HHS Public Access

Author manuscript

*Acta Biomater*. Author manuscript; available in PMC 2021 November 01.

Published in final edited form as:

*Acta Biomater*. 2020 November ; 117: 77–92. doi:10.1016/j.actbio.2020.09.050.

# Bioadhesives for Musculoskeletal Tissue Regeneration

**Solaiman Tarafder**, **Ga Young Park**, **Jeffrey Felix**, **Chang H. Lee***

Regenerative Engineering Laboratory, Center for Dental and Craniofacial Research, Columbia University Irving Medical Center, 630 West 168th Street, VC12-211B, New York, NY 10032

## Abstract

Natural or synthetic materials designed to adhere to biological components, bioadhesives, have received significant attention in clinics and surgeries. As a result, there are several commercially available, FDA-approved bioadhesives used for skin wound closure, hemostasis, and sealing tissue gaps or cracks in soft tissues. Recently, the application of bioadhesives has been expanded to various areas including musculoskeletal tissue engineering and regenerative medicine. The instant establishment of a strong adhesion force on tissue surfaces has shown potential to augment repair of connective tissues. Bioadhesives have also been applied to secure tissue grafts to host bodies and to fill or seal gaps in musculoskeletal tissues caused by injuries or degenerative diseases. In addition, the injectability equipped with the instant adhesion formation may provide the great potential of bioadhesives as vehicles for localized delivery of cells, growth factors, and small molecules to facilitate tissue healing and regeneration. This review covers recent research progress in bioadhesives as focused on their applications in musculoskeletal tissue repair and regeneration. We also discuss the advantages and outstanding challenges of bioadhesives, as well as the future perspective toward regeneration of connective tissues with high mechanical demand.

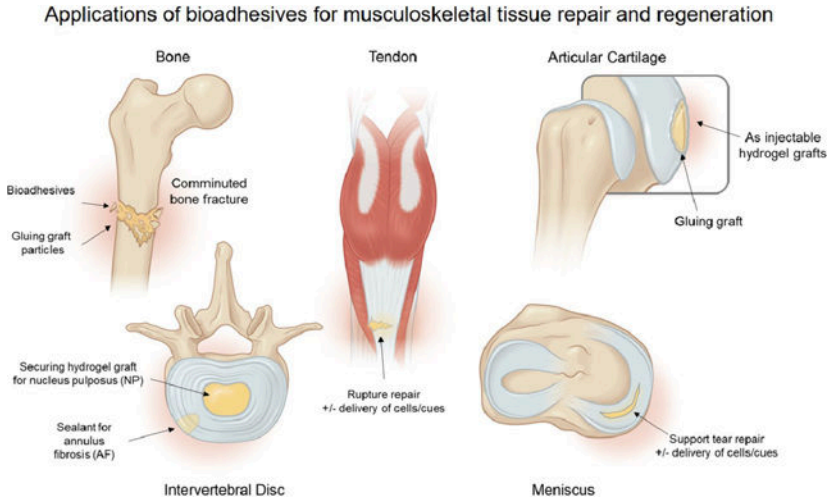
## Graphical abstract

*Corresponding author. chl2109@cumc.columbia.edu.



Declaration of Competing Interest

None.




**Keywords**

Bioadhesives; tissue adhesive; musculoskeletal regeneration; bioactive materials; controlled delivery

## 1 Introduction

Bioadhesives are often referred to as natural or synthetic materials that adhere to biological components such as cells, tissues, and organs through physical or chemical conjugation. Bioadhesives have been widely applied as tissue adhesives to bind tissues together in soft tissue wound healing [1–3]. Some types of bioadhesives have been used as a hemostatic agent to stop bleeding during surgical operations or as a tissue sealant for secure gaps or cracks to prevent leakage of liquid or air [3–5]. A number of different bioadhesives have been investigated as a tissue glue for skin wound closure to replace suture or wound dressing [1, 2, 6]. Internal medicine has also utilized various bioadhesives as hemostasis, graft fixation, and sealants in support of surgical treatments [3].

The key properties considered for such bioadhesives, include but not limited to, biocompatibility, biodegradability, toxicity, adhesion strength on target surface, and duration of cross-linking [1, 3]. Although each aspect may be considered on different weight depending on target application, the adhesive property is likely the most important feature for the abovementioned applications of bioadhesives [1, 3]. Functionality of most bioadhesives is highly attributed to its adhesion properties on tissues, grafts, or materials, providing secure graft fixation, wound closure or dressing. Recently, the application of bioadhesives has been expanded for tissue repair, tissue engineering, and regenerative medicine [7–14]. To support repair and healing, particularly for musculoskeletal tissues, the adhesion strength of bioadhesives is being challenged to move up to the next level [8, 9, 14–16]. The majority of bioadhesives have adhesion strength at a magnitude of kPa but connective tissues such as tendon and knee meniscus show tensile modulus and strength in a MPa range [8, 9, 14–16]. Such high mechanical demands in musculoskeletal tissues also requires a further improvement not only in the adhesion strength but also in bulk mechanical

properties of bioadhesives [8, 9, 14–16]. Besides the physical support, bioadhesives have been evolved to deliver bioactive cues and/or cells in addition to provide tissue adhesion [13, 14, 17–19]. Bioadhesives applied on injured site can serve as effective localized delivery vehicles as secured *in situ* [13, 14, 17–19] or providing physical and/or biochemical environment promoting tissue repair [20, 21]. Recent improvements in the design and synthesis of bioadhesives have made steps closer to successful applications for musculoskeletal tissues in high mechanical demand.

As per PubMed literature search from 2000 to 2019, the number of peer-reviewed publications hit by a keyword “tissue adhesives” had increased by 2012 and started to decline from 2013 (Fig. 1A). The number of papers with a keyword “tissue adhesives *in vivo*” appears to be steady over last decade but only account for ~6.7% of total papers searched by “tissue adhesives” (Fig. 1A). The yearly number of publications hit by “bioadhesives” shows a steady increase in last 20 years (Fig. 1B). The number of papers hit by “bioadhesives *in vivo*” also show a continuous increase (Fig. 1B) that accounts for over 20% of total papers with “bioadhesives”. These observations in literature search may suggest a growing research interest in adhesive biomaterials containing a designed biological function beyond the traditional role as “tissue adhesive” providing physical bonding, hemostasis, or sealing. Tissue specifically, the publication number of tissue adhesives and bioadhesives was the highest for bone, followed by cartilage, tendon, meniscus and intervertebral discs (IVD) (Fig. 1C).

This review summarizes various types of existing bioadhesives and their adhesion mechanisms. It covers the recent advancements in bioadhesives for tissue repair and regeneration, focusing on musculoskeletal tissues. The advantages and outstanding limitations of bioadhesives in musculoskeletal repair and regeneration are also discussed with regard to potential and perspective.

## 2 Types of adhesive

### 2.1 Cyanoacrylates

Cyanoacrylates or acrylic tissue adhesives, synthesized by condensation of a cyanoacetate with formaldehyde [22, 23], have been used as a surgical glue for over 50 years [22, 24]. The cyanoacrylate monomers polymerize very rapidly (5–60 s) on contact with tissue surfaces to form a film that bonds the apposed wound edges. As summarized in Fig. 2, this polymerization is an exothermic reaction triggered by the hydroxyl groups present on the tissue surface or from the moisture [1, 25, 26]. Participation from amino groups on the tissue surface can also take place during the polymerization resulting in a strong bond with the tissue. The general chemical name and formula of cyanoacrylates are alkyl-2-cyanoacrylates and $CH_2$=C(CN)-COOR, respectively, where R could be any alkyl group ranging from methyl to decyl [25, 27]. The first developed cyanoacrylate adhesive was methyl-2-cyanoacrylate (R= $-CH_3$) (known as Eastman 910) with the shortest chain derivative[22]. It was found that the longer the alkyl chain (the R group) the lower the tissue toxicity from the cyanoacrylate adhesives [3, 28, 29]. While the cyanoacrylate with the shortest alkyl group ($-CH_3$) produces a rigid polymer, flexibility can be improved with the longer alkyl chain and adding plasticizer as well. As a result, many cyanoacrylates with longer chain

derivatives have been developed such as ethyl-2-cyanoacrylate (Epiglu®; Meyer-Haake, Ober-Morlen, Germany) & Krazy Glue® (Elmer's Products Inc, Columbus, OH), butyl-2-cyanoacrylate (Trufill®; Codman & Shurtleff, Inc., Raynham MA), Indermil® (Connexicon Medical Ltd., Dublin, Ireland), Histoacryl® (B. Braun AG, Melsungen, Germany), and 2-octyl-cyanoacrylate (Dermabond®; Ethicon US, LLC., a Johnson & Johnson Company, Cincinnati, OH) & Surgiseal (Adhezion Biomedical, Wyomissing, PA)[22, 29, 30]. Although cyanoacrylates have been used as tissue adhesives or sealants for decades outside the U.S., the first cyanoacrylate that was approved by FDA (in 1998) to be used as tissue adhesive was 2-octyl-cyanoacrylate (Dermabond®) [1, 22].

Some of the benefits of cyanoacrylate tissue adhesives are the ease of application for first aid, quick adhesion or sealing of wounded tissues, excellent hemostasis, and potential bacteriostatic or microbial barrier properties [1, 31]. Despite these benefits, cyanoacrylate and its degradation by-products may cause cytotoxicity, foreign body reactions, tissue necrosis, and inflammatory responses [32, 33]. Cyanoacrylates degrade via hydrolysis resulting in toxic cyanoacetates and formaldehydes as degradation by-products [26, 33]. The inherent brittleness is another setback for cyanoacrylates. Significant efforts have been made to mitigate such brittleness and cytotoxicity by introducing longer alkyl chain derivatives. Because of their cytotoxic and inflammation prone nature, a limited number of cyanoacrylates are approved by FDA, predominately for topical use.

### 2.2 Fibrin

Fibrin tissue adhesives or tissue sealants are the most widely used bioadhesives in the U.S. since their first approval by FDA in 1998 [27, 30, 34, 35]. Fibrin sealants, also known as fibrin glue, contain two key components derived from plasma coagulation proteins, (i) fibrinogen and (ii) thrombin. Upon mixture, these two components mimic the body's natural blood clotting cascades, as thrombin converts soluble fibrinogen into crosslinked, insoluble fibrin [1, 34]. Calcium is often added to thrombin to further catalyze the clot formation. Although clotting occurs rapidly (within seconds), the clotting time can vary depending on the concentrations of fibrinogen and thrombin and the presence of other catalyzing and stabilizing components. Fibrin is the only material that is currently FDA approved for use as a hemostat, tissue adhesive, and tissue sealant [36].

Fibrin sealant has a wide range of applications. For example, orthopaedic surgeons frequently use fibrin sealant in autologous chondrocyte implantation (ACI) treatment, where culture-expanded chondrocytes are delivered into a cartilage defect confined by a periosteal or collagenous membrane fixated by sutures, followed by sealing the defect boundaries with fibrin sealant. In the suture-free matrix-induced ACI (MACI), type I/type III collagen bilayer seeded with chondrocytes is secured directly to the defect site by fibrin glue [37, 38]. Repair of delaminated acetabular articular cartilage using fibrin adhesive was shown to be a useful technique for the early cartilage damage treatment strategy [23]. A cadaveric study showed that improved press-fit fixation of osteochondral scaffolds can be achieved using fibrin glue[39]. Fibrin glue can improve the meniscus healing when applied to outer zone meniscus defect compared to defect only repair [40, 41]. Even better meniscus healing was observed when fibrin was mixed with bone marrow cells in a rabbit model [40]. A long term follow-

up (average of 8 years) of 40 patients showed better repair and healing of arthroscopically repaired meniscal tears using fibrin glue with comparable recurrence rate (10%) compared to repair with suturing [40].

Fibrin is a unique biopolymer with unique biological and physical characteristics. Fibrin sealants exhibit excellent biocompatibility, biodegradability, deformability, and elasticity. In addition to that, fibrin adhesives do not trigger any inflammatory responses, foreign body reactions, tissue necrosis, or extensive fibrosis. However, in spite of having all these benefits, fibrin glues have low bond strengths (0.005–0.17 MPa) compared to synthetic tissue adhesives [1, 35]. This limits their application to the defect site undergoing significant tensile loads. Fibrin glue can degrade very rapidly even before the healing process begins because of the proteolytic activity in the musculoskeletal joints [42, 43]. This is one of the major reasons because of their limited applicability for musculoskeletal tissue repair or regeneration associated with synovial joints. In addition to their uses as sealants and tissue adhesives, fibrin alone, or in combination with other polymers, has also been extensively used for tissue engineering and regenerative medicine applications.

### 2.3 Aldehyde based bioadhesives

Another family of commonly used bioadhesive is based on aldehyde. For example, gelatin-resorcinol cross-linked with formaldehyde (GRF) and GRFG (GRF with glutaraldehyde) adhesives are the most commonly applied aldehyde based formulations. Originally developed in Europe in the 1960s, GRF/GRFG have been widely used in Europe and Japan for the past few decades for vascular, thoracoscopic, gastrointestinal, lever, and urinary track surgeries [1, 44, 45]. The idea of having both formaldehyde and glutaraldehyde in the same formulation is to obtain the initial strong bonding from formaldehyde and the high *in vivo* stability from glutaraldehyde. Gelatin contributes to the biodegradability and elasticity of the GRF/GRFG glue. Since gelatin crosslinked by formaldehyde/glutaraldehyde performs poorly in wet condition, resorcin, a phenolic component (1,3-benzenediol), is added to GRFG formulation to improve its strength by minimizing the negative effect from an aqueous environment [1] (Fig. 3). Formaldehyde and (or) glutaraldehyde act as crosslinking agents for both gelatin and resorcin. Aldehyde groups from formaldehyde and glutaraldehyde react with the amine group from gelatin, in addition to the amine group present in the tissue, and thus form a strong bond between GRF/GRFG and tissue. Bonding strength of GRF/GRFG can be achieved to the level of cyanoacrylates. In spite of its excellent hemostatic and adhesive properties and widespread usage in Europe and Japan for decades, GRF/GRFG glues have not been approved by FDA to be used for clinical applications in the U.S.[3] This is likely due to the potential cytotoxicity, mutagenicity, and carcinogenicity caused by formaldehydes, which either can be caused by the residue of unreacted formaldehyde molecules or by the degradation byproducts [46, 47]. As a result, some formulations with less toxic glutaraldehyde glyoxal or glutaric acid may improve the biosafety of GRF/GRFG [1, 48, 49].

