Polysaccharide-Based Composite Scaffolds for Osteochondral and Enthesis Regeneration

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Abstract

The rotator cuff and Achilles tendons along with the anterior cruciate ligament (ACL) are frequently injured with limited healing capacity. At the soft-hard tissue interface, enthesis is prone to get damaged and its regeneration in osteochondral defects is essential for complete healing. The current clinical techniques used in suturing procedures to reattach tendons to bones need much improvement for the generation of the native interface tissue, i.e., enthesis, for patients to regain their full functions. Recently, inspired by the composite native tissue, much effort has been made to fabricate composite scaffolds for enthesis tissue regeneration. The current review first focuses on the studies that used composite scaffolds for the regeneration of enthesis. Then, the use of polysaccharides for osteochondral tissue engineering is reviewed and their potential for enthesis regeneration is presented based on their supporting effects on osteo- and chondrogenesis. Gellan gum is selected and reviewed as a promising polysaccharide due to its unique osteogenic and chondrogenic activities that help avoid the inherent weakness of dissimilar materials in composite scaffolds.

Keywords: tissue engineering, tendon-bone, enthesis, gellan gum, rhamnose, polysaccharides

Impact Statement

Enthesis regeneration is essential for complete and functional healing of tendon and ligaments tissues. Current suturing techniques to reattach the tendon/ligament to bones have high failure rates. This review highlights the studies on biomimetic scaffolds aimed to regenerate enthesis. Additionally, the potential of using polysaccharides to regenerate enthesis is discussed based on their ability to regenerate osteochondral tissues. Gellan gum is presented as a promising biopolymer that can be modified to simultaneously support bone and cartilage regeneration by providing structural continuity for the scaffold.

1. Introduction

The rotator cuff and Achilles tendons along with the anterior cruciate ligament (ACL) are one of the most frequently injured musculoskeletal tissues. Over 250,000 rotator cuff tendon repairs and more than 100,000 ACL reconstructions are performed annually in the United States.[1-3] In clinical settings, the suturing technique is applied to reattach the tendon/ligaments to bones but these tissues rarely regain their full function with a failure rate of 94%.[4-6] The tissue-associated inherent limitations including low cellularity and vascularization are considered as the primary source for limited healing and functionality.[5,7,8] Yet, in recent years, the prominent studies demonstrated that the lack of transition and integration at the soft-hard tissue interface are the important contributor for limited functionality.[9-13]

The enthesis, a connective tissue between tendon or ligament and bone, transfers the mechanical loading from soft tissue to bone for locomotion. The enthesis provides a smooth stress transition from soft tissue to hard tissue (bone) through its four distinct but structurally continuous matrix zones including tendon, unmineralized fibrocartilage, mineralized fibrocartilage, and bone. The soft tissue connects to the hard tissue through a gradual transition from tendon/ligament to non-mineralized fibrocartilage tissue followed by mineralized fibrocartilage tissue that connects to bone (Figure 1).[14] The structurally gradual transition in enthesis material composition is crucial in minimizing stress concentrations at the interface and allows forces to be dispersed properly from tendon to bone.[15] Thus, mimicking the gradual matrix transition between the zones is essential for complete and functional healing of tendon and ligament tissues.

Towards enthesis tissue regeneration, several prominent research groups have utilized single-phase scaffolds with or without cells and growth factors and demonstrated promising results to some degree in forming single-type tissue systems.[16-19] However, the enthesis has a complex structure with longitudinal variations in mineral content, collagen alignment, and extracellular matrix composition, which presents gradual differences in functional and mechanical characteristics.[20,21] Thus, inspired by the multiphasic inherent structure of native enthesis, multiphasic scaffolds, stratified or gradient, have been investigated to mimic the native variation in material composition of the enthesis matrix.[4,22-29] Although promising, gradient scaffolds that mimic the gradual transition in mineralization still lack the ability to provide physiologically relevant transitional tissue thickness in the order of micro- or nanometers.[21,24,30-33] The mineralization exponentially increases across the mineralized fibrocartilage region toward bone in bovine tibiofemoral ligament-bone insertion[34], while the increase in mineralization is linear over a distance of 120 μm in rat rotator-cuff tendon-bone insertion.[21] The rapid increase in mineralization along the enthesis suggests that scaffold stratification is a promising method for enthesis regeneration. It has been suggested that stratified scaffold phases be fabricated from the same biomaterial to provide the structural continuity and avoid the inherent weakness of sharp transitions of dissimilar materials.[35,36] It is critical that the base biomaterial can be modified such that it supports the formation of cartilage and bone for a regenerative enthesis healing.

The polysaccharides and ECM proteins are attractive scaffold materials for enthesis repair because they are able to form a 3-D matrix containing water and facilitating transportation of nutrients/waste exchange and signaling molecules.[37-47] Among many biomaterials, polysaccharides have been used as effective scaffolds for various purposes in tissue engineering.[48-52] Polysaccharides are long carbohydrate molecules of monosaccharide units joined together by glycosidic bonds.[53-55] In living organisms, polysaccharides such as pectin, cellulose, chitin, and agar support the tissue structure, while other polysaccharides including starch and glycogen serve as storage units. Polysaccharides also play critical roles in cell signaling and cell adhesion processes that modulate cell behavior.[48,56] In tissue engineering, the most commonly used polysaccharides include hyaluronic acid, alginate, chitosan, starch, cellulose, dextran,

and pullulan.[57-69] The use of polysaccharides in drug delivery and tissue engineering has been reviewed and compared in previous works.[70-78] It is noted that rhamnose-containing polysaccharides have shown to stimulate cell proliferation, collagen biosynthesis, and modulate matrix biosynthesis.[79-86] The addition of rhamnose-containing polysaccharides to fibroblasts demonstrated stimulation of calcium-signaling pathway to induce increases in Ca^{2+} influx and intracellular free Ca^{2+} levels.[87,88]

The current review first highlights the studies on composite scaffolds for enthesis regeneration. Then, the use of polysaccharides in osteochondral regeneration is reviewed and its potential for enthesis regeneration is presented. Gellan gum is focused due to its positive effects on osteochondral tissue engineering and suggested as a promising polysaccharide for enthesis scaffolds.

2. Synthetic composite scaffolds for enthesis regeneration

Soft tissues in musculoskeletal structures such as tendons that connect muscle to bone comprise of units of collagen bundles (150-1000 μm), which are assembled from collagen fibers (1-300 μm) that are made of collagen fibrils (10-500 nm).[89,90] These collagen bundles populate the extracellular matrix of connective tissues. Upon injury, collagen fibers decrease in diameter, exhibit a disorganization in structure, and the total area of collagen fibrils becomes significantly smaller than healthy collagen fibers.[91,92] Consequently, scaffold fiber diameter is a critical design parameter that regulates the response of human tendon fibroblasts.[92] Following the hypothesis that aligned nanofibers would guide deposition of aligned collagen fibers, Lipner et al. fabricated composite scaffolds using layers of poly-(lactic-co-glycolic acid) (PLGA) nanofibers (diameters of 400–900 nm), and the layers are further added with fibrin hydrogel layers seeded with stromal cells transduced with an adenovirus that leads them to produce bone morphogenetic protein-2 (BMP-2).[93] However, the composite scaffold showed a negative effect on rat supraspinatus enthesis restoration by exhibiting scar-mediated healing rather than regeneration. As mentioned by the authors, the rationale behind the negative effect of the PLGA nanofiber scaffold was attributed to low cell density seeded in the scaffold as well as the implantation of the scaffold as a patch over the repair site rather than at the interface of tendon and bone. Polyglycolic acid (PGA) nanofibers have been also used

and combined with poly-L-lactide-co-ε-caprolactone (PLCL) to fabricate a biphasic scaffold for integrative repair of rotator cuff injuries on sheep infraspinatus tendon-to-bone insertion.[94] In this work, the tendon was sharply transected and immediately reattached with the scaffold sutured between the tendon and the bone. Compared to the suture-only group, the scaffold group exhibited an increase in ultimate failure in load and in stress. Histologically, perforating collagen fibers were present and extended through a region of calcified fibrocartilage attaching to the humerus. Intense inflammatory response was also triggered in the scaffold group that was not observed in the suture-only group. The impact of this inflammatory response on the translation to human enthesis healing is not yet understood.

Another research group used PLGA in microspheres to encapsulate connective tissue growth factor (CTGF), transforming growth factor beta 3 (TGFβ3) and BMP-2 in order to regenerate the fibrocartilaginous tissue in the tendon-to-bone interface.[95] The encapsulating microspheres were embedded in polycaprolactone (PCL) micro-strands such a way that CTGF was on the top layer (tendon side), BMP-2 was on the bottom layer (bone side), and CTGF + TGFβ3 microspheres in the middle layer for a fibrocartilage interface layer between tendon and bone. The scaffold was implanted at the interface between the supraspinatus tendon and the humeral head in a rat rotator cuff repair model for 4 weeks. Enhanced healing of the enthesis was observed with greater fibrocartilaginous tissue formation and a higher bone volume compared to the scaffold without growth factors (control). PCL has been also investigated for enthesis regeneration in a rat patellar tendon avulsion model. Kim et al. designed asymmetrically porous membranes prepared by mixing PCL and Pluronic F127. Platelet-derived growth factor (PDGF) and BMP-2 were immobilized in the mixture to induce tenogenic differentiation and osteogenic differentiation, respectively.[96] One side of the membrane has nano-size pores designed to prevent scar tissue infiltration into bone-tendon interface injury site, while the other side of membrane was designed to provide nutrient permeation through micron-size pores, which would enhance the adhesion of the membrane with the defect site and act as a scaffold to guide bone-tendon interface regeneration.[97] The study demonstrated that PCL/Pluronic F127 accelerates the regeneration of the tendon-bone interface due to the continuous release of both growth factors and their complementary

effects on creating a multiphasic structure. Although promising, the use of growth factors has several limitations including short effective half-life, low recombinant expression yield, suboptimal efficacy, and high cost of research and quality control.[98-102] These limitations lead to multiple administrations or high doses to sustain an effective concentration of growth factors which often results in ectopic tissue formation, abnormal growth, inflammatory complications, and toxicity.[103-106] Long term storage of growth factors is also challenging largely due to poor protein stability which can be affected by temperature, pH, hydrolysis or oxidation of amino acid side chains, and freeze-thawing and freeze-drying.[107-110]

Cai et al. developed a dual-layer aligned-random nanofibrous scaffold (ARS) using silk fibroin-blended poly(l-lactic acid-co-ε-caprolactone) (PLLA-PCL) in a rabbit extra-articular model.[111] Autologous Achilles tendon was wrapped with the ARS and passed through a bone tunnel and sutured to the adjacent soft tissue. New bone formation was observed at 12 weeks along with a formation of fibrocartilage and collagen organization. However, as mentioned by the authors, this tendon-bone healing model was different from that used in humans, and the sample size of the study was too small with a short observation period. Additionally, the use of autologous grafts is known to be limited by donor site co-morbidity and can lead to postoperative chronic pain and poor muscle function.[112,113]

In an effort to mimic mineralized and non-mineralized fibrocartilage of enthesis, Li et al. fabricated a dual-layer of flexible bipolar fibrous membrane with a gradient microstructure for enthesis regeneration using a poly-L-lactic acid (PLLA) fibrous membrane as the upper layer (fiber diameter = 1.64 ± 0.62 μm) and a nanohydroxyapatite- poly-L-lactic acid (nHA-PLLA) fibrous membrane as the lower layer (fiber diameter = 1.47 ± 0.51 μm), respectively.[114] Using a rabbit rotator cuff tear model, they demonstrated an improved collagen organization, bone formation, and fibrillogenesis with the dual-layer membrane compared to the single-layer PLLA membrane. Load-of-failure and stiffness measurements showed greater values in the dual-layer membrane compared to the single-layer one, but still inferior to the normal uninjured tendon. There was no significant difference between experimental and control groups in bone mineral density (BMD) and bone volume fraction (bone volume/total volume; BV/TV). The

biodegradability and tailorable mechanical properties of PLLA make it advantageous to use in tissue engineering. It is noted that limitations of PLLA include low cell adhesion because of its hydrophobicity, acidic degradation by-products, and lack of cell differentiation properties.[115-117] These shortcomings of PLLA may obstruct its application to osteochondral generation where specific interactions between cells and implants are necessary.[118,119]

Table 1 summarizes the in vivo studies of composite scaffolds for enthesis regeneration. Although tissue formation is promising, it remains challenging to achieve biological healing of a multi-tissue transition at the tendon-to-bone interface. The use of big animal injury model and the repairing methods that are physiologically relevant to humans are needed for further evaluation. Additionally, creating a physiologically relevant scale of the mineral gradient is still technologically challenging for in vivo investigations.

