligament tissue. Now a days, the most studied ligament tissue engineering projects are fibrous stents and heterogeneous

interface stents, which facilitate the reconstruction of the tendon – bone interface and ultimately anchor the regenerated ligament in the bone. In recent years, due to retaining the structure, composition and mechanical properties of the original tissue, scaffolds derived from biological tissue have been studied more and more in ligament or interface tissue engineering, among which book acellular scaffolds are more innovative [10].

Seed Cells

Immune rejection and disease transmission. Adult bone marrow is a collection of bone marrow recharge stem cells, and Bone Marrow Recharge Stem Cells (BMSC) can self-renew and have the ability to differentiate into various mesenchymal cells [11,12]. Several remarkable properties of BMSCs make them a clear choice for various tissue engineering: (1) they can form a single colony of fibroblasts that can be isolated and expanded according to their ability to adhere to a solid surface to contain large numbers of cells; (2) the massive amplification of BMSCs *in vitro* has no obvious pathological effect on the eventual formation of ligament tissue *in vivo* and can differentiate into ligaments and tendons, and it is precisely because of this property that it is a popular seed cell in tissue engineering ligament construction; (3) by setting the same culture conditions can induce the differentiation of BMSCs into different mesenchymal phenotypes, including osteoblasts, chondroblasts, adipoblasts, and tendon/ligament cells; (4) the tissue-engineered ligaments formed by BMSCs can be used as a suitable autograft without surgical resection. No risk of an immune response (an allograft may occur) [13].

Growth Factors

Growth factors play an important role in inducing and/or supporting the expression of specific mesenchymal cell lines. Oxygen can influence the rate of extracellular matrix synthesis and the development of tissue-engineered tissues *in vitro*. Whether oxygen affects stem cell differentiation into ligamentous fibroblasts is unknown. Fermor et al. [14] showed that high oxygen concentration (21%) maximizes the differentiation of ACL fibroblasts, while low oxygen concentration (10%) promotes extracellular collagen synthesis. As a long-acting source of vitamin C, phosphate vitamin C has significantly increased the viability of cultured cells *in vitro* and increased the expression level of type I collagen by. Epidermal birth Growth Factors such as long factor (EGF), basic Fibroblast Growth Factor (bFGF), Insulin-like Growth Factor-II (IGF-II), and Transforming Growth Factor- (TGF-) can enhance cell proliferation. Furthermore, insulin, TGF-, and IGF- can promote connective tissue protein expression and extracellular matrix production. Its effect on the proliferation and differentiation of bone marrow mesenchymal stem cells is also related to concentration, but the specific dose-effect relationship needs to be further studied. TGF- and EGF as well as TGF- and insulin can synergistically stimulate the proliferation of fibroblasts and stromal cells. BMSCs were cultured *in vitro* using Advanced DMEM (ADMEM) medium with or without added growth factors by Morrow et al. [15]. The expression level of collagen type I was all significantly increased in BMSCs when compared with DMEM medium. The combination of EGF and TGF s (in sequence) maximizes cell proliferation and type I collagen expression. The effect of growth factors on BMSCs is dose-dependent. Hankemeier et al. [16] have shown that low-dose bFGF (3 ng/mL) significantly promotes the differentiation of BMSCs into fibroblasts and significantly increases ligament and tendon-specific extracellular matrix expression of cytoskeletal components, but has no effect at high doses of bFGF (30) ng/ml) [16-18] (Table 1).

Mechanical Stimulation

Mechanical signals that affect ligament growth and development *in vivo* also play an important role in tissue-engineered ligament cultures *in vitro*. The application of stress can affect tissue development in at least two ways: (1) enhancing the matrix (2) directly stimulating cells. Mechanical stimulation of cultured ligamentous fibroblasts enhanced the expression of type I and type III collagen [19], fibronectin, and mucin-C. In the absence of specific growth factors and regulators, human MSCs can differentiate into ligament-like cells by exposing human MSCs to physiological circulatory stress. Stress can alter the autologous and the fineness of the tissue-engineered ligaments. The extracellular environment, which directly affects cell shape and fibrillary clearance, or enhances mass delivery in and out of the cell through fluid flow. Mechanical pressure stress directly acting on the cellular level can stimulate the proliferation and regeneration of BMSCs and promote bone marrow mesenchymal stem cells to repair ligament damage.

