



## Review Article

# Flowable placental connective tissue matrices for tendon repair: A review

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**Keywords:** Biomaterials; Connective tissue matrix; Extracellular matrix; Flowable; Placenta; Tendon repair

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## Abstract

Tendon injuries are associated with considerable pain and disability. Owing to the hypovascularity and hypocellularity of the tissue, natural tendon healing is slow and ineffective. Traditional conservative and surgical treatment options fail to address the underlying pathology. As a result, the healed tendon is mechanically incompetent and prone to degeneration and rupture. Therefore, new biological methods have been suggested to enhance tendon repair and regeneration. Flowable Placental Connective Tissue Matrices (FP-CTMs) represent a promising means to promote tendon healing. Like non-flowable placental scaffolds, FP-CTMs possess the innate healing properties of the placenta and provide structural and biochemical extracellular matrix components. Unlike their non-flowable counterparts, FP-CTMs have the added benefits of minimal invasiveness and the capacity to fill irregular spaces. FP-CTMs can enhance tendon repair by providing a three-dimensional extracellular matrix for cellular attachment and proliferation while decreasing inflammation and limiting adhesion formation. The present report reviews tendon biology, pathology, healing, and current treatment modalities, followed by a comprehensive literature review, evaluating the clinical application of FP-CTMs for tendon repair. Recent research suggests that the use of FP-CTMs in tendon repair is safe and efficacious and further indicates that FP-CTMs can modulate the tendon repair environment and improve clinical outcomes. However, the existing clinical evidence is limited to retrospective case series with no control group. Therefore, additional work must be performed to better understand the clinical applications and therapeutic benefits of FP-CTM in tendon repair compared with conventional treatments.

## Key Points

- The hypocellularity and hypovascularity of tendons make the healing process slow and ineffective.
- Current conservative and surgical treatments fail to address the underlying pathology, and as a result, the healed tendon is mechanically incompetent and prone to degeneration and rupture.
- Flowable Placental Connective Tissue Matrices (FP-CTMs) may enhance tendon repair by providing a three-dimensional extracellular matrix for cellular attachment and proliferation while decreasing inflammation and limiting adhesion formation.
- Although FP-CTMs represent a distinct group of scaffolds, each commercial product is sourced from various placental tissues and subjected to different processing procedures, impacting the biological and mechanical properties of the tissue, which may impact tenocyte-CTM interactions and clinical outcomes.
- Available evidence shows that the use of FP-CTMs for tendon repair is safe and efficacious and has further demonstrated significant improvements in pain and function. However, the clinical research is limited to retrospective case series. Therefore, additional research is needed to evaluate the appropriate clinical applications and associated outcomes.

## Abbreviations

AATB, American Association of Tissue Banks; A-CTM, AmnioFill® Connective Tissue Matrix; ADLs, Activities of Daily Living; AM, Amniotic Membrane; B-CTM, BioRenew® Connective Tissue Matrix; bFGF, basic Fibroblast Growth Factor; DNA, Deoxyribonucleic Acid; ECM, Extracellular Matrix; FP-CTM, Flowable Placental Connective Tissue Matrix; GAGs, Glycosaminoglycans; HCT/P, Human Cell, Tissue, or Cellular or Tissue-based Product; I-CTM, Interfytl® Connective Tissue Matrix; IL-6, InterLeukin-6; IL-1 $\beta$ , Interleukin 1 beta; LE, Lower Extremity; LOE, Level Of Evidence; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs); NRS, Numerical Rating Scale; PDGF, Platelet-Derived Growth Factor; SMR<sup>2</sup>T™, Selective Membrane of Reparative and Reconstructive Tissue; TGF- $\beta$ , Transforming Growth Factor-Beta;  $\mu$ HACM, micronized dehydrated Human Amnion/Chorion Membrane; VAS, Visual Analog Scale; VEGF, Vascular Endothelial Growth Factor.

## Introduction/background

Tendon disorders have become increasingly common and account for a substantial portion of musculoskeletal injuries [1-4]. The number of people affected by tendinopathies and subsequent tendon tears is rapidly increasing [5]. This is attributed to the combined effects of population growth and aging as well as increased participation in extreme/competitive sports. The magnitude of treating these injuries presents a major clinical and financial burden to modern medicine [6].

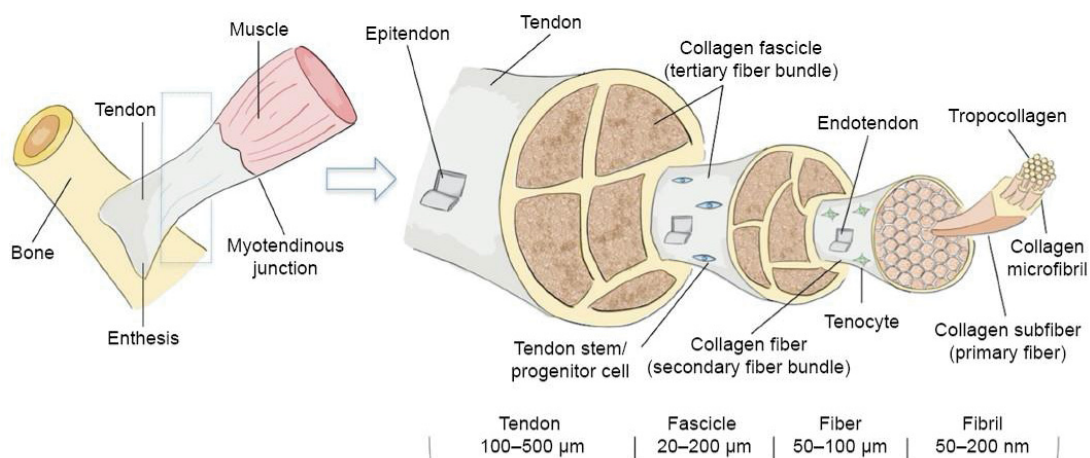
After the injury, the mechanical competence of the native tendon is never restored. The healed tissue is burdened by the formation of adhesions, which disrupt the Extracellular Matrix (ECM) and increase the risk of further degeneration and rupture [7]. Even after surgical repair, the tendon is predisposed to re-rupture [8]. Thus, researchers and clinicians are interested in new methods to augment tendon repair. While exogenous scaffolds have shown promise [9-11], Flowable