3. Polysaccharides in osteochondral tissue engineering

Polysaccharides are natural materials that mimic the physiological structure of the ECM and provide glycosaminoglycan (GAG)-like environments with nontoxic degradation products. One of them is alginate that is a naturally occurring anionic disaccharide with repeating units of 1-4 linked D-mannuronic acid and L-guluronic acid. Alginate is one of the most widely used materials for tissue engineering because of its biocompatibility and biodegradability with tunable mechanical properties.[120,121] Encapsulation of cells and growth factors in alginate gels has been demonstrated successfully in vitro.[122] In vivo, bone marrow stromal cells (BMSCs) have been encapsulated in ultra-purified alginate gels and injected into full-thickness osteochondral defects of 5 mm in diameter and 3 mm in depth in the patella groove of rabbit knees.[123] The alginate gels histologically and mechanically improved the repaired tissue in the 12-week study period. Additionally, alginate, alone or in combination with other materials such as hyaluronic acid[124], chitosan[125], and gellan gum[126], was investigated with acellular approaches with promising results. For example, Chen et al. applied a combination of alginate and hyaluronic acid scaffold to osteochondral defects in the patella groove of rat knees.[127] The results showed simultaneous regeneration of cartilage and subchondral bone in the 8-week study period.

Hyaluronic acid is a disaccharide with glucuronic acid and N-acetylglucosamine repeating units linked via alternating β-1,4 and β-1,3 glycosidic bonds. In a human case report, umbilical cord blood-derived MSCs were encapsulated in hyaluronic acid hydrogel and applied to a large osteochondral defect (5 mm diameter and 5 mm deep) of the knee with a follow-up period of 5 years.[128] The underlying bone was only partially restored as bony tissue, while the superficial portion near the articular cartilage was restored as cartilaginous tissue. It was concluded that the composite hydrogel is a viable therapeutic option that can be performed through a one-stage arthrotomy. Recently, Hwang et al. injected an acellular hyaluronic acid weekly for 3 weeks on osteochondral lesions of the talus after a failed microfracture surgery.[129] On average, symptoms, pain, and quality of life were improved between the pre-injection and the last follow-up visit of patients. However, the treatment failed in one-third of the patients. The authors concluded that hyaluronic acid injections may possibly be a safe and effective alternative as a secondary operative treatment after a failed primary operative intervention.

Another well-known polysaccharide used in osteochondral regeneration is chitosan. Chitosan is a linear positively charged polysaccharide with repeating units of β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. Chitosan is known to mimic the structure of the glycosaminoglycan.[130] Chitosan-based materials have been extensively investigated in osteochondral tissue engineering.[131] Rajagopal et al. encapsulated rabbit bone MSCs in a multi-layered aligned chitosan-gelatin scaffold that resembles the ECM and the native collagen architecture.[132] The scaffold was applied to osteochondral defects (4 mm diameter and 3 mm deep) in the patella groove of rabbit knees. The scaffold supported the differentiation of MSCs to chondrocytes and the regenerated cartilage and subchondral bone were greater in volume in the aligned group compared to the randomly aligned group. In another study, chitosan in combination with icariin-conditioned serum was intraarticularly injected into osteochondral defects in rabbit knees.[133] More cartilage and subchondral bone regeneration were observed in the chitosan-serum group than serum-only group.

Long-term studies are needed to demonstrate the efficacy of polysaccharides on long-lasting repair of osteochondral defects. Additionally, identifying the metabolic pathway underlying the activity of regeneration is critical for future clinical translation. The

dependency of healing on the size of the defect also remains ambiguous. Table 2 summarizes the in vivo studies of polysaccharide-based materials for osteochondral regeneration.

4. Gellan gum in osteochondral tissue engineering

Gellan gum is a naturally occurring polysaccharide with repeating units of D-glucose, D-glucuronic acid, and L-rhamnose [-(→3)-β-D-Glc*p*-(1,4)-β-D-Glc*p*A-(1,4)-β-D-Glc-(1,4)-α-L- Rha-(1→)-], with two acyl substituents, L-glyceryl and acetyl, that are attached to the C-2 and C-6 positions of the O-3- linked 1,3-D-glucose residue[142] (Figure 2). Deacylation in alkaline solution yields low acyl gellan gum.[143,144] Since both substituents are bulky, the gellan polymer chains are unable to form close double-helix formation between them. Therefore, the native form of gellan gels are weak, soft, and elastic.[145] Low acyl gellan gum, on the other hand, is firm, non-elastic, and brittle.[146] At high temperatures, low acyl gellan gum is present as a random coil conformation, which converts to an ordered, cross-linked double helix conformation upon cooling.[147] As a result, controlled drug release can be achieved by varying the degree of cross-linking.[148,149] Due to its biocompatibility and biodegradability, gellan gum has been investigated in biomedical applications[150-152], food processing[153], pharmaceutics[154], drug delivery[155,156] and tissue engineering[157-162].

For bone regeneration purposes, gellan gum has been blended with various materials including bioglass, polydopamine, gold, hyaluronic acid, methacrylate, demineralized bone powder, silk fibroin, collagen, and hydroxyapatite, as well as physical modification by enzymatic or thermal hydrolysis as summarized in Table 3. Jung et al.[163] prepared a gellan gum/tuna skin gelatin film to guide bone regeneration using β-tricalcium phosphate as bone graft in an artificial bone defect on parietal bones of rabbits. The film had a positive effect on the formation of new bone, and degradation of the film was observed. Kim et al.[164] used bone defects in a rat model to study the effects of gellan gum-demineralized bone powder scaffold on bone regeneration. It was found that the scaffold was biocompatible, and it facilitated the cell adhesion and proliferation of BMSCs and regeneration of bone tissue. Similarly, investigations on cartilage regeneration have used gellan gum successfully to support chondrogenesis with or without cells.[165,166] Acellular

approaches have been performed using a rabbit model with articular cartilage defects that were treated with intra-articular injections of three different polysaccharides, gellan gum, alginate, and agarose.[165] The results were compared with hyaluronic acid, which is frequently used in cartilage tissue engineering. Gellan gum and agarose groups were covered with regenerated tissues comparably to the hyaluronic acid group. In vitro, the expressions of NF-jB and Cox-2 decreased and those of IjBa, Sox-9, aggrecan, and type II collagen increased in gellan gum, alginate, and hyaluronic acid. It was concluded that gellan gum improves cartilage regeneration by suppressing inflammatory mediators and inducing cartilage formation and autophagy-related gene expression, indicating its potential for cartilage tissue engineering. Pereira et al.[167] developed an acellular bi-layered scaffold of gellan gum/gellan gum-hydroxyapatite to produce cartilage-like and bone-like layers, respectively. After soaking in a simulated body fluid solution up to 14 days, it was found that the hydroxyapatite layer formation is limited to the bone-like layer of the bi-layered scaffold. This result indicates that gellan gum-based scaffolds can provide the mineral disparity between layers and may be used as a base biomaterial with the ability to support the regeneration of bone and cartilage simultaneously. Vuornos et al. co-cultured human adipose stem cells and human umbilical vein endothelial cells in gellan gum-collagen scaffold using two different media, endothelial growth medium-2 (EGM-2) and bioactive glass extract-based endothelial and osteogenic medium (BaG EM-OM).[168] In both media, osteogenic and endothelial marker gene expression were supported as well as the formation of reticulated cellular structures. Hydroxyapatite mineralization was detected only in BaG EM-OM medium. This result indicates that gellan gum-based scaffolds support the proliferation and differentiation of multiple cell populations. Table 3 summarizes studies targeting bone and cartilage regeneration using gellan gum-based scaffolds.

Based on these promising results in osteochondral applications, gellan gum-based materials targeting enthesis may overcome the limitations of synthetic polymers and the problems of their toxic degradation products. They would make it possible to avoid the use of growth factors and thereby prevent abnormal and ectopic tissue formation. Moreover, polysaccharides may help the mineralization be restricted to certain layers of the composite while the other layers can stay unmineralized, which is critical for the

regeneration of enthesis. Supporting proliferation of multiple cell populations is also advantageous in creating cartilaginous and osseous tissues simultaneously.

In our preliminary investigations, low acyl gellan gum (KELCOGEL® F, CP Kelco, USA) gels with different molecular weights were prepared at 1% (w/v) and placed in a 24-well plate. LA-GAGR refers to low acyl gellan gum (MW= 200-300 kDa) and mini-GAGR (MW_v= 25 kDa) is an enzymatic hydrolysis product of LA-GAGR.[190] Pre-osteoblast cells (MC3T3-E1, ATCC, USA) were seeded on top of the gels and a complete α-MEM medium containing 10% fetal bovine serum, ascorbic acid (50 µg/mL), and 1% penicillin streptomycin was used for cell culture. The control consisted of wells without gels. After 3 days of culture, extracellular collagen type I concentration was measured using an Enzyme Linked Immunosorbent Assay (ELISA) kit (MyBioSource, USA) according to the manufacturer's instructions. Figure 3 shows that both LA-GAGR and mini-GAGR supported the synthesis of extracellular collagen I which supports previous findings that gellan gum enhances extracellular matrix production by the cells. After 7 days of culture, total RNA was extracted using miRNeasy Mini extraction kit (Qiagen, USA) according to the manufacturer's instructions. RT^2 First Strand kit (Qiagen, USA) was used for cDNA synthesis and genomic DNA elimination. The relative expression levels of osteogenic marker genes were investigated by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) using the human osteogenesis RT^2 Profiler™ PCR array (Qiagen, USA). Figure 4 shows the relative expression levels of osteogenic marker genes. It is noted TGFB3 and SP7 show a 3 ± 0.1-fold and 12 ± 0.1-fold increase, respectively, for the LA-GAGR group. Also, the TGFB3 and SP7 gene markers show a 1.3 ± 0.1-fold and 20 ± 0.1-fold increase, respectively, for the mini-GAGR group. These results suggest a positive initiation of bone osteogenesis since it has been shown that SP7 acts as a master regulator of bone formation during both embryonic development and the homeostatic maintenance of bone in adults.[191] Transforming growth factor-beta (TGFB), also, is well known for its many functions in skeletogenesis and osteogenesis, including skeletal morphogenesis, growth plate development, and osteoblast differentiation.[192] In addition, TGFBs play an important role in bone remodelling by regulating osteoblast and osteoclast differentiation.