Stent Material

The scaffold materials involved in ligament or tendon tissue engineering are generally divided into synthetic polymers, natural polymers, inorganic osteoconductive materials and biological tissue-derived materials. Synthetic polymers mainly include Polycaprolactone (PCL), Poly-L-Lactic Acid (PLLA), Polyglycolic Acid (PGA), Poly-L-Lactide-Co-Caprolactone (PLCL), Polylactic Acid-Co-Glycolide (PLGA), Polyethylene Terephthalate (PET), etc. Natural macromolecules mainly include Collagen (COL), Silk Fibroin (SF), silk, chitosan, Hyaluronic Acid (HA), Chondroitin Sulfate (CS), fiber glucosamine, Glycosaminoglycan (GAG), alginate, Gelatin (GEL), etc. [20]. Inorganic osteoinductive materials mainly include Hydroxyapatite (HAp), phosphoric acid Tricalcium (TCP), etc. Materials derived from biological tissues include acellular cortical bone, tendon, fibrocartilage, fat or bone-fibrocartilage-tendon composite tissue (Table 2).

Stent Manufacturing Technology

Fiber structures are often used in the design of tissue engineering scaffolds for ligaments or tendons. Traditional electrospinning techniques can spray material solutions into filaments by applying an electric field, and the arrangement or diameter of the fibers can be tuned by multiple parameters. Melt electrospinning can generate more precise and repeatable fiber patterns, such as sinusoidal fibers with specific wavelengths and amplitudes, mimicking the coiled structure of collagen fibers, further enhancing the guiding effect of fiber topology on cells. The wet spinning technology is to extrude the spinning solution and then shape the fibers in a coagulation bath, which can produce nano-to-micron fibers. The process is simple, but the accuracy of fiber arrangement and graphic design is insufficient [21]. Fiber manufacturing technologies such as electrospinning can be combined with technologies such as twisting, weaving, and knitting. 3D bracket, characteristic. Based on fibrous or braided structures, gel materials are used to assemble or chemically modify scaffolds through various cross-linking techniques, while porous sponge structures are formed by freeze-drying [22]. The currently emerging Three-Dimensional (3D) printing technology is used for the fabrication of multi-panel tissue engineering scaffolds due to its precise structuring and 3D structural control capabilities, but has no advantages in the fabrication of fibrous structures and thus

Table 1: Application of growth factors in the construction of engineering ligaments.

Clan	Growth factors	Effect	Acts on cells	Reference
bFGF	bFGF-1	Promotes cell proliferation and synthesizes ligament-related matrix proteins	Mouse BMSCs	Liu et al.
	bFGF	Promotes tendon healing, improves cell proliferation and biological activity	Rabbit BMSCs, osteoblasts, ligament cells	Cheng et al.
	bFGF	Promotes cell proliferation and enhances ligament mechanical activity	Mouse ligament cells	Leong et al.
	bFGF	Accelerates healing after tendon injury and promotes cell proliferation and differentiation	Murine tendon cells	Tang et al.
TGF-β	TGF- β1	Promotes ECM production and upregulates collagen and fibronectin Expresses, inhibits ECM degradation	Human fibroblasts	Lee et al.
	TGF- β1	Upregulate ligament-specific gene expression and increase ligament specificity Protein synthesis	Human amniotic mesenchymal stem cells	Jin et al.
	TGF- β1	Induces the encoding of the LOX gene and promotes the interbreeding of collagen and elastin join, increase ECM generation	Human ACL and MCI	Xie et al.
BMP	BMP-2	Has a bone-forming effect and promotes tendon healing	Rabbit ACL cells	Takigami et al.
	BMP-2	Delay the ACL degeneration process	Human ACL tissues and cells	Ruscke et al.
	BMP-2	Used in combination with VEGF to promote ligament and fibrocartilage attachment points the tendon bones heal	Rabbit ACL cells	Cheng et al.
	BMP-12	Promotes regenerative healing of tendon ligament-like tissue	Rabbit ACL cells	Kuroda et al.
IGF	IGE	Positive modulating effect of early tendon strain. inhibition of the inflammatory response	Human ACL and MCL	Herchenhan et al.
GDF	GDF	Promotes the repair of MCI space damage and promotes the differentiation of ligament cells Synthesis of ECM proteins	Human ACL tissues and cells	Tashiro et al.
EGF	EGF	Promotes rapid proliferation of ACL cells	Human ACL tissues and cells	Woo et al.
PDGF	PDGF	Promotes healing of ligament cells and tissues and blood vessel formation	Human ACL tissues and cells	Bisel et al.
VEGF	VEGF	Promotes cell proliferation and improves the mechanical tensile force of ligaments	Human ACL tissues and cells	Tang et al.
PRP	PRP	Synthesis of matrix proteins, catalyzing collagen and fibronectin Crosslinking	Human ACL tissues and cells	Komzak et al.

