The Placenta

Applications in Orthopaedic Sports Medicine

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Background: Placenta has a long history of use for treating burns and wounds. It is a rich source of collagen and other extracellular matrix proteins, tissue reparative growth factors, and stem cells, including mesenchymal stem cells (MSCs). Recent data show its therapeutic potential for orthopaedic sports medicine indications.

Purpose: To provide orthopaedic surgeons with an anatomic description of the placenta, to characterize its cellular composition, and to review the literature reporting the use of placenta-derived cells and placental tissue allografts for orthopaedic sports medicine indications in animal models and in humans.

Study Design: Systematic review.

Methods: Using a total of 63 keyword combinations, the PubMed and MEDLINE databases were searched for published articles describing the use of placental cells and/or tissue for orthopaedic sports medicine indications. Information was collected on placental tissue type, indications, animal model, study design, treatment regimen, safety, and efficacy outcomes. Results were categorized by indication and subcategorized by animal model.

Results: Outcomes for 29 animal studies and 6 human studies reporting the use of placenta-derived therapeutics were generally positive; however, the placental tissue source, clinical indication, and administration route were highly variable across these studies. Fourteen animal studies described the use of placental tissue for tendon injuries, 13 studies for osteoarthritis or articular cartilage injuries, 3 for ligament injuries, and 1 for synovitis. Both placenta-derived culture-expanded cells (epithelial cells or MSCs) and placental tissue allografts were used in animal studies. In all human studies, commercial placental allografts were used. Five of 6 human studies examined the treatment of foot and ankle pathological conditions, and 1 studied the treatment of knee osteoarthritis.

Conclusion: A review of the small number of reported studies revealed a high degree of variability in placental cell types, placental tissue preparation, routes of administration, and treatment regimens, which prohibits making any definitive conclusions. Currently, the clinical use of placenta is limited to only commercial placental tissue allografts, as there are no placenta-derived biological drugs approved for the treatment of orthopaedic sports medicine conditions in the United States. However, this review shows that the application of placental cells or tissue allografts appears to be safe and has potential to improve outcomes for orthopaedic sports medicine indications.

Keywords: placenta; amniotic membrane; umbilical cord; stem cells; regenerative medicine

The investigational use of adult mesenchymal stem cells (MSCs) in sports medicine has increased rapidly in the past 2 decades. MSCs were discovered by Friedenstein et al[19] in mouse bone marrow and by Caplan[9] in human bone marrow. Accumulated data show that MSCs are present and can be isolated from other tissues including adipose,[82] bone,[9] synovial tissue,[14] and placenta.[37] A growing body of evidence points to the broad regenerative potential of MSCs including the field of orthopaedic sports medicine.

Placental tissues have been used to treat burns and wounds for over a century.[64] The regenerative potential of placental tissues has been studied in hepatic, cardiac, and neurological disorders.[54] Only recently has placental tissue use been considered as a clinical alternative for musculoskeletal injuries.[5,69] The human placenta is a rich source

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of MSCs.[70] Placenta-derived MSCs (P-MSCs), which have a fetal origin, show higher proliferative potential and greater differentiation plasticity.[52,56,65] In addition to serving as a stem cell source, placental tissues contain a collagen-rich extracellular matrix (ECM) and a variety of growth factors known to be beneficial for tissue repair and regeneration.[7] Additionally, the low immunogenicity of placental tissue allows for its banking and use "off the shelf" as an allograft.[1]

Several anatomic parts of the placenta, including amniotic fluid/membrane,[12,44] chorion,[56] umbilical cord,[61] and umbilical cord blood,[23] have been investigated for potential use in orthopaedic surgery and postoperative wound healing.[7] Clinical indications that have been explored within the orthopaedic field using placental tissues include ligamentous injuries, tendinous injuries, muscular injuries, osteoarthritis, and chondral lesions. However, the human literature is scarce, and all currently available products on the market in the United States are human tissue allografts. There are no placenta-derived biological drugs or devices marketed in the United States. Therefore, most scientific studies of tissue allografts or placenta-derived cell therapies have been conducted in animal models.

This review will provide a detailed analysis of published human and animal studies that assess the safety and efficacy of placental cells and/or placental tissue allografts for orthopaedic sports medicine applications.

STEM CELLS IN PLACENTAL TISSUE

The International Society for Cellular Therapy (ISCT) established the immunophenotype of MSCs.[17] Cells must express CD105, CD73, and CD90 and lack the expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR to be considered multipotent MSCs.[17] With the rapidly growing number of stem cell–containing products, particularly for orthopaedic applications, it is important that orthopaedic surgeons be aware of the immunophenotype of MSCs to assess the composition of cellular products marketed as stem cell–based products and the legitimacy of potential treatments.

Figure 1 shows the structure of the human placenta at full term with labels for anatomic placental parts of fetal origin: the amniotic membrane, chorionic membrane, amniotic fluid, and umbilical cord.

Placentas collected from full-term pregnancies represent a noncontroversial source of neonatal stem cells. Neonatal MSCs can be isolated from amniotic fluid, amnion, chorion, umbilical cord tissue, and blood and be rapidly expanded ex vivo for therapeutic use. Furthermore, neonatal P-MSCs have higher proliferative and differentiation potential than adult stem cells isolated from other types of tissues.[52] The cellular composition and number of cells in different parts of the placenta are described below and summarized in Table 1.

Amniotic Membrane

The amniotic membrane is the inner lining of the placenta that forms the tough amniotic sac and holds amniotic fluid,

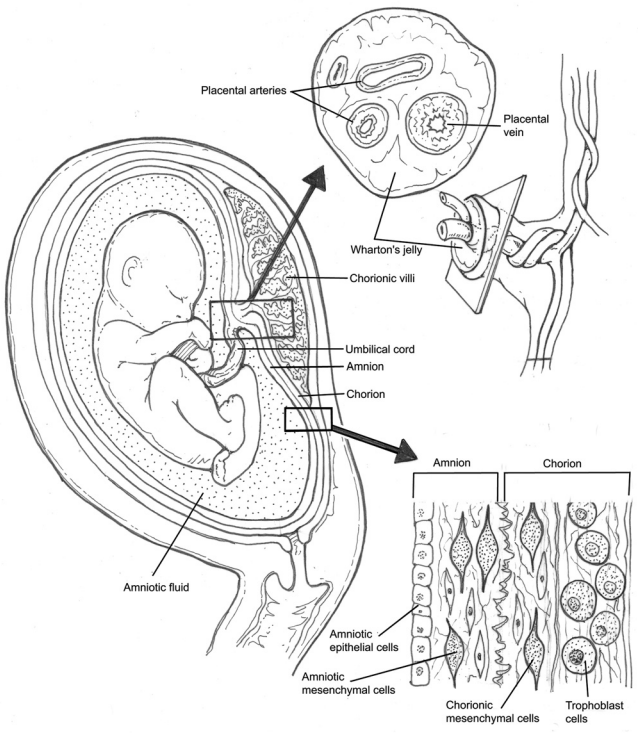

Figure 1. Labeled structure of human placenta at full term.