Placental Connective Tissue Matrices (FP-CTMs) have gained increasing interest [12]. Like non-flowable scaffolds, FP-CTMs provide structural and biochemical ECM components. In contrast with their non-flowable counterparts, FP-CTMs offer the added benefits of minimal invasiveness and the capacity to fill irregular spaces. Clinicians are considering the use of FP-CTMs for the treatment of tendinopathy/tendinitis [13-15] as well as the surgical repair and reconstruction of periarticular soft tissues [12].

Within, tendon biology, pathology, healing, and current treatment modalities are presented, followed by a review of the clinical application of FP-CTMs for tendon repair. A comprehensive literature search was performed to identify the available clinical evidence. Specifically, the PubMed database was queried for the terms: “placental tissue matrix,” “micronized,” “human,” and “tendon.” Inclusion criteria included studies reporting on the use of FP-CTMs for tendon repair, clinical outcomes, and human subjects. Exclusion criteria included animal data, basic science studies, review articles, and non-English language literature. A date range was not defined. This review uniquely demonstrates the gaps in the literature as well as potential directions for future work relative to the application of FT-CTMs for tendon repair.

## Tendon structure and function

Tendons are comprised of dense fibrous connective tissue, which connects muscle to bone. The interface where the tendon attaches to the muscle is known as the myotendinous junction, and the interface where it attaches to the bone is known as the osteotendinous junction or enthesis (Figure 1). The primary function of a tendon is to transmit forces produced by a muscular contraction to the skeletal system to enable movement. Tendons have higher tensile strength compared with muscle, which enables them to withstand significant amounts of tension and protect the muscle from external forces [16].



**Figure 1:** Tendon anatomy and organization. The interface where the tendon attaches to the muscle is known as the myotendinous junction, and the interface where it attaches to the bone is known as the osteotendinous junction or enthesis. Tendons have a hierarchical structure. As shown, collagen molecules assemble to form subunits of increasing diameter: tropocollagen, fibrils, fibers, bundles, and fascicles. The figure is used with permission from the original publisher. ‘Drug Design, Development, and Therapy 2018 12 591-603’ Originally published by and used with permission from Dove Medical Press Ltd [96].

The ECM of tendons is composed of collagen, elastin, proteoglycans, and glycoproteins (Figure 2) [17]. Collagen is the most abundant molecule in the ECM, accounting for 60% to 85% of the dry weight of the tissue [18]. Type I collagen fibers are organized along the long-axis of the tissue, which affords tendons excellent uniaxial mechanical strength [19], although there are small amounts of type II collagens in the epitenonium/endotenonium and type III collagens in the fibrocartilaginous areas of the osteotendinous junction [16]. The organization of the tendon is hierarchical; collagen fibrils join together to form collagen fibers, which join together to form bundles, the bundles join together to form fascicles, and finally, the fascicles join together to form the whole tendon (Figure 1). The epitenon and endotenon are connective tissue sheaths, which permit smooth movements against adjacent structures and provide blood vessels, lymphatics, and nerves. The epitenon surrounds each tendon, and the endotenon encloses fibers. The elastic fibers contain elastin, which is responsible for the extensibility of the tendon [20]. Interspersed throughout the collagen hierarchy, there are non-collagenous matrix components, commonly grouped into proteoglycans, glycoproteins, and glycoconjugates [19]. Proteoglycans are responsible for the viscoelastic properties of the tendon. There are two predominant proteoglycans in tendons: decorin, a member of the small leucine-rich proteoglycan (SLRP) family accounting for approximately 80% of the total proteoglycan content in tendons, and versican, a large aggregating proteoglycan [21]. Various glycoproteins have been identified in tendon tissue, including collagen oligomeric matrix component (COMP) [22], tenascin-C [23], and tenomodulin [23].

There are two specialized fibroblast cells in tendon tissue: tenoblasts and tenocytes. They comprise 90% to 95% of

the cells within the tendon. Chondrocytes, synovial cells, and vascular cells make up the remaining 5% to 10% [24]. Tenoblasts are immature tendon cells with an ovoid shape. As they mature, they transform into tenocytes with an elongated spindle shape [16]. Tenocytes are responsible for maintaining and synthesizing the components of the ECM and are intimately involved in tendon repair. Tenocytes respond to mechanical loading through the modulation of the ECM [25]. Loading, therefore, is essential to tendon homeostasis, but can also readily promote remodeling or degeneration [26]. Exposure to elevated mechanical stresses can place tendon tissues at risk of damage, and overloading is widely considered a causative factor in the onset of tendon injuries [26].