is not suitable for ligament or tendon tissues [23]. In engineering, it is mainly used to study tendon-bone interface reconstruction, facet joint ligament reconstruction and tendon repair. Using cell-loaded bioinks for scaffold printing can save time for cell seeding and adhesion, and directly achieve uniform cell distribution without obvious cytotoxicity, but improper scaffold pore size or connectivity may inhibit cell proliferation.

The development direction of 3D printing in ligament tissue engineering is more focused on printing consumables, and the bone tissue engineering scaffold printed by a single material often has limitations in performance, and the composite material composed of two or more different materials, such as metal and bioceramics, polymeric materials and bioceramics, can give full play to the complementary role between materials, improve the biocompatibility and mechanical properties of the scaffold, and gradually become the preferred printing consumables for bone tissue engineering scaffolds.

- Fiber scaffolds have the following advantages for biological tissues:Chemical resistance
- Heat resistance
- Good tissue compatibility
- Does not lead to coagulation and hemolysis.

Fiber Support

The design of fibrous scaffolds is related to the collagen fiber composition and structure of the ligament, from the initial use of a single material to create parallel fiber structures through spinning techniques, to the progressive use of hybrid or composite materials combined with weaving and knitting through biomimetic aspects of structure and material with the continuous in-depth exploration of characteristics, the performance of fiber scaffolds has been greatly improved [24].

Composite or hybrid material fiber stents

Ligament ECM contains collagen, elastin, proteoglycan and other components, each of which has its own function. At present, in the research of ligament tissue engineering, composite materials or hybrid material scaffolds also tend to be studied, because these materials have their own advantages and can complement each other [25]. Mixed material stent is to stir and mix several raw materials in proportion, and then make a stent. Several studies used PLLA/COL-I solutions with different mixing ratios to spin fiber bundles that mimic the structure and diameter of native tendons, and found that the Young's modulus and tensile strength of the fiber bundles with a ratio of 75/25 were stronger than 50/25, respectively, 50 groups [26]. The stiffness and toughness of cross-linked fiber bundles are similar to tendons, and the biocompatibility is good. Similar studies also used different types and ratios of synthetic/natural polymer hybrid materials to prepare fiber scaffolds, such as PCL/cellulose, PCL/COL, and found that the hybrid material fiber scaffolds had advantages over pure synthetic polymer scaffolds. Under a certain mixing ratio, the adhesion and proliferation of cells are more obvious [27]. Some studies have added inorganic particles, such as silica or hydroxyapatite, to synthetic polymer materials, and it can be seen that these particles are uniformly distributed on the fiber surface, and cells exhibit a "beaded" structure and proliferation ability to fibers. Better adhesion promotes ECM deposition [28]. The composite scaffold framework system refers to the chemical or physical combination of multiple materials with different properties to form a multiphase system. The composite material also has excellent performance in improving the mechanical properties and bioactivity of scaffolds, such as two layers of nano-ordered PLGA fiber sheets sandwiched in coaxially arranged PLLA microfiber sheets, which become columnar after fracture load. The stent measured on rolling was approximately 200 N, and the initial fracture load of the graft (130 N) and the deformation (2 mm to 8 mm) after reconstruction of the rabbit Anterior Cruciate Ligament

Table 2: Common stent material for constructing engineering ligaments.