Several studies have shown that two main pathways cause the induction of the SP7 transforming growth factor, one of them is through DLX5 gene regulation.[193] Figure 4 shows an upregulation of DLX5 of almost 3.5 ± 0.1-fold and a 1.5 ± 0.1-fold increase for the LA-GAGR and mini-GAGR group, respectively. Once the expression of SP7 is triggered, a slew of mature osteoblast genes is induced such as collagen type-I and osteonectin which are necessary for productive osteoblasts during bone ossification.[191] The osteogenic markers, CDH11 and SOX9 were upregulated for the both groups. The transcription factor SOX9, which plays a central role in chondrocyte differentiation, and the CDH11 are key transcription factors for BMSCs. Several studies have shown that SOX9 enhanced the chondrogenesis of BMSCs, playing an important role during inhibition of chondrocyte proliferation thus enhancing osteogenesis, and preventing chondrocyte differentiation.[194] CDH11 is a pro-osteogenic and anti-adipogenic marker that promotes the osteogenic differentiation of BMSCs. Granulocyte colony-stimulating factor (G-CSF) has many functions including induction of proliferation, viability, and differentiation of osteoblasts, as well as mobilization of bone marrow cells.[195] As shown in Figure 4, CSF2 and CSF3 were upregulated showing an increase of almost 10 ± 0.1-fold and 19 ± 0.1-fold for the LA-GAGR group, respectively. The expression of both CSF2 and CSF3 was also upregulated for the mini-GAGR group with a 6 ± 0.1-fold and 8 ± 0.1-fold increase, respectively.

Fibroblast growth factor (FGF) and insulin growth factors (IGF) are molecules associated with bone regeneration. In our study, FGFR1 and IGF1R markers were upregulated for both experimental groups. The increase of FGFR1 of almost 10 ± 0.1-fold and 13 ± 0.1-fold and the increase of IGF1R of almost 3 ± 0.1-fold and 1.5 ± 0.1-fold for the LA-GAGR and mini-GAGR group respectively demonstrated the effective conditions the study had on the cells for the initiation of bone healing and regeneration. Many of these growth factors and osteogenic markers play important roles in natural bone formation and regeneration.

Polysaccharides have attracted attention not only as scaffolds but also as bioactive natural macromolecules. The bioactivity of polysaccharides has been studied in antioxidant activity[190], neuroprotective activity[151], immunoregulatory and anti-inflammatory activities.[196-199] The bioactivity of polysaccharides has been shown to

depend on the structure of the polysaccharides including the monosaccharide repeating unit, molecular weight, functional groups, and the types of linkages.[200] Several research results demonstrated that rhamnose-containing polysaccharides like gellan gum induce apoptosis in osteosarcoma cells and exhibit anti-osteoporosis activity by inhibiting the formation of osteoclasts, decrease osteoclast differentiation, and increase osteoblast activity.[87,201] The bioactivity and cell-signaling pathways of rhamnose-containing polysaccharides on bone and cartilage formation remains to be investigated. It is necessary to better understand the underlying mechanisms of rhamnose-containing polysaccharides in enhancing bone and cartilage formation.

5. Conclusion and future directions

Efforts to regenerate tendon-bone interface tissue are ongoing, with limited success. The current approaches to regeneration of enthesis still lack the recovery of comparable tissue as the native enthesis in terms of biological and biomechanical properties. The gradual changes in mineral content, collagen alignment, and ECM inspired the use of composite scaffolds to mimic these unique structural and compositional variations in the enthesis matrix. Such composites should be fabricated from one base biomaterial which supports the regeneration of bone and cartilage simultaneously.

Rhamnose-containing polysaccharides such as gellan gum fulfil several main roles in osteochondral tissue engineering, especially after modification and blending with other materials. Fabricating biomimetic composite scaffolds for enthesis regeneration with gellan gum may possibly provide the needed structure with longitudinal disparity in mineral content and collagen alignment to support multiple cell populations and specific tissue formation. Further research is needed to better understand the underlying mechanisms by which gellan gum regulates cellular response and differentiation, and tissue regeneration. Currently, in vivo studies on gellan gum are scarce for tissue engineering and are limited to small animal models. The use of appropriate animal and injury models need to be considered to ensure that the evaluations are justified in physiologically relevant environments to humans. Currently mimicking the mineral gradient of enthesis for small animals is technologically challenging to realize in fabricated scaffolds. Big animal models can potentially overcome this limitation although the costs

for investigation may be an obstacle. Establishing a standard animal injury model for enthesis regeneration is necessary for fair evaluations of various scaffolds for potential clinical translation. The review is summarized as follows:

- Enthesis regeneration efforts are ongoing with limited success.
- Fabricating biomimetic scaffolds using polysaccharide-based materials offer promising results.
- The underlying mechanisms of bioactivity of rhamnose-containing polysaccharides for osteochondral tissue regeneration should be further investigated.
- Elucidation of a standard animal and injury model for enthesis regeneration is yet to be attained.

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Authors' contributions

Abdullah Baawad: Conceptualization, Methodology, Data curation, Formal analysis, Writing-original draft preparation, Software. Diego Jacho: Formal analysis, Data curation, Writing-editing. Taijah Hamil*:* Investigation, Writing-review & editing. Eda Yildirim-Ayan: Conceptualization, Validation, Data curation, Writing-reviewing & editing. Dong-Shik Kim: Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing-reviewing & editing, Funding acquisition.

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List of abbreviations

PGA: polyglycolic acid

PLCL: poly-L-lactide-co-ε-caprolactone

PLGA: poly-(lactic-co-glycolic acid)

PCL: polycaprolactone

nHA-PLLA: nanohydroxyapatite-poly-L-lactic acid

F127: Pluronic F127

BMP-2: bone morphogenetic protein-2

PDGF: platelet-derived growth factor

CTGF: connective tissue growth factor

TGF, β3: transforming growth factor beta 3

ARS: aligned-random nanofibrous scaffold

BMD: bone mineral density

BV/TV: bone volume/total volume

GAG: glycosaminoglycan

BMSCs: bone marrow stromal cells

BER: berberine

β-TCP: beta-tricalcium phosphate

HPMC: hydroxypropylmethylcellulose

ICS: icariin-conditioned serum

EGM-2: endothelial growth medium-2

BaG: bioactive glass

EM-OM: endothelial and osteogenic medium

GG: gellan gum

MSCs: mesenchymal stem cells

ALP: alkaline phosphatase

PDA: polydopamine

ASCs: adipose-derived stem cells

MA: methacrylated

CHX: chlorhexidine

GBR: guided bone regeneration

SBF: simulated body fluid

PL: pullulan

Ty: tyramine

LG_{NF}: lignocellulose nanofibrils

FS: forsterite

MEL: melatonin

PEGDA: polyethylene glycol diacrylate.

References

1. Pedowitz RA, Yamaguchi K, Ahmad CS, et al. Optimizing the management of rotator cuff problems. *J Am Acad Orthop Surg* 2011;19:368-79. doi:10.5435/00124635-201106000-00007
2. Owings M, Kozak LJ. Ambulatory and inpatient procedures in the United States, 1996. In: Vital and health statistics Series 13, Data from the National Health Servey pp. 1-119. 1998.
3. Miller MD, American Orthopaedic Society for Sports M, American Academy of Orthopaedic S. *Orthopaedic knowledge update, OKU 5*. 2016.
4. Font Tellado S, Bonani W, Balmayor ER, et al. Fabrication and characterization of biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering. *Tissue Eng Part A* 2017;23:859-872. doi:10.1089/ten.TEA.2016.0460
5. Galatz LM, Ball CM, Teefey SA, et al. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am* 2004;86:219-24. doi:10.2106/00004623-200402000-00002
6. Cole BJ, McCarty LP, 3rd, Kang RW, et al. Arthroscopic rotator cuff repair: prospective functional outcome and repair integrity at minimum 2-year follow-up. *J Shoulder Elbow Surg* 2007;16:579-85. doi:10.1016/j.jse.2006.12.011
7. Lu HH, Thomopoulos S. Functional attachment of soft tissues to bone: development, healing, and tissue engineering. *Annu Rev Biomed Eng* 2013;15:201-26. doi:10.1146/annurev-bioeng-071910-124656
8. Shen H, Yoneda S, Sakiyama-Elbert SE, et al. Flexor tendon injury and repair. the influence of synovial environment on the early healing response in a canine model. *J Bone Joint Surg Am* 2021;103:e36. doi:10.2106/JBJS.20.01253
9. Genin GM, Thomopoulos S. The tendon-to-bone attachment: Unification through disarray. *Nat Mater* 2017;16:607-608. doi:10.1038/nmat4906
10. Deymier AC, An Y, Boyle JJ, et al. Micro-mechanical properties of the tendon-to-bone attachment. *Acta Biomater* 2017;56:25-35. doi:10.1016/j.actbio.2017.01.037
11. Saadat F, Deymier AC, Birman V, et al. The concentration of stress at the rotator cuff tendon-to-bone attachment site is conserved across species. *J Mech Behav Biomed Mater* 2016;62:24-32. doi:10.1016/j.jmbbm.2016.04.025
12. Hu Y, Birman V, Deymier-Black A, et al. Stochastic interdigitation as a toughening mechanism at the interface between tendon and bone. *Biophys J* 2015;108:431-7. doi:10.1016/j.bpj.2014.09.049
13. Deymier-Black AC, Pasteris JD, Genin GM, et al. Allometry of the tendon enthesis: mechanisms of load transfer between tendon and bone. *J Biomech Eng* 2015;137:111005. doi:10.1115/1.4031571
14. Baldino L, Cardea S, Maffulli N, et al. Regeneration techniques for bone-to-tendon and muscle-to-tendon interfaces reconstruction. *Br Med Bull* 2016;117:25-37. doi:10.1093/bmb/ldv056
15. Shaw HM, Benjamin M. Structure-function relationships of entheses in relation to mechanical load and exercise. *Scand J Med Sci Sports* 2007;17:303-15. doi:10.1111/j.1600-0838.2007.00689.x