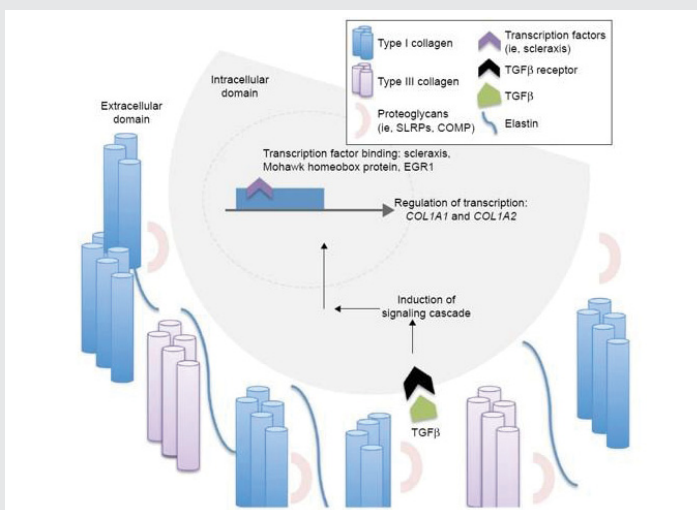
## Tendon pathology

Tendon injuries are common disorders of the musculoskeletal system that are associated with considerable pain and disability, affecting both the athletic and general populations [27–31]. Tendon injuries often occur secondary to overuse, traumatic injury, or intrinsic age-related degeneration. Tendon injuries can be classified into one of two groups: acute or chronic. Acute injuries occur instantaneously, whereas chronic injuries develop over time.

The terminology and definitions relating to tendon injury are ever-changing [32]. Generally, the term “tendinopathy” describes a broad spectrum of tendon pathologies that are associated with pain, swelling, and impaired function [33–35]. It is often used to describe a chronic tendon injury in the absence of a partial or complete tear. The term “tendinitis” describes tendon pathology that has an inflammatory component [32]. The term “tendinosis” describes a tendon with impaired tendon healing, devoid of inflammatory cells. Tendon “tears” or “ruptures” refer to the separation of the tendon from the tissue to which it is attached. Spontaneous tendon tears occur without prior symptoms.

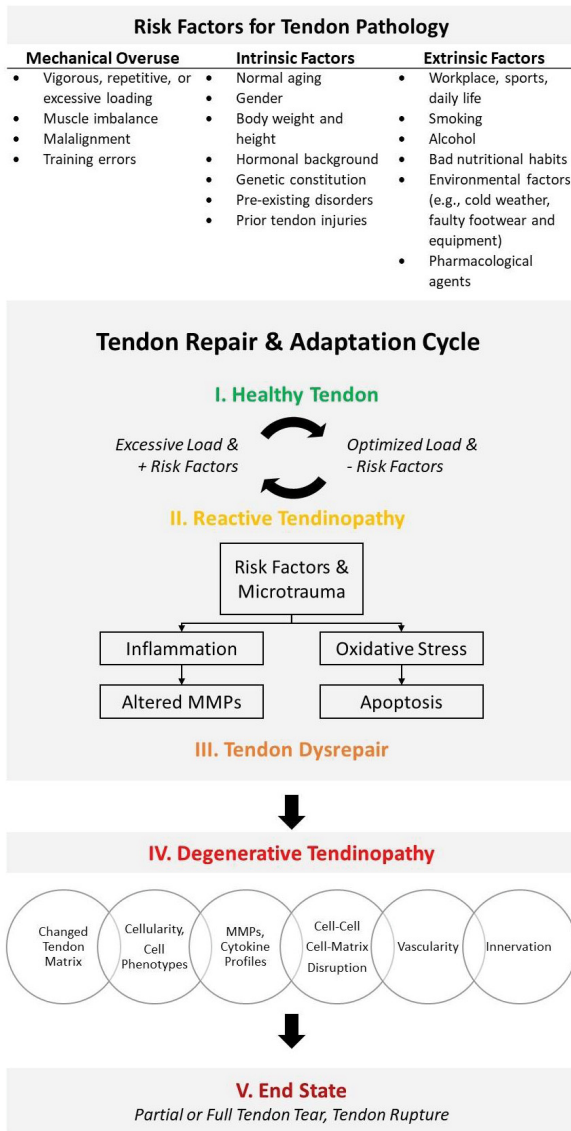
Tendinopathy has a multifactorial etiology, arising from biological and lifestyle-related factors as well as the use of pharmacologic agents. Like others, Steinmann and colleagues [36] grouped risk factors for chronic tendon pathology into three buckets: 1) mechanical overuse; 2) intrinsic factors (i.e., acting from within the body); and 3) extrinsic factors (i.e., acting on the body) (Figure 3). The onset of tendinopathy is often associated with a mechanical event, such as overuse or overloading. However, there is variation in how much load an individual can endure before developing tendinopathy. Likewise, the treatments required for recovery vary by individual. Identifying and understanding the risk factors may assist in understanding the progression of this multifaceted disease.