BioGlue® (Cryolife, Kennesaw, GA), a protein-aldehyde system (PAS), is a commercially available glutaraldehyde-based formulation. It has two components, bovine serum albumin (BSA) and glutaraldehyde, and the gluing mechanism is similar to GRF/GRFG. BioGlue®

has been approved by FDA in 1999 to be used in the U.S. as adjunct to suturing or stapling for acute thoracic aortic dissection and cardiac surgery [27, 35]. In vivo degradation rate of BioGlue® is slower than GRF/GRFG. However, the potential cytotoxicity of glutaraldehyde has led to the use of alternative crosslinking agents in other albumin based formulations. For instance, PreveLeak™ (Baxter Healthcare, Deerfield, IL) is composed of BSA and polyaldehyde[50] and Progel® (Neomend, Inc., Irvine, CA) is composed of human serum albumin and a polyethyleneglycol (PEG) crosslinker functionalized with succinate groups ($PEG\text{-}(SS)_2$), where N-hydroxysuccinimide (NHS) ester groups are attached to each end of the PEG[51]. Both PreveLeak and Progel® are FDA approved for vascular reconstructions and intraoperative use during pulmonary resection, respectively.

### 2.4 Polyethylene Glycol (PEG) Based Adhesives

Polyethylene Glycol (PEG) based adhesives are a highly water-absorptive hydrogel which have been widely used as fluid barriers and hemostatic adhesives. The first commercially available PEG based adhesive was FocalSeal® (Genzyme Biosurgery Inc., Cambridge, MA) [52]. It was activated by light and intended to be used as a lung sealant. However, FocalSeal® is no longer available in the market due to its difficulty to use. Currently there are two FDA approved PEG based adhesives available in the market, Coseal (Baxter International Inc., Deerfield, IL) and DuraSeal® (Integra LifeSciences, Princeton, NJ)[53–55]. Coseal is a fully synthetic adhesive that contains two biocompatible functionalized polyethylene glycols (PEG), tetra-succinimidyl (4S) and tetra-thiol (4T)-derivatized polyethyleneglycol (4S-PEG and 4T-PEG) [52]. A covalently bonded hydrogel forms when 4S-PEG and 4T-PEG are mixed together. Gel formation occurs through the reaction between the thiol groups and the carbonyl groups of the succinimidyl esters resulting in the formation of a thio-ester covalent network between PEG molecules (Fig. 4). Free N-hydroxy-succinimide molecules are liberated from the reactions. It is indicated for use in vascular reconstructions to achieve adjunctive hemostasis by mechanically sealing areas of leakage. Duraseal contains polyethylene glycol (PEG) ester solution and a trilysine amine solution. This has been used as an adjunct for dural closure to prevent cerebrospinal fluid (CSF) leakage during brain and spine surgeries. PEG based adhesives are hydrophilic, biocompatible, and biodegradable. However, these adhesives exhibit high swelling ratio of up to 400% and thus need to be very cautious for closed space applications to avoid pressure build up on surrounding tissues [1, 27, 56].

### 2.5 Nature-inspired bioadhesives

In order to improve wet adhesion and bonding strength, some bioadhesives adopted chemical formulations inspired by nature. Mussel adhesive proteins are one example that have received significant attentions in the field of bioadhesives. Mussels secrete a proteinaceous fluid known as mussel adhesive proteins (MAPs), also known as mussel foot proteins (MFPs), that enables them to form byssal threads and adhesive plaques to anchor themselves onto a wide variety of underwater surfaces in harsh environment [32, 57]. This feature has inspired the scientists and tissue engineers to design a bioadhesive that can strongly adhere to wet biological surfaces [58–63]. Excellent wet adhesion of MAPs can primarily be attributed to the presence of a unique catechol containing amino acid known as L-3,4-dihydroxyphenylalanine (L-DOPA). The catechol moieties (the hydroxyl groups) of

DOPA are able to generate strong non-covalent interactions to various surfaces [64]. These catechol hydroxyl groups can convert to ortho-quinones under oxidizing or alkaline conditions that trigger covalent self-crosslinking between MAPs [57]. Oxidized DOPA can also form covalent bonds with −NH2, −SH, −OH, and −COOH groups present on tissue surfaces resulting in strong adhesion[57, 65, 66]. Moreover, catechol groups of DOPA also interact among themselves through reversible interactions in the presence of metal ions from ambient environment, such as $Fe^{3+}$, $Cu^{2+}$, $Ti^{3+}$, $V^{3+}$[48, 57, 67]. These catechol-metal ion complexes further strengthen the previously formed self-crosslinking between MAPs to achieve the final hardness. Inspired by these adhesion mechanisms summarized in Fig. 5, researchers have developed diverse mussel-inspired wet-resistant bioadhesive formulations by incorporating and adopting DOPA as a functional component for strong tissue adhesion [1, 68–70]. Another example based on mussel adhesive strategies, is the injectable citrate-enabled mussel inspired bioadhesives (iCMBAs)[1, 68]. The iCMBAs were synthesized by polycondensation reaction using citric acid, PEG, and dopamine/L-DOPA. The wet adhesion property, bonding strength, and shear modulus and strengths of iCMBAs are superior to those of fibrin sealants, suggesting its potential as a tissue glue for wound healing [1, 68]. Since the first development, iCMBAs have significantly evolved to equip with anti-bacterial properties [71] or to further improve bonding by implementing click chemistry [6].

Similarly, Gecko's unique capability of climbing surfaces via fast detaching and reattaching to surfaces has provided a useful inspiration to bioadhesives research. Gecko's extraordinary adhesion feature is attributed to millions of nanostructured hairs covering gecko's soles [72]. Capillary forces and van der Waals interactions are the main mechanisms for adhesion to hydrophilic and hydrophobic materials, respectively [72]. Inspired by geckos, flexible polyimide films with sub-micron pillars were fabricated using electron-beam lithography and dry etching in oxygen plasma [72] of which adhesion strength is proportional to the number of foot-hairs [72]. In another study, the gecko-inspired nanoscale pillars were combined with a mussel-mimetic polymer film to create an adhesive with the capability of reversibly adhering to different surfaces in dry and wet condition [73]. To date, there is no commercially available bioadhesives inspired by nature.

## 3 Bioadhesives for musculoskeletal tissues

### 3.1 Bone

Various structural scaffolds have been fabricated as biomaterial grafts for large sized bone defects [74–77]. However, such bulk scaffolds are not appropriate to augment healing of fragmented bone defects such as comminuted fractures [74, 76, 77]. Thus, bioadhesives have been developed as an injectable bone implant that are readily applied to fragmented bone defects [76, 77]. As another mode of application, bioadhesives can also be used as a glue to fix other types of bone grafts to host tissues[74]. Bulk bone grafts, either autologous or bioengineered, are frequently fixed to host tissues in aids of metal screw, wire, and/or locking plates [75]. However, such graft fixation strategy is hardly applicable for fragmented or powdered bone grafts to support healing of comminuted bone fractures or small defects [74]. A number of bioadhesives showed their potential as a biocompatible, biodegradable and mechanically stable glue to secure bone grafts.

For example, chitosan and oxidized dextran were composed into biocompatible and degradable bioadhesives for bone regeneration through covalent crosslinking [74]. L-DOPA was conjugated in oxidized dextran to replicate the gluing mechanism of mussel. The bone adhesives composed of chitosan and dextran exhibited minimal *in vitro* cytotoxicity and a bonding strength 3 times higher than fibrin [75]. Despite the significant improvement over fibrin, the bonding strength of the chitosan/dextran bone adhesives is much lower than that of cyanoacrylate and likely not sufficient for functional integration of bone grafts under high mechanical demand. To further enhance the bonding strength and mechanical properties of injectable bone adhesives, different formulations of bioadhesives were tested. A composite of mussel-inspired iCMBAs and hydroxyapatite (HA) was prepared for bone replacement and tested both *in vitro* and *in vivo* [76]. The addition of HA significantly improved compressive modulus and lap shear strength in comparison with iCMBAs [76]. *In vitro*, iCMBA/HA also promoted osteogenic differentiation of human bone marrow derived mesenchymal stem/progenitor cells (MSCs) [76]. In addition, iCMBA/HA showed suitable injectability and crosslinking when applied to a rabbit comminuted radial fracture model [76]. *In vivo* delivery of iCMBA/HA significantly improved bone formation with markedly enhanced bending strength as compared to control up to 12 weeks [76].

Another research group also adopted the mussel gluing mechanism to establish bone adhesives for xenograft bone substitute [75]. DOPA-containing mussel adhesive protein was prepared as a bone adhesive and demonstrated a promising efficiency to maintain adhesion of deproteinized bovine bone mineral (DBBM) particles [75]. DOPA-containing mussel adhesion (MAP) protein showed improved osteogenic differentiation of MC3T3-E1 osteoblasts as compared to controls including tissue culture plate, MAP without DOPA, and poly-L-lactide (PLLA) [75]. *In vivo* delivery of DBBM aggregates formed with DOPA-containing MAP into critical sized bone defects in rat calvaria significantly enhanced bone formation in comparison with DMMB alone or untreated control by 8 weeks follow-up [75].

Chondroitin sulfate (CS), another type of biologically derived adhesive, has been applied to enhance integration of bone grafts for bone regeneration [77]. CS is a major component of glycosaminoglycans (GAGs), and CS modified with N-hydroxysuccinimide (NHS) can serve as an efficient bioadhesives that forms chemical bonds with various tissues and matrices, including bone marrow, platelet-rich plasma, cartilage, eye, bone, and skin [77]. When Bioactive ceramics in particulate form, such as Bioglass® (BG) 45S5 CS-NHS, are aggregated with CS-NHS, bone marrow (BM) can be encapsulated into BG-CS that in turn form mechanically stable constructs. The BG-CS-BM constructs demonstrated a significantly improved integrity and successfully enhanced healing of critical-size distal femoral bone defects in rabbits by 6 weeks, in comparison with BG alone [77].

As summarized above, various bioadhesives have been constructed either as an injectable bone implant or as glue for fragmented or powdered bone grafts. Recent advancements in the chemical formulation have promoted biocompatibility, safety, degradation properties, and osteo-conductivity/inductivity, consequently leading to improved bone healing. In general, bone bioadhesives are advantageous for treatment of small or fragmented bone defects. Outstanding challenges in bone adhesives include mechanical properties and long-term degradation associated with bone remodeling, which are important factors to be

considered for regeneration of load-bearing long bone in pre-clinical large animal model and human patients.

### 3.2 Intervertebral disc (IVD)

Over 80% of the U.S. population suffers from back pain, and approximately 90% of spinal disorders are caused by the intervertebral disc (IVD) [78, 79]. As fibrocartilaginous tissue lying between vertebrae, the IVD consist of 1) the central gel-like nucleus pulposus (NP) with abundant collagen type II (Col-II) and proteoglycan, 2) the outer annulus fibrosus (AF) in collagen and elastic, and 3) the thin layers of endplates bound above and below to the adjacent vertebral bodies [80]. The flexibility and mechanical stability of the IVD is attributed to the confinement of the NP by the AF, thus the structural injury of the IVD leads to the dislocation of the NP through defective parts of the AF and compresses the adjacent spinal nerves [81].

For IVD regeneration, bioadhesives have been applied in two different modes. First, adhesive properties were incorporated into hydrogel-based scaffolds for NP regeneration [82]. Various hydrogels have been injected into degenerating NP to support cell viability and restore the mechanical stability [83]. Application of bioadhesives for such scaffolds was intended to provide an adhesive interface to surrounding tissues, further securing hydrogel material inside NP cavity [84]. For example, poly(N-isopropylacrylamide) (PNIPAAm) was copolymerized with poly(ethylene glycol) (PEG) and blended with poly(ethylene imine) (PEI) as an injectable adhesive scaffold for NP regeneration [85]. PNIPAAm-PEG/PEI showed improved mechanical properties and tissue adhesion force when glutaraldehyde was injected into the gel core [85]. In another study, PNIPAAm was grafted with chondroitin sulfate (CS) or aldehyde-modified CS to form injectable thermogelling hydrogel scaffold for NP, also forming covalent bonding with surrounding tissue upon contact [86]. In comparison with PNIPAAm, PNIPAAm-g-CS and PNIPAAm-g-CS with CS aldehyde showed increased adhesion strength, suggesting their potential as an injectable, adhesive scaffold for NP restoration[86].

As the other application mode for IVD regeneration, various bioadhesives have been investigated as a sealant for AF. Defects on outer parts of AF, either by degeneration or surgical removal, can cause IVD herniation associated with inflammation and mechanical instability [87, 88]. Thus, sealing the damaged AF can support functional restoration of the herniated IVD and mitigate the pain [89]. Injectable bioadhesives for AF repair have been considered given their advantages over non-injectable approaches, such as sutures and plugs, which fail to restore intradiscal pressure [89] and to prevent NP extrusion[89], respectively.

Genipin-Crosslinked fibrin gel (FibGen) is one of the bioadhesives that have been extensively investigated as a sealant for augmented AF repair [90, 91]. Genipin crosslinking was applied to enhance mechanical properties and to slower degradation of fibrin [92]. Several previous studies suggested the potential of FibGen as an efficient AF sealant [93]. When applied to repair AF defects in bovine coccygeal functional spine units (FSU) *ex vivo*, FibGen significantly enhanced functional properties of IVD, superior to a clinically available BioGlue® [83]. AF repaired with FibGen also led to a significant improvement in functional restoration of bovine IVD as compared to AF repaired with bulk space-filling

scaffold such as poly(trimethylene carbonate) (PTMC) [94]. FibGen resulted in meaningful restoration of torsional stiffness, bending range of motion and disc height, with minimal risk of herniation and failure in comparison with PTMC scaffold-based AF repairs[94]. In addition, various doses of genipin were tested to enhance mechanical properties of FibGen to more closely match compressive, tensile, and shear properties of native AF [92]. Reinforcement with fibrous poly(D,L-lactide-co-glycolide) (PDLGA) scaffolds was also implemented to further enhance mechanical properties of FibGen bioadhesives [92].