16. Yokoya S, Mochizuki Y, Natsu K, et al. Rotator cuff regeneration using a bioabsorbable material with bone marrow-derived mesenchymal stem cells in a rabbit model. *Am J Sports Med* 2012;40:1259-68. doi:10.1177/0363546512442343
17. Zhao S, Zhao J, Dong S, et al. Biological augmentation of rotator cuff repair using bFGF-loaded electrospun poly(lactide-co-glycolide) fibrous membranes. *Int J Nanomedicine* 2014;9:2373-85. doi:10.2147/IJN.S59536
18. Su W, Wang Z, Jiang J, et al. Promoting tendon to bone integration using graphene oxide-doped electrospun poly(lactic-co-glycolic acid) nanofibrous membrane. *Int J Nanomedicine* 2019;14:1835-1847. doi:10.2147/IJN.S183842
19. Lee KW, Lee JS, Jang JW, et al. Tendon-bone interface healing using an injectable rhBMP-2-containing collagen gel in a rabbit extra-articular bone tunnel model. *J Tissue Eng Regen Med* 2017;11:1435-1441. doi:10.1002/term.2041
20. Thomopoulos S, Williams GR, Gimbel JA, et al. Variation of biomechanical, structural, and compositional properties along the tendon to bone insertion site. *J Orthop Res* 2003;21:413-9. doi:10.1016/S0736-0266(03)00057-3
21. Wopenka B, Kent A, Pasteris JD, et al. The tendon-to-bone transition of the rotator cuff: a preliminary Raman spectroscopic study documenting the gradual mineralization across the insertion in rat tissue samples. *Appl Spectrosc* 2008;62:1285-94. doi:10.1366/000370208786822179
22. Subramanian GG. *Mechanically-conditioned biphasic composite scaffolds to augment healing of tendon-bone interface*. University of Toledo; 2017.
23. Su M, Zhang Q, Zhu Y, et al. Preparation of decellularized triphasic hierarchical bone-fibrocartilage-tendon composite extracellular matrix for enthesis regeneration. *Adv Healthc Mater* 2019;8:e1900831. doi:10.1002/adhm.201900831
24. Dickerson DA, Misk TN, Van Sickle DC, et al. In vitro and in vivo evaluation of orthopedic interface repair using a tissue scaffold with a continuous hard tissue-soft tissue transition. *J Orthop Surg Res* 2013;8:18. doi:10.1186/1749-799X-8-18
25. Spalazzi JP, Dagher E, Doty SB, et al. In vivo evaluation of a multiphased scaffold designed for orthopaedic interface tissue engineering and soft tissue-to-bone integration. *J Biomed Mater Res A* 2008;86:1-12. doi:10.1002/jbm.a.32073
26. Moffat K, Cassilly R, Subramony S, et al. In vivo evaluation of a bi-phasic nanofiber-based scaffold for integrative rotator cuff repair. Transactions of the 56th Orthopaedic Research Society; 2010.
27. Yang G, Lin H, Rothrauff BB, et al. Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering. *Acta Biomater* 2016;35:68-76. doi:10.1016/j.actbio.2016.03.004
28. Phillips JE, Burns KL, Le Doux JM, et al. Engineering graded tissue interfaces. *Proceedings of the National Academy of Sciences* 2008;105:12170-12175. doi:10.1073/pnas.0801988105
29. Zhu C, Qiu J, Thomopoulos S, et al. Augmenting tendon-to-bone repair with functionally graded scaffolds. *Adv Healthc Mater* 2021;10:e2002269. doi:10.1002/adhm.202002269
30. Li X, Xie J, Lipner J, et al. Nanofiber scaffolds with gradations in mineral content for mimicking the tendon-to-bone insertion site. *Nano Lett* 2009;9:2763-8. doi:10.1021/nl901582f

31. Liu W, Lipner J, Xie J, et al. Nanofiber scaffolds with gradients in mineral content for spatial control of osteogenesis. *ACS Appl Mater Interfaces* 2014;6:2842-9. doi:10.1021/am405418g
32. Schwartz AG, Pasteris JD, Genin GM, et al. Mineral distributions at the developing tendon enthesis. *PLoS One* 2012;7:e48630. doi:10.1371/journal.pone.0048630
33. Milz S, Rufai A, Buettner A, et al. Three-dimensional reconstructions of the Achilles tendon insertion in man. *J Anat* 2002;200:145-52. doi:10.1046/j.0021-8782.2001.00016.x
34. Spalazzi JP, Boskey AL, Pleshko N, et al. Quantitative mapping of matrix content and distribution across the ligament-to-bone insertion. *PLoS One* 2013;8:e74349. doi:10.1371/journal.pone.0074349
35. Lee N, Robinson J, Lu H. Biomimetic strategies for engineering composite tissues. *Curr Opin Biotechnol* 2016;40:64-74. doi:10.1016/j.copbio.2016.03.006
36. Patel S, Caldwell JM, Doty SB, et al. Integrating soft and hard tissues via interface tissue engineering. *J Orthop Res* 2018;36:1069-1077. doi:10.1002/jor.23810
37. Wei F, Liu S, Chen M, et al. Host response to biomaterials for cartilage tissue engineering: key to remodeling. *Front Bioeng Biotechnol* 2021;9:664592. doi:10.3389/fbioe.2021.664592
38. Romo-Valera C, Guerrero P, Arluzea J, et al. Cytocompatibility and suitability of protein-based biomaterials as potential candidates for corneal tissue engineering. *Int J Mol Sci* 2021;22:doi:10.3390/ijms22073648
39. Sensini A, Massafra G, Gotti C, et al. Tissue engineering for the insertions of tendons and ligaments: an overview of electrospun biomaterials and structures. *Front Bioeng Biotechnol* 2021;9:645544. doi:10.3389/fbioe.2021.645544
40. Shi H, Wang C, Ma Z. Stimuli-responsive biomaterials for cardiac tissue engineering and dynamic mechanobiology. *APL Bioeng* 2021;5:011506. doi:10.1063/5.0025378
41. Irastorza-Lorenzo A, Sanchez-Porras D, Ortiz-Arrabal O, et al. Evaluation of marine agarose biomaterials for tissue engineering applications. *Int J Mol Sci* 2021;22:doi:10.3390/ijms22041923
42. Rico-Llanos GA, Borrego-Gonzalez S, Moncayo-Donoso M, et al. Collagen type I biomaterials as scaffolds for bone tissue engineering. *Polymers (Basel)* 2021;13:doi:10.3390/polym13040599
43. Chen ZY, Gao S, Zhang YW, et al. Antibacterial biomaterials in bone tissue engineering. *J Mater Chem B* 2021;9:2594-2612. doi:10.1039/d0tb02983a
44. Parekh M, Romano V, Hassanin K, et al. Biomaterials for corneal endothelial cell culture and tissue engineering. *J Tissue Eng* 2021;12:2041731421990536. doi:10.1177/2041731421990536
45. Youn J, Choi JH, Lee S, et al. Pluronic F-127/silk fibroin for enhanced mechanical property and sustained release drug for tissue engineering biomaterial. *Materials (Basel)* 2021;14:doi:10.3390/ma14051287
46. Liu J, Chen H, Wang Y, et al. Flexible water-absorbing silk-fibroin biomaterial sponges with unique pore structure for tissue engineering. *ACS Biomater Sci Eng* 2020;6:1641-1649. doi:10.1021/acsbiomaterials.9b01721

47. Sordi MB, da Cruz ACC, Aragones A, et al. PLGA+HA/betaTCP scaffold incorporating simvastatin: a promising biomaterial for bone tissue engineering. *J Oral Implantol* 2021;47:93-101. doi:10.1563/aaid-joi-D-19-00148
48. Zhang LT, Liu RM, Luo Y, et al. Hyaluronic acid promotes osteogenic differentiation of human amniotic mesenchymal stem cells via the TGF-beta/Smad signalling pathway. *Life Sci* 2019;232:116669. doi:10.1016/j.lfs.2019.116669
49. Xu D, Cheng G, Dai J, et al. Bi-layered composite scaffold for repair of the osteochondral defects. *Adv Wound Care (New Rochelle)* 2021;10:401-414. doi:10.1089/wound.2019.1140
50. Komur B, Akyuva Y, Karaslan N, et al. Can a biodegradable implanted bilayered drug delivery system loaded with BMP-2/BMP-12 take an effective role in the biological repair process of bone-tendon injuries? A preliminary report. *J Pharm (Cairo)* 2017;2017:7457865. doi:10.1155/2017/7457865
51. Matsusaki M, Ikeguchi H, Kubo C, et al. Fabrication of perfusable pseudo blood vessels by controlling sol-gel transition of gellan gum templates. *ACS Biomater Sci Eng* 2019;5:5637-5643. doi:10.1021/acsbiomaterials.8b01272
52. Thorvaldsson A, Silva-Correia J, Oliveira JM, et al. Development of nanofiber-reinforced hydrogel scaffolds for nucleus pulposus regeneration by a combination of electrospinning and spraying technique. *J Appl Polym Sci* 2013;128:1158-1163. doi:10.1002/app.38316
53. Zhang Y, Zhang Z, Liu H, et al. A natural selenium polysaccharide from Pleurotus ostreatus: structural elucidation, anti-gastric cancer and anti-colon cancer activity in vitro. *Int J Biol Macromol* 2022;201:630-640. doi:10.1016/j.ijbiomac.2022.01.101
54. Yu-Hao D, Chun C, Qiang H, et al. Study on a novel spherical polysaccharide from Fructus Mori with good antioxidant activity. *Carbohydr Polym* 2021;256:117516. doi:10.1016/j.carbpol.2020.117516
55. Zhu YM, Pan LC, Zhang LJ, et al. Chemical structure and antioxidant activity of a polysaccharide from Siraitia grosvenorii. *Int J Biol Macromol* 2020;165:1900-1910. doi:10.1016/j.ijbiomac.2020.10.127
56. Kawano M, Ariyoshi W, Iwanaga K, et al. Mechanism involved in enhancement of osteoblast differentiation by hyaluronic acid. *Biochem Biophys Res Commun* 2011;405:575-80. doi:10.1016/j.bbrc.2011.01.071
57. Zuliani CC, Damas, II, Andrade KC, et al. Chondrogenesis of human amniotic fluid stem cells in chitosan-xanthan scaffold for cartilage tissue engineering. *Sci Rep* 2021;11:3063. doi:10.1038/s41598-021-82341-x
58. Yang Y, Campbell Ritchie A, Everitt NM. Recombinant human collagen/chitosan-based soft hydrogels as biomaterials for soft tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2021;121:111846. doi:10.1016/j.msec.2020.111846
59. Qin X, He R, Chen H, et al. Methacrylated pullulan/polyethylene (glycol) diacrylate composite hydrogel for cartilage tissue engineering. *J Biomater Sci Polym Ed* 2021;1-14. doi:10.1080/09205063.2021.1899888
60. Mousa HM, Hussein KH, Sayed MM, et al. Development and characterization of cellulose/iron acetate nanofibers for bone tissue engineering applications. *Polymers (Basel)* 2021;13:1339. doi:10.3390/polym13081339