Material	Strengths	Shortcoming	Reference
Decellular scaffolding	Good cell compatibility, low immunogenicity, firm healing of the tendon bone solid and can withstand the stress before healing	The tensile strength is average, and it is difficult to achieve complete Decellularization extent	Yang et al.
collagen	Good biocompatibility, low antigenicity, no rejection in the implanted body react	Mechanical tensile strength is weak	Sun et al.
silk	Good cell adsorption, maintain normal cell function, degradation Slow, high-strength mechanical stretching capability	Immunogenic, antigenic, present with host immunity Epidemic exclusion	Wang et al.
Chitosan	Good biocompatibility and degradability, good cell adsorption Effect, can promote cell proliferation and differentiation	Mechanical tensile strength is weak	Pinese et al.
Fucoic acid	Good biocompatibility, the scaffold is gelatinous, and the specific surface area is relative Large, with good mechanical compressive strength	The mechanical tensile strength is weak, and the preparation steps of the stent are complicated trifling	Khojasteh et al.
hyaluronic acid	Good biocompatibility, strong plasticity, short degradation cycle	Mechanical tensile strength is weak	Kahn et al.
PLA	High mechanical tensile properties, strong resistance to internal pressure and high stitching degree	Biocompatibility is low, biological activity is weak, and it is easy to degrade	Stolzel et al.
PLGA	Good biocompatibility and biodegradability, first beauty The National FDA approved biodegradable materials for clinical use	The biological activity is low, the rate of degradation is fast, and acids are produced Sexual metabolites	Toosi et al.
PGA	Good cell adhesion, widely used in suture making	The rate of biodegradation is too fast, resulting in acidic metabolic production, mechanical tensile strength is weaker, biological activity is lower	Macarini et al.
PLLA	Mechanical tensile strength is high, degradation rate is moderate, good cells Adhesion, easy to make	The biological activity is low, producing acidic metabolites	Deepthi et al.

(ACL) were similar to those of the native ACL [29]. Munsterb For the first time, 4 PET and 20 PLLA wires were used to construct a "Tiger" scaffold, a composite gradient mesh, and the mechanical strength of the composite scaffold reached the maximum peak load (996 N) in the current rabbit ACL reconstruction study. Two mechanically enhanced scaffolds were used for ACL reconstruction in rabbits, and the scaffold structure remained intact and supported tissue ingrowth at 12 weeks postoperatively. Liu used SF/PLCL composite braid based on multilayer braided scaffolds to improve the pore size of SF braided scaffolds by surface modification of L-lysine and hyaluronic acid on the adhesion, migration and proliferation activities of Mesenchymal Stem Cells (MSCs), rate and mechanical strength. Zhang et al. performed 3 chemical optimizations on the ordered PLLA fibrous scaffolds and found that surface modification of COL on the scaffolds significantly improved cell penetration, allowing uniform distribution within a week, and then used fibroblast growth factor and transforming sequential biochemical stimulation of growth factors significantly improves ECM synthesis and organization, while fibrin glue can bind between fibrous layers, increase scaffold thickness to some extent, and synergize with growth factors to promote ECM synthesis [29-31]. In two rabbit ACL reconstruction studies, the knitted silk/COL-sponge composite scaffolds exhibited good biocompatibility and mechanical strength, combined with cellularization or vascularization, and simultaneously achieved ligamentization and ligamentization of the tendon-bone interface. Reconstruction [32], whether it is a hybrid scaffold or a composite scaffold, the materials have overlapping advantages and complementary defects, and are superior to single-material scaffolds in terms of biocompatibility, mechanical properties, and bioinduction.