In a 2009 review, Cook and Purdam introduced a continuum model of tendinopathy [37]. This model suggests that in response to an excessive load (i.e., volume, intensity, frequency), tendons enter a pathologic continuum that consists of three continuous stages: 1) reactive tendinopathy; 2) tendon disrepair (i.e., failed to heal); and 3) degenerative tendinopathy. According to Cook and Purdam, adding or removing load moves



**Figure 2:** Schematic representation of tendon extracellular matrix components. Tendon extracellular matrix components include collagen, elastin, and proteoglycans and glycoproteins, such as SLRPs and COMP. In addition, the interaction between the tendon extracellular matrix and cellular signal transduction is shown. The figure is used with permission from the original publisher. ‘Drug Design, Development, and Therapy 2018 12 591-603’ Originally published by and used with permission from Dove Medical Press Ltd [96].

Abbreviations: COL1A1, Collagen, type I, Alpha 1; COL1A2, Collagen, type I, Alpha 2; COMP, Collagen Oligomeric Matrix Protein; SLRP, Small Leucine-Rich Proteoglycan; TGFβ, Transforming Growth Factor Beta.



**Figure 3:** Schematic representation of tendinopathy pathogenesis. Individual risk factors may predispose a tendon to injury and impair tendon healing, resulting in degeneration and ultimately, tendon rupture. Tendons in the early reactive tendinopathy still possess the capacity to heal. Accumulation of risk factors leads to tendon disrepair and eventually tendon degeneration. Tendon degeneration is associated with structural and compositional changes, making the tendon more prone to further injury and rupture. The figure is adapted with permission from Steinmann et al. 2020 [36] and is based on the work of Cook & Purdham, 2009 [37], Shearn et al. 2011 [97], and Steinmann et al. 2020 [36]. Abbreviations: MMPs, Matrix Metalloproteinases.

is unknown and is likely influenced by various risk factors, such as age, gender, biomechanics, activity level, and nutritional habits [36].

### Incidence of tendon tears

Although there are almost 4,000 tendons in the human body, certain tendons are more prone to rupture than others (Table 1).

The rotator cuff experiences more tendon tears than any other tendon in the human body [38]. Rotator cuff tears are multifactorial in origin, often a combination of age-related and degenerative changes from micro- and macro-trauma [39]. For example, 13% of individuals in their fifties [40] and 50% of individuals in their eighties experience a rotator cuff tear [41]. The Achilles tendon, the largest and strongest tendon in the human body, is the most common tendon to rupture in the lower extremity [42]. Rupture of the Achilles tendon most commonly affects middle-aged men, who participate in sports [43-45]. The third most common group of tendons to rupture are the flexor and extensor tendons of the hand and wrist. Injury to the flexor and extensor tendons of the hand and wrist most commonly occurs in males between the ages of 20-29 years of age. Twenty-five percent of these injuries are work-related, most commonly occurring in construction and extraction occupations [46].

### Tendon healing

The natural process of tendon healing is slow and ineffective. The hypovascular and hypocellular nature of tendons limits their intrinsic capacity for healing [47]. Existing degenerative pathology and repetitive injury cause excessive inflammation, further impairing the healing process [48]. Even when a tendon successfully progresses through the healing cascade, the result is a mechanically and histologically inferior tendon compared with its native counterpart [11].

Tendon healing is controlled by tenocytes and their surrounding ECM [50] and is orchestrated through three multifaceted, overlapping stages: inflammatory, proliferative, and remodeling [49] (Table 2).

The initial inflammatory stage begins with a vascular and cellular response to injury. Vascular permeability increases, followed by an influx of red blood cells, white blood cells, and platelets. The invading inflammatory cells secrete a variety of cytokines and growth factors throughout the healing cascade. Initially, proinflammatory Interleukin-6 (IL-6) and Interleukin 1 Beta (IL-1 $\beta$ ) are released, and later, in the reparative stage, Transforming Growth Factor Beta (TGF- $\beta$ ), Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), and basic Fibroblast Growth Factor (bFGF) are released [50]. During the inflammatory stage, macrophages and tenocytes are recruited to the site of injury. Macrophages digest necrotic materials, and tenocytes are activated and begin proliferating. The release of angiogenic factors also initiates the formation of a vascular network in the healing tissue [51]. In normal healing, the inflammatory phase lasts between 3 and 7 days.

the tendon forward or backward along the continuum. Building on the work of Cook and Purdham, Steinmann and colleagues [36] suggest that risk factors can act as triggers, predisposing the tendon to injury and impairing proper tendon healing. In their model, a tendon in the early reactive tendinopathy stage still possesses the capacity for healing. However, as risk factors increase, the tendon enters a state of disrepair, and eventually, tendon degeneration occurs. The tendon undergoes structural and compositional changes with alterations in biology and biomechanics. Cell-to-cell and cell-to-matrix communication are distorted, disrupting the organization of the ECM, making the tendon prone to further injury and tendon rupture [36]. The rate of progression through the various stages of tendinopathy



During the proliferative stage, also known as the reparative stage, tenocytes synthesize ECM components, including proteoglycans and collagen. The collagen is primarily type III collagen in random organization [52]. The formation of granular tissue, neovascularization, and epithelialization are the notable characteristics of the proliferative stage, which lasts for several weeks.