the developing embryo, and eventually the fetus. This membrane distinguishes the amniotic cavity from the chorion and maternal vasculature and consists of 2 cellular layers: a single layer of amniotic epithelial cells (AECs) called the epithelial layer, which is attached to the basement membrane, and an outer mesenchymal layer that contains fibroblasts and amniotic membrane MSCs (AM-MSCs).[50] Stem cells have been isolated from both the epithelial layer (AECs) and the mesenchymal cell layer (AM-MSCs). AECs are clonogenic, can differentiate into cells of all 3 germline lineages, and express cell surface markers that are accepted as stem cell markers.[4,26,50,59] AECs exhibit an epithelial morphology, while AM-MSCs show a fibroblastic morphology. AM-MSCs also express stem cell markers on their surface and have demonstrated a higher proliferative rate than MSCs from adult sources.[59]

Amniotic Fluid

Amniotic fluid is critical to the health, protection, and development of a fetus. It is composed of water, waste excreted from the fetus, and nutrients essential for fetal growth and health.[68] In 2001, Kaviani and colleagues[29] were among the first to identify MSCs in amniotic fluid and attempt to use them for tissue engineering. Roubelakis et al[59] recently performed a comprehensive review of the characteristics of amniotic fluid–derived MSCs and found that, in addition to having the ability to differentiate into cells of adipogenic, osteogenic, myogenic, and endothelial lineages, amniotic fluid–derived MSCs expressed markers such as CD73, CD90, and CD105 and lacked the expression

TABLE 1
Cellular Composition and Number of Cells in Fetal Placental Tissue at Term[a]

Placental Tissue Type	Structural Composition	Tissue Cell Type	No. of MSCs	References
Amniotic membrane	Epithelial and mesenchymal layers	Epithelial cells, fibroblasts, macrophages, epithelial stem cells and MSCs	10-40,000/cm^2 (mesenchymal layer)	Bieback and Brinkmann,[6] Ilancheran et al,[26] Toda et al[66]
Chorionic membrane	Mesenchymal and trophoblast layers	Fibroblasts, macrophages, trophoblast cells, MSCs	10-40,000/cm^2 (mesenchymal layer)	Bieback and Brinkmann,[6] Ilancheran et al[26]
Umbilical cord tissue	Epithelial and Wharton's jelly	Epithelial cells, fibroblasts, macrophages, epithelial stem cells and MSCs	10-50,000/cm	Bieback and Brinkmann[6]
Umbilical cord blood	Cells suspended in plasma	Blood cells, hematopoietic cells, endothelial cells, neural stem cells, MSCs and other types of stem cells	Rare cells, 1/108 frequency	Bieback and Brinkmann[6]
Amniotic fluid	Cells shed from amniotic membrane and from fetus suspended in liquid	Epithelial cells and stem cells, MSCs	3/mL	Sessarego et al,[62] Delo et al[15]

[a]MSC, mesenchymal stem cell.

of hematopoietic markers (CD34 and CD45), endothelial markers (CD31), and the HLA-DR antigen.[21] This marker display is consistent with the ISCT's minimal criteria for defining MSCs, providing further evidence that amniotic fluid contains MSCs.

Chorionic Membrane

The chorionic membrane is the outermost layer of placental tissue consisting of the chorionic mesenchyme and trophoblast layer that encapsulates the amniotic tissue and embeds into the mother's endometrium during development. Blood, oxygen, and nutrient exchanges between the mother and developing embryo occur within the villous trophoblast area, which is a part of the chorion. Stem cells are isolated from both the chorionic membrane[2] and chorionic villi.[25] The chorionic membrane is attractive for application to a wide array of orthopaedic sports medicine indications because it contains cells that can differentiate into a variety of cells of mesenchymal lineages.[1] Human chorionic MSCs have higher proliferative potential compared with bone marrow–derived MSCs (BM-MSCs).[56] A recent publication from Gonzalez et al[22] demonstrated that MSCs of fetal origin isolated from the chorionic membrane showed superior immunosuppressive and angiogenic potential compared with fetal umbilical cord tissue and maternal decidual cells, although the other 2 cell types demonstrated a higher proliferative capacity. The immunophenotypic characterization and morphology of these cells have proven to be similar to those of BM-MSCs and AM-MSCs, although the morphology appears to be different than that of AM-MSCs after longer culture periods.[63]

Umbilical Cord Tissue

The umbilical cord is tissue between the mother and fetus of extraembryonic origin. The primary function of the umbilical cord is as a protective barrier for the blood vessels inside, which serve as a "superhighway" on which oxygen and nutrients travel from the mother to the developing fetus and waste products and carbon dioxide travel from the fetus back to the mother. The umbilical cord is composed of the umbilical epithelium and connective tissue called Wharton's jelly. Umbilical cord MSCs (UC-MSCs) have been isolated from all subsections of the umbilical cord[13,60,67] in addition to several subsections of Wharton's jelly.[42] A recent article reported that Wharton's jelly has the highest concentration of MSCs among all MSC sources.[70] UC-MSCs have repeatedly proven to have an immunosuppressive capacity and multilineage differentiation potential while lacking tumorigenicity.[8,75] UC-MSCs also express biomarkers consistent with BM-MSCs such as CD73, CD90, and CD105 while lacking hematopoietic markers CD34 and CD45.[42,72] Although there are reports noting the lower differentiation potential of UC-MSCs toward cell types relevant to orthopaedic surgery indications,[41,57] others have demonstrated an equal or even superior differentiation potential in comparison to MSCs derived from other tissue sources.[3,33,42]

Umbilical Cord Blood

Umbilical cord blood cells exchange oxygen and nutrients between the mother and fetus. The major population of stem cells in umbilical cord blood is CD34-positive hematopoietic stem cells. Although present in much lower numbers

TABLE 2
Literature Review Results[a]

	Indication							
Tissue Type	Orthopaedic	Sports Medicine	Musculoskeletal Injury	Knee	Shoulder	Elbow	Ankle	Total
Placenta	68	21	21	34	2	131	160	437
Wharton's jelly	7	0	1	3	0	11	18	40
Amnion	3	1	0	1	0	4	2	11
Amniotic membrane	36	2	1	8	5	1	15	68
Amniotic fluid	23	3	8	18	2	75	55	184
Chorion	60	1	4	34	1	57	111	268
Chorionic membrane	8	0	0	2	1	1	4	16
Umbilical cord	4	0	1	1	1	4	11	22
Umbilical cord blood	4	1	7	6	2	24	18	62
Total	213	29	43	107	14	308	394	1108

[a]The literature search was conducted by selecting 1 term from the indication group, followed by 1 term from the tissue type group. Values represent the total number of publications obtained using the 2 search terms.

as compared with bone marrow, adipose tissue, and other sources,[30,70] MSCs can be isolated from umbilical cord blood and may have a higher expansion potential than MSCs isolated from other tissues. Lee and colleagues[36] demonstrated that multipotent umbilical cord blood–derived MSCs (UCB-MSCs) express the common characteristics of BM-MSCs.