Structure of the fiber scaffold

Ordered fibers: Since the collagen fibers of ligaments are parallel and ordered, the ordered structural fibers produced by spinning technology have become the most widely used structural design in ligament tissue engineering research. Recent studies have found that the assembly of PCL and PLGA fibers can enhance the axial mechanical strength of fiber scaffolds, promote the axial stretching and alignment of cells, induce tendon differentiation and ECM deposition of MSCS, and even alleviate inflammatory conditions (FBS) by inhibiting ECM deterioration. By simulating the diameter and structure of natural collagen fibers, Lu prepared SF membranes with parallel groove comb-like structures with groove widths ranging from 5 μm to 20 μm. They found that the ordered topology of the grooves has an obvious contact guiding effect on the biological behavior of MSCS, and the grooves of 10 μm have a clear contact guiding effect. Cells, representatives may be associated with activation of focal adhesion kinase [33-36]. In one study, ordered PCL nanofiber bundles were implanted in sheep cadavers for ACL reconstruction, and mechanical testing showed that the scaffold was similar to ACL in terms of fatigue resistance, toe stiffness, and post-implantation anterior-posterior stability. Petrilliano Rat ACL reconstruction using ordered PCL nanofiber scaffolds. After 12 weeks, collagen deposition was found on the scaffolds in the bone tunnel and the intra-articular area [37], smaller than natural ligaments. Leong's team fabricated a PCL/COL ordered fibrous scaffold for rat ACL reconstruction and found that the initial maximum load and stiffness of the graft *in vivo* were 13.5% and 15.7% of that of native ACL, respectively, and increased by 28.2% over 31.3% postoperatively, 16 weeks %. A similar scaffold was then fabricated with super-polymer PCL for rat ACL reconstruction. *In vitro* mechanical properties were not tested. Although the mechanical strength of the ultra-high polymer PCL graft was found to be stronger than that of ordinary PCL at 16 weeks after surgery, its ultimate load and stiffness only reached 41.9% and 21.3% of that of the native ACL. Taken together, it is found that although ordered fibrous scaffolds made of such synthetic polymers can induce tendon differentiation and ECM deposition *in vitro* and *in vivo*, the mechanical strength after implantation is still much lower than that of ACL [38-39].

Core-sheath structure: The ligament has a multi-layered structure such as collagen fibers and fiber bundles. The fiber bundles at all levels are wrapped by appropriate endothelium and tendon sheaths. The mesh structure also improves the overall mechanical strength of the ligament. Some scientists fabricated PLLA nanofiber mats by electrospinning and rolled them into fiber bundles. After bundling 100 parallel fiber bundles together, the disordered PLLA fiber sheath was attached to the surface, successfully simulating the gradient. Fiber and core sheath structure. The frame stress-strain curve has a good toe-in and linear range. Similar stiffness to tape, but lower tensile strength. FB can penetrate between fibers and perform well [40], proliferation activity. The Teuschl et al. used degummed silk fibers

for graded twisting and braiding, wrapped two inner braided ropes with a tubular braided outer sheath, and constructed a core-sheath structural framework based on the braided fibers, with a maximum load and stiffness of 1,450 N and 1,300 N, respectively. It is 450 N. 200 N/mm, all within the range of mechanical strength of sheep cruciate ligament. One year after the operation, it was found that the fibrous filaments of the intra-articular skeleton were significantly reduced, to a large extent, the new ligament tissue grew in, and the graft was connected to the spherical new bone at the junction of the bone tunnel. The use of autologous vascular stromal tissue has no long-term effect. Cai et al. [41] first fabricated PCL fiber bundles wrapped with PLCL/SF nanofibers, and then weaved the core-sheath-structured wires into a 3D scaffold with peak load and elastic modulus of 77 N and 91 MPa, respectively, which are suitable for *in vitro* studies. The tendon differentiation of MSCs was significantly increased. When the scaffold was used to repair the patellar ligament defect in rabbits, the histological and mechanical properties of the repaired tissue were better than those of the PCL fiber scaffold at 6 months after surgery, and were similar to the natural tissue. It can be seen that the sheathed graded fiber scaffold improves the integrity and mechanical strength of the scaffold by mimicking the natural ligament structure while supporting cell and tissue ingrowth [42].