The remodeling stage begins 6 to 8 weeks after injury and can take more than a year to complete [52]. During the remodeling phase, the collagen reorganizes along the longitudinal axis of the tendon, thereby restoring tendon stiffness and tensile strength. In addition, tenocytes synthesize and degrade the ECM, replacing the mechanically inferior type III collagen with type I collagen. The ECM continues to mature as collagen fibril crosslinking occurs, and the tissue gains biomechanical strength. However, successful restoration of native tendon structure and function does not occur due to the formation of scar tissue.

Previous reports have suggested that there are two cellular mechanisms of tendon healing, known as intrinsic and extrinsic healing [49]. Intrinsic healing occurs with the proliferation and migration of tenocytes from the epitendon and endotenon, preventing the formation of adhesions [53]. Extrinsic healing, on the other hand, occurs with the invasion of cells from outside the tendon [53]. Although these mechanisms were once believed to be independent of one another, researchers now believe that they must be balanced to optimize tendon healing [53].

### Treatment of tendon injuries

The treatment of tendon injuries is conservatively focused. The goal is pain management, rather than treatment of the underlying pathology [35]. The mainstays of conservative treatment include rest or activity modification, physical therapy, immobilization, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), and/or corticosteroid injections [35,54,55]. Although both NSAIDs and corticosteroid injections provide short-term pain relief, long-term NSAIDs are discouraged, due to the associated risks, including gastrointestinal toxicity, renal damage, and increased cardiovascular risk [56,57], and corticosteroid injections have shown no intermediate or long-term benefit [58,59]. Additionally, there is evidence to suggest that corticosteroids may predispose tendons to rupture, especially in weight-bearing joints [60]. Surgical intervention is often not recommended until conservative treatment options have been exhausted [61]. Even with surgical repair, however, clinical outcomes are less than optimal with reported re-tear rates as high as 94% [8].

For this reason, many biological treatments have been suggested for the management of tendon injuries (Table 3) [61]. Flowable placental connective tissue matrices represent a promising solution. The flowable ECM-like material is designed to fill the deficits within the tendon, facilitate cellular attachment and proliferation, attenuate the inflammatory response, and limit adhesion formation [12].

### FP-CTMs

Flowable placental connective tissue matrices are sourced from various placental tissues, such as the amniotic membrane, the chorionic membranes, the umbilical cord, or a combination of these sources. Placental tissues possess anti-inflammatory [62-65], anti-bacterial [66-70], anti-viral [68,69], anti-fibrotic [62,68,71], and immunomodulatory properties [72,73] that are innate to healing (Table 4). In addition to their well-recognized biological properties, placental tissue also has notable mechanical properties, including elasticity, stiffness, and tensile strength [74]. The placenta has a collagen-rich ECM and contains key bioactive molecules, such as fibronectin, laminin, Glycosaminoglycans (GAGs), and elastin, which contribute to its biomechanical properties [75]. The presence of elastin and type III collagen gives the tissue its elasticity; the presence of elastin and interstitial collagens (types I and II), its stiffness; and the lattice-like orientation of collagen bundles, its mechanical strength. The structure of the placental ECM is presumed to promote cell attachment, proliferation,

**Table 1:** Incidence of tendon tears.

| Tendon                                      | Incidence<br>(Per 100,000 person-years) | References |
|---|---|------------|
| Achilles tendon                             | 18.0                                    | [42]       |
| Biceps tendon                               | 5.4                                     | [98]       |
| Flexor & extensor tendons of hand and wrist | 33.2                                    | [46]       |
| Patellar tendon                             | 0.7                                     | [99]       |
| Quadriceps tendon                           | 1.4                                     | [99]       |
| Rotator cuff tendons                        | 83.1                                    | [38]       |

**Table 2:** Tendon healing continuum.

| Stage         | Events   | Duration  |
|---------------|--|-----------|
| Inflammatory  | <ul style="list-style-type: none"> <li>Innate immune response</li> <li>Formation of a vascular network</li> <li>Tenocyte proliferation</li> </ul>                            | 3-7 days  |
| Proliferative | <ul style="list-style-type: none"> <li>Formation of granular tissue</li> <li>Neovascularization</li> <li>Epithelialization</li> </ul>  | 6-8 weeks |
| Remodeling    | <ul style="list-style-type: none"> <li>Collagen organization</li> <li>Replacement of type III collagen with type I collagen</li> <li>Collagen fibril crosslinking</li> </ul> | 1+ years  |

**Table 3:** Biomaterials for the treatment of tendon injury.

- Platelet-rich plasma therapy
- Cell therapy
- Protein delivery
- Gene delivery
- Injectable systems
- Implantable systems

**Table 4:** Innate healing properties of placental tissue.

| Biological Properties  | Mechanical Properties   |
|--|---|
| <ul style="list-style-type: none"> <li>• Anti-inflammatory</li> <li>• Anti-bacterial</li> <li>• Anti-viral</li> <li>• Anti-fibrotic</li> <li>• Immunomodulatory</li> </ul> | <ul style="list-style-type: none"> <li>• Elasticity</li> <li>• Stiffness</li> <li>• Tensile Strength</li> </ul> |



differentiation, epithelialization, and other aspects of healing [76–78]. Despite extensive research documenting the inherent ability of placental tissue to aid in healing, the acquisition and processing of the tissue are constantly evolving, and differences in tissue source and processing have the potential to influence both the biological and mechanical properties of the tissue.