LITERATURE REVIEW

Methods

A comprehensive search of the literature was carried out in May 2016 (Table 2). Electronic databases (PubMed, MEDLINE, Cochrane Library) were utilized to identify published studies related to placental tissues and orthopaedic surgery. Search terms were paired into the following 2 groups: tissue type and indication. The 9 search terms in the tissue type group were "placenta," "Wharton's jelly," "amnion," "amniotic membrane," "amniotic fluid," "chorion," "chorionic membrane," "umbilical cord," and "umbilical cord blood." The 7 search terms in the indication group were "orthopaedic," "sports medicine," "musculoskeletal injury," "knee," "shoulder," "elbow," and "ankle." Every search term from the tissue type group was paired with every search term from the indication group, totaling 63 possible search combinations that were used to identify and extract publications from the electronic databases listed above.

This search yielded 1108 results. All duplicate (n = 274) and non-English studies (n = 74) were eliminated. Studies describing the use of placenta for non–orthopaedic sports indications (ie, cranial defects, spine pathological conditions, fractures, etc) (n = 734) were further excluded. This search yielded 6 published human studies and 25 animal studies that investigated the safety and/or efficacy of placental tissues or cells as a therapeutic agent. The bibliographies of these reports were reviewed, and 9 relevant studies were identified, bringing the total number of publications included for analysis to 35 (Figure 2).

Information was collected on the tissue type, animal model, indication, number of cells, study design, treatment regimen, treatment safety, and study outcomes (Appendix Table A1, available in the online version of this article).

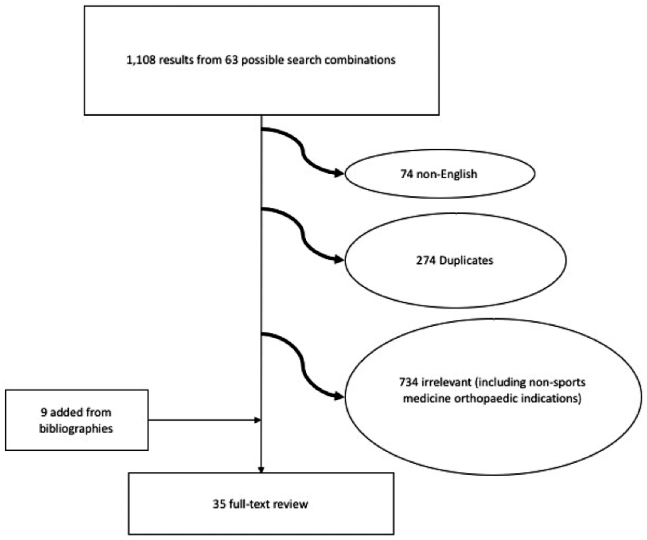

Figure 2. Flowchart describing literature evaluation methods.

These results were organized into 4 groups by orthopaedic surgical indication: tendon injury, osteoarthritis/articular cartilage injury, ligament injury, and synovitis. The same data were collected for the 6 human studies (Appendix Table A2, available online).

Results

Of the 29 publications describing the use of placenta for orthopaedic sports medicine indications in animal models, 14 studied tendon injuries, 13 studied osteoarthritis or articular cartilage injuries, 3 studied ligament injuries, and 1 studied synovitis. Of the 6 human studies describing the use of placenta for orthopaedic sports medicine indications, 5 studied foot/ankle injuries, and 1 studied knee osteoarthritis. Of all 35 studies, there were 26 studies in

which placenta-derived culture-expanded cells alone or in combination with carriers or scaffolds were applied (11 of which provided cell counts) and 9 studies that utilized acellular placental tissue, the ECM of placental tissue, or culture medium containing biologically active factors released by placental cells. In the 35 reviewed human and animal studies, 25 different placental treatments were used. There were no placental treatment–related adverse events reported in human studies.

ANIMAL MODELS

Tendon Injury

Rat Model. Coban et al[12] used the human amniotic membrane and/or amniotic fluid to treat Achilles tendon ruptures after local corticosteroid-induced Achilles tendinosis in rats. Human amniotic fluid was collected between the 16th and 24th weeks of gestation and stored at –20°C before use. The amniotic membrane was dissected from the placenta, stored at 4°C, and used within 4 hours after preparation. The characterization of amniotic fluid and membrane was not performed; however, the storage of amniotic fluid at –20°C without cryopreservation agents should result in cell death, whereas short refrigeration of the amniotic membrane should retain tissue cell viability. Thus, in this study, devitalized amniotic fluid and viable amniotic membrane cells were used. Seventy-two tendons in 36 rats received an intratendinous injection of 0.1 mL betamethasone sodium phosphate every 3 days for 4 weeks to induce tendon degeneration. On the 30th day, all rats underwent surgery. After segmental transection, rats were randomized into 3 repair groups (12 rats, 24 tendons/group). Group 1 had the tendon repaired by suturing only, group 2 had the tendon sutured and 0.3 mL of devitalized amniotic fluid injected under the paratenon after repair, and group 3 had the tendon repaired plus an injection of 0.3 mL of devitalized amniotic fluid and an amniotic membrane wrapped around the repaired tendon. The rats in each group were further divided randomly into 3 subgroups (8 rats, 16 tendons/subgroup) for histopathological and biomechanical testing. Histopathological analysis of tendons was performed at 1 and 2 weeks after repair, and biomechanical testing was performed 3 weeks after tendon repair. Although there was a statistical significant difference between the histopathological scores of groups 2 and 3 at the first week, the authors concluded that the human amniotic membrane and fluid did not significantly contribute to the healing process of Achilles tendon ruptures in the early phase.

Chicken Model. Two studies utilizing a chicken model described the results of 2 distinct products. In 2002, Demirkan and colleagues[16] used a human amniotic membrane patch for flexor tendon repair. Although the authors did not specify the viability of the tissue patch, storage of the fresh amniotic membrane at 4°C for 24 hours suggests that the graft contained viable cells. Seventy-two 1-year-old leghorn chickens were randomized into 1 of 3 treatment groups: group 1 had the flexor tendon repaired, group 2 had the tendon and the sheath repaired, and group 3 had the tendon and the sheath repaired and then wrapped with the amniotic membrane. Postoperative tendon adhesions were measured by gross and histological examinations at 3, 6, and 12 weeks after surgery. The tendons that were wrapped with the amniotic membrane demonstrated a significant reduction of postoperative adhesions when compared with the other 2 groups.

Ozgenel[48] investigated the effects of hyaluronic acid (HA), the human amniotic membrane, and their combination on the repair of deep flexor tendons. The chickens' bilateral long toes were operated on, providing a total of 144 tendons for gross and histological evaluations at 3 and 6 weeks postoperatively and a biomechanical evaluation at 20 weeks postoperatively. Chickens were randomized into the control group (50% partial tenotomy and repair alone), repair with an HA injection, repair with the human amniotic membrane wrapped around the repair site with care taken to have the mesenchymal side of the human amniotic membrane in contact with the repair, and repair with a combination of HA and human amniotic membrane. The HA + human amniotic membrane group had a significantly lower number of adhesions and significantly better range of motion than the other 3 groups. However, no significant differences were noted in healing and tensile strength among the 4 groups at week 20 postoperatively.