61. Huang K, Yang MS, Tang YJ, et al. Porous shape memory scaffold of dextran and hydroxyapatite for minimum invasive implantation for bone tissue engineering applications. *J Biomater Appl* 2021;35:823-837. doi:10.1177/0885328220950062
62. Hong JK, Cooke SL, Whittington AR, et al. Bioactive cellulose nanocrystal-poly(epsilon-caprolactone) nanocomposites for bone tissue engineering applications. *Front Bioeng Biotechnol* 2021;9:605924. doi:10.3389/fbioe.2021.605924
63. Chen S, Guo R, Liang Q, et al. Multifunctional modified polylactic acid nanofibrous scaffold incorporating sodium alginate microspheres decorated with strontium and black phosphorus for bone tissue engineering. *J Biomater Sci Polym Ed* 2021;1-20. doi:10.1080/09205063.2021.1927497
64. Alshemary AZ, Bilgin S, Isik G, et al. Biomechanical evaluation of an injectable alginate / dicalcium phosphate cement composites for bone tissue engineering. *J Mech Behav Biomed Mater* 2021;118:104439. doi:10.1016/j.jmbbm.2021.104439
65. Wen X, Shen M, Bai Y, et al. Biodegradable cell-laden starch foams for the rapid fabrication of 3D tissue constructs and the application in neural tissue engineering. *J Biomed Mater Res B Appl Biomater* 2020;108:104-116. doi:10.1002/jbm.b.34370
66. Seker S, Elcin AE, Elcin YM. Macroporous elastic cryogels based on platelet lysate and oxidized dextran as tissue engineering scaffold: in vitro and in vivo evaluations. *Mater Sci Eng C Mater Biol Appl* 2020;110:110703. doi:10.1016/j.msec.2020.110703
67. Silva CR, Babo PS, Gulino M, et al. Injectable and tunable hyaluronic acid hydrogels releasing chemotactic and angiogenic growth factors for endodontic regeneration. *Acta Biomater* 2018;77:155-171. doi:10.1016/j.actbio.2018.07.035
68. Ju D, Han L, Li Z, et al. Porous poly(L-lactic acid) sheet prepared by stretching with starch particles as filler for tissue engineering. *Carbohydr Polym* 2016;142:222-9. doi:10.1016/j.carbpol.2016.01.038
69. Atila D, Keskin D, Tezcaner A. Crosslinked pullulan/cellulose acetate fibrous scaffolds for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2016;69:1103-15. doi:10.1016/j.msec.2016.08.015
70. Zeng K, Groth T, Zhang K. Recent advances in artificially sulfated polysaccharides for applications in cell growth and differentiation, drug delivery, and tissue engineering. *Chembiochem* 2019;20:737-746. doi:10.1002/cbic.201800569
71. Vandghanooni S, Eskandani M. Electrically conductive biomaterials based on natural polysaccharides: challenges and applications in tissue engineering. *Int J Biol Macromol* 2019;141:636-662. doi:10.1016/j.ijbiomac.2019.09.020
72. Tchobanian A, Van Oosterwyck H, Fardim P. Polysaccharides for tissue engineering: Current landscape and future prospects. *Carbohydr Polym* 2019;205:601-625. doi:10.1016/j.carbpol.2018.10.039
73. Malviya R, Sharma PK, Dubey SK. Modification of polysaccharides: pharmaceutical and tissue engineering applications with commercial utility (patents). *Mater Sci Eng C Mater Biol Appl* 2016;68:929-938. doi:10.1016/j.msec.2016.06.093
74. Bacakova L, Novotna K, Parizek M. Polysaccharides as cell carriers for tissue engineering: the use of cellulose in vascular wall reconstruction. *Physiol Res* 2014;63:S29-47. doi:10.33549/physiolres.932644

75. Khan F, Ahmad SR. Polysaccharides and their derivatives for versatile tissue engineering application. *Macromol Biosci* 2013;13:395-421. doi:10.1002/mabi.201200409
76. Silva TH, Alves A, Popa EG, et al. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. *Biomatter* 2012;2:278-89. doi:10.4161/biom.22947
77. Senni K, Pereira J, Gueniche F, et al. Marine polysaccharides: a source of bioactive molecules for cell therapy and tissue engineering. *Mar Drugs* 2011;9:1664-81. doi:10.3390/md9091664
78. Farion IA, Burdukovskii VF, Kholkhoev BC, et al. Unsaturated and thiolated derivatives of polysaccharides as functional matrixes for tissue engineering and pharmacology: a review. *Carbohydr Polym* 2021;259:117735. doi:10.1016/j.carbpol.2021.117735
79. Andres E, Molinari J, Peterszegi G, et al. Pharmacological properties of rhamnose-rich polysaccharides, potential interest in age-dependent alterations of connectives tissues. *Pathol Biol (Paris)* 2006;54:420-5. doi:10.1016/j.patbio.2006.07.004
80. Ravelojaona V, Molinari J, Robert L. Protection by rhamnose-rich polysaccharides against the cytotoxicity of Maillard reaction products. *Biomed Pharmacother* 2006;60:359-62. doi:10.1016/j.biopha.2006.06.019
81. Ravelojaona V, Robert AM, Robert L, et al. Collagen biosynthesis in cell culture: comparison of corneal keratocytes and skin fibroblasts. Effect of rhamnose-rich oligo- and polysaccharides. *Pathol Biol (Paris)* 2008;56:66-9. doi:10.1016/j.patbio.2007.10.003
82. Zeng D, Hu C, Li R-L, et al. Polysaccharide extracts of Astragalus membranaceus and Atractylodes macrocephala promote intestinal epithelial cell migration by activating the polyamine-mediated K+ channel. *Chinese Journal of Natural Medicines* 2018;16:674-682. doi:10.1016/S1875-5364(18)30107-9
83. Zhang S, Li Z, Wang X, et al. Isolation, structural elucidation, and immunoregulation properties of an arabinofuranan from the rinds of Garcinia mangostana. *Carbohydr Polym* 2020;246:116567. doi:10.1016/j.carbpol.2020.116567
84. Hsu HY, Jeyashoke N, Yeh CH, et al. Immunostimulatory bioactivity of algal polysaccharides from Chlorella pyrenoidosa activates macrophages via Toll-like receptor 4. *J Agric Food Chem* 2010;58:927-36. doi:10.1021/jf902952z
85. Pageon H, Azouaoui A, Zucchi H, et al. Potentially beneficial effects of rhamnose on skin ageing: an in vitro and in vivo study. *Int J Cosmet Sci* 2019;41:213-220. doi:10.1111/ics.12523
86. Ghosh S, Khatua S, Acharya K. Crude polysaccharide from a wild mushroom enhances immune response in murine macrophage cells by TLR/NF-kappaB pathway. *J Pharm Pharmacol* 2019;71:1311-1323. doi:10.1111/jphp.13104
87. Faury G, Ruszova E, Molinari J, et al. The alpha-L-Rhamnose recognizing lectin site of human dermal fibroblasts functions as a signal transducer: modulation of Ca2+ fluxes and gene expression. *Biochim Biophys Acta* 2008;1780:1388-94. doi:10.1016/j.bbagen.2008.07.008
88. Faury G, Molinari J, Rusova E, et al. Receptors and aging: structural selectivity of the rhamnose-receptor on fibroblasts as shown by Ca(2+)-mobilization and gene-

expression profiles. *Arch Gerontol Geriatr* 2011;53:106-12. doi:10.1016/j.archger.2010.05.017
89. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports* 2000;10:312-20. doi:10.1034/j.1600-0838.2000.010006312.x
90. Yang L, van der Werf KO, Fitie CF, et al. Mechanical properties of native and cross-linked type I collagen fibrils. *Biophys J* 2008;94:2204-11. doi:10.1529/biophysj.107.111013
91. Majima T, Yasuda K, Tsuchida T, et al. Stress shielding of patellar tendon: effect on small-diameter collagen fibrils in a rabbit model. *J Orthop Sci* 2003;8:836-41. doi:10.1007/s00776-003-0707-x
92. Erisken C, Zhang X, Moffat KL, et al. Scaffold fiber diameter regulates human tendon fibroblast growth and differentiation. *Tissue Eng Part A* 2013;19:519-28. doi:10.1089/ten.tea.2012.0072
93. Lipner J, Shen H, Cavinatto L, et al. In vivo evaluation of adipose-derived stromal cells delivered with a nanofiber scaffold for tendon-to-bone repair. *Tissue Eng Part A* 2015;21:2766-74. doi:10.1089/ten.TEA.2015.0101
94. Romeo A, Easley J, Regan D, et al. Rotator cuff repair using a bioresorbable nanofiber interposition scaffold: a biomechanical and histologic analysis in sheep. *J Shoulder Elbow Surg* 2022;31:402-412. doi:10.1016/j.jse.2021.07.018
95. Tarafder S, Brito JA, Minhas S, et al. In situ tissue engineering of the tendon-to-bone interface by endogenous stem/progenitor cells. *Biofabrication* 2019;12:015008. doi:10.1088/1758-5090/ab48ca
96. Kim JH, Oh SH, Min HK, et al. Dual growth factor-immobilized asymmetrically porous membrane for bone-to-tendon interface regeneration on rat patellar tendon avulsion model. *J Biomed Mater Res A* 2018;106:115-125. doi:10.1002/jbm.a.36212
97. MacGillivray JD, Fealy S, Terry MA, et al. Biomechanical evaluation of a rotator cuff defect model augmented with a bioresorbable scaffold in goats. *J Shoulder Elbow Surg* 2006;15:639-44. doi:10.1016/j.jse.2005.11.009
98. Arakawa T, Prestrelski SJ, Kenney WC, et al. Factors affecting short-term and long-term stabilities of proteins. *Advanced Drug Delivery Reviews* 2001;46:307-326. doi:10.1016/S0169-409X(00)00144-7
99. Carragee EJ, Chu G, Rohatgi R, et al. Cancer risk after use of recombinant bone morphogenetic protein-2 for spinal arthrodesis. *J Bone Joint Surg Am* 2013;95:1537-45. doi:10.2106/JBJS.L.01483
100. Carreira AC, Alves GG, Zambuzzi WF, et al. Bone Morphogenetic Proteins: structure, biological function and therapeutic applications. *Arch Biochem Biophys* 2014;561:64-73. doi:10.1016/j.abb.2014.07.011
101. Hermonat PL, Li D, Yang B, et al. Mechanism of action and delivery possibilities for TGFbeta1 in the treatment of myocardial ischemia. *Cardiovasc Res* 2007;74:235-43. doi:10.1016/j.cardiores.2007.01.016
102. Saik JE, Gould DJ, Watkins EM, et al. Covalently immobilized platelet-derived growth factor-BB promotes angiogenesis in biomimetic poly(ethylene glycol) hydrogels. *Acta Biomater* 2011;7:133-43. doi:10.1016/j.actbio.2010.08.018
103. James AW, LaChaud G, Shen J, et al. A review of the clinical side effects of bone morphogenetic protein-2. *Tissue Eng Part B Rev* 2016;22:284-97. doi:10.1089/ten.TEB.2015.0357