Multiphase Tendon Bone Interface Stent the Tendon

Bone interface is composed of tendon, fibrocartilage, calcified fibrous cartilage, and bone tissue junctions, and the mechanical performance differences and structural gradients of this composition formation can avoid tissue damage caused by stress concentration during stress conduction. The difficulty of interface tissue engineering lies in the simultaneous regeneration of multiple tissue types and restoring their unique gradient structure and mechanical properties, while the multiphase scaffold designed based on biomimetic principles brings hope for the regeneration of such complex tissues. Different regions on the scaffold have different representative phases depending on the structure or composition, such as the typical three-phase tendon-bone interface scaffold usually including the ligamentous phase, fibrlochondral phase, and bone phase [43]. Inorganic materials are commonly used bone conduction materials in bone tissue engineering, and growth factors have the ability to induce multidirectional differentiation. Recent studies of interface tissue engineering often construct heterogeneous scaffold [44] based on the constituent gradients of both. TCP has become a commonly used material in bone and interface tissue engineering due to its inherent bone-induction properties and an appropriate degradation rate. In one study, 3D printing was used to create a porous PCL (tendon region) -tubular PCL/TCP (fibrocartilage region) -porous PCL/TCP (bone region) three-phase scaffold forming a TCP gradient from the bone region to the tendon showing good pore connectivity at both stages, supporting the adhesion and proliferation of FB, Bone Marrow Mesenchymal Stem Cells (BMSCs) and osteoblasts, respectively, and chondrogenesis in the scaffold fibrocartilage region 21 days after chondrogenesis induction and culture. After implantation in mice at 8 weeks after subcutaneous injection, marker genes were identified from tendons, cartilage, and bone during the scaffold stage, and the upregulation of COL-X further clarified the formation of calcified cartilage [45]. In another study, a 3D-printed PCL/PLGA/-TCP stent was wrapped and secured in the femoral and tibial tunnel to complete the rabbit ACL reconstruction, forming a TCP gradient-visible bone junction tissue between the stent and the autologous tendon, rich in fibrocartilage, and the bone tunnel area was significantly reduced by [46]. Interestingly, some scientists used polyethylene glycol gel to connect the COL/GAG at both ends and the calcified COL/GAG material at both ends to form a three-phase scaffold, which enhanced the toughness of the gel by optimizing the gel viscoelastic transition process, and the stress concentration became the interface framework designed to combine the mineral gradient with the stress distribution mechanism of [47].

Both HAp and TCP are calcium phosphate materials, with a long degradation cycle and good bone conductivity, which are often used in the design of interface tissue engineering scaffolds. The three-phase woven fiber scaffold, designed by Calejo was woven from wet-spun PCL/GEL ordered fibers and PCL/GEL/HAp disordered fibers into the middle and deposited COL-II and COL-X, suggesting bone and cartilage matrix synthesis, and another study found that the deposition of COL-I, GAG + COL-II and calcium in human Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) was similar to the composition gradient of the tendon-bone interface matrix 8 weeks after implantation [48]. Rat rotator cuff injury to mature collagen fibers and fibrocartilage structures were clearly visible in the repair group.

Biological Tissue-Derived Scaffolds

Biological tissue scaffolds are obtained directly taken from tissues or organs, preserve ECM after physical and chemical cell removal, and scaffolds are more similar in structure, composition, and function to native tissues than other types of scaffolds, and low immunogenicity ensures biocompatibility. Recently, several studies have used single tissue or composite tissue to prepare de-acellular scaffolds for tendon or ligament tissue engineering, and achieved some results by cell replantation, mechanical stimulation or material addition, but there are also insufficient [49]. Studies have shown that the fiber parallel structure and some mechanical properties (tensile strength, stiffness, fracture elongation) of decellularized tendon do not differ significantly different compared with before decellularization. In the study of rabbit ACL xenocellular tendon reconstruction, it was found that the cell replanting of the scaffold combined with mechanical stimulation significantly increased the mechanical strength after the graft, while the HAp/gold nanoparticle composite scaffold could promote regeneration, however, the host tissue remodeling had varying degrees of inflammation and graft degradation or necrosis. In interface tissue engineering, the un-celled composite bone-fibrocartilage-tendon tissue is a multiphase scaffold with natural structural and compositional gradients. Su et al. took the tendon bone junction complex [50] in the porcine Achilles tendon. After decellularization, they found that the structure of collagen fibers and cartilage fibers were basically retained, while the COL and GAG were partially retained, similar to the human Achilles tendon. Mouse BMSCs showed excellent adhesion, infiltration, and proliferative activity on the scaffold, and the tendon and bone marrow genes were upregulated in the corresponding regions 2 weeks later. After the re transplantation of the cells to the scaffold, the rabbit medial collateral ligament significantly promoted the osseointegration within the tunnel as compared with the cell-free tendon alone. Another study decellularized the canine patella-patellar ligament complex and confirmed that the retention of fibrocartilage matrix and collagen fiber axial structures promoted the infiltration, axial arrangement and tendon differentiation of BMSC under traction stimulation, but not obvious cartilage differentiation. In recent years, Professor Tang's et al. has been committed to the study of the cell-