FibGen sealant has also been considered as an adhesive carrier for cells and bioactive factors [84, 93]. However, an *in vitro* study showed that increasing dose of genipin improves mechanical properties of FibGen but reduces cell viability [93], necessitating a balanced genipin crosslinking to serve as an efficient cell carrier. Another study incorporated collagen I hollow spheres in FibGen to deliver anti-TNFα drug [84]. FibGen with collagen spheres successfully provided sustained release of anti-TNFα drug that, in turn, resulted in sustained reduction of pro-inflammatory cytokines produced by AF cells [84]. More recently, FibGen was tested as a delivery carrier for transforming growth factor beta-3 (TGFβ3) [95]. TGFβ3 loaded in FibGen encapsulated with AF cells showed sustained release for 16 days *in vitro* that resulted in enhanced matrix synthesis as compared to FibGen alone [95]. Despite the above-described advantages of FibGen, the outstanding challenges for FibGen to augment AF repair include the suboptimal mechanical properties and the low cell viability caused by genipin crosslinking [84, 95].

Other bioadhesives investigated as AF sealants include the copolymer of PEG with trimethylene carbonate (TMC) and hexamethylene diisocyanate (HDI) end-groups [88]. The TMC-based adhesives showed high adhesion strength to AF tissue and slow degradation by 3 weeks *in vitro* [88]. Despite the lower compressive strength compared to native AF, the TMC adhesive displayed promising shear moduli similar to AF tissues[88]. Hybrid hydrogel of decellularized AF matrix (DAFM) and chitosan, crosslinked with genipin, also showed potential to support matrix synthesis from AF stem/progenitor cells *in vitro* [96]. Another group has been investigating a high-density collagen (HDC) gel seeded with cells for augmented AF repair [97–99]. HDC seeded with AF cells significantly improved healing of punctured rat tail discs as compared to punctured control and HDC without cells [100]. When HDC seeded with mesenchymal stem cells (MSCs) was delivered to sheep AF rupture models, the disc height index was significantly increased as compared to the untreated control by 6 wks follow-up [101]. Those data suggest that HDC is a promising carrier for cells, but its bonding strength to AF tissue requires further enhancement for functional restoration of AF. To facilitate adhesion of HDC to AF tissue, chondroitinase ABC (C-ABC) was applied to AF tissues to expose binding site by digesting proteoglycan, which led to a promising improvement with marginal effect on cell viability [102].

To sum, important research progress has been made for development of an injectable bioadhesive sealant for AF repair with ultimate goals to restore functional properties of IVD, to prevent disc herniation, and to guide AF regeneration. Various hydrogel formulations and crosslinkers showed some meaningful outcomes in functional properties as AF sealant. However, the mechanical properties of the existing bioadhesives are far lower than those of native AF tissues and the adhesion strength needs further enhancement for functional

restoration of IVD upon AF rupture. Moreover, the majority of previous works predominantly focused on the mechanical properties of bioadhesives *in vitro* with limited analyses for biological aspects and *in vivo* efficacy.

### 3.3 Articular cartilage

Focal cartilage lesions hardly heal and frequently progress into degenerative changes in the joint. Accordingly, tissue engineering approaches using cells, biomaterial scaffolds, and/or bioactive cues have been widely applied toward cartilage regeneration [103]. Engineering cartilage constructs *in vitro* followed by *in vivo* implantation, as well as applying hydrogel-based scaffolds with or without cell and/or growth factors, have been popular regenerative approaches to overcome limitations of the current treatments for cartilage defects [104]. Despite successful engineering of functional cartilaginous tissues *in vitro* [105], integration with adjacent host tissue has been one of the outstanding challenges to achieve long-lasting success in cartilage regeneration [106]. Similarly, the integration issue was also involved with the injectable hydrogels with or without cells and/or bioactive cues [107].

Thus, bioadhesives have been applied to facilitate integration between cartilage implants and host tissues [108]. For example, BioGlue® was applied to secure autologous cartilage grafts to repair 6-mm focal defects on rabbit femoral condyles [109]. Application of BioGlue® along with cartilage grafts improved cartilage healing by 60 days as compared to grafts without BioGlue® [109]. Collagen adhesion protein was used to provide secure adhesion of a poly(vinly alcohol) (PVA) implant, with a promising outcome to improve the adhesion strength between PVA scaffold and articular cartilage tissues [110]. In other studies, CS bioadhesives were applied to glue PEG diacrylate (PEGDA) hydrogel scaffolds for cartilage regeneration [103, 111, 112]. PEGDA hydrogel provides appropriate 3D environment for chondrocytes culture and differentiation but suffers from poor integration with adjacent tissue given its intrinsic non-adherent characteristics [103, 111, 112]. Application of CS adhesives on surface of cartilage lesions prior to application of PEGDA hydrogel significantly improved the initial bonding of scaffolds as well as cartilage healing *in vivo* [103]. The PEGDA hydrogel scaffolds covalently bonded to articular cartilage by CS adhesives was then tested in human patients [111]. After performing microfracture procedure, CS adhesives were applied on surface of cartilage lesion, followed by application of PEDGA to be supplemented with bone marrow [111]. The phase I clinical trials with 15 patients and 6-months follow-up resulted in an improved clinical outcome as compared to control without treatment [111].

Other groups modified CS bioadhesives to form CS-cysteine conjugate (CS-cys) to enhance adhesion and mechanical properties of CS [113]. CS-cys was synthesized by forming bonding between the primary amine of cysteine and the carboxylic acid group of CS that led to enhanced adhesion strength on porcine cartilage as compared to unmodified CS [113]. A recent study has fabricated tyramine-modified hyaluronic acid (HA-Tyr) hydrogels as bioadhesives cell carrier for cartilage regeneration [114]. Although its bonding strength to cartilage explant was at the level of fibrin glue, HA-Tyr hydrogel encapsulated with MSCs showed potential to facilitate chondrogenic differentiation stimulated by mechanical loading [114]. A recently reported bioadhesive, polycaprolactone-β-cyclodextrin (PCL-CD)

polymersome, was designed as a carrier for co-delivery of hydrophilic and hydrophobic drug molecules for cartilage healing [115]. PCL-CD polymersomes showed shear thinning, efficient self-healing and tissue adhesion via host-guest complexation process [115]. Intra-Articular injection of PCL-CD polyersomes loaded with TGFβ1 and/or kartogenin reduced aberrant subchondral bone formation and attenuated articular cartilage degeneration in animal osteoarthritic knees by 6 weeks [115].

The aforementioned works consistently suggest that hydrogel-based bioadhesives have significant potential to support tissue engineering approaches for regeneration of focal cartilage lesions. Injectability of such bioadhesives is likely appropriate for filling cartilage defects as restoring surface congruency. In addition, the adhesive feature seems to be necessary to fulfill the clinical needs to establish functional tissue integration. Outstanding challenges in application of bioadhesives for cartilage regeneration include the limited mechanical properties of hydrogel-based materials, unexplored process of material degradation followed by tissue remodeling, and unknown effects of bioadhesives on friction coefficient of repaired articular surface.

### 3.4 Knee meniscus

Knee meniscus is an inhomogeneous fibrocartilaginous tissue, playing essential roles in congruence, shock absorption, lubrication, stability, and load transmission. These roles are critical to joint health and function, and are dependent upon maintenance of normal meniscal viability, composition, architecture, and geometry. Normal meniscus is defined by its multiphase biochemical composition and structure. The vascularized outer third zone of meniscus is constituted with dense fibrous matrix populated with fibroblast-like cells, the middle zone is fibrocartilaginous matrix with co-residing of fibroblast-like cells and rounded chondrocyte-like cells, and the avascular inner third zone is more like cartilage populated with chondrocyte-like cells. While tears in the vascularized outer third region of meniscus can often successfully heal after suture repair, tears in the inner avascular region rarely heal due to poor intrinsic healing capacity. As such, these tears frequently propagate and lead to meniscus deterioration, degeneration, and whole-joint disease [116–118]. Thus, bioadhesives have been considered to augment healing of such tears in the meniscus avascular zone that cannot be suture-repaired or to fix grafts replacing damaged meniscus parts [119].

In 1995, cyanoacrylate glue was first tested for augmentation of meniscus repair *in vitro* [120]. Cyanoacrylate applied together with suture improved adhesion strength between bone meniscus tissue strips [120]. Another study also showed potential of a modified cyanoacrylate (Histoacryl) to glue bovine meniscal tissues [121]. However, cyanoacrylate has been rarely used for *in vivo* meniscus repair likely due to outstanding limitations including cytotoxicity and inflammation [119]. An *in vivo* study applied cyanoacrylate to glue meniscus grafts in rabbits but ended with a poor outcome due to a severe inflammatory response [122]. Fibrin, a biologically derived hydrogel, also has a long histology of investigation as augmentation for meniscal repair [123]. Over 30 years ago, fibrin glue was used to augment suture repair of outer vascularized zone tears [41]. Although clinical data suggest fibrin augmentation somewhat enhanced healing of outer zone tears [41], we do not

have sufficient experimental data to estimate the potential efficacy of fibrin glue to augment meniscus repair in the inner avascular zone. Fibrin gel gluing two meniscal tissue strips with and without porcine meniscal cells showed an improved tissue integration and new matrix formation when the fibrin-tissue constructs were implanted subcutaneously in nude mice [123]. However, an application of fibrin glue *in situ* for meniscal repair or graft implantation resulted in poor healing outcome [124, 125], likely due to fast intra-synovial degradation and weak mechanical properties of fibrin [123].

Despite the weak mechanical and bonding properties, fibrin may work as an efficient carrier for cells and growth factors to facilitate meniscus healing [126]. Connective tissue growth factor (CTGF) delivered via fibrin gel resulted in an improved healing of avascular meniscus tears in rabbits by promoting matrix formation [125]. Although being tested in an ectopic implantation model, meniscal cells delivered via fibrin glue supported integration of meniscus tissue strips [123]. More recently, our group has used fibrin glue as an injectable carrier for controlled delivery of CTGF and TGFβ3 microspheres for avascular meniscus healing by endogenous stem/progenitor cells [126, 127]. Fast release of CTGF from successfully recruited endogenous synovial MSCs into meniscus defects and slow release of TGFβ3 from biodegradable microspheres led to integrated fibrocartilaginous healing of avascular meniscus tears in rabbits [126]. Despite the promising *in vivo* outcome with small animal models, it is imperative to address the limitations of fibrin including fast *in vivo* degradation, weak adhesion strength and bulk mechanical properties in order to move forward to large animal model and clinical application. FibGen, as extensively investigated for IVD sealant, may have potential to overcome such limitations of fibrin glue for supporting repair/healing of avascular meniscus tears.

Besides the fibrin-based glue, there have been a few other types of bioadhesives introduced for augmented repair of avascular meniscus tears [126]. One example is CS-based bioadhesives that have been widely applied for cartilage healing [112]. CS modified with n-hydroxysuccinimide hydrogel (NHS) was mixed with bone marrow aspirates to form CS-BM tissue adhesive for meniscus repair [112]. CS-BM hydrogel showed appropriate viability of meniscus fibrochondrocytes and improved compressive and shear moduli as compared to CS-PEG hydrogel [112]. In addition, CS-BM supported cell migration, fibrocartilaginous differentiation *in vitro* and fusion of meniscal tissues implanted subcutaneously in athymic rats [112]. Despite the promising outcome from *in vitro* experiments and ectopic implantation, there is yet any pre-clinical data or clinical trials for CS-based bioadhesives for meniscus repair. Another group developed hyper-branched tissue adhesives for repair of meniscus tears [119]. Copolymers based on PEG, trimethylene carbonate (TMC) and citric acid (CA) were synthesized, followed by end-functionalization with hexamethylene diisocyanate. The CA-PEG-TMC hydrogel showed the lap shear strength to bovine meniscus tissue at 4 – 8 fold of fibrin glue and the elastic modulus at the level of native meniscus [119]. The CA-PEG-TMC was then further modified with 2,2-dimorpholinodiethylether (DMDEE) or 1,4-diazabicyclo [2.2.2] octane (DABCO) to obtain fast curing [128].

A lacked vascularization, a scarcity of cells, an abundance of cartilaginous matrix, a complex anatomical structure, and a high physiological loading are among the many features

that make a suture-repair nearly impossible for meniscus tears in avascular zone [64]. Thus, injectable bioadhesives in combination with cells and/or bioactive cues may serve as an efficient tool to induce regenerative healing of inner meniscus tears only if successful in functional restoration and long-term sustainability. Despite the meaningful research progress in the field, the existing bioadhesives have yet to reach the important milestones.

### 3.5 Tendon

Tendons are dense connective tissues with the primary function of transferring mechanical forces from muscle to bone. Tendon injuries are highly prevalent, caused by laceration, contusion, or tensile overload [127, 129]. The primary treatment option for tendon rupture is suture-repair, and various suture techniques are being implemented depending on types of tendon and injuries. Unfortunately, the rate of tendon re-rupture is high because repaired tendons hardly recover the original mechanical strength [130]. Few bioadhesive materials have been investigated to enhance the success rate of suture-repair, consequently reducing the re-tear rate [90]. An *ex vivo* study examined tensile properties of sheep Achilles tendons suture repaired or glued with commercially available bioadhesives, including BioGlue® and Tissucol®, a fibrin sealant [90]. The tested bioadhesives showed significantly inferior tensile strength to sutures[90].

Another *in vitro* study applied a MAP-mimicking bioadhesive film to wrap around transected porcine Achilles tendons after suture repair [131]. Wrapping repaired tendons with bioadhesive film increased tensile stiffness, failure load, and energy to failure in comparison with suture alone group [131]. In contrast, *ex vivo* application of bioadhesives (BioGlue®) on suture repaired flexor tendons failed to improve tensile properties [130]. When bioadhesives were applied to sutures instead of tendons, repaired tendons with bioadhesives-coated sutures increased the tensile properties of repaired tendon likely attributed to shear lag effect [132]. Strong adhesives such as cyanoacrylates resulted in higher improvement in tensile load and stiffness as compared to other bioadhesives including but not limited to BioGlue® and poly(dopamine) [132].

Previously tested bioadhesives showed marginal effect on improving tensile properties of suture repaired tendons *ex vivo*. These outcomes are not surprising given the tensile properties of typical suture materials at higher order of magnitude as compared to most of bioadhesives materials. In consideration of very high tensile modulus and strength of tendons in parallel to the collagen alignment, the existing bioadhesives may not be ideal for mechanical augmentation. Regardless, bioadhesives potentially serve as control-delivery vehicles adherent on target surface of tendons for treatment of degenerative tendon diseases (e.g. tendinopathy), which likely represents the current research direction in tendon bioadhesives.