104. Ren X, Zhao M, Lash B, et al. Growth factor engineering strategies for regenerative medicine applications. *Front Bioeng Biotechnol* 2019;7:469. doi:10.3389/fbioe.2019.00469
105. Mitchell AC, Briquez PS, Hubbell JA, et al. Engineering growth factors for regenerative medicine applications. *Acta Biomater* 2016;30:1-12. doi:10.1016/j.actbio.2015.11.007
106. Yamakawa S, Hayashida K. Advances in surgical applications of growth factors for wound healing. *Burns Trauma* 2019;7:10. doi:10.1186/s41038-019-0148-1
107. Mao AS, Mooney DJ. Regenerative medicine: Current therapies and future directions. *Proc Natl Acad Sci U S A* 2015;112:14452-9. doi:10.1073/pnas.1508520112
108. McClain AK, McCarrel TM. The effect of four different freezing conditions and time in frozen storage on the concentration of commonly measured growth factors and enzymes in equine platelet-rich plasma over six months. *BMC Vet Res* 2019;15:292. doi:10.1186/s12917-019-2040-4
109. Sonker A, Dubey A. Determining the effect of preparation and storage: an effort to streamline platelet components as a source of growth factors for clinical application. *Transfus Med Hemother* 2015;42:174-80. doi:10.1159/000371504
110. Tang R, Lu Y, Yin R, et al. The effects of storage time and repeated freeze-thaw cycles on intact fibroblast growth factor 23 levels. *Biopreserv Biobank* 2021;19:48-52. doi:10.1089/bio.2020.0073
111. Cai J, Wang J, Ye K, et al. Dual-layer aligned-random nanofibrous scaffolds for improving gradient microstructure of tendon-to-bone healing in a rabbit extra-articular model. *Int J Nanomedicine* 2018;13:3481-3492. doi:10.2147/IJN.S165633
112. Duquin TR, Wind WM, Fineberg MS, et al. Current trends in anterior cruciate ligament reconstruction. *J Knee Surg* 2009;22:7-12. doi:10.1055/s-0030-1247719
113. Mahalingam VD, Behbahani-Nejad N, Horine SV, et al. Allogeneic versus autologous derived cell sources for use in engineered bone-ligament-bone grafts in sheep anterior cruciate ligament repair. *Tissue Eng Part A* 2015;21:1047-54. doi:10.1089/ten.TEA.2014.0422
114. Li X, Cheng R, Sun Z, et al. Flexible bipolar nanofibrous membranes for improving gradient microstructure in tendon-to-bone healing. *Acta Biomater* 2017;61:204-216. doi:10.1016/j.actbio.2017.07.044
115. Al Tawil E, Monnier A, Nguyen QT, et al. Microarchitecture of poly(lactic acid) membranes with an interconnected network of macropores and micropores influences cell behavior. *Eur Polym J* 2018;105:370-388. doi:10.1016/j.eurpolymj.2018.06.012
116. Arora B, Bhatia R, Attri P. Bionanocomposites: Green materials for a sustainable future. In: New Polymer Nanocomposites for Environmental Remediation (Hussain CM, Mishra AK, eds.), pp. 699-712. Elsevier, 2018.
117. Shebi A, Lisa S. Pectin mediated synthesis of nano hydroxyapatite-decorated poly(lactic acid) honeycomb membranes for tissue engineering. *Carbohydr Polym* 2018;201:39-47. doi:10.1016/j.carbpol.2018.08.012
118. Sartore L, Inverardi N, Pandini S, et al. PLA/PCL-based foams as scaffolds for tissue engineering applications. *Materials Today: Proceedings* 2019;7:410-417. doi:10.1016/j.matpr.2018.11.103

119. Liu S, Qin S, He M, et al. Current applications of poly(lactic acid) composites in tissue engineering and drug delivery. *Composites Part B: Engineering* 2020;199:108238. doi:10.1016/j.compositesb.2020.108238
120. Stagnaro P, Schizzi I, Utzeri R, et al. Alginate-polymethacrylate hybrid hydrogels for potential osteochondral tissue regeneration. *Carbohydr Polym* 2018;185:56-62. doi:10.1016/j.carbpol.2018.01.012
121. Harris E, Liu Y, Cunniffe G, et al. Biofabrication of soft tissue templates for engineering the bone-ligament interface. *Biotechnol Bioeng* 2017;114:2400-2411. doi:10.1002/bit.26362
122. Yu J, Lee S, Choi S, et al. Fabrication of a polycaprolactone/alginate bipartite hybrid scaffold for osteochondral tissue using a three-dimensional bioprinting system. *Polymers (Basel)* 2020;12:2203. doi:10.3390/polym12102203
123. Igarashi T, Iwasaki N, Kasahara Y, et al. A cellular implantation system using an injectable ultra-purified alginate gel for repair of osteochondral defects in a rabbit model. *J Biomed Mater Res A* 2010;94:844-55. doi:10.1002/jbm.a.32762
124. Filardo G, Perdisa F, Gelinsky M, et al. Novel alginate biphasic scaffold for osteochondral regeneration: an in vivo evaluation in rabbit and sheep models. *J Mater Sci Mater Med* 2018;29:74. doi:10.1007/s10856-018-6074-0
125. Reed S, Lau G, Delattre B, et al. Macro- and micro-designed chitosan-alginate scaffold architecture by three-dimensional printing and directional freezing. *Biofabrication* 2016;8:015003. doi:10.1088/1758-5090/8/1/015003
126. Xing J, Peng X, Li A, et al. Gellan gum/alginate-based Ca-enriched acellular bilayer hydrogel with robust interface bonding for effective osteochondral repair. *Carbohydr Polym* 2021;270:118382. doi:10.1016/j.carbpol.2021.118382
127. Chen P, Xia C, Mo J, et al. Interpenetrating polymer network scaffold of sodium hyaluronate and sodium alginate combined with berberine for osteochondral defect regeneration. *Mater Sci Eng C Mater Biol Appl* 2018;91:190-200. doi:10.1016/j.msec.2018.05.034
128. Park YB, Ha CW, Lee CH, et al. Restoration of a large osteochondral defect of the knee using a composite of umbilical cord blood-derived mesenchymal stem cells and hyaluronic acid hydrogel: a case report with a 5-year follow-up. *BMC Musculoskelet Disord* 2017;18:59. doi:10.1186/s12891-017-1422-7
129. Hwang YG, Lee JW, Park KH, et al. Intra-articular injections of hyaluronic acid on osteochondral lesions of the talus after failed arthroscopic bone marrow stimulation. *Foot Ankle Int* 2020;41:1376-1382. doi:10.1177/1071100720945944
130. Mohan N, Mohanan PV, Sabareeswaran A, et al. Chitosan-hyaluronic acid hydrogel for cartilage repair. *Int J Biol Macromol* 2017;104:1936-1945. doi:10.1016/j.ijbiomac.2017.03.142
131. Liu J, Fang Q, Yu X, et al. Chitosan-based nanofibrous membrane unit with gradient compositional and structural features for mimicking calcified layer in osteochondral matrix. *Int J Mol Sci* 2018;19:doi:10.3390/ijms19082330
132. Rajagopal K, Ramesh S, Walter NM, et al. In vivo cartilage regeneration in a multi-layered articular cartilage architecture mimicking scaffold. *Bone Joint Res* 2020;9:601-612. doi:10.1302/2046-3758.99.BJR-2019-0210.R2

133. Zhang J, Ming D, Ji Q, et al. Repair of osteochondral defect using icariin-conditioned serum combined with chitosan in rabbit knees. *BMC Complement Med Ther* 2020;20:193. doi:10.1186/s12906-020-02996-3
134. Onodera T, Baba R, Kasahara Y, et al. Therapeutic effects and adaptive limits of an acellular technique by ultrapurified alginate (UPAL) gel implantation in canine osteochondral defect models. *Regen Ther* 2020;14:154-159. doi:10.1016/j.reth.2020.01.001
135. Ruan SQ, Yan L, Deng J, et al. Preparation of a biphase composite scaffold and its application in tissue engineering for femoral osteochondral defects in rabbits. *Int Orthop* 2017;41:1899-1908. doi:10.1007/s00264-017-3522-2
136. Zhai C, Fei H, Hu J, et al. Repair of articular osteochondral defects using an integrated and biomimetic trilayered scaffold. *Tissue Eng Part A* 2018;24:1680-1692. doi:10.1089/ten.TEA.2018.0086
137. Olive M, Boyer C, Lesoeur J, et al. Preliminary evaluation of an osteochondral autograft, a prosthetic implant, and a biphasic absorbable implant for osteochondral reconstruction in a sheep model. *Vet Surg* 2020;49:570-581. doi:10.1111/vsu.13373
138. Chen B, Liang Y, Bai L, et al. Sustained release of magnesium ions mediated by injectable self-healing adhesive hydrogel promotes fibrocartilaginous interface regeneration in the rabbit rotator cuff tear model. *Chem Eng J* 2020;396:125335. doi:10.1016/j.cej.2020.125335
139. Tahta M, Akkaya M, Gursoy S, et al. Arthroscopic treatment of osteochondral lesions of the talus: nanofracture versus hyaluronic acid-based cell-free scaffold with concentration of autologous bone marrow aspirate. *J Orthop Surg (Hong Kong)* 2017;25:2309499017717870. doi:10.1177/2309499017717870
140. Yontar NS, Aslan L, Can A, et al. One step treatment of talus osteochondral lesions with microfracture and cell free hyaluronic acid based scaffold combination. *Acta Orthop Traumatol Turc* 2019;53:372-375. doi:10.1016/j.aott.2019.04.002
141. Sahin AA, Degirmenci E, Ozturan KE, et al. Effects of adipose tissue-derived stromal vascular fraction on osteochondral defects treated by hyaluronic acid-based scaffold: an experimental study. *Jt Dis Relat Surg* 2021;32:347-354. doi:10.52312/jdrs.2021.19
142. Kuo M-S, Mort AJ, Dell A. Identification and location of l-glycerate, an unusual acyl substituent in gellan gum. *Carbohydr Res* 1986;156:173-187. doi:10.1016/S0008-6215(00)90109-5.
143. Kang KS, Veeder GT, Mirrasoul PJ, et al. Agar-like polysaccharide produced by a pseudomonas species: production and basic properties. *Appl Environ Microbiol* 1982;43:1086-91.
144. Prajapati VD, Jani GK, Zala BS, et al. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydr Polym* 2013;93:670-678. doi:10.1016/j.carbpol.2013.01.030
145. Mao R, Tang J, Swanson BG. Texture properties of high and low acyl mixed gellan gels. *Carbohydr Polym* 2000;41:331-338. doi:10.1016/S0144-8617(99)00108-3
146. Ohtsuka A, Watanabe T. The network structure of gellan gum hydrogels based on the structural parameters by the analysis of the restricted diffusion of water. *Carbohydr Polym* 1996;30:135-140. doi:Doi 10.1016/S0144-8617(96)00104-X