free scaffold structure of the cell layer. The book structure cuts the whole scaffold into a page structure. The side of the tissue is cut off, and the opposite side is connected to the spine to facilitate the binding between the scaffolds or with the cell sheet. They successively prepared fibrocartilage and tendon tissue from rabbit bone, and demonstrated their good biocompatibility and ability to induce MSCs to differentiate into scaffold original tissue lineages and bind to MSCs *in vitro*. The rabbit patellar tendon junction and Achilles tendon defects were repaired, respectively, with good tissue structure and function. The team recently designed a book-like composite scaffold that combines acellular bone, fibrocartilage, and tendons, which cross-connects between pages to form four transition areas similar to the tendon-bone interface, attaching the BMSC cell layer evenly to the [51] between them. After 2 weeks, the expression of bone, cartilage and ligament marker genes and proteins in each region, and then the scaffold/cell constructs were implanted in the rabbit patellar-patellar-tendon defect, which was locally formed at 16 weeks after surgery, retained the continuous bone-fibrocartilage-tendon interface tissue, histological and biochemical analysis showed structural and composition gradient, the mechanical strength of the interface is also significantly higher than the control [52]. Biological tissue-derived scaffolds were found to have good biocompatibility because the original tissue ECM microenvironment is preserved to varying degrees and has the potential to induce MSCs to differentiate into native lineages of scaffolds and undergo tissue regeneration. However, such scaffolds still have the problem of cell residues leading to an inflammatory response of the immune system or variable loss of components, causing its mechanical strength and diminished ability to induce differentiation. Some studies improved the retention of ECM components and growth factors by optimizing the sodium dodecyl sulfate concentration and de-cellulation time with sufficient cell removal, and the scaffold showed a more significant differentiation induction on MSCs. Therefore, while further optimizing the cellular desalination technique, a comprehensive evaluation of the decentralized frame structure, composition, and mechanical properties of the [53-59] should be completed in the future.

Summary and Outlook

The study of ligament tissue engineering scaffolds involves a wide range of materials and manufacturing techniques, whose bionics is embodied in achieving similar mechanical properties and physicochemical environments by mimicking the composition and macroscopic and microstructures of ligaments with endogenous tissues. *In vivo* animal studies show that such scaffolds still have problems such as insufficient mechanical strength, fatigue resistance and tissue regeneration ability, and composite biomimetic multi-phase scaffolds are still the main idea of ligament tissue engineering scaffold design in the future. Bionics in structure and composition is difficult to achieve. The advent of biological tissue scaffolds yields a simplified scheme for the construction of scaffolds, and their inherent biomimetic properties in composition and structure confer a favorable bio-induced environment and mechanical properties, including ligament creep resistance and stress distribution at the interface. Subcellular matrix components and matrix degradation caused by inflammation *in vivo* can affect scaffold properties. Future research direction of ribbon tissue engineering scaffold can use the bionic advantage of biological tissue scaffold, integrate different structures of natural, synthetic polymers or inorganic materials, build enhanced mechanical and biological properties, and combine mechanical stimulation, growth factors, MSCs to promote tissue regeneration, improve the inflammatory environment, while realizing the reconstruction of joint area ligament and tunnel tendon bone interface.

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