Rabbit Model. Ozgenel and colleagues[49] tested the topical application of human amniotic fluid on peritendinous adhesion formation and tendon healing in rabbits. Each rabbit (n = 32, 256 long flexor tendons) was assigned to 1 of 4 groups: excision of the tendon sheath, excision of the tendon sheath and a local injection of 0.3 mL of human amniotic fluid at the surgical site, repair of the tendon sheath, and repair of the sheath with an injection of 0.3 mL of human amniotic fluid. Rabbits were sacrificed at 12 weeks, at which time adhesions and histology were evaluated, and at 20 weeks, when biomechanical strength of the repaired tendon was assessed. There were significantly less adhesions in the sheath repair and human amniotic fluid injection group compared with the other 3 groups and significantly greater tensile strength in tendons after the injection of human amniotic fluid than tendons without the human amniotic fluid injection.

Ozboluk et al[47] examined the effects of the human amniotic membrane and periosteal autografts on flexor digitorum fibularis tendon healing after being cut and repaired with a modified Kessler technique. Fresh amniotic membranes were obtained from cesarean sections of seronegative mothers and stored in a sterile saline solution containing 1% streptomycin for 24 hours at 4°C before experiments. Although the authors did not report tissue cell viability, this preparation of the human amniotic membrane suggests the retention of viable cells. Forty-two rabbits were randomly divided into 3 groups (n = 14): tendon repair alone, tendon repair wrapped in the human amniotic membrane, or tendon repair wrapped in the periosteal autograft harvested from the iliac crest of each rabbit. All animals were immobilized in a plaster cast for 2 weeks after surgery. Half of the animals in each group were euthanized at 2 weeks and another half at 6 weeks for histological and

biomechanical evaluations. At week 2, tendons treated with the human amniotic membrane were biomechanically stronger than those undergoing repair alone but were not as strong as those treated with the periosteal autograft. At week 6, there was no difference between tendons treated with the human amniotic membrane and repair alone, although periosteal autograft tendons were still significantly stronger. Adhesion formation was similar in all groups at week 2, although adhesions in the repair-only group were much more severe than the other 2 groups at week 6.

Park et al[51] studied the efficacy of an ultrasound-guided injection of human UCB-MSCs in the treatment of rotator cuff tendon tears. Full-thickness subscapularis tendon tears measuring 5 mm were created in rabbits and immediately confirmed by ultrasound. Thirty rabbits were randomly assigned to 1 of 3 treatment groups and received a 0.1-mL ultrasound-guided injection of the following: (1) human UCB-MSCs with HA, (2) HA alone, or (3) normal saline. Four weeks after ultrasound-guided injections, gross morphological and histological examinations of repaired tendons were performed. Tendon functionality was assessed via video tracking of rabbit gait. The presence of human UCB-MSCs at the site of the injection was evaluated via tissue staining with an HLA-ABC antibody. Rabbits in group 1, which received human UCB-MSCs with HA, had notably better results in this study. Seven of 10 full-thickness tears improved to partial-thickness tears in group 1 as compared with none of the rabbits in the 2 other groups. Repaired tendons stained positively for collagen type I, which indicates proper deposition of a new tendon ECM. The gait analysis revealed that the walking distance, fast walking time, and mean walking speed at 4 weeks were significantly longer, faster, and higher in group 1. Cell tracking revealed that the human UCB-MSCs were present at the tendon tear site at 4 weeks after the injection.

Sheep Model. Barboni et al[4] investigated the use of ovine AECs in treating sheep Achilles tendon ruptures. The ovine AECs were obtained via enzymatic digestion of the amniotic epithelial layer of the ovine placenta and were characterized for the expression of surface markers using flow cytometry. After characterizing these cells in vitro, the authors evaluated the use of ovine AECs for repairing 3-mm full-thickness punch defects in bilateral Achilles tendons of 23 sheep (46 tendons). Each sheep received 1 of 2 treatments: 4×10^6 ovine AECs embedded in fibrin glue or fibrin glue alone. Eighteen animals were randomly divided into subgroups for morphological and histological evaluations and in vivo cell tracking at 7, 14, and 28 days. The last subgroup was used for a biomechanical evaluation of the tendons at 28 days. Histology results showed that at early time points, ovine AECs accelerated tendon healing compared with the control group. The biomechanical properties of ovine AEC–treated tendons were also significantly improved over the contralateral control tendons at 28 days postoperatively.

Horse Model. Muttini et al[45] investigated the use of ovine AEC xenotransplantation in 6 horses with acute (n = 4) or chronic (n = 2) superficial digital flexor tendon lesions. Ultrasound results confirmed the presence of bilateral superficial digital flexor tendon lesions in horses and a normal contralateral tendon. A total of 7 million ovine AECs were either injected into the defect for acute lesions or injected into the area of greatest tendinopathy for chronic cases. Horses were rested for 7 days and then underwent a rehabilitation protocol. During the study, follow-up ultrasound was performed 6 times. At day 180, tendon samples were collected for a histological examination. Ultrasound showed that the affected areas in acute lesions increased in cross-sectional area at 30 days. At 60 days and beyond, the echo texture gradually became more regular, the cross-sectional area decreased, and collagen fibers became more parallel. In the chronic lesions, damaged sections appeared less hyperechoic but showed no change in cross-sectional area at 30 days. However, an improvement in tendons was detected after 60 days with a normal appearance at day 180. The histological examination showed that tendon tissue integrity was restored in both acute and chronic cases. Immunohistochemistry showed that injected ovine AECs lost their epithelial marker and significantly increased the expression of the collagen type I gene: COL1. The authors concluded that the use of xenographic ovine AECs is a promising approach for tendon lesions.

Osteoarthritis/Chondral Injury

Models of osteoarthritis and acute focal cartilage defects have been used to evaluate cartilage reparative effects of placental cells or placental extract in reviewed studies.[31,61,77]

Rat Model. In a monoiodoacetate-induced rat model of osteoarthritis, Kim et al[31] demonstrated that human aqueous placental extract (HPE) had a chondroprotective effect. HPE was prepared from human full-term placentas derived from donors tested for adventitious agents. Placentas were cut into pieces and extracted with water through enzymatic molecular separation and chemical hydrolysis. The composition of HPE was characterized by amino acid content. Insoluble macromolecules (polysaccharides, polynucleotides, etc) were excluded from HPE. On day 14 of the monoiodoacetate injections, the rats were split into 6 groups that received different doses of HPE ranging from 0.022 to 0.4 mL/kg or 3 mg/kg diclofenac or saline. Intra-articular knee injections were performed once daily for 14 days. Rats were sacrificed at day 28 for gross, histopathological, and radiographic evaluations. The results of this study led the authors to conclude that HPE might reduce cartilage degradation through the suppression of MMP-2 and MMP-9, the activation of which is associated with progressive destruction of the cartilage matrix in early osteoarthritis. The authors did not discuss which components of HPE might have such effects; however, results indicate that the placenta has water-soluble factors that inhibit protease activity, such as tissue inhibitors of metalloproteinases.