147. Morris ER, Gothard MGE, Hember MWN, et al. Conformational and rheological transitions of welan, rhamsan and acylated gellan. *Carbohydr Polym* 1996;30:165-175. doi:10.1016/S0144-8617(96)00059-8
148. Babu RJ, Sathigari S, Kumar MT, et al. Formulation of controlled release gellan gum macro beads of amoxicillin. *Curr Drug Deliv* 2010;7:36-43. doi:10.2174/156720110790396445
149. Matricardi P, Cencetti C, Ria R, et al. Preparation and characterization of novel gellan gum hydrogels suitable for modified drug release. *Molecules* 2009;14:3376-91. doi:10.3390/molecules14093376
150. Makani V, Jang YG, Christopher K, et al. BBB-permeable, neuroprotective, and neurotrophic polysaccharide, midi-GAGR. *PLoS One* 2016;11:e0149715. doi:10.1371/journal.pone.0149715
151. Murphy K, Llewellyn K, Wakser S, et al. Mini-GAGR, an intranasally applied polysaccharide, activates the neuronal Nrf2-mediated antioxidant defense system. *J Biol Chem* 2018;293:18242-18269. doi:10.1074/jbc.RA117.001245
152. Chakraborty S, Jana S, Gandhi A, et al. Gellan gum microspheres containing a novel alpha-amylase from marine Nocardiopsis sp. strain B2 for immobilization. *Int J Biol Macromol* 2014;70:292-9. doi:10.1016/j.ijbiomac.2014.06.046
153. León PG, Rojas AM. Gellan gum films as carriers of l-(+)-ascorbic acid. *Food Res Int* 2007;40:565-575. doi:10.1016/j.foodres.2006.10.021
154. Crescenzi V, Dentini M, Segatori M, et al. Synthesis and preliminary characterisation of new esters of the bacterial polysaccharide gellan. *Carbohydr Res* 1992;231:73-81. doi:10.1016/0008-6215(92)84009-h
155. Prezotti FG, Cury BS, Evangelista RC. Mucoadhesive beads of gellan gum/pectin intended to controlled delivery of drugs. *Carbohydr Polym* 2014;113:286-95. doi:10.1016/j.carbpol.2014.07.021
156. Ahuja M, Yadav M, Kumar S. Application of response surface methodology to formulation of ionotropically gelled gum cordia/gellan beads. *Carbohydr Polym* 2010;80:161-167. doi:10.1016/j.carbpol.2009.11.005
157. Smith AM, Shelton RM, Perrie Y, et al. An initial evaluation of gellan gum as a material for tissue engineering applications. *J Biomater Appl* 2007;22:241-54. doi:10.1177/0885328207076522
158. Bellini D, Cencetti C, Meraner J, et al. An in situ gelling system for bone regeneration of osteochondral defects. *Eur Polym J* 2015;72:642-650. doi:10.1016/j.eurpolymj.2015.02.043
159. Oliveira JT, Santos TC, Martins L, et al. Gellan gum injectable hydrogels for cartilage tissue engineering applications: in vitro studies and preliminary in vivo evaluation. *Tissue Eng Part A* 2009;16:343-353. doi:10.1089/ten.tea.2009.0117
160. Lee S, Choi JH, Park A, et al. Advanced gellan gum-based glycol chitosan hydrogel for cartilage tissue engineering material. *Int J Biol Macromol* 2020;158:452-460. doi:10.1016/j.ijbiomac.2020.04.135.
161. Stevens LR, Gilmore KJ, Wallace GG, et al. Tissue engineering with gellan gum. *Biomater Sci* 2016;4:1276-90. doi:10.1039/c6bm00322b
162. Kuo SM, Chang SJ, Wang HY, et al. Evaluation of the ability of xanthan gum/gellan gum/hyaluronan hydrogel membranes to prevent the adhesion of

postrepaired tendons. *Carbohydr Polym* 2014;114:230-237. doi:10.1016/j.carbpol.2014.07.049
163. Jung S, Oh HK, Kim MS, et al. Effect of gellan gum/tuna skin film in guided bone regeneration in artificial bone defect in rabbit calvaria. *Materials (Basel)* 2020;13:doi:10.3390/ma13061318
164. Kim D, Thangavelu M, Cheolui S, et al. Effect of different concentration of demineralized bone powder with gellan gum porous scaffold for the application of bone tissue regeneration. *Int J Biol Macromol* 2019;134:749-758. doi:10.1016/j.ijbiomac.2019.04.184
165. Heo DN, Kim HJ, Lee D, et al. Comparison of polysaccharides in articular cartilage regeneration associated with chondrogenic and autophagy-related gene expression. *Int J Biol Macromol* 2020;146:922-930. doi:10.1016/j.ijbiomac.2019.09.215
166. Vilela CA, Correia C, da Silva Morais A, et al. In vitro and in vivo performance of methacrylated gellan gum hydrogel formulations for cartilage repair. *J Biomed Mater Res A* 2018;106:1987-1996. doi:10.1002/jbm.a.36406
167. Pereira DR, Canadas RF, Silva-Correia J, et al. Gellan gum-based hydrogel bilayered scaffolds for osteochondral tissue engineering. *Key Eng Mater* 2014;587:255-260. doi:10.4028/www.scientific.net/KEM.587.255
168. Vuornos K, Huhtala H, Kaariainen M, et al. Bioactive glass ions for in vitro osteogenesis and microvascularization in gellan gum-collagen hydrogels. *J Biomed Mater Res B Appl Biomater* 2020;108:1332-1342. doi:10.1002/jbm.b.34482
169. Douglas TE, Piwowarczyk W, Pamula E, et al. Injectable self-gelling composites for bone tissue engineering based on gellan gum hydrogel enriched with different bioglasses. *Biomed Mater* 2014;9:045014. doi:10.1088/1748-6041/9/4/045014
170. Douglas TE, Wlodarczyk M, Pamula E, et al. Enzymatic mineralization of gellan gum hydrogel for bone tissue-engineering applications and its enhancement by polydopamine. *J Tissue Eng Regen Med* 2014;8:906-18. doi:10.1002/term.1616
171. Vieira S, Vial S, Maia FR, et al. Gellan gum-coated gold nanorods: an intracellular nanosystem for bone tissue engineering. 10.1039/C5RA13556G. *RSC Advances* 2015;5:77996-78005. doi:10.1039/C5RA13556G
172. Gantar A, da Silva LP, Oliveira JM, et al. Nanoparticulate bioactive-glass-reinforced gellan-gum hydrogels for bone-tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2014;43:27-36. doi:10.1016/j.msec.2014.06.045
173. Oliveira MB, Custodio CA, Gasperini L, et al. Autonomous osteogenic differentiation of hASCs encapsulated in methacrylated gellan-gum hydrogels. *Acta Biomater* 2016;41:119-32. doi:10.1016/j.actbio.2016.05.033
174. Kundu B, Brancato V, Oliveira JM, et al. Silk fibroin promotes mineralization of gellan gum hydrogels. *Int J Biol Macromol* 2020;153:1328-1334. doi:10.1016/j.ijbiomac.2019.10.269
175. Xu L, Bai X, Yang J, et al. Preparation and characterisation of a gellan gum-based hydrogel enabling osteogenesis and inhibiting Enterococcus faecalis. *Int J Biol Macromol* 2020;165:2964-2973. doi:10.1016/j.ijbiomac.2020.10.083
176. Gong Y, Wang C, Lai RC, et al. An improved injectable polysaccharide hydrogel: modified gellan gum for long-term cartilage regeneration in vitro. 10.1039/B818090C. *J Mater Chem* 2009;19:1968-1977. doi:10.1039/B818090C

177. Tang Y, Sun J, Fan H, et al. An improved complex gel of modified gellan gum and carboxymethyl chitosan for chondrocytes encapsulation. *Carbohydr Polym* 2012;88:46-53. doi:10.1016/j.carbpol.2011.11.058
178. Mouser VH, Melchels FP, Visser J, et al. Yield stress determines bioprintability of hydrogels based on gelatin-methacryloyl and gellan gum for cartilage bioprinting. *Biofabrication* 2016;8:035003. doi:10.1088/1758-5090/8/3/035003
179. Bonifacio MA, Cochis A, Cometa S, et al. From the sea to the bee: gellan gum-honey-diatom composite to deliver resveratrol for cartilage regeneration under oxidative stress conditions. *Carbohydr Polym* 2020;245:116410. doi:10.1016/j.carbpol.2020.116410
180. Bonifacio MA, Cometa S, Cochis A, et al. Antibacterial effectiveness meets improved mechanical properties: Manuka honey/gellan gum composite hydrogels for cartilage repair. *Carbohydr Polym* 2018;198:462-472. doi:10.1016/j.carbpol.2018.06.115
181. Lee S, Choi JH, Park A, et al. Advanced gellan gum-based glycol chitosan hydrogel for cartilage tissue engineering biomaterial. *Int J Biol Macromol* 2020;158:452-460. doi:10.1016/j.ijbiomac.2020.04.135
182. Park A, Choi JH, Lee S, et al. Application of double network of gellan gum and pullulan for bone marrow stem cells differentiation towards chondrogenesis by controlling viscous substrates. *J Tissue Eng Regen Med* 2020;14:1592-1603. doi:10.1002/term.3116
183. Oliveira IM, Goncalves C, Shin ME, et al. Enzymatically crosslinked tyramine-gellan gum hydrogels as drug delivery system for rheumatoid arthritis treatment. *Drug Deliv Transl Res* 2021;11:1288-1300. doi:10.1007/s13346-020-00855-9
184. Kouhi M, Varshosaz J, Hashemibeni B, et al. Injectable gellan gum/lignocellulose nanofibrils hydrogels enriched with melatonin loaded forsterite nanoparticles for cartilage tissue engineering: fabrication, characterization and cell culture studies. *Mater Sci Eng C Mater Biol Appl* 2020;115:111114. doi:10.1016/j.msec.2020.111114
185. Learmonth DA, Costa PM, Veloso TR, et al. Synthesis and biological evaluation of a bioinspired, tissue-adhesive gellan gum-based hydrogel designed for minimally invasive delivery and retention of chondrogenic cells. *Biomater Sci* 2020;8:3697-3711. doi:10.1039/d0bm00286k
186. Oliveira JT, Santos TC, Martins L, et al. Gellan gum injectable hydrogels for cartilage tissue engineering applications: in vitro studies and preliminary in vivo evaluation. *Tissue Eng Part A* 2010;16:343-53. doi:10.1089/ten.TEA.2009.0117
187. Oliveira JT, Gardel LS, Rada T, et al. Injectable gellan gum hydrogels with autologous cells for the treatment of rabbit articular cartilage defects. *J Orthop Res* 2010;28:1193-9. doi:10.1002/jor.21114
188. Li W, Wu D, Hu D, et al. Stress-relaxing double-network hydrogel for chondrogenic differentiation of stem cells. *Mater Sci Eng C Mater Biol Appl* 2020;107:110333. doi:10.1016/j.msec.2019.110333
189. Choi JH, Park A, Lee W, et al. Preparation and characterization of an injectable dexamethasone-cyclodextrin complexes-loaded gellan gum hydrogel for cartilage tissue engineering. *J Control Release* 2020;327:747-765. doi:10.1016/j.jconrel.2020.08.049
190. Baawad A, Rice C, Hamil T, et al. Molecular weight effects of low acyl gellan gum on antioxidant capacity and rheological properties. *J Food Sci* 2021;86:4275-4287. doi:10.1111/1750-3841.15887

191. Toosi S, Behravan J. Osteogenesis and bone remodeling: A focus on growth factors and bioactive peptides. *Biofactors* 2020;46:326-340. doi:10.1002/biof.1598
192. Wu M, Chen G, Li YP. TGF-beta and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Res* 2016;4:16009. doi:10.1038/boneres.2016.9
193. Hojo H, Ohba S, He X, et al. Sp7/Osterix Is Restricted to Bone-Forming Vertebrates where It Acts as a Dlx Co-factor in Osteoblast Specification. *Dev Cell* 2016;37:238-53. doi:10.1016/j.devcel.2016.04.002
194. Zheng J, Lin Y, Tang F, et al. Promotive Role of CircATRNL1 on Chondrogenic Differentiation of BMSCs Mediated by miR-338-3p. *Arch Med Res* 2021;52:514-522. doi:10.1016/j.arcmed.2021.02.003
195. Ishida K, Matsumoto T, Sasaki K, et al. Bone regeneration properties of granulocyte colony-stimulating factor via neovascularization and osteogenesis. *Tissue Eng Part A* 2010;16:3271-84. doi:10.1089/ten.tea.2009.0268
196. Zhang M, Wu J, Han J, et al. Isolation of polysaccharides from Dendrobium officinale leaves and anti-inflammatory activity in LPS-stimulated THP-1 cells. *Chem Cent J* 2018;12:109. doi:10.1186/s13065-018-0480-8
197. Wang Y, Liu Y, Kirpich I, et al. Lactobacillus rhamnosus GG reduces hepatic TNFalpha production and inflammation in chronic alcohol-induced liver injury. *J Nutr Biochem* 2013;24:1609-15. doi:10.1016/j.jnutbio.2013.02.001
198. Lee SK, Yang KM, Cheon JH, et al. Anti-inflammatory mechanism of Lactobacillus rhamnosus GG in lipopolysaccharide- stimulated HT-29 cell. *Korean J Gastroenterol* 2012;60:86-93. doi:10.4166/kjg.2012.60.2.86
199. Yu Y, Shen M, Wang Z, et al. Sulfated polysaccharide from Cyclocarya paliurus enhances the immunomodulatory activity of macrophages. *Carbohydr Polym* 2017;174:669-676. doi:10.1016/j.carbpol.2017.07.009
200. Ferreira SS, Passos CP, Madureira P, et al. Structure-function relationships of immunostimulatory polysaccharides: a review. *Carbohydr Polym* 2015;132:378-96. doi:10.1016/j.carbpol.2015.05.079
201. Chen J, Yao D, Yuan H, et al. Dipsacus asperoides polysaccharide induces apoptosis in osteosarcoma cells by modulating the PI3K/Akt pathway. *Carbohydr Polym* 2013;95:780-4. doi:10.1016/j.carbpol.2013.03.009

Table 1. In vivo studies utilizing composite scaffolds for enthesis (tendon-bone) regeneration.