## 4 Summary and perspectives

In the last decades, our scientific community has made significant progress in development of regenerative bioadhesives for musculoskeletal tissues. As summarized in Table 3, successful bioadhesives for musculoskeletal tissue repair and regeneration must exhibit appropriate physical properties, outside of just adhesion strength, including a bulk modulus

and strength that meets the mechanical needs of the target tissues. Implementation of various chemical modifications, including but not limited to co-polymerization, cross-linking, blending, and surface modification, has demonstrated meaningful improvements in the essential physical properties. Despite the promising enhancement of adhesion strength as well as bulk modulus, the existing bioadhesives yet achieved mechanical properties sufficient to instantly restore functional properties of a majority of musculoskeletal tissues. Such suboptimal functional properties of bioadhesives might have been attributed to the limited number of *in vivo* studies for their efficacy in tissue repair and regeneration. Outstanding challenges for improving mechanical properties are closely associated with biocompatibility. For example, cyanoacrylates exhibit a strong tissue adhesion but causes excessive inflammation and necrosis. Although genipin crosslinking led to functional properties of fibrin meeting the mechanical needs for AF, an excess in genipin also results in an extremely dense matrix causing cell apoptosis. Thus, one must comprehensively evaluate biocompatibility of bioadhesives in regard to cell viability, cell migration and proliferation, tissue ingrowth and remodeling, and angiogenesis if needed for a target tissue. Besides, cytotoxicity must be carefully examined not only *in vitro* with direct cell contact but also *in vivo* long-term follow-up in consideration of potential harmful effect of any degradation bi-products.

Application of bioadhesives as a delivery vehicle is an emerging idea in the field [7, 13, 14, 19]. Even with suboptimal mechanical properties compared to target tissues, the intrinsic adhesive force on a tissue surface is likely beneficial for a localized delivery of cells, growth factors and small molecules on the target area. If a bioadhesive was only to provide a controlled delivery rather than supporting cell and tissue ingrowth, it would be more feasible to achieve high adhesion strength and mechanical properties that would be an effective treatment option for degenerative musculoskeletal diseases such as tendinopathy [133]. As bioadhesives are mostly hydrogel-based, it is practically appropriate to design and implement various modalities to control entrapment and release of growth factors and small molecules [133, 134]. There is a growing interest in development and application of bioadhesives as controlled delivery vehicles with significant clinical impact [133, 134].

One of the important but understudied areas in the field is the *in vivo* degradation of bioadhesives. Either for mechanical support, cell and tissue ingrowth, or controlled delivery, the *in vivo* functionality of a bioadhesive is closely connected to its degradation rate. Bioadhesives securing tissue grafts or filling tissue defects must undergo degradation as balanced with new tissue formation and remodeling. Similarly, release kinetics of bioactive cues are largely regulated by degradation of vehicles. Given inevitable difference between *in vitro* and *in vivo* in regard to biochemical and mechanical environment, *in vitro* degradation of biodegradable materials often hardly corresponds to that of *in vivo* [135–139]. Despite the importance, the *in vivo* degradation rate of bioadhesives has been rarely addressed in previous works. Accordingly, we expect more attention in the controlling and tracing of in vivo degradation of bioadhesives. Fortunately, advanced imaging technologies and imaging modalities as incorporated into various biomaterials are being developed for various applications such as cell tracing, drug delivery, and cancer tracing [140, 141]. Once incorporated with a dye or a fluorophore, a remaining amount of *in vivo* delivered bioadhesives can be traced and quantified in real time via a non- or minimally-invasive *in*

*vivo* imaging system [140, 141]. Understanding *in vivo* degradation rates and patterns would provide essential data to tune a bioadhesive's degradation fit for each target tissue.

To sum, bioadhesives have a number of unique advantages to facilitate repair and regeneration of musculoskeletal tissues including but not limited to bone, IVD, cartilage, knee meniscus, and tendon. They can provide an augmentation for tissue repair, a secured filling of tissue defects, a sealing of tissue gaps, and a regenerative stimulation by releasing bioactive cues. Despite the outstanding challenges in regard to the suboptimal adhesion and mechanical properties and the lacked understanding of *in vivo* degradation, our continuous and dedicated efforts in the field hold a great potential to develop clinically applicable bioactive adhesives promoting musculoskeletal repair and regeneration.

## Acknowledgements

We thank Sharmin Hossain for her administrative support. The schematic art work describing use of bioadhesives was created by Lauren Hugdahl. Funding: This work was supported by the National Institutes of Health 5R01AR071316-04 and 1R01DE029321-01A1.

## Reference

1. Mehdizadeh M, Yang J, Design Strategies and Applications of Tissue Bioadhesives, Macromol Biosci. 2013;13:271–288. [PubMed: 23225776]
2. Zhang H, Bre LP, Zhao TY, Zheng Y, Newland B, Wang WX, Mussel-inspired hyperbranched poly(amino ester) polymer as strong wet tissue adhesive, Biomaterials. 2014;35:711–719. [PubMed: 24140046]
3. Zhu WZ, Chuah YJ, Wang DA, Bioadhesives for internal medical applications: A review, Acta Biomater. 2018;74:1–16. [PubMed: 29684627]
4. Qiu L, Qi See AA, Steele TWJ, Kam King NK, Bioadhesives in neurosurgery: a review, J Neurosurg. 2019 1–11.
5. Scognamiglio F, Travan A, Rustighi I, Tarchi P, Palmisano S, Marsich E, Borgogna M, Donati I, de Manzini N, Paoletti S, Adhesive and sealant interfaces for general surgery applications, J Biomed Mater Res B Appl Biomater. 2016;104:626–639. [PubMed: 25891348]
6. Guo J, Kim GB, Shan D, Kim JP, Hu J, Wang W, Hamad FG, Qian G, Rizk EB, Yang J, Click chemistry improved wet adhesion strength of mussel-inspired citrate-based antimicrobial bioadhesives, Biomaterials. 2017;112:275–286. [PubMed: 27770631]
7. Abou-ElNour M, Soliman ME, Skouras A, Casettari L, Geneidi AS, Ishak RAH, Microparticles-in-Thermoresponsive/Bioadhesive Hydrogels as a Novel Integrated Platform for Effective Intra-articular Delivery of Triamcinolone Acetonide, Mol Pharm. 2020;17:1963–1978. [PubMed: 32271590]
8. Agnol LD, Dias FTG, Nicoletti NF, Marinowic D, Moura ESS, Marcos-Fernandez A, Falavigna A, Bianchi O, Polyurethane tissue adhesives for annulus fibrosus repair: Mechanical restoration and cytotoxicity, J Biomater Appl. 2019;34:673–686. [PubMed: 31354030]
9. Allon AA, Ng KW, Hammoud S, Russell BH, Jones CM, Rivera JJ, Schwartz J, Hook M, Maher SA, Augmenting the articular cartilage-implant interface: Functionalizing with a collagen adhesion protein, J Biomed Mater Res A. 2012;100:2168–2175. [PubMed: 22615182]
10. Bochynska AI, Hannink G, Janssen D, Buma P, Grijpma DW, Development of a fast curing tissue adhesive for meniscus tear repair, J Mater Sci Mater Med. 2017;28:1. [PubMed: 27866344]
11. Bochynska AI, Van Tienen TG, Hannink G, Buma P, Grijpma DW, Development of biodegradable hyper-branched tissue adhesives for the repair of meniscus tears, Acta Biomater. 2016;32:1–9. [PubMed: 26689469]
12. Tanideh N, Dehghani Nazhvani S, Mojtahed Jaberi F, Mehrabani D, Rezazadeh S, Pakbaz S, Tamadon A, Nikahval B, The healing effect of bioglue in articular cartilage defect of femoral

condyle in experimental rabbit model, Iran Red Crescent Med J. 2011;13:629–633. [PubMed: 22737537]

13. Tarafder S, Gulko J, Kim D, Sim KH, Gutman S, Yang J, Cook JL, Lee CH, Effect of dose and release rate of CTGF and TGFbeta3 on avascular meniscus healing, J Orthop Res. 2019;37:1555–1562. [PubMed: 30908692]
14. Tarafder S, Gulko J, Sim KH, Yang J, Cook JL, Lee CH, Engineered Healing of Avascular Meniscus Tears by Stem Cell Recruitment, Sci Rep. 2018;8:8150. [PubMed: 29802356]
15. Bochyska AI, Hannink G, Grijpma DW, Buma P, Tissue adhesives for meniscus tear repair: an overview of current advances and prospects for future clinical solutions, J Mater Sci Mater Med. 2016;27:85. [PubMed: 26970767]
16. Bulman SE, Coleman CM, Murphy JM, Medcalf N, Ryan AE, Barry F, Pullulan: a new cytoadhesive for cell-mediated cartilage repair, Stem Cell Res Ther. 2015;6:34. [PubMed: 25889571]
17. Likhitpanichkul M, Kim Y, Torre OM, See E, Kazezian Z, Pandit A, Hecht AC, Iatridis JC, Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFalpha drug, Spine J. 2015;15:2045–2054. [PubMed: 25912501]
18. Panebianco CJ, DiStefano TJ, Mui B, Hom WW, Iatridis JC, Crosslinker concentration controls TGFbeta-3 release and annulus fibrosus cell apoptosis in genipin-crosslinked fibrin hydrogels, Eur Cell Mater. 2020;39:211–226. [PubMed: 32396210]
19. Zhu M, Wei K, Lin S, Chen X, Wu CC, Li G, Bian L, Bioadhesive Polymersome for Localized and Sustained Drug Delivery at Pathological Sites with Harsh Enzymatic and Fluidic Environment via Supramolecular Host-Guest Complexation, Small. 2018;14.
20. Nukaga T, Sakai D, Schol J, Sato M, Watanabe M, Annulus fibrosus cell sheets limit disc degeneration in a rat annulus fibrosus injury model, JOR Spine. 2019;2:e1050. [PubMed: 31463464]
21. Vernengo J, Fussell GW, Smith NG, Lowman AM, Synthesis and characterization of injectable bioadhesive hydrogels for nucleus pulposus replacement and repair of the damaged intervertebral disc, J Biomed Mater Res B Appl Biomater. 2010;93:309–317. [PubMed: 20225214]
22. Mattamal GJ US FDA perspective on the regulations of medical-grade polymers: cyanoacrylate polymer medical device tissue adhesives, Expert Rev Med Devices. 2008;5:41–49. [PubMed: 18095895]
23. Stafford GH, Bunn JR, Villar RN, Arthroscopic repair of delaminated acetabular articular cartilage using fibrin adhesive. Results at one to three years, Hip Int. 2011;21:744–750. [PubMed: 22117261]
24. Eaglstein WH, Sullivan T, Cyanoacrylates for skin closure, Dermatol Clin. 2005;23:193–+. [PubMed: 15837150]
25. Stavropoulou C, Atout RN, Brownlee M, Schroth RJ, Kelekis-Cholakis A, A randomized clinical trial of cyanoacrylate tissue adhesives in donor site of connective tissue grafts, J Periodontol. 2019;90:608–615. [PubMed: 30517975]
26. Tighe BJ, Mann A, Adhesives and interfacial phenomena in wound healing, in: Woodhead Publ Mater, 2011, pp. 247–283.
27. Spotnitz WD, Burks S, Hemostats, sealants, and adhesives: components of the surgical toolbox, Transfusion. 2008;48:1502–1516. [PubMed: 18422855]
28. Park DH, Kim SB, Ahn KD, Kim EY, Kim YJ, Han DK, In vitro degradation and cytotoxicity of alkyl 2-cyanoacrylate polymers for application to tissue adhesives, J Appl Polym Sci. 2003;89:3272–3278.
29. Mobley SR, Hilinski J, Toriumi DM, Surgical tissue adhesives, Facial Plast Surg Clin North Am. 2002;10:147–154. [PubMed: 15062317]
30. Heher P, Ferguson J, Redl H, Slezak P, An overview of surgical sealant devices: current approaches and future trends, Expert Rev Med Devic. 2018;15:747–755.
31. Singer AJ, Thode HC, A review of the literature on octylcyanoacrylate tissue adhesive, Am J Surg. 2004;187:238–248. [PubMed: 14769312]
32. Bhagat V, Becker ML, Degradable Adhesives for Surgery and Tissue Engineering, Biomacromolecules. 2017;18:3009–3039. [PubMed: 28862846]