Willett and colleagues[77] evaluated differences between knee injections of saline versus a micronized, dehydrated, devitalized human amniotic and chorionic membrane (mdHACM) in both naïve rats and rats that had undergone medial meniscal transection. Analysis was performed at 3 or 21 days after treatment. Synovial fluid was collected at

the time of animal sacrifice and analyzed for 10 inflammatory cytokines using an enzyme-linked immunosorbent assay kit. Histology of the synovium and cartilage was also performed. Micro–computed tomography (CT) at day 21 was performed to evaluate cartilage morphology and composition on the tibial plateau. Histology in all groups revealed the presence of amnion fragments in the synovium at 3 days after the injection, but the number of amniotic fragments diminished by day 21. There was significantly lower cartilage attenuation, fewer incidences of erosion, and no lesions in medial meniscus–transected joints in the group of animals treated with mdHACM. There was not a significant difference in average attenuation values between medial meniscus–transected joints treated with mdHACM and naïve joints, leading the authors to believe that mdHACM may have a positive effect on proteoglycan content in the joint.

Two studies on acute chondral injuries in a rat model came from the same group in South Korea. In both studies, human UCB-MSCs were isolated and expanded to be used for treatment. In their first study,[11] full-thickness articular cartilage defects 2 mm in diameter were created in the trochlear groove of both femurs in 20 rats, which were randomly assigned to 1 of 4 treatment groups. Right knees (experimental knees) underwent implantation of a mixture of human UCB-MSCs (1.0×10^7 cells/mL) and different hydrogels, and hydrogel only was used for the treatment of left knees (control knees). The 4 different hydrogels were as follows: group A, 4% HA; group B, 3% alginate:30% pluronic (1:1, v/v); group C, 4% HA:3% alginate:20% pluronic (2:1:1, v/v); and group D, 4% HA:3% alginate:20% pluronic:chitosan (4:1:1:2, v/v). At 16 weeks after surgery, cartilage was analyzed grossly and histologically. The right (treatment) knees of group A (human UCB-MSCs and HA only) rats showed superior cartilage repair histologically and morphologically compared with all other treatments. Additionally, knees that were treated with human UCB-MSCs and hydrogel, as opposed to hydrogel alone, showed superior cartilage repair, including increased type II collagen content on immunohistochemical staining. This led the authors to conclude that human UCB-MSCs could be used to repair articular cartilage defects in vivo and that HA is a promising carrier to use in combination with human UCB-MSCs.

The group performed another study[53] with a similar experimental design, which confirmed previously obtained results.[11] Full-thickness articular cartilage defects were created in the same fashion as mentioned above in both knees of 15 rats, which were randomly assigned to 3 treatment groups. Each animal had a treatment knee and a control knee. Human UCB-MSCs and 3 different hydrogel composites (hydrogel A: 4% HA/30% pluronic (1:1, v/v); hydrogel B: 4% HA; and hydrogel C: 4% HA/30% pluronic/chitosan (1:1:2, v/v)) were implanted into the experimental knee (right knee), and hydrogels without human UCB-MSCs were implanted into the control knee (left knee). At 8 weeks after surgery, experimental (right) knees treated by a combination of cells with a hydrogel showed significantly better cartilage repair both grossly and histologically compared with the opposite control (left) knee treated with a hydrogel only. Consistent with the previously mentioned study, treatment with human UCB-MSCs and 4% HA had a superior outcome.

In a recently published study, Nogami et al[46] studied the effect of the ECM from human AM-MSCs coated on a polylactic-co-glycolic acid (PLGA) scaffold to treat acute chondral injuries. The ECM-PLGA scaffold was generated by incubating the PLGA scaffold with a cell suspension of immortalized human amniotic mesenchymal cells overnight, followed by 14 days of culture. The medium was then changed to a chondrogenic medium supplemented with BMP-2 and cultured for another 14 days before being irradiated for decellularization of the scaffold. The cell-free ECM-PLGA scaffold was then washed 3 times with phosphate buffered saline and stored at –80°C until use. At the time of surgery, chondral defects were created in the bilateral trochlear grooves of 26 rats (52 knees), and each knee was randomly assigned to 1 of 3 groups: empty (control) group, PLGA implant only, or ECM-PLGA scaffold. Examination of the macroscopic degree of cartilage repair, histological evaluation, and immunohistochemistry staining for type I and II collagen were performed at 4, 12, or 24 weeks after surgery. The PLGA scaffold was present in the subchondral region at 4 and 12 weeks, indicating slow biodegradation and slow cartilage repair. However, in the ECM-PLGA group, immunohistochemistry revealed that the regenerated tissue had strong staining for type II collagen at week 24. Such staining was not present in the empty control and PLGA alone groups. The macroscopic cartilage score was significantly different between the empty control and ECM-PLGA groups at week 24 but was not significantly different among the 3 groups at any other time point.

Rabbit Model. Yan and Yu[79] compared 4 types of cells for their cartilage regenerative potential in full-thickness cartilage defects. Bilateral (72 knees) full-thickness chondral defects were created in 36 rabbits. Rabbits were randomly assigned to 1 of 5 treatments: allogenic chondrocytes, allogenic BM-MSCs, allogenic fibroblasts, human UCB-MSCs, or no implanted cells (control group). Cells were seeded on a polylactic acid matrix before implantation. Each rabbit received the same type of graft for both knees according to the treatment group assignment. Gross, histological, and biochemical evaluations of cartilage were performed at 6 and 12 weeks after implantation. Using a semiquantitative histological scoring system, it was observed that the repaired tissue found in the chondrocyte and BM-MSC groups was significantly better than in the other 3 groups. The cartilage tissue in the human UCB-MSC group was significantly better than in the fibroblast and control groups. Results of this study suggest that human UCB-MSCs are not the best cell source for cartilage repair.

Li et al[38] studied the use of P-MSCs embedded in a silk fibroin scaffold for articular cartilage defects. These cells were isolated from full-term whole placentas after mechanical mincing and enzymatic digestion of the tissue. Twelve rabbits had full-thickness chondral defects created surgically that were filled with the silk fibroin biomaterial and P-MSCs. Cartilage analysis was performed at 4, 8, and 12 weeks after implantation. Gross and histological results demonstrated that the repair tissue filling the defects

contained hyaline cartilage, which was integrated well with the surrounding host cartilage. Despite the use of xenogeneic P-MSCs (human cells into rabbits), lymphocytes and leukocytes did not infiltrate the defect, and the silk fibroin biomaterial was remodeled by 12 weeks. The histological evaluation showed that implanted P-MSCs were able to survive 8 weeks and, when combined with silk fibroin, contributed to cartilage repair. Although the authors did not specify the anatomic origin of the P-MSCs, the method of cell isolation suggests that a mixture of maternal and fetal P-MSCs was used.