Following stringent donor screening and selection, the tissue is procured and processed. Manufacturers sterilize and preserve the tissue to minimize the risk of disease transmission and to allow prolonged storage, respectively. Tissue preservation is often achieved using one of several preservation methods, most commonly through cryopreservation, drying, or lyophilization [66]. Although these conventional preservation techniques render the amniotic epithelial cells nonviable, the sterilization process is incomplete as evidenced by immunogenic responses in non-decellularized AM [79, 80]. Consequently, removing the cellular content from natural tissue-derived matrices has been suggested to promote healing, and integration with host tissues, and to avoid rejection [81].

Endogenous cells are a contaminant and have the potential to induce host reactivity, including an immune reaction and inflammation, leading to implant rejection. Decellularization is a process whereby endogenous cells, cell debris, and Deoxyribonucleic Acid (DNA) remnants are removed to prevent an immune response while retaining the natural structural and chemical elements of the ECM [82]. Decellularization occurs through mechanical, enzymatic, and chemical means, although it is not rigidly defined [81]. As with the preservation of tissue, decellularization can also affect the structures and entities within the ECM. Therefore, a successful preservation-decellularization protocol must be designed to delicately balance the removal of cellular material as well as retain the innate properties and functional characteristics of the ECM [81–83]. This balance, however, is particularly elusive and is dependent upon tissue source and application.

### Commercially available FP-CTMs

Commercially available FP-CTMs are sourced from various placental tissues and are subjected to different processing methodologies (Table 5). The products listed in Table 5 are processed from human tissue according to the American

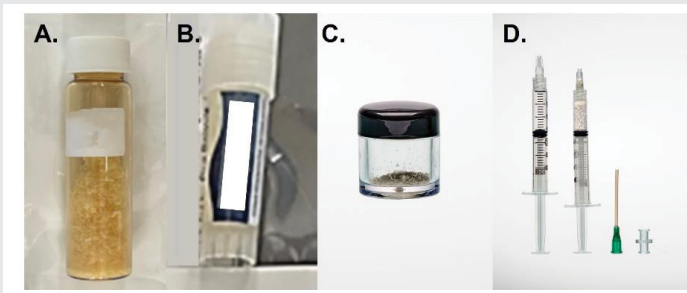
Association of Tissue Banks (AATB) standards and are regulated as a Human Cell, Tissue, or Cellular or Tissue-based Product (HCT/P) by the US FDA under section 361 of the Public Health Service act as HCT/P (21 CFR, Part 127.10a). Indications for use include supplementation or replacement of inadequate or damaged integumental tissue, providing a framework for incorporation by the recipient's tissues and cells. AmnioFill® (A-CTM) is a minimally manipulated, non-viable cellular tissue matrix allograft that retains ECM proteins, growth factors, cytokines, and other specialty proteins of the placenta [84,85] (Figure 4A). AmnioFix® is a micronized dehydrated human amnion/chorion membrane allograft that also retains ECM proteins, growth factors, cytokines, and other specialty proteins of the placenta. Both products are manufactured using a selective membrane of reparative and reconstructive tissue (SMR<sup>2</sup>T™) technology and patented PURION® processing. According to the manufacturer, the PURION® process involves the gentle separation of placental tissue layers, cleaning, and tissue reassembly, followed by dehydration of the tissue [86]. During this process, blood components are removed, and the ECM remains intact [86]. BioRenew™ (B-CTM) is an all-natural placental tissue treatment that retains powerful growth factors, cytokines, collagens, tissue inhibitors of metalloproteinases, and bioactive molecules known to modulate the immune system [87,88] (Figure 4B). Interfyl® (I-CTM) is an allogenic decellularized particulate human FP-CTM consisting of natural human structural and biochemical ECM components [12, 62] (Figure 4C & 4D). Contrary to the other FP-CTMs, the decellularization process removes residual cells, cell debris, growth factors, and cytokines, while retaining an ECM structure with high collagen content and key bioactive molecules, such as fibronectin, laminin, GAGs, and elastin [75].

### *In vitro* research supporting FP-CTMs in tendon repair

In 2022, Moreno and colleagues [64] conducted an *in vitro* investigation to evaluate the effects of micronized dehydrated Human Amnion/Chorion Membrane (µdHACM, MiMedx, Marietta, GA) on the inflammatory environment and hypervascularity associated with tendinopathy. Treatment with µdHACM was found to neutralize proinflammatory cytokines and proteases and regulate angiogenesis. These findings suggest that µdHACM may reduce the influx of inflammatory cells, attenuate inflammation, and improve ECM restoration,