33. Leggat PA, Smith DR, Kedjarune U, Surgical applications of cyanoacrylate adhesives: A review of toxicity, Anz J Surg. 2007;77:209–213. [PubMed: 17388821]
34. Martinowitz U, Saltz R, Fibrin sealant, Curr Opin Hematol. 1996;3:395–402. [PubMed: 9372108]
35. Shah NV, Meislin R, Current State and Use of Biological Adhesives in Orthopedic Surgery, Orthopedics. 2013;36:945–956. [PubMed: 24579215]
36. Spotnitz WD, Fibrin Sealant: The Only Approved Hemostat, Sealant, and Adhesive-a Laboratory and Clinical Perspective, ISRN Surg. 2014;2014:203943. [PubMed: 24729902]
37. Bartlett W, Skinner JA, Gooding CR, Carrington RW, Flanagan AM, Briggs TW, Bentley G, Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study, J Bone Joint Surg Br. 2005;87:640–645. [PubMed: 15855365]
38. Basad E, Ishaque B, Bachmann G, Sturz H, Steinmeyer J, Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study, Knee Surg Sports Traumatol Arthrosc. 2010;18:519–527. [PubMed: 20062969]
39. Filardo G, Drobnic M, Perdisa F, Kon E, Hribernik M, Marcacci M, Fibrin glue improves osteochondral scaffold fixation: study on the human cadaveric knee exposed to continuous passive motion, Osteoarthr Cartilage. 2014;22:557–565.
40. Ishimura M, Ohgushi H, Habata T, Tamai S, Fujisawa Y, Arthroscopic meniscal repair using fibrin glue .1. Experimental study, Arthroscopy. 1997;13:551–557. [PubMed: 9343641]
41. Ishimura M, Tamai S, Fujisawa Y, Arthroscopic meniscal repair with fibrin glue, Arthroscopy. 1991;7:177–181. [PubMed: 2069630]
42. Jackson MR, Fibrin sealants in surgical practice: An overview, Am J Surg. 2001;182:1s–7s.
43. Radosevich M, Goubran HI, Burnouf T, Fibrin sealant: scientific rationale, production methods, properties, and current clinical use, Vox Sang. 1997;72:133–143. [PubMed: 9145483]
44. Bonchek LI, Braunwald NS, Experimental evaluation of a cross-linked gelatin adhesive in gastrointestinal surgery, Ann Surg. 1967;165:420–424. [PubMed: 6019317]
45. Nomori H, Horio H, Gelatin-resorcinol-formaldehyde-glutaraldehyde glue-spread stapler prevents air leakage from the lung, Ann Thorac Surg. 1997;63:352–355. [PubMed: 9033299]
46. Ennker IC, Ennker J, Schoon D, Schoon HA, Rimpler M, Hetzer R, Formaldehyde-free collagen glue in experimental lung gluing, Ann Thorac Surg. 1994;57:1622–1627. [PubMed: 8010812]
47. Witter K, Tonar Z, Matejka VM, Martinca T, Jonak M, Rokosny S, Pirk J, Tissue reaction to three different types of tissue glues in an experimental aorta dissection model: a quantitative approach, Histochem Cell Biol. 2010;133:241–259. [PubMed: 19902233]
48. Bao Z, Gao M, Sun Y, Nian R, Xian M, The recent progress of tissue adhesives in design strategies, adhesive mechanism and applications, Mater Sci Eng C Mater Biol Appl. 2020;111:110796. [PubMed: 32279807]
49. Suzuki S, Ikada Y, Sealing effects of cross-linked gelatin, J Biomater Appl. 2013;27:801–810. [PubMed: 22274878]
50. Murdock MH, Chang JT, Luketich SK, Pedersen D, Hussey GS, D'Amore A, Badylak SF, Cytocompatibility and mechanical properties of surgical sealants for cardiovascular applications, J Thorac Cardiov Sur. 2019;157:176–183.
51. Fuller C, Reduction of intraoperative air leaks with Progel in pulmonary resection: a comprehensive review, J Cardiothorac Surg. 2013;8. [PubMed: 23317447]
52. Bu YZ, Zhang LC, Sun GF, Sun FF, Liu JH, Yang F, Tang PF, Wu DC, Tetra-PEG Based Hydrogel Sealants for In Vivo Visceral Hemostasis, Advanced Materials. 2019;31.
53. Kim KD, Wright NM, Polyethylene glycol hydrogel spinal sealant (DuraSeal Spinal Sealant) as an adjunct to sutured dural repair in the spine: results of a prospective, multicenter, randomized controlled study, Spine (Phila Pa 1976). 2011;36:1906–1912. [PubMed: 22008746]
54. Spotnitz WD, Tissue Adhesives: Science, Products, and Clinical Use, in: Pietrzak WS (Ed.) Musculoskeletal Tissue Regeneration. Orthopedic Biology and Medicine, Humana Press, 2008, pp. 531–546.

55. Tan C, Utley M, Paschalides C, Pilling J, Robb JD, Harrison-Phipps KM, Lang-Lazdunski L, Treasure T, A prospective randomized controlled study to assess the effectiveness of CoSeal(R) to seal air leaks in lung surgery, Eur J Cardiothorac Surg. 2011;40:304–308. [PubMed: 21288733]
56. Wheat JC, Wolf JS Jr., Advances in bioadhesives, tissue sealants, and hemostatic agents, Urol Clin North Am. 2009;36:265–275, x. [PubMed: 19406326]
57. Lee BP, Messersmith PB, Israelachvili JN, Waite JH, Mussel-Inspired Adhesives and Coatings, Annu Rev Mater Res. 2011;41:99–132. [PubMed: 22058660]
58. Horsch J, Wilke P, Pretzler M, Seuss M, Melnyk I, Remmler D, Fery A, Rompel A, Borner HG, Polymerizing Like Mussels Do: Toward Synthetic Mussel Foot Proteins and Resistant Glues, Angew Chem Int Ed Engl. 2018;57:15728–15732. [PubMed: 30246912]
59. Saiz-Poseu J, Mancebo-Aracil J, Nador F, Busque F, Ruiz-Molina D, The Chemistry behind Catechol-Based Adhesion, Angew Chem Int Ed Engl. 2019;58:696–714. [PubMed: 29573319]
60. Waite JH, Mussel adhesion - essential footwork, J Exp Biol. 2017;220:517–530. [PubMed: 28202646]
61. Wei K, Senturk B, Matter MT, Wu X, Herrmann IK, Rottmar M, Toncelli C, Mussel-Inspired Injectable Hydrogel Adhesive Formed under Mild Conditions Features Near-Native Tissue Properties, ACS Appl Mater Interfaces. 2019;11:47707–47719. [PubMed: 31765122]
62. Xu YJ, Wei K, Zhao P, Feng Q, Choi CK, Bian L, Preserving the adhesion of catechol-conjugated hydrogels by thiourea-quinone coupling, Biomater Sci. 2016;4:1726–1730. [PubMed: 27722561]
63. Yu J, Wei W, Danner E, Ashley RK, Israelachvili JN, Waite JH, Mussel protein adhesion depends on interprotein thiol-mediated redox modulation, Nat Chem Biol. 2011;7:588–590. [PubMed: 21804534]
64. Lee H, Dellatore SM, Miller WM, Messersmith PB, Mussel-inspired surface chemistry for multifunctional coatings, Science. 2007;318:426–430. [PubMed: 17947576]
65. Waite JH, Qin X, Polyphosphoprotein from the adhesive pads of Mytilus edulis, Biochemistry. 2001;40:2887–2893. [PubMed: 11258900]
66. Waite JH, Tanzer ML, The bioadhesive of Mytilus byssus: a protein containing L-dopa, Biochem Biophys Res Commun. 1980;96:1554–1561. [PubMed: 7447941]
67. Harrington MJ, Masic A, Holten-Andersen N, Waite JH, Fratzl P, Iron-clad fibers: a metal-based biological strategy for hard flexible coatings, Science. 2010;328:216–220. [PubMed: 20203014]
68. Brubaker CE, Kissler H, Wang LJ, Kaufman DB, Messersmith PB, Biological performance of mussel-inspired adhesive in extrahepatic islet transplantation, Biomaterials. 2010;31:420–427. [PubMed: 19811819]
69. Murphy JL, Vollenweider L, Xu F, Lee BP, Adhesive performance of biomimetic adhesive-coated biologic scaffolds, Biomacromolecules. 2010;11:2976–2984. [PubMed: 20919699]
70. Xie D, Guo J, Mehdizadeh M, Tran RT, Chen R, Sun D, Qian G, Jin D, Bai X, Yang J, Development of Injectable Citrate-Based Bioadhesive Bone Implants, J Mater Chem B. 2015;3:387–398. [PubMed: 25580247]
71. Ma C, Gerhard E, Lu D, Yang J, Citrate chemistry and biology for biomaterials design, Biomaterials. 2018;178:383–400. [PubMed: 29759730]
72. Geim AK, Dubonos SV, Grigorieva IV, Novoselov KS, Zhukov AA, Shapoval SY, Microfabricated adhesive mimicking gecko foot-hair, Nat Mater. 2003;2:461–463. [PubMed: 12776092]
73. Lee BP, Konst S, Novel hydrogel actuator inspired by reversible mussel adhesive protein chemistry, Adv Mater. 2014;26:3415–3419. [PubMed: 24596273]
74. Hoffmann B, Volkmer E, Kokott A, Augat P, Ohnmacht M, Sedlmayr N, Schieker M, Claes L, Mutschler W, Ziegler Gn, Characterisation of a new bioadhesive system based on polysaccharides with the potential to be used as bone glue, Mater Med. 2009 2001–2009.
75. Choi BH, Cheong H, Ahn JS, Zhou C, Kwon JJ, Cha HJ, Jun SH, Engineered mussel bioglue as a functional osteoinductive binder for grafting of bone substitute particles to accelerate in vivo bone regeneration, J Mater Chem B. 2015;3:546–555. [PubMed: 32262336]
76. Xie D, Guo J, Mehdizadeh MR, Tran RT, Chen R, Sun D, Qian G, Jin D, Bai X, Yang aJ, Development of injectable citrate-based bioadhesive bone implants, Materials Chemistry B. 2015387–398.

77. Yang S, Qiongyu Guo, Shores LS, Ahmed Aly, Ramakrishnan M, Kim Ga Hye L Q, Su L, Elisseef JH, Use of a chondroitin sulfate bioadhesive to enhance integration of bioglass particles for repairing critical-size bone defects, Journal of Biomedical biomaterials research part A. 2015 235–242.

78. Yang L, Kandel RA, Chang G, Santerre JP, Polar surface chemistry of nanofibrous polyurethane scaffold affects annulus fibrosus cell attachment and early matrix accumulation, Wiley InterScience. 2008.

79. Xiao L, Ding M, Saadoon O, Vess E, Fernandez A, Zhao P, Jin L, Li X, A Novel Culture Platform for Fast Proliferation of Human Annulus Fibrosus Cells, Cell Tissue Res. 2017339–350.

80. Sharifi S, Kooten TGv, Kranenburg H-JC, Meij BP, Behl M, Lendlein A, Grijpma DW, An annulus fibrosus closure device based on a biodegradable shape-memory polymer network, Biomaterials. 20138105–8113. [PubMed: 23932501]

81. Agnol LD, Dias FTG, Nicoletti NaF, Falavigna A, Bianchi Oa, Polyurethane as a strategy for annulus fibrosus repair and regeneration: a systematic review, Regenerative Medicine. 2018;13:611–626. [PubMed: 30132392]

82. Wiltsey C, Kubinski P, Christiani T, Toomer K, Sheehan J, Branda A, Kadlowec J, Iftode C, Vernengo J, Characterization of injectable hydrogels based on poly (N-isopropylacrylamide)-g-chondroitin sulfate with adhesive properties for nucleus pulposus tissue engineering, Materials Medical. 2013 837–847.

83. ScheiblerID A-G, tschi TG, Widmer J, Holenstein C, Steffen T, CamenzindID RS, Snedeker JG, Farshad M, Feasibility of the annulus fibrosus repair with in situ gelating hydrogels – A biomechanical study, PLOS ONE. 2018.

84. Likhitpanichkul M, Dreischarf M, Illien-Junger S, Walter BA, Nukaga T, Long RG, Sakai D, Hecht AC, Iatridis JC, Fibrin-Genipin Adhesive Hydrogel for Annulus Fibrosus Repair: Performance Evaluation with Large Animal Organ Culture, In Situ Biomechanics, and In Vivo Degradation Tests, Eur Cell Mater. 2015;28:25–28.

85. Vernengo J G WF, Smith NG 2,Lowman AM 2, Synthesis and Characterization of Injectable Bioadhesive Hydrogels for Nucleus Pulposus Replacement and Repair of the Damaged Intervertebral Disc, Journal of Biomedical Materials Research Part B Applied Biomaterials 2010 309–317.

86. Wiltsey C, Christiani T, Williams J, Scaramazza J, Van Sciver C, Toomer K, Sheehan J, Branda A, Nitzl A, England E, Kadlowec J, Iftode C, Vernengo J, Thermogelling bioadhesive scaffolds for intervertebral disk tissue engineering: Preliminary in vitro comparison of aldehyde-based versus alginate microparticle-mediated adhesion, Acta Biomater. 2015;16:71–80. [PubMed: 25641647]

87. Likhitpanichkul M, Kim Y, Torre OM, See E, Kazezian Z, Pandit A, Hechta AC, Iatridis JC, Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFα drug, Spine J. 2015;15:2045–2054. [PubMed: 25912501]

88. Long RG, Rotman SG, Hom WW, Assael DJ, Illien-Jünger S, Grijpma DW, Iatridis JC, In vitro and biomechanical screening of polyethylene glycol and poly(trimethylene carbonate) block copolymers for annulus fibrosus repair, J TIssue Eng Regen Med. 2018 727–736. [PubMed: 28603879]

89. Agnol LD, Dias FTG, Nicoletti NlF, Marinowic D, Silva SMe, Marcos-Fernandez A, Falavigna A, Bianchi Ov, Polyurethane tissue adhesives for annulus fibrosus repair: Mechanical restoration and cytotoxicity, BIomaterials applications. 2019;34:673–686. [PubMed: 31354030]

90. Schneppendahl J, Thelen S, Schek A, Bala I, Hakimi M, Grassmann J-P, Eichler C, Windolf J, Wild M, Initial stability of two different adhesives compared to suture repair for acute Achilles tendon rupture—A biomechanical evaluation, International Orthopaedics (SICOT) (2012;36:627–632.

91. Guterl CC, Torre OM, Purmessur D, Dave K, Likhitpanichkul M, Hecht AC, Nicoll SB, Iatridis JC, Characterization of Mechanics and Cytocompatibility of Fibrin-Genipin Annulus Fibrosus Sealant with the Addition of Cell Adhesion Molecules, Tissue Engineering: Part A. 2014;20.

92. Cruz MA, McAnany S, Gupta N, Long RG, Nasser P, Eglin D, Hecht AC, Illien-Junger S, Iatridis JC, Structural and Chemical Modification to Improve Adhesive and Material Properties of Fibrin-Genipin for Repair of Annulus Fibrosus Defects in Intervertebral Disks, Journal of Biomechanical Engineering. 2017;139.