Saulnier et al[61] used neonatal equine UC-MSCs derived from umbilical cord Wharton's jelly in a rabbit model of osteoarthritis. Osteoarthritis was induced by medial meniscal release in 30 rabbit knees. Rabbits were given a single intra-articular injection of 3.5×10^6 UC-MSCs at 3 (early) or 15 (delayed) days after surgery with a time-matched saline injection as a control. The rabbits were sacrificed at day 15 or 56, and osteoarthritis grading was performed along with analysis of the gene expression of inflammatory cytokines and metalloproteinases in synovial tissue. As part of the same study, paracrine effects of UC-MSCs were investigated using an umbilical cord–conditioned versus control medium on rabbit primary synoviocytes stimulated with IL-1β in vitro. An early (day 3) intra-articular injection of equine UC-MSCs was effective in preventing osteoarthritic signs in rabbit knees after medial meniscal release. This study confirms that the synovium is a major target of MSC therapy. MSCs modulate the expression of matrix-degrading enzymes and promote an anticatabolic environment.

Liu and colleagues[39] investigated the use of the human acellular amniotic membrane with and without rabbit BM-MSCs in a rabbit articular cartilage defect model. The human acellular amniotic membrane was prepared by separating the human amniotic membrane from chorion by blunt dissection, followed by marking of the epithelial surface. It was then washed with sterile saline and digested with 0.25% trypsin for 30 minutes, and remaining AECs were gently removed with a blunt instrument. Allogenic rabbit BM-MSCs were inoculated into the human acellular amniotic membrane over 7 to 8 days of culture. Chondral defects measuring 4 mm in diameter were created in the nonweightbearing area of 24 rabbits' bilateral femoral condyles (48 knees). Experimental (left) knees were randomly assigned into 2 groups. Group A had the human acellular amniotic membrane inoculated with rabbit BM-MSCs implanted in the defects, and group B had the human acellular amniotic membrane implanted alone. Control (right) knees in both groups did not have any material implanted. At 8 and 12 weeks after surgery, gross observation, histological evaluation, and cartilage defect scoring were performed. In group A, histology and gross observation showed cartilage-like cells that stained positive for type II collagen at 8 weeks, which increased at week 12. No such cells were present in group B and control defects at any time point. Cartilage scores in group A were significantly better than those in the control and group B at 12 weeks.

Minipig Model. Ha et al[23] tested the cartilage reparative effect of a mixture of 3 human UCB-MSC cell lines with 4% HA in minipigs. A full-thickness chondral injury was intentionally created in the trochlear groove of each knee in 6 minipigs. Three weeks later, an osteochondral defect, 5 mm wide by 10 mm deep, was created, followed by 8 mm–wide and 5 mm–deep reaming. A mixture of human UCB-MSCs (0.5×10^7 cells/mL) and 4% HA hydrogel composite was then transplanted into the defect on the right knee. Each cell line was used in 2 minipigs. The control (left) knees had no treatment. The degree of cartilage regeneration was assessed by gross and histological analyses at 12 weeks after treatment. The knees in the treatment group showed increased cartilage regeneration compared with the defect alone and showed significantly better histological scores than the control knees. The cartilage regenerated in the treatment group stained highly positive for type II collagen, leading the authors to conclude that the regenerated tissue was hyaline cartilage. Interestingly, the degree of cartilage regeneration for the 3 tested cell lines correlated with chondrogenic differentiation of these cell lines.

Ligament Injury

Rabbit Model. Jang et al[27] evaluated the difference of bone-tendon junction healing after anterior cruciate ligament reconstruction using a hamstring autograft supplemented with Cartistem. Cartistem is composed of culture-expanded allogenic human UCB-MSCs and is manufactured by Medipost and approved in South Korea for the treatment of knee cartilage defects as a result of degenerative osteoarthritis or repeated trauma. Four million Cartistem cells were mixed with fibrin glue for a total volume of 250 μL and injected into the knees of 15 rabbits. Another 15 rabbits underwent anterior cruciate ligament reconstruction with a hamstring autograft only. Gross and histological evaluations of the knee joint, as well as micro-CT to assess bone tunnel enlargement, were performed at 4, 8, and 12 weeks. The total histological scores of bone-tendon healing in the treatment group were significantly higher at all time points than those scores in the control group. Immunohistochemical staining showed a significantly higher amount of type II collagen present in the treatment group than in the control group. The tibial tunnel size as measured on micro-CT was smaller in the treatment group than in the control group at 12 weeks, but no such difference was noted at earlier time points. The femoral tunnel size was significantly smaller in the treatment group at the 8- and 12-week time points but not at 4 weeks. Results of this study concluded that allogenic human UCB-MSCs (Cartistem) support bone-tendon interface healing, which is a difficult-to-heal zone, often resulting in poor-quality tissue formation with a high risk of reinjuries.

Horse Model. Lange-Consiglio et al[34,35] published 2 studies in 2013 about a new treatment of unilateral ligament and/or tendon injuries in horses. In one study,[35] they compared the treatment of such injuries with allogenic equine AM-MSCs to autologous BM-MSCs. In this study, 51 horses were treated with ultrasound-guided injections of AM-MSCs, and 44 horses were treated with

BM-MSCs. The animals were followed up for approximately 2 years, with clinical and ultrasound measurements at regular intervals. The reinjury rate of horses treated with AM-MSCs was 4.00% (n = 2) compared with 23.08% (n = 10) in those treated with BM-MSCs. Horses treated with AM-MSCs returned to activity in 4 to 5 months, while those treated with BM-MSCs returned in 4 to 12 months.

In another study by the same group,[34] the culture medium derived from AM-MSCs was injected under ultrasound guidance to treat 13 horses (10 case, 3 control) with unilateral ligament or tendon injuries. The average incidence of reinjuries was 15%, and approximately half of all horses returned to activity within 4 to 5 months.

Synovitis

Horse Model. Williams et al[78] used UCB-MSCs to treat lipopolysaccharide (LPS)–induced synovitis. Feasibility studies were performed to determine that (1) LPS administration reproducibly triggers acute synovitis and that (2) the allogenic administration of UCB-MSCs did not induce significant adverse reactions. The administration of LPS induced a mild, transient inflammatory reaction. However, the inflammatory effect of LPS lasted only 24 to 48 hours, which limits the clinical applicability of the study's findings. Additionally, while cells did not cause any significant adverse events, the administration of UCB-MSCs did induce a moderate, transient inflammatory reaction in all animals.