**Table 5:** Commercially available flowable placental connective tissue matrices.

| FP-CTM                                    | Manufacturer                         | Source                                  | Processing  | Decellularized   | References |
|---|--------------------------------------|---|---|--|------------|
| AmnioFill® Placental Tissue Allograft     | MiMedx Group, Inc., Marietta, GA     | Amnion and chorion membranes            | <ul style="list-style-type: none"> <li>SMR<sup>2</sup>T™ Technology</li> <li>PURION® processing</li> <li>Terminally sterilized</li> </ul>   | No   | [84, 85]   |
| AmnioFix®                                 | MiMedx Group, Inc., Marietta, GA     | Amnion and chorion membranes            | <ul style="list-style-type: none"> <li>SMR<sup>2</sup>T™ Technology</li> <li>PURION® processing</li> <li>Terminally sterilized</li> </ul>   | No   | [84, 85]   |
| BioRenew™ Placental Tissue Matrix Therapy | Skye Biologics, Inc., El Segundo, CA | Various tissue from the placental organ | <ul style="list-style-type: none"> <li>HydraTek® Processing</li> <li>Terminally sterilized with e-beam radiation</li> </ul>   | No   | [87, 88]   |
| Interfyl® Human Connective Tissue Matrix  | Celularity Inc., Florham Park, NJ    | Chorionic plate                         | <ul style="list-style-type: none"> <li>Washed and scraped to remove extraneous tissues and cells</li> <li>Dried</li> <li>Terminally sterilized with e-beam irradiation</li> </ul> | Yes, using an osmotic shock followed by a mild detergent treatment | [62]       |



**Figure 4:** Commercially available FP-CTM products. Three commercially available human FP-CTM products are shown: (A) a minimally manipulated, non-viable cellular tissue matrix; (B) a liquid placental tissue treatment; and (C & D) a decellularized particulate.

Abbreviations: FP-CTM, Flowable Placental Connective Tissue Matrix.

which may give rise to a more structurally sound tendon. While encouraging, additional *in vivo* study is necessitated.

Our research group recently published an *in vitro* investigation, evaluating the direct interaction between tenocytes and FP-CTMs. The study compared three human FP-CTMs to determine which interacted more favorably with human tenocytes [20]. The FP-CTMs included 1) A-CTM, a minimally manipulated, non-viable cellular particulate; 2) B-CTM, a liquid matrix; and 3) I-CTM, a decellularized flowable particulate. Readouts included tenocyte adhesion and proliferation, cell migration, phenotype maintenance, and inflammatory response. In line with the hypothesis, the study demonstrated that I-CTM, the decellularized FP-CTM, provided a more cell-friendly matrix to support tenocyte function. Although tenocyte attachment was significantly higher on A-CTM, I-CTM supported greater tenocyte proliferation. In addition, I-CTM significantly increased tenocyte migration, whereas A-CTM was comparable to the control. The presence of I-CTM also prevented the loss of the tenocyte phenotype and attenuated the inflammatory response [20]. These findings demonstrate that the direct interaction of tenocytes with FP-CTMs positively modulates the tendon repair environment.

Collectively, the results from these two *in vitro* reports show promise for the use of FP-CTMs in the treatment of tendon injuries. However, the latter report also suggests that the decellularization of placental tissue may enhance the regulation of inflammatory processes by human tenocytes. Although decellularization is performed to reduce immune response, the process of removing the cellular components can affect the structures and entities within the ECM, disrupting its functional characteristics [1,6,17]. However, it appears as though the decellularization of I-CTM balances the removal of cellular content with the retention of necessary regulatory proteins. This is evident based on I-CTM attenuating the inflammatory response beyond that of other non-decellularized FP-CTMs [62]. More research is needed to determine if the observations are in fact due to decellularization or to other differences in tissue processing. Moreover, the clinical translation of these findings remains to be understood.

### **In vivo application of FP-CTMs**

A comprehensive literature search was conducted to identify studies evaluating the clinical application of FP-CTM for tendon repair [13-15] (Table 6).

In 2015, Lullove published a pilot study reviewing ten patients who received a flowable placental tissue matrix injection (Human Regenerative Technologies LLC, Redondo Beach, CA) to treat tendon or muscular injuries of the lower extremity [13]. These included posterior tibial tendonitis, peroneal tendonitis, anterior tibial tendonitis, extensor muscles of the foot, plantar musculature of the foot, excluding the plantar fascia, and Achilles tendonitis. Ultrasound guidance was used to target the site of injury when administering the injection. Outcome measures included pain and ultrasound evaluation of the tendon/muscle at four and six weeks. By week four, 8/10 patients reported no pain, and by week five, all patients were pain-free. No adverse events or side effects were reported. No standard of the care treatment group was included for comparison.

In 2017, Gellhorn and Han reported a case series to evaluate the use of  $\mu$ dHACM allograft injection (MiMedx, Marietta, GA) for the treatment of tendinopathy or arthritis [15]. Forty patients were included; 20 were treated for tendon pathology and 20 for joint pathology. All patients received an ultrasound-guided injection of  $\mu$ dHACM. Outcomes measures included pain and function, which were measured at 1, 2, and 3 months after the injection. Statistically significant reductions in pain and statistically significant improvements in function were observed. Localized pain at the injection site was common, but no other adverse events or side effects were reported. From this, the authors conclude that  $\mu$ dHACM injection is clinically effective in reducing pain and improving function.

And most recently, in 2020, a retrospective case series was published by Spector and colleagues evaluating  $\mu$ dHACM allograft injection (MiMedx, Marietta, GA) as a treatment for Achilles tendinopathy. Patients were seen twice after the injection within a 45-day observation window. The examined outcome variables included changes in the reported level of pain and treatment-associated adverse events. In the 45-day period, 66% of patients reported complete symptom resolution, while the remaining patients reported symptom improvement without complete resolution. After injection, there were two patients who reported muscle tightness.