93. Cruz MA, Hom WW, DiStefano TJ, Merrill R, Torre OM, MEng, Lin HA, Hecht AC, Illien-Junger S, Iatridis aJC, Cell-Seeded Adhesive Biomaterial for Repair of Annulus Fibrosus Defects in Intervertebral Discs, Tissue Engineering: Part A. 2018;24. [PubMed: 29490603]
94. Long RG, Bürki A, Zysset P, Eglin D, Grijpma DW, Blanquer SB, Hecht AC, Iatridis JC, Mechanical Restoration and Failure Analyses of a Hydrogel and Scaffold Composite Strategy for Annulus Fibrosus Repair, Acta Biomater. 2016;30:116–125. [PubMed: 26577987]
95. Panebianco CJ, DiStefano TJ, Mui B, Hom WW, Iatridis JC, CROSSLINKER CONCENTRATION CONTROLS TGFβ−3 RELEASE AND ANNULUS FIBROSUS CELL APOPTOSIS IN GENIPINCROSSLINKED FIBRIN HYDROGELS, European Cells and Materials. 2020;39:2111–2226.
96. Liu C, Jin Z, Ge X, Zhang Y, Xu H, Decellularized Annulus Fibrosus Matrix/Chitosan Hybrid Hydrogels with Basic Fibroblast Growth Factor for Annulus Fibrosus Tissue Engineering, Tissue Engineering: Part A. 2019;25.
97. Hussain I, Sloan SR, Wipplinger C, Navarro-Ramirez R, Zubkov M, Kim E, Kirnaz S, Bonassar LJ, Hartl R, Mesenchymal Stem Cell-Seeded High-Density Collagen Gel for Annular Repair: 6-Week Results From In Vivo Sheep Models, Neurosurgery. 2019;85:E350–E359. [PubMed: 30476218]
98. Jiang EY, Sloan SR Jr., Wipplinger C, Kirnaz S, Hartl R, Bonassar LJ, Proteoglycan removal by chondroitinase ABC improves injectable collagen gel adhesion to annulus fibrosus, Acta Biomater. 2019;97:428–436. [PubMed: 31425894]
99. Moriguchi Y, Borde B, Berlin C, Wipplinger C, Sloan SR, Kirnaz S, Pennicooke B, Navarro-Ramirez R, Khair T, Grunert P, Kim E, Bonassar L, Hartl R, In vivo annular repair using high-density collagen gel seeded with annulus fibrosus cells, Acta Biomater. 2018;79:230–238. [PubMed: 29981494]
100. Moriguchi Y, Borde B, Berlin C, Wipplinger C, Sloan SR, Kirnaz S, Pennicooke B, Navarro-Ramirez R, Khair T, Grunert P, Kim E, Bonassar L, Härtl R, In vivo annular repair using high-density collagen gel seeded with annulus fibrosus cells, Acta Biomater. 2018;79:230–238. [PubMed: 29981494]
101. Hussain I, Sloan SR, Christoph Wipplinger, Navarro-Ramirez R, Zubkov M, Kim E, Kirnaz S, Bonassar LJ, Härtl R, Mesenchymal Stem Cell-Seeded High-Density Collagen Gel for Annular Repair: 6-Week Results From In Vivo Sheep Models, NEUROSURGERY. 2019;85.
102. Jiang EY, S SR Jr, Wipplinger C, Kirnaz S, Härtl R, Bonassar LJ, Proteoglycan removal by chondroitinase ABC improves injectable collagen gel adhesion to annulus fibrosus, Acta Biomater. 2019;97:428–436. [PubMed: 31425894]
103. WANG D-A, VARGHESE S, SHARMA B, STREHIN I, FERMANIAN S, GORHAM J, FAIRBROTHER DH, CASCIO B, ELISSEEFF JH, Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration, nature materials. 2007;6:385–392. [PubMed: 17435762]
104. Tanideh N, Nazhvani SD, Jaberi FM, Mehrabani D, Rezazadeh S, Pakbaz S, Tamadon A, Nikahval B, farahmand M, The Healing Effect of Bioglue in an Experimental Rabbit Model of Femoral Condyle Articular Cartilage Defect, Iranian Red Crescent Medical Journal. 2011641–646. [PubMed: 22737538]
105. Bulman SE, Coleman CM, Murphy JM, Medcalf N, Ryan AE, Barry F, Pullulan: a new cytoadhesive for cell-mediated cartilage repair, Stem Cell Research & Therapy 2015.
106. Zhu W, Iqbal J, Wang D-A, A DOPA-functionalized chondroitin sulfate-based adhesive hydrogel as a promising multi-functional bioadhesive, Matierials Chemistry B. 2018.
107. Abou-ElNour M, Soliman ME, Skouras A, Casettari L, Geneidi AS, Ishak RAH, Microparticles-in-Thermoresponsive/Bioadhesive Hydrogels as a Novel Integrated Platform for Effective Intra-articular Delivery of Triamcinolone Acetonide, Molecular pharmaceutics. 2020 1963–1978. [PubMed: 32271590]
108. Vahdati A, Zhao Y, Ovaert TC, Wagner DR, Computational Investigation of Fibrin Mechanical and Damage Properties at the Interface Between Native Cartilage and Implant, Journal of Biomechanical Engineering. 2012;134.
109. Tanideh N, Nazhvani SD, Jaberi FM, Mehrabani D, Rezazadeh S, Pakbaz S, Tamadon A, Nikahval B, The Healing Effect of Bioglue in Articular Cartilage Defect of Femoral Condyle in

Experimental Rabbit Model, Iranian Red Crescent Medical Journal. 2011 629–636. [PubMed: 22737537]

110. Allon AA, Ng KW, Hammoud S, Russell BH, Jones CM, Rivera JJ, Schwartz J, Hook M, Maher SA, Augmenting the articular cartilage-implant interface: functionalizing with a collagen adhesion protein, BIomedical Materials Research A. 2012;100(8):2168–2175.
111. Sharma B, Fermanian S, Gibson M, Unterman S, Herzka DA, Cascio B, Coburn J, Hui AY, Marcus N, Gold GE, Elisseeff JH, Human Cartilage Repair with a Photoreactive Adhesive-Hydrogel Composite, Tissue Engineering. 2013;5.
112. Simson JA, Strehin IA, Allen BW, Elisseeff JH, Bonding and Fusion of Meniscus Fibrocartilage Using a Novel Chondroitin Sulfate Bone Marrow Tissue Adhesive, Tissue Eng Pt A. 2013;19:1843–1851.
113. Suchaoin W, Bonengel S, Grießinger JA, Sousa IPd, Hussain S, Huck CW, Bernkop-Schnürch A, Novel bioadhesive polymers as intra-articular agents: Chondroitin sulfate-cysteine conjugates, European Journal of Pharmaceutics and Biopharmaceutics. 201625–32.
114. Behrendt P, Ladner Y, Stoddart MJ, Lippross S, Alini M, Eglin D, Armiento AR, Articular Joint-Simulating Mechanical Load Activates Endogenous TGF-b in a Highly Cellularized Bioadhesive Hydrogel for Cartilage Repair, The American Journal of Sports Medicine. 2020210–221. [PubMed: 31877102]
115. Zhu M, Wei K, Lin S, Chen X, Wu C-C, Li G, Bian aL, Bioadhesive Polymersome for Localized and Sustained Drug Delivery at Pathological Sites with Harsh Enzymatic and Fluidic Environment via Supramolecular Host–Guest Complexation, Small. 2018;14.
116. Athanasiou KA, Sanchez-Adams J, Engineering the Knee Meniscus, Morgan and Claypool Publishers, 2009.
117. Baker BM, Gee AO, Sheth NP, Huffman GR, Sennett BJ, Schaer TP, Mauck RL, Meniscus tissue engineering on the nanoscale: from basic principles to clinical application, J Knee Surg. 2009;22:45–59. [PubMed: 19216353]
118. Noyes FR, Barber-Westin SD, Repair of complex and avascular meniscal tears and meniscal transplantation, J Bone Joint Surg Am. 2010;92:1012–1029. [PubMed: 20360529]
119. Bochyńka AI, Hannink G, Grijpma DW, Buma P, Tissue adhesives for meniscus tear repair: an overview of current advances and prospects for future clinical solutions, J Mater Sci: Mater Med. 2016.
120. Koukoubis TD, Glisson RR, Feagin JA, Seaber AV, Vailt TP, Augmentation of rneniscal repairs with cyanoacrylate glue, BIomaedical Materials Research. 1995;29:715–720.
121. Ayan I, Colak M, Comelekoglu U, Milcan A, Ogenler O, Oztuna V, Kuyurtar F, Histoacryl glue in meniscal repairs (a biomechanical study), International Orthopaedics (SICOT). 2007;31:241–246.
122. Reckers LJ, Fagundes DJ, Cohen M, Raymundo JLP, Moreira MB, CarlaPaiva V, Medial meniscus transplantation using cyanoacrylate in rabbits, Acta Cirúrgica Brasileira. 2006;21. [PubMed: 16491218]
123. Scotti C, Pozzi A, Mangiavini L, Vitari F, Boschetti F, Domeneghini C, Fraschini G, Peretti GM, Healing of meniscal tissue by cellular fibrin glue: an in vivo study, Knee Surg Sports Traumatol Arthrosc. 2009;17:645–651. [PubMed: 19296087]
124. Reckers LJ, Fagundes DJ, Cohen M, The ineffectiveness of fibrin glue and cyanoacrylate on fixation of meniscus transplants in rabbits, The Knee. 2009 290–294. [PubMed: 19155176]
125. Wei H, Yu-jie L, Zhi-gang W, Zi-kuan G, Ming-xin W, Ning W, Enhancement of meniscal repair in the avascular zone using connective tissue growth factor in a rabbit model, Chinese Medical Journal. 2011;124:3968–3975. [PubMed: 22340326]
126. Tarafder S, Gulko J, Sim KH, Yang J, Cook JL, Lee CH, Engineered Healing of Avascular Meniscus Tears by Stem Cell Recruitment, Nature. 2018.
127. Tarafder S, Gulko J, Kim D, Sim KH, Gutman S, Yang J, Cook JL, Lee CH, Effect of Dose and Release Rate of CTGF and TGFb3 on Avascular Meniscus Healing, JOURNAL OF ORTHOPAEDIC RESEARCH. 2019.
128. Bochyńska AI, Hannink G, Janssen D, Buma P, Grijpma SW, Development of a fast curing tissue adhesive for meniscus tear repair, J Mater Sci: Mater Med. 2017.

129. Lee H-R, Shon O-J, Park S-I, Kim H-J, Kim S, Ahn M-W, Do SH, Platelet-Rich Plasma Increases the Levels of Catabolic Molecules and Cellular Dedifferentiation in the Meniscus of a Rabbit Model, International JOurnal of Molecular Sciences. 2016.
130. Jordan MC, Schmitt V, Dannigkeit S, Schmidt K, Meffert RH, Hoelscher-Doht S, Surgical adhesive BioGlue™ does not benefit tendon repair strength: an ex vivo study, The Journal of Hand Surgery. 2015 700–704.
131. Brodie M, Vollenweider L, Murphy JL, Xu F, Lyman A, Lew WD, Lee BP, Biomechanical properties of Achilles tendon repair augmented with a bioadhesive-coated scaffold, Biomedical materials. 2011.
132. Lindermana SW, Kormpakis I, Gelberman RH, Birman V, Wegst UGK, Genine GM, Thomopoulos S, Shear lag sutures: Improved suture repair through the use of adhesives, Acta Biomater. 2015 229–239. [PubMed: 26022966]
133. Li J, Mooney DJ, Designing hydrogels for controlled drug delivery, Nat Rev Mater. 2016;1.
134. Kumar VA, Taylor NL, Shi S, Wickremasinghe NC, D'Souza RN, Hartgerink JD, Self-assembling multidomain peptides tailor biological responses through biphasic release, Biomaterials. 2015;52:71–78. [PubMed: 25818414]
135. Lee CH, Cook JL, Mendelson A, Moioli EK, Yao H, Mao JJ, Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study, Lancet. 2010;376:440–448. [PubMed: 20692530]
136. Lee CH, Hajibandeh J, Suzuki T, Fan A, Shang P, Mao JJ, Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex, Tissue Eng Part A. 2014;20:1342–1351. [PubMed: 24295512]
137. Lee CH, Rodeo SA, Fortier LA, Lu C, Erisken C, Mao JJ, Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep, Science translational medicine. 2014;6:266ra171.
138. Lee CH, Shah B, Moioli EK, Mao JJ, CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model, The Journal of clinical investigation. 2010;120:3340–3349. [PubMed: 20679726]
139. Tarafder S, Koch A, Jun Y, Chou C, Awadallah MR, Lee CH, Micro-precise spatiotemporal delivery system embedded in 3D printing for complex tissue regeneration, Biofabrication. 2016;8:025003. [PubMed: 27108484]
140. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H, Quantum dot bioconjugates for imaging, labelling and sensing, Nat Mater. 2005;4:435–446. [PubMed: 15928695]
141. Ye L, Yong KT, Liu L, Roy I, Hu R, Zhu J, Cai H, Law WC, Liu J, Wang K, Liu J, Liu Y, Hu Y, Zhang X, Swihart MT, Prasad PN, A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots, Nat Nanotechnol. 2012;7:453–458. [PubMed: 22609691]

## Statement of Significance

Bioadhesives are a unique type of biomaterials that has been investigated in various biomedical fields, including tissue engineering and regenerative medicine. This manuscript provides a comprehensive review for the recent advancements in bioadhesives for tissue repair and regeneration, focusing on musculoskeletal tissues. We also discuss the advantages and outstanding limitations of each type of bioadhesives for repair and regeneration of different types of musculoskeletal tissues.

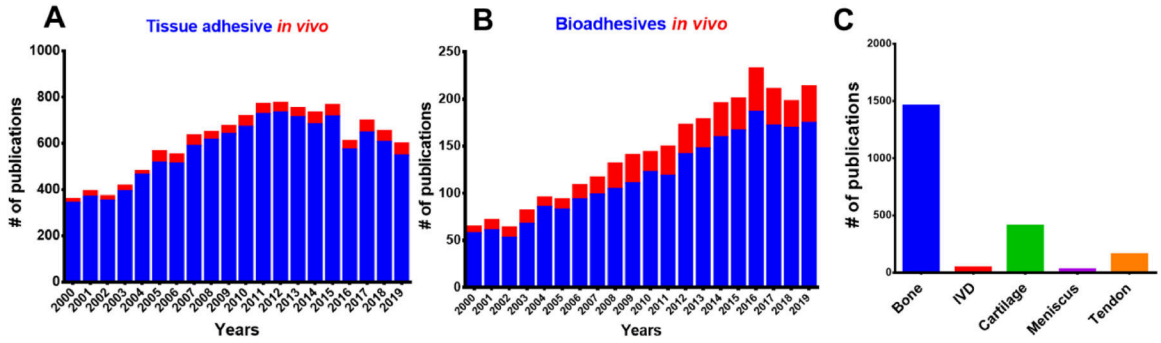



**Fig 1.**

PubMed literature search from 2009 to 2019 with selected key words (A and B) and specific tissue target (C).