After a 4-week washout period, the same 6 horses that were used in the feasibility study were given a unilateral injection of LPS and 10 million cryopreserved equine UCB-MSCs. The contralateral joints were injected with LPS and saline as a control. Synovial fluid aspiration, joint circumference measurement, and lameness grading were performed. There was a decrease in neutrophil and mononuclear cell numbers after the administration of UCB-MSCs, which suggests that UCB-MSCs may have therapeutic potential for treating joint inflammation. However, synovial fluid biomarker levels (glycosaminoglycan, PGE2, CP-II, C2C, and CS-846) after LPS/equine UCB-MSC injections were not significantly different than those produced by LPS-only injections. The authors explained that the significant elevation in biomarker levels caused from LPS alone and/or the small sample size may have prevented them from detecting differences between LPS/UCB-MSC and LPS-only injections. It is also possible that equine UCB-MSCs did not affect the cartilage structure because of the dose, timing, or treatment frequency that was used in this study.

HUMAN MODELS

Foot/Ankle Pathological Conditions

Lullove[40] published a single-site, retrospective case series. In this study, 10 patients with acute or chronic tendon or muscular injuries of the lower extremity were identified and included posterior tibial tendinitis, peroneal tendinitis, anterior tibial tendinitis, Achilles tendinitis, and injuries to the extensor muscles of the foot or plantar musculature of the foot excluding the plantar fascia. Patients received an ultrasound-guided injection of a commercially available flowable placental tissue matrix allograft. The flowable tissue matrix allograft is derived from human placental connective tissue and was recently made available for the minimally invasive treatment of damaged or inadequate tissue (PX50; Human Regenerative Technologies). The description, composition, and features of the placental product used in the study are not provided. As of the writing of this review, the company website of Human Regenerative Technologies shows that 2 flowable placental tissue matrix products are available; however, no details except the storage temperature are provided. In this retrospective study, visual analog scale (VAS) scores were obtained at baseline and then weekly for 6 weeks. Ultrasound of the nonvascular extremity was performed at the time of the injection and at weeks 4 and 6. Patients were given a short-leg walking boot to wear while weightbearing for 2 weeks after the injection. Eight of the 10 patients reported VAS pain scores of 0 by week 4, and all reported scores of 0 by week 5.

Warner and Lasyone[73] published a single-site, retrospective case series. Fourteen patients underwent open foot and ankle surgical repair supplemented with CLARIX CORD 1K (Amniox Medical Inc) for the treatment of a variety of conditions in which there was tendon and/or nerve involvement. CLARIX CORD 1K contains a cryopreserved human amniotic membrane and umbilical cord, which is bioactive, according to the manufacturer's product information. Fourteen patients were included in this study, 9 of whom underwent revision surgery. American Orthopaedic Foot and Ankle Society (AOFAS) Ankle-Hindfoot Scale and pain numerical rating scale scores were measured preoperatively and postoperatively to assess outcomes. At a mean of 15 weeks of follow-up (range, 4-32 weeks), mean postoperative AOFAS and numerical rating scale scores improved significantly when compared with preoperative scores. No adverse events were reported to be related to the use of a cryopreserved human amniotic membrane and umbilical cord.

Werber[76] conducted a prospective, open-label case series using an allograft called PalinGen SportFlow (Amnio Technology) to treat chronic plantar fasciosis and Achilles tendinosis. PalinGen SportFlow is composed of a cryopreserved human amniotic membrane and amniotic fluid, which according to the manufacturer's product information has amniotic fluid–derived cells and is stored at –80°C before use. Forty-four patients who were unresponsive to standard therapies for chronic plantar fasciosis and Achilles tendinosis for a minimum of 6 months were selected to receive 1 ultrasound-guided human amniotic membrane and amniotic fluid injection around the plantar fascia or along the Achilles paratenon. Patients with chronic plantar fasciosis received injections of 0.5 mL of a human amniotic membrane and amniotic fluid mixed with 0.5 mL of 1% lidocaine, and patients with Achilles tendinosis received injections of 1 mL of a human amniotic

membrane and amniotic fluid mixed with 1 mL of 1% lidocaine. VAS pain scores were collected before the injection and every 2 weeks after the injection for 12 weeks. All patients had significantly reduced pain by 4 weeks after the injection and mild pain (VAS score <4) at week 12. No adverse reactions were reported in any of the patients.

Zelen et al[81] conducted a randomized, controlled, single-blind study to compare a low and high dose of mdHACM to a saline placebo in patients with chronic refractory plantar fasciitis. The placental tissue was processed using a proprietary method involving cleaning, sterilizing, and drying the human amniotic/chorionic membrane obtained from screened and tested donors. Because of the nature of the process, mdHACM has no viable cells. In this study, 45 patients were randomized to receive an injection of 2 mL of 0.5% Marcaine plain and then either 1.25 mL of saline (control), 0.5 mL of mdHACM, or 1.25 mL of mdHACM. Patients were instructed to wear a cam boot in the daytime for 2 weeks after the injection. Follow-up visits were once weekly for 6 weeks, with a final follow-up at 8 weeks. The AOFAS Ankle-Hindfoot Scale and Wong-Baker FACES Pain Rating Scale were administered at each study visit, and the SF-36 was used at baseline and 8 weeks. No adverse events related to the treatment were recorded. Results showed significant improvement in both AOFAS and Wong-Baker FACES scores at all time points in both mdHACM groups as compared with saline. No differences were noted between the high- and low-dose mdHACM groups. Both the physical and mental component scores of the SF-36 were significantly improved at 8 weeks as compared with baseline in the study groups, although no differences were noted between the groups.

Hanselman et al[24] conducted a randomized, controlled, double-blind pilot study to compare the injection of a cryopreserved human amniotic membrane to a corticosteroid injection (control) for the treatment of plantar fasciitis. The human amniotic membrane (AM3, now known as Clarix FLO; Amniox Medical Inc) is a cryopreserved, micronized amniotic membrane, which according to the manufacturer's product information has no living cells and is stored at –80°C before use. There were 23 patients in this study, and 14 were randomized into the steroid injection group, while 9 were randomized to receive a human amniotic membrane injection. Patients received an injection at baseline and were evaluated at 6, 12, and 18 weeks with the option to receive a second injection (same as first injection) at the 6-week visit. There were 3 patients in each group who received 2 injections. Six patients received a single injection of the human amniotic membrane, and 11 patients received a single corticosteroid injection. Patient outcomes were analyzed between 2 groups for the 2 different cohorts (1 injection vs 2 injections). The primary outcome measure was the Foot Health Status Questionnaire (FHSQ), and secondary outcomes were the VAS and verbal percentage improvement from baseline. No adverse events were reported. There were no significant differences in the VAS, FHSQ foot pain, or FHSQ general foot health scores in the group receiving only 1 injection. Some variables showed greater improvement in the control group, although it was not significant. There was significant improvement in the FHSQ foot pain score in the group receiving 2 injections of the human amniotic membrane as compared with 2 injections of corticosteroid. Verbal percentage improvement at 12 weeks was statistically greater in the steroid group than in the human amniotic membrane group.