### **Summary & next steps**

Placental tissues offer vast clinical utility due to their unique structure, low immunogenicity, and biological properties. The amniotic membrane (AM) of the placenta was first used as a biomaterial for surgical reconstruction in 1910 as a substrate for skin transplantation [89]. However, only fresh AM was available at that time, which was difficult to obtain and carried a risk of disease transmission. Several decades later, with the introduction of better tissue processing techniques and preservation methods, placental tissue has regained popularity and is now used in several specialties, including wound and skin care [12], ophthalmology [90], gynecology [66], and orthopedics [91].

In this report, the literature was reviewed to better understand the clinical application of FP-CTMs in the setting of tendon repair. While the use of FP-CTMs is expanding [91-94], there is a paucity of clinical data evaluating its application



**Table 6:** Clinical studies evaluating the *in vivo* application of FP-CTMs in tendon repair.

| Study                     | Study Design & LOE   | Purpose   | Product & Manufacturer                     | Patients  | Outcomes   | Results Summary  |
|---------------------------|--|---|--|---|--|--|
| Pullover et al. 2015 [13] | Retrospective case series<br>Level IV                                  | To evaluate the use of a flowable tissue matrix allograft injection for the treatment of various tendon/muscle injuries of the LE | PX50®, Human Regenerative Technologies LLC | 10 patients with various acute or chronic tendon or muscular injuries | Pain: assessed with VAS pain scale weekly<br>Tendon Integrity: assessed with ultrasound evaluation at weeks 0, 4, and 6  | 8 of 10 patients reported no pain by week 4 and 10 of 10 patients reported no pain by week 5   |
| Gellhorn & Han, 2017 [15] | Expanded medical record review with a structured interview<br>Level IV | To evaluate the clinical effectiveness of µdHACM injection in patients with chronic tendinopathy and arthropathy                  | AmnioFix®, MiMedx                          | 40 patients (50% with tendon pathology and 50% with joint pathology)  | Pain: assessed with an NRS at 1, 2, and 3 months<br>Change in ADLs and sports/recreation function: assessed with a patient-specific functional scale at 1, 2, and 3 months<br>More than a 30% improvement in pain and function were considered clinically meaningful | Pain scores significantly declined (p < 0.001)<br>Impairment in ADLs and sports/recreation function significantly declined (p < 0.001)<br>75% of patients had a clinically meaningful reduction in pain at 1 month, 84% at 2 months, and 91% at 3 months |
| Spector et al. 2020 [14]  | Retrospective case series<br>Level IV                                  | To report the experience and short-term outcomes associated with using µdHACM injection as a treatment for Achilles tendinopathy  | AmnioFix®, MiMedx                          | 45 patients with Achilles tendinopathy                                | Pain level: assessed at two postoperative follow-up visits within 45 days of the procedure<br>Treatment-associated adverse events  | At the second follow-up visit, 72% of patients reported no pain, 25% reported mild pain, 3% reported moderate pain, and 0 reported severe pain<br>Two patients reported calf/quadricep pain/tightness after injection                                    |

in the treatment of tendon pathology. Three studies were identified that used an FP-CTM injection for the treatment of tendonitis/tendinopathy [13-15]. Although the results from these studies demonstrate the safety and efficacy of FP-CTM injection, all three studies are retrospective case series (level IV) that reviewed the results of a single investigator. To systematically evaluate the safety and efficacy of FP-CTMs in tendon healing, a prospective clinical trial is needed.

Prior evidence suggests that differences in processing methodology could influence the direct interaction between tenocytes and FP-CTMs [4,62], potentially causing variation in clinical outcomes. Two of the three clinical case series evaluated the injection of AmnioFix® for the treatment of tendinopathy [14,15], whereas the pilot study from 2015 evaluated the use of PX50® (Human Regenerative Technologies LLC, Redondo Beach, CA). PX50® and DX100® are the flowable forms of BioRenew® PTM™ therapy [95]. All three studies reported statistically significant reductions in pain [13-15], and the two studies evaluating AmnioFix® reported statistically significant improvements in function [14,15]. Both products evaluated were thought to retain ECM proteins, growth factors, collagens, and bioactive molecules found in human placental tissues [84,85,87]. To date, no clinical research studies have evaluated the use of a decellularized FP-CTM to augment tendon healing. This is an important next step, as *in vitro* evidence suggests that decellularization of the placental tissue may improve tenocyte function and phenotype maintenance and also attenuate the inflammatory response [62].

Two clinical applications have been suggested for the use of FP-CTM in tendon repair [12-15]. The first is for the treatment of tendinitis/tendinopathy with an ultrasound-guided

injection [13-15]. The second is an injection directly into the tendon or tendon sheath during the surgical repair of ruptured tendons. At this time, the available clinical evidence is limited to the former method. Additional clinical research is needed to evaluate the outcomes associated with the surgical repair of the ruptured tendon using FP-CTMs.

Although the existing data appears to support the clinical use of FP-CTMs for tendon repair [13-15,62, 91], additional clinical research is needed to more fully assess the safety and efficacy of FP-CTMs in this application. Once the safety and efficacy have been demonstrated, additional randomized, controlled trials are needed to determine if this treatment strategy improves clinical outcomes both in the treatment of tendinosis and tendon tears.

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