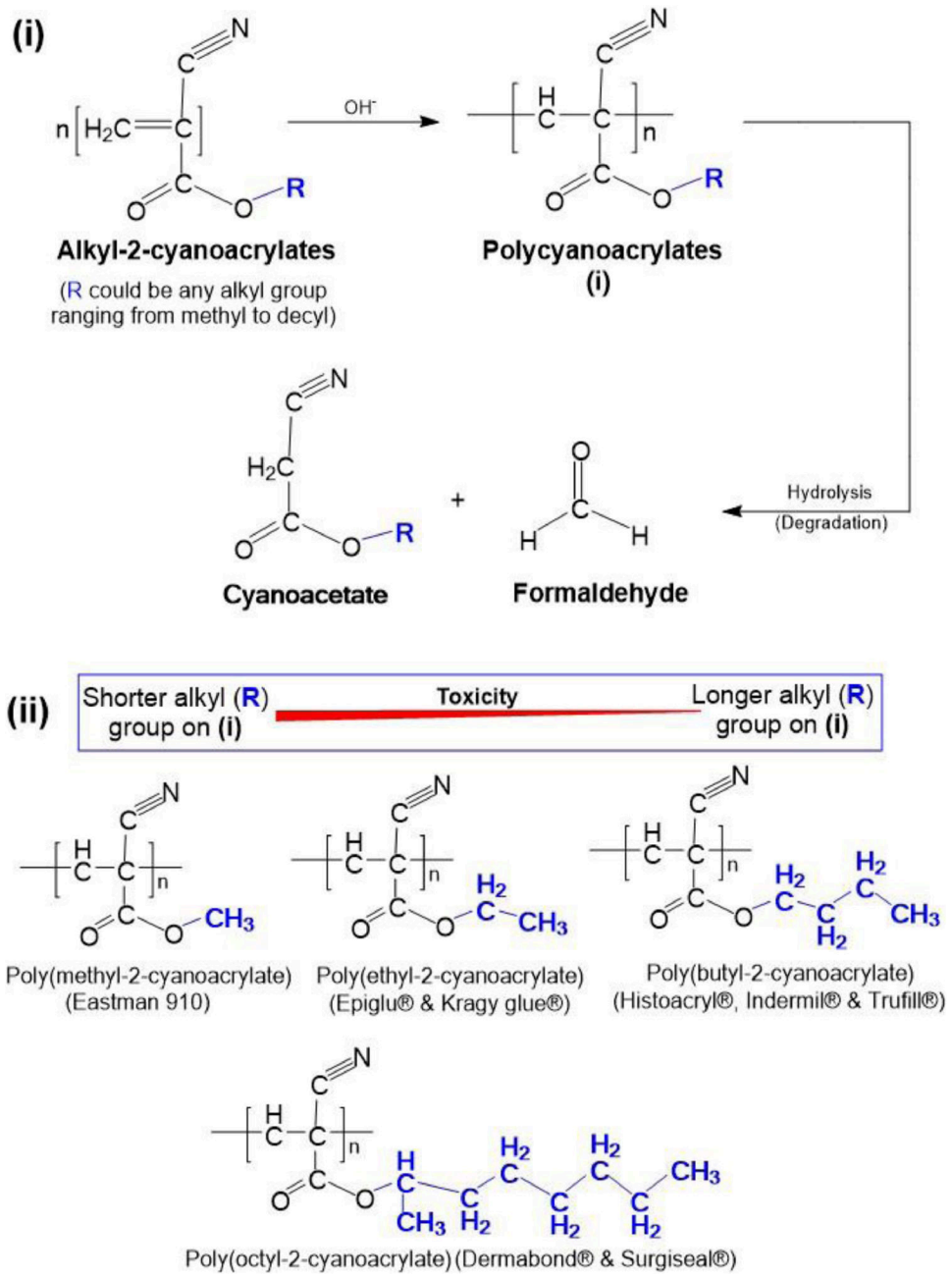



**Figure 2.**

Cyanoacrylate adhesivies: formation of polycyanoacrylates from alkyl-2-cyanoacrylate monomeric units, and the resulting byproducts from the degradation of polycyanoacrylates (i); some of the shorter and longer alkyl chain derivatives and their commercial names (ii).

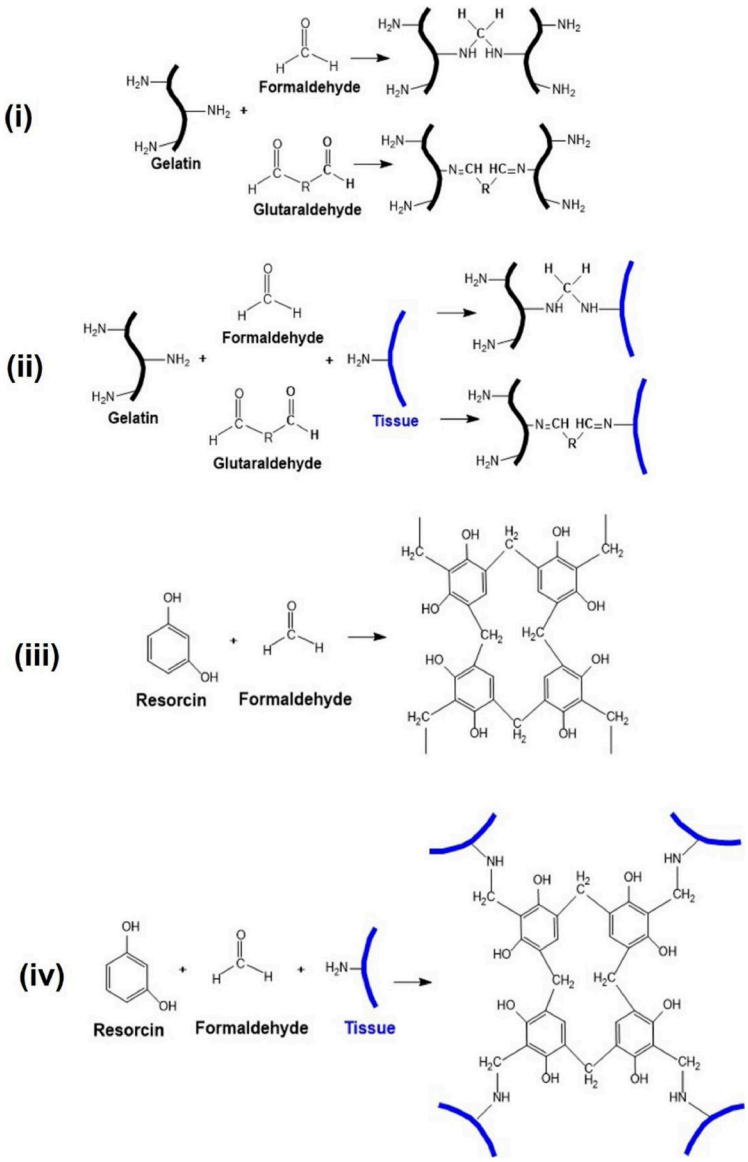



**Figure 3.**

Aldehyde based adhesives: formaldehyde and glutaraldehyde act as cross-linkers between gelatin molecules (i) and gelatin and tissue or biological surfaces (ii); crosslinking between resorcin and formaldehyde (iii) and resorcin, formaldehyde and tissue (iv).

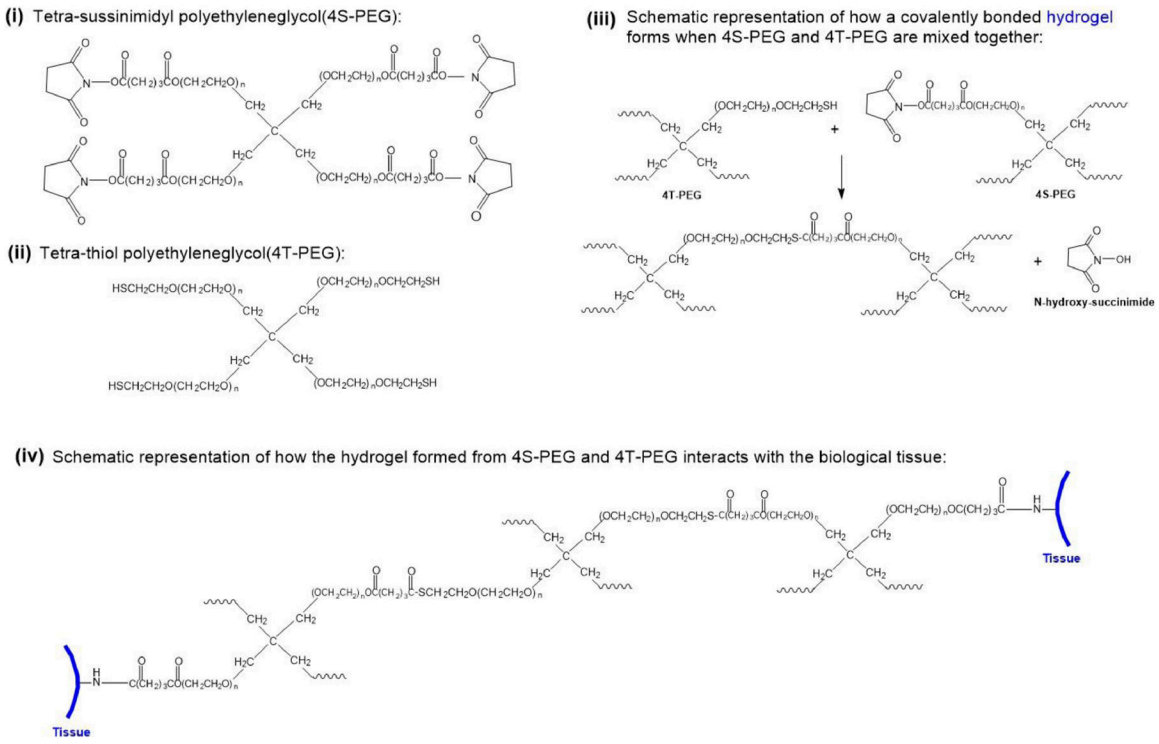



**Figure 4.**

Polyethylene glycol (PEG) based adhesives: Chemical structure of tetra-succinimidyl (4S) and tetra-thiol (4T)-derivatized polyethyleneglycol 4S-PEG (i) and 4T-PEG (ii); crosslinking between 4S-PEG and 4T-PEG (iii); and interactions between tissue/biological surfaces with the hydrogel formed from 4S-PEG and 4T-PEG (iv).

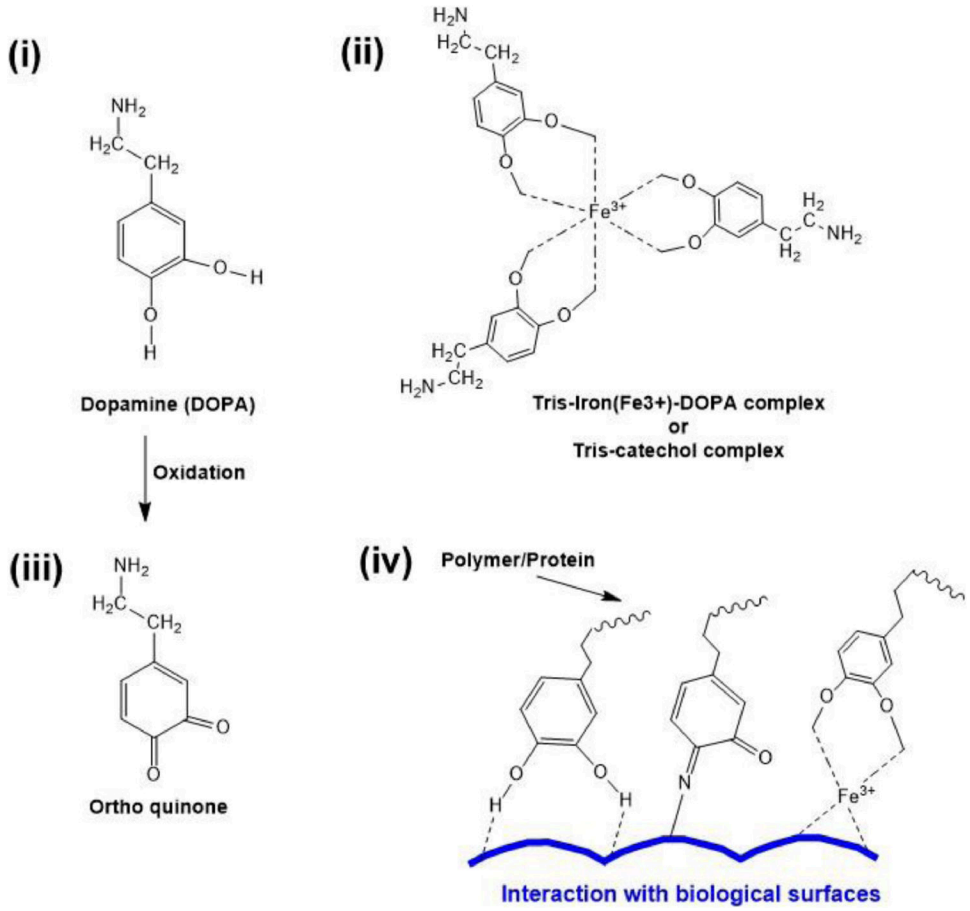



**Figure 5.**

Mussel inspired adhesives: catechol containing amino acid known as 3,4-dihydroxyphenylalanine (DOPA) (i); catechol (hydroxyl) groups of DOPA can interact reversibly with metal ions from ambient environment (ii); formation of ortho quinone resulting from the oxidation of DOPA; and interactions between catechol containing adhesives and tissue/biological surfaces (iv).