Scaffold materials	Induction factor	Cell source	Animal model	Outcome	Reference
PGA and PLCL nanofibers	-	-	- Sheep (infraspinatus)	Fibrocartilage	94
PLGA nanofibers with fibrin layers	BMP-2	Rat ADSCs	Rat (supraspinatus)	Fibrovascular scar	93
PLLA and nHA-PLLA layers	-	-	Rabbit (supraspinatus)	Fibrocartilage	114
Asymmetrically porous PCL/F127 membranes	PDGF + BMP-2	-	Rat (patellar)	Fibrocartilage, mineralized fibrocartilage, bone	96
Aligned-random dual layers of PLLA/PCL/silk fibroin	Autologous Achilles tendon	-	Rabbit (patellar)	Fibrocartilage, bone	111
PLGA microspheres embedded in PCL microstrands	CTGF + TGF β3 + BMP-2	Endogenous stem cells	Rat (supraspinatus)	Fibrocartilage, bone	95

Note: PGA: polyglycolic acid; PLCL: poly-L-lactide-co-ε-caprolactone; PLGA: poly-(lactic-co-glycolic acid); PCL: polycaprolactone; nHA-PLLA: nanohydroxyapatite-poly-L-lactic acid; F127: Pluronic F127; PDGF: Platelet-derived growth factor; BMP-2: bone morphogenetic protein-2; CTGF: connective tissue growth factor; TGF β3: transforming growth factor beta 3.

Table 2. In vivo studies utilizing polysaccharide-based materials for osteochondral regeneration.

Scaffold materials	Animal model	Impact	Ref
Ultra-purified alginate gel	Rabbit (patellar)	The purification of alginate significantly enhanced the cellular proliferation and chondrogenic differentiation of BMSCs and improved the reparative tissue of osteochondral defects.	123
Hyaluronic acid-alginate + BER	Rat (patellar)	The scaffold promoted the regeneration of cartilage and bone tissues.	127
Hyaluronic acid-alginate layer and HA-alginate layer	Sheep (patellar)	Incomplete bone formation due to slow resorption rate of the scaffold. Cartilage repair and integration with the scaffold was observed.	124
Ultra-purified alginate gel	Canine (patellar)	Hyaline-like cartilage and fibrocartilage tissue formation were observed. Compared to untreated defects, gel implantation significantly enhanced osteochondral repair.	134
Silk fibroin-chitosan layer and silk fibroin-chitosan-nHA layer	Rabbit (patellar)	Cartilage and subchondral bone tissues formed with complete filling of the lesion site.	135
β-TCP layer, high-concentration chitosan-gelatin layer, and low-concentration chitosan-gelatin layer	Goat (femoral condyle)	The tri-layered scaffold prevented cartilage infiltration into bone with better cartilage repair than bi-layered scaffold.	136
Calcium phosphate granules layer and chitosan-HPMC layer	Sheep (femoral condyle)	Hyaline cartilage and subchondral bone regeneration were observed but achieved incomplete restoration of articular cartilage.	137
Tri-layered chitosan-gelatin scaffold	Rabbit (patellar)	The scaffold supported glycosaminoglycan (GAG) deposition and facilitated the articular cartilage repair.	132
Injectable ICS combined with chitosan	Rabbit (femoral condyle)	Cartilage and subchondral bone formation were enhanced by the addition of chitosan to ICS.	133
Magnesium-encapsulated chitosan/ Pluronic F127 composite	Rabbit (rotator cuff)	The composite enhanced BMSCs adhesion and chondrogenic differentiation in vitro. The composite significantly increased the fibrocartilage interface regeneration in vivo.	138

Hyaluronic acid scaffold in combination with an autologous bone marrow aspirate	Human (osteochondral lesions of the talus)	Effective pain relief and good clinical results. A hyaline-like chondral tissue and integration of the regenerated tissue was complete in 82% of the cases. Well organized regenerated tissue but relatively non-homogeneous and minimally edematous.	139
Hyaluronic acid hydrogel	Human (knee joint)	Effective pain relief and function of the knee joint. A hyaline-like cartilage filled the defect and was integrated with the surrounding normal cartilage.	128
Hyaluronic acid hydrogel	Human (osteochondral lesions of the talus)	No postoperative complications including nerve injury, infection, and delayed wound healing. The overall patient satisfaction rate was 90%.	140
Injectable hyaluronic acid	Human (osteochondral lesions of the talus)	Symptom, pain, activities of daily living, and quality of life were improved over a mean follow-up period of 2 years. No adverse effects related to the injections were reported.	129
Hyaluronic acid scaffold	Rabbit (femoral condyles)	Stable and organized cartilage formation. The surface was smooth and integrated with the surrounding cartilage.	141

Note: BER: berberine; nHA: nano-hydroxyapatite; β-TCP: beta-tricalcium phosphate; HPMC: Hydroxypropyl methylcellulose; ICS: icariin-conditioned serum.

Table 3. In vitro and in vivo studies utilizing gellan gum for bone and cartilage tissue engineering

Materials/modification	Cell source	Animal model	Target tissue	Impact	Reference
Injectable GG-Bioglass	Rat MSCs	-	Bone	Addition of bioglass increased mechanical strength and mineralization of the scaffold. Cell behavior and antibacterial activity were influenced by the type of bioglass.	169
GG-ALP + PDA	Osteoblastic cell line MC3T3-E1	-	Bone	Addition of ALP induced apatite-like mineral formation and increased scaffold stiffness. Cell attachment and proliferation increased by incorporating PDA.	170
GG-Coated gold nanorods	Human osteoblast-like cell line SaOS-2	-	Bone	Increased mineralization	171
GG-Bioglass	Human ASCs	-	Bone	Addition of bioglass improved microstructure and the mechanical properties. Cells adhered and spread.	172
GG-Hyaluronic acid-$CaCl_2$	Human primary osteoblasts	-	Osteochondral	Promoted cell survival and osteoblastic progression and produced mineralized nodules	158
Injectable GG-MA	Human ASCs	-	Bone	Cells were able to osteodifferentiated.	173
GG-Demineralized bone powder	-	Rat calvarial bone	Bone	Bone tissue formed	164
GG-Silk fibroin-$CaCl_2$	Human ASCs	-	Bone	Increased mineralization	174
GG-Collagen	Human ASCs	-	Bone	Cells were able to osteodifferentiate. Microvessel-like network formation was observed.	168
GG-nHA-CHX	BMSCs	-	Bone	Addition of nHA improved mechanical, biodegradable, and osteogenic properties. Incorporating CHX inhibited *E. faecalis*.	175
TCP bone graft with GG-Tuna skin gelatin film for GBR	-	Rabbit calvarial bone	Bone	The film protected the bone defects from soft tissue invasion, and bone regeneration was observed.	163

GG molecular weight reduction	Porcine articular cartilage chondrocytes	-	Cartilage	Controlled gelation temperature, cell proliferation and specific matrix formation	174
Oxidized GG-Carboxymethyl chitosan	Rabbit articular cartilage chondrocytes	-	Cartilage	Enhanced the viability and proliferation of cells.	176
Bilayered GG/GG-HAp soaked in SBF	-	-	Osteochondral	Distinct cartilage-like and bone-like layers	167
GG-Gelatine methacrylamide	Equine joints chondrocytes	-	Cartilage	Addition of GG increased stiffness of constructed and supported matrix production by cells.	178
GG-Manuka honey composite	hMSCs	-	Cartilage	Suitable mechanical properties, antibacterial activity, high synthesis of collagen II, GAGs and proteoglycans	179, 180
GG-Glycol chitosan	Rabbit leg cartilage chondrocytes	-	Cartilage	GAG synthesis and mRNA expression of cartilage-specific genes.	181
GG/PL hydrogel	Rabbit BMSCs	-	Cartilage	Chondrogenesis promotion of BMSC	182
Betamethasone-loaded Ty-GG hydrogels	Rabbit chondrogenic primary cells	-	Cartilage	Healthy proliferation and survival of chondrogenic primary cells	183
Injectable GG/LG_{NF}/FS-MEL	Human articular chondrocytes	-	Cartilage	Cell adhesion, proliferation and gene expression of cartilage-specific genes	184
Dopamine-modified GG	Human nasal cartilage	-	Cartilage	Up-regulation of cartilage-specific genes	185
GG-encapsulated cells	Rabbit ASCs	Rabbit articular cartilage defects	Cartilage	Hyaline-like cartilage tissue formation	186, 187
GG-MA	Autologous rabbit ASCs	Rabbit with induced chondral lesions	Cartilage	Regeneration of critical size lesions with good integration with native cartilage	166
Injectable GG	-	Rabbit medial parapatellar arthrotomy	Cartilage	Suppressed inflammatory mediators, induced cartilage formation and autophagy-related gene expression	165
GG-PEGDA	BMSCs	Mouse subcutaneous	Cartilage	Chondrogenic differentiation of BMSCs	188
Injectable GG/dexamethasone-cyclodextrin hydrogel	Rabbit chondrocytes	Rabbit cartilage defect	Cartilage	Enhanced expression levels of cartilage-related genes, and improved anti-inflammatory response	189

Note: GG: gellan gum; MSCs: mesenchymal stem cells; ALP: alkaline phosphatase; PDA: polydopamine; ASCs:

adipose-derived stem cells; MA: methacrylated; nHA: nano-hydroxyapatite; CHX: chlorhexidine; BMSCs: bone marrow mesenchymal stem cells; GBR: guided bone regeneration; SBF: simulated body fluid; PL: pullulan; Ty: tyramine; LG_{NF}: lignocellulose nanofibrils; FS: forsterite; MEL: melatonin; PEGDA: polyethylene glycol diacrylate.