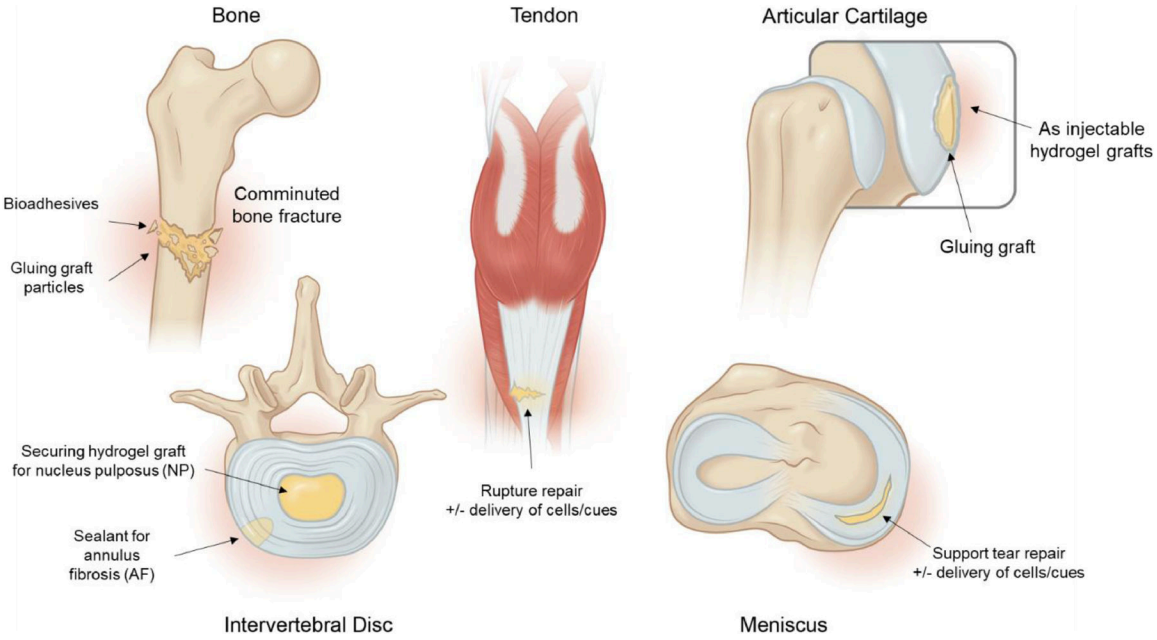



**Figure 6.**
Application of Bioadhesives for repair and regeneration of musculoskeletal tissues.

## Table 1

Commercially available bioadhesives

| Commercial name | Components | Gelation time | Mechanism of action | Target application |
|---|---|---|---|---|
| TISSEEL® (Baxter International Inc., Deerfield, IL) | Contains human fibrinogen, human thrombin, $CaCI_2$, and synthetic aprotonin as fibrinolysis inhibitor. | Instantly | When fibrinogen and thrombin mixed together, mimic the body's natural blood clotting cascades. Thrombin converts soluble fibrinogen into insoluble fibrin, and this process is independent of the body's own clotting cascades. Sometimes calcium is used to catalyze the clot formation. | As an adjunct to hemostasis. |
| COSEAL® (Baxter International Inc., Deerfield, IL) | Contains two biocompatible synthetic derivatized polyethylene glycols (PEG), tetra-succinimidyl (4S) and tetra-thiol (4T)-derivatized polyethyleneglycol (4S-PEG and 4T-PEG). A covalently bonded hydrogel forms when these two components (dissolved in hydrogen chloride solution) are mixed together. | −60 sec | A covalently bonded hydrogel forms when 4S-PEG and 4T-PEG (dissolved in hydrogen chloride solution) are mixed together. Gel formation occurs through the reaction between the thiol groups and the carbonyl groups of the succinimidyl ester resulting in the formation of a thio-ester covalent network between PEG molecules. Free N-hydroxy-succinimide molecules are liberated from the reactions. | In vascular reconstructions to achieve adjunctive hemostasis by mechanically sealing areas of leakage. |
| BIOGLUE® (Cryolife Inc, Kennesaw, Ca) | Coontains bovine serum albumin (BSA) and glutaraldehyde. | −2 min | Aldehyde groups from glutaraldehyde react with the amine group from BSA, they also react with the amine groups present in the tissue resulting a strong bond between BioGlue and tissue. | As an adjunct to standard methods of surgical repair (such as sutures, staples, electrocautery, and/or patches) to bond, seal, and/or reinforce soft tissue. |
| PREVELEAK® (Mallinckrodt Pharmaceuticals, St Louis, Mo) | Contains BSA and a polyaldehyde | 10 – 15 sec | Polyaldehyde crosslink molecules react with the lysine residues in the BSA and forms crosslinking between BSA molecules. Polyaldehyde molecules can form crosslinking between BSA and the tissue by reacting with the tissue resident amine groups. | For vascular reconstructions to achieve adjunctive haemostasis |
| TRIDYNE® (BD, Franklin Lakes, NJ) | Contains a proprietary formulation of polyethylene glycol (PEG) and human serum albumin. | −2 min | Upon application, PEG and human serum albumin forms a strong, flexible seal, even in anticoagulated patients. | In aortic surgery when adjunctive measures to achieve hemostasis are required by mechanically sealing areas of leakage. |
| DURASEALI® (Integra LifeSciences, Princeton, NJ) | Contains proprietary formulation PEG ester solution and a trilysine amine solution, and FD&C blue #1 colorant. | Instantly (<3.5 sec) | Precursor mixer solution diffuses into tissue crevices and cross-links immediately to form hydrogel sealant upon application. The blue colorant in DuraSeal allows the surgeons with excellent visualization of gel coverage and thickness. | As an adjunct to sutured dural repair during brain and spine surgeries to provide watertight closure. |
| PROGEL® (Neomend, Inc., Irvine, CA) | Human serum albumin and polyethyleneglycol (PEG) cross-linker, functionalized with succinate groups (PEG-$(SS)_2$), where N-hydroxysuccinimide (NHS) ester groups are attached to each end of the PEG. | Instantly (<20 sec) | Gel is formed due to the amide bonds formation between albumin and the crosslinkers, PEG-$(SS)_2$. The crosslinkers can form amide bonds between tissue and the albumin resulting in strong adhesion. The reaction takes place in basic condition and N-Hydroxysuccinimide (NHS) is the reaction byproduct. | For intraoperative use of alveolar air leaks sealing resulting from surgical lung resection |

## Table 2

Bioadhesives investigated for musculoskeletal tissue regeneration.

| Bioadhesives | Target tissue | Experimental model | Key outcome | Ref |
|---|---|---|---|---|
| BioGlue® | Articular cartilage | Rabbit model with articular cartilage defect of femoral condyle | Microscopic and macroscopic investigations showed that bioglue had a significant healing effect in the femoral condyle | 101 |
| | Tendon | In vitro biomechanical study with sheep Achilles tendons; compared with fibrin sealant (Tissucol®) | Ultimate failure loads of sutures are significantly superior compared to the use of bioadhesives BioGlue® and Tissucol® | 90 |
| | Tendon | 60 porcine flexor tendons separated into 3 groups; static and axial load testing followed | BioGlue did not improve the tensile strength when added to a conventional core and suture repair | 127 |
| Cyanoacrylate | Knee meniscus | In vitro biomechanical tests with bovine meniscus tissues | Combination of cyanoacrylate glue and suture resulted in a significantly higher peak load to failure than cyanoacrylate alone, but no significant difference from suture alone. | 117 |
| | Knee meniscus | Cyanoacrylate glue was applied to glue rabbit meniscus graft transplantation in vivo. | Euthanasia occurred earlier than expected due to complications; necrosis was observed | 119 |
| Histoacryl (cyanoacrylate, N-asetil 2 butyl sistein) | Articular cartilage, meniscus | Application of Histoacryl with sutures, Histoacryl alone, and suture alone to 3 groups of bovine medial menisci; followed with biomechanical force studies | Biomechanical force was significantly high in all groups when vertical suture and Histoacryl glue were used together | 118 |
| Fibrin | Knee meniscus | Fibrin containing articular chondrocytes was applied to glue pig meniscal slices, followed by 4 wks subcutaneous implantation in mouse. | Fibrin with cells showed a better gross binding than fibrin alone. A fibrocartilaginous tissue was found at the interface between the meniscal slices, partially penetrating the native meniscus tissue | 120 |
| | Knee meniscus | Fibrin was used to glue rabbit allografts and compared with cyanoacrylate in vivo. | Fibrin reduced severe inflammation and necrosis by 4 weeks as compared to cyanoacrylate. | 121 |
| Fibrin, loaded with CTGF and TGF$\beta$3 encapsulated in PLGA μS | Knee meniscus | Bovine menisci explant healing model; In vivo critical sized, avascular zone meniscus defects in rabbits | Successful recruitment and induction of synovial MSCs, as well as fibrocartilaginous differentiation for improved healing of avascular meniscus tears both in vitro and in vivo | 14 |
| | Knee meniscus | Bovine meniscus explant healing model; study for dose and release rate of CTGF and TGF$\beta$3. | High CTGF dose and slow TGF$\beta$3 release showed to be most effective for integrated healing of avascular meniscus, demonstrated by alignment of collagen fibers, fibrocartilaginous matrix, and enhanced mechanical properties. | 13 |
| Fibrin crosslinked with genipin (FibGen) | Annulus fibrosis (AF) | Ex vivo large AF defect repair model of bovine caudal IVD; Subcutaneous implantation in rats. | Injectable Fib-Gen successfully sealed large AF defects, promoted functional restoration with improved motion segment biomechanics, and served as a biocompatible adhesive biomaterial that had greatly enhanced in vivo longevity compared to fibrin. | 84 |
| | | Ex vivo biomechanical study of FibGen applied to bovine coccygeal functional spine units (FSU) with a comparison with BioGlue® | Most FibGen repaired AF endured the entire biomechanical testing procedure while only a small number of BioGlue repaired AF failed; FibGen demonstrated a promising prevention of re-herniation. | 83 |
| | | Bovine coccygeal IVD repair model | Fibrin-genipin hydrogel restored some torsional stiffness, bending range of motion (ROM) and disc height loss, with negligible herniation risk, as compared to scaffold-based repairs. | 94 |
| High-density collagen (HDC) gel | AF | Punctured rat tail discs with HDCs seeded with AF cells, only crosslinked HDCs, and controls | AF cell-laden HDCs retained disc height, NP size, and hydration more than comparison groups at 1 and 5 weeks | 97 |

| Bioadhesives | Target tissue | Experimental model | Key outcome | Ref |
|---|---|---|---|---|
| | | Sheep lumbar IVDs randomized groups: intact, injury only, injury and acellular HDC gel treatment, or injury and MSC-seeded HDC gel treatment | Improved outcome in MSC-seeded HDC gel treatment group; statistically significant enhanced disc height index in MSC-seeded HDC gel treatment compared to other groups | 98 |
| Poly(N-isopropylacrylamide) (PNIPAAm) branched with poly(ethylene glycol) (PEG) blended with poly(ethylene imine) (PEI) | Nucleus pulposus (NP) | Physical and mechanical characterization of PNIPAAm-PEG/PEI in vitro. | Bioadhesive forces with porcine skin displayed a significant increase in the mean maximum force of detachment for PNIPAAm-PEG/PEI gels when glutaraldehyde was injected into the gel core | 21 |
| PNIPAAm grafted CS (PNIPAAM-g-CS) | NP | In vitro biomechanical analysis | Gel blends of PNIPAAm-g-CS and CS aldehyde displayed increased adhesive strength compared to PNIPAAm-g-CS alone; Addition of gelatin-loaded liposomes decreased adhesion strength; PNIPAAm-g-CS alone and PNIPAAm-g-CS with CS aldehyde showed increased adhesion strength compared to PNIPAAm | 86 |
| Poly(ethylene glycol) diacrylate (PEGDA) hydrogel with CS | Articular cartilage | Pilot clinical study of 18 patients; application of PEGDA with CS adhesive in combination with standard microfracture surgery to focal cartilage defects on the medial femoral condyle | 6 month follow-up: MRI displayed significantly higher tissue fill in treated patients compared to control (microfracture surgery alone); treated patients had less pain; knee function scores were similar between groups | 108 |
| Chondroitin sulfate succinimidyl succinate (CS-NHS) and bone marrow aspirate hydrogels (CS-BM) | Knee meniscus | in vitro analysis of MFC migration in bovine meniscus cultured for 2 and 4 weeks; studied in vivo performance using a subcutaneous bovine meniscus adhesion model in athymic rats for 3 months | In vivo subcutaneous model showed that fusion of meniscus tissue at 12 weeks only occurred with the highest BM % volume and that these migrated cells continued to form new matrix (Type-1 collagen), which generated a “nearly indiscernible interface” | 109 |
| Chondroitin sulfate- (CS-) based bioglass (BG) composite | Bone | In vivo rabbit femoral defect model | Significantly greater bone growth in BG-CS-BM as compared to bioglass-only and the empty control after 4 weeks implantation | 77 |
| Injectable citrate-based mussel-inspired bioadhesive hydroxyapatite (iCMBA/HA) | Bone | In vivo rabbit comminuted radial fracture model | Delivery of iCMBA/HA significantly increased bone formation with markedly enhanced three-point bending strength compared to the negative control. Neovascularization, bone ingrowth, and highly organized bone formation were also observed | 70 |
| Muscle adhesive protein (MAP) | Bone | In vitro cell attachment, proliferation, spreading, and differentiation; In vivo rat calvarial bone healing model | Application of MAP significantly improved new bone formation compared to controls. In vitro results support MAP’s osteoconductivity. | 75 |
| | Tendon | In vitro biomechanical test with suture-repaired porcine Achilles tendons | Wrapping MAP-mimicked bioadhesive film around transected porcine Achilles tendons increased tensile strength stiffness, energy to failure, and failure load in treated group compared to control group of sutures alone | 128 |
| Polycaprolactone-$\beta$-cyclodextrin (PCL-CD) polymersome | Articular cartilage | ACL tendon and medial meniscus resected rat knee model; injected drug-loaded PCL-CD polymersome at 7 day post-op | Injected PCL-CD polymersomes promote retention of cargo molecules in rat osteoarthritic knee model; drug delivery of Celecoxib and TGF-beta1 by PCL-CD polymersomes reduced aberrant subchondral bone formation and reduced articular artilage degeneration by 6 weeks | 19 |

**Table 3**

Criteria for successful bioadhesives for masculoskeletal tissue repair and regeneration

| | | |
|---|---|---|
| Physical properties | Adhesion strengtd | Sufficient and sustainable adhesion strengtd to secure repaired tissues or implanted grafts. |
| | | Adhesion efficacy to provide secure sealing (e.g. annulus fibrosis). |
| | Bulk modulus and strengt | Compressive, tensile and/or shear modulus and strength to support functional restoration of tissue repair/healing. |
| | Ultimate strain | Maximum displacement prior to breakdown is an important milestone to maintain the functionality of repaired tissues (e.g. IVD, meniscus, and cartilage). |
| | Injectability | Injectability is an important criterion for certain target applications (e.g. NP, and meniscus) |
| Bio/ chemical properties | In vivo degradation rate | Rate of in vivo degradation needs to be balanced with new tissue formation and integration |
| | Load and release of bioactive cues | Loading efficiency of cytokines, growth factors or small molecules and their release kinetics should be coordinated with the process and timeline of tissue repair and healing. |
| | Cross-linking | Appropriate mode of cross-linking to be considered fitting to each application. |
| Safety | Biocompatibility | Support cell and tissue ingrowth, angiogenesis, new tissue formation and tissue remodeling. |
| | Cytotoxicity | Minimal cytotoxicity of material itself as well as degradation bi-products needs to be confirmed. |