Knee Osteoarthritis

Vines and colleagues[71] performed a prospective, open-label case series as a feasibility study for a larger clinical trial. Six patients with Kellgren-Lawrence grades 3 and 4 tibiofemoral osteoarthritis were given a single intra-articular injection of a commercially available amniotic suspension allograft (ASA) and followed up for 12 months. The ASA contains a cryopreserved, particulated human amniotic membrane and human amniotic fluid containing cells (ReNu; NuTech Medical). Patients were assessed before treatment and prospectively evaluated at 1 and 2 weeks and at 3, 6, and 12 months after treatment for safety and efficacy. A comprehensive blood analysis including blood counts, metabolic profiles, creatinine and liver enzymes, inflammatory markers, and immunological parameters was performed for the assessment of ASA injection safety. Knee injury and Osteoarthritis Outcome Score, International Knee Documentation Committee, and Single Assessment Numeric Evaluation scores were collected at baseline, at 1 and 2 weeks, and at 3, 6, and 12 months. Statistical significance was not calculated because of the small sample size. There was a general trend of improvement in all patient-reported outcomes. There was a statistically significant increase in immunoglobulin G and immunoglobulin E levels in approximately 15% of samples at the 12-month time point, but the reported levels were still within the normal reference range. Two adverse events were reported during the study, but they were transient increases in pain that resolved by the 2-week follow-up. This study demonstrated the feasibility of using an intra-articular injection of a cryopreserved ASA for knee osteoarthritis. This study provides a foundation for a large, multisite, placebo-controlled trial of intra-articular ASA injections for symptomatic knee osteoarthritis.

DISCUSSION

The composition of placental tissue is optimal for supporting healing of the injury site. Placental tissue includes a collagen-rich structural matrix, a cocktail of biologically active factors, and endogenous viable cells such as epithelial cells, fibroblasts, and MSCs. Placental tissue, mostly the amniotic membrane, has a long history of clinical use for burns and wounds; however, recent data suggest potential benefits of the placenta for orthopaedic sports medicine indications.

The review of published articles presented here reveals that in the majority of animal model studies, isolated, culture-expanded P-MSCs alone or in combination with HA or other matrices were investigated. At the present time, there are no published reports of using culture-expanded

P-MSCs in clinical cases or clinical trials for sports medicine indications. Among the reviewed publications, we identified one study in which a commercial stem cell product, Cartistem, was tested in a rabbit model of ligament injuries. Cartistem, a culture-expanded allogenic human UCB-MSC product, is manufactured by Medipost and approved in South Korea for the treatment of knee osteoarthritis. At the present time, Medipost has announced the completion of a phase 1/2a clinical trial in the United States; however, results of the trial are not yet available.

Both placenta-derived culture-expanded cells and placental allografts demonstrated anticatabolic properties for osteoarthritis, the regeneration of hyaline cartilage in articular cartilage defects, and improved ligament and tendon healing. All reviewed publications demonstrated some degree of effectiveness in treating orthopaedic sports medicine indications when compared with controls, if a control was used. However, the majority of studies compared the therapy to a control/sham procedure. Only a few studies included a placebo control, and only one study compared the effectiveness of different placental cell types. Thirty-four reviewed studies were focused on the treatment of lower extremities. Only 1 of the 35 reviewed studies examined a sports medicine indication in the upper extremity.[51]

Given the small number of preclinical animal studies published thus far and the high variability of animal models used, indications, placental cell types, tissue preparation and preservation, doses, and administration routes, it is not possible to perform a comparative analysis.

Published articles describing the use of the placenta in humans are limited to commercial tissue allografts. Tissue allografts are regulated by the Food and Drug Administration (FDA) as Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) as defined in Title 21 (Part 1271) of the Code of Federal Regulations and Section 361 of the Public Health Service Act.[18] In contrast to devices and drugs, tissue allografts, including placenta-derived products, need only satisfy 4 criteria to be considered an HCT/P: (1) minimal manipulation, (2) homologous use, (3) not combined with drugs or devices, and (4) not reliant on cell metabolic activity as a primary function. HCT/Ps do not require premarket approval and therefore have the shortest path to commercialization. The isolation and culture expansion of cells from placental tissues are considered beyond minimal manipulation.

Products that do not meet the definition of an HCT/P are regulated by the FDA as biological drugs (Section 351 of the Public Health Service Act). To be able to obtain a Biologics License Application for product marketing, companies must file an Investigational New Drug Application and conduct phase I to III clinical trials. This drug/biologics pathway is expensive and time consuming, which may explain why there are no approved placenta-based biological drugs in the United States.

All 6 human clinical trials reviewed here used a flowable (or injectable) placental allograft formulation for orthopaedic indications. There are 14 companies or distributors who offer commercial amniotic membrane products alone, not including ones that utilize other placental tissues.[58] None of the placenta-derived treatments offered by these companies are covered by insurance, and 2 of these companies have recently been notified by the FDA that their flowable placental allograft formulation does not meet HCT/P requirements. While there is growing interest in flowable placental allografts among companies and health care providers, the lack of reimbursement, which forces patients to pay "out of pocket," and regulatory uncertainty have limited the development and use of these products.

Data in the literature show that aging negatively affects the functionality of stem cells.[80] Therefore, the young cells obtained from the placenta and umbilical cord blood may have advantages over older cells obtained from bone marrow, adipose tissue, and other types of adult tissue.

Using placental cells might concern patients who believe that they are embryonic. However, placental and umbilical cord blood cells are collected from full-term normal pregnancies up to 4 weeks after birth and are not embryonic. While the placenta is a novel treatment option, it is not a controversial source of tissues or cells. Orthopaedic surgeons must ensure that patients are informed regarding the origin, collection, and properties of placental tissues and cells.

This review included 6 publications investigating the use of placental allograft products in humans. Five studies described the use of placental tissue allografts for the foot and ankle and 1 for the knee; however, none of these studies used the same product. All of these studies had several flaws in the clinical study design, including being retrospective (2 studies), open-label rather than controlled (3 studies), underpowered (4 studies), or case series (2 studies), making it difficult to draw conclusions. One randomized, controlled, double-blind pilot study was performed in which corticosteroid injections were compared with injections of a cryopreserved, micronized, devitalized human amniotic membrane (Clarix FLO). Patient-reported outcomes failed to reach statistical significance likely because of the small number of patients in the study.

The placenta from full-term normal pregnancies is a noncontroversial source of young potent MSCs, which shows promise for orthopaedic sports medicine. MSCs are easy to isolate from amniotic fluid but are present at very low numbers. P-MSCs can be expanded in culture, preserved, stored for long times, and used in humans as an "off-the-shelf" biological drug. There are no approved P-MSC–based biological drugs in the United States; however, several clinical trials are underway for a variety of different indications (www.clinicaltrials.gov). All currently available commercial placental products in the United States are tissue allografts that are regulated as HCT/Ps and do not require premarket approval. However, some uncertainty exists around the regulation of flowable or micronized placental allograft formulations, which happen to be the only formulations reported for clinical use in orthopaedic applications.

Additionally, the majority of placental tissue allografts available contain no living cells as a result of processing, preservation, and sterilization methods. The observed beneficial effects of such products are not mediated by MSCs but might be linked to the placental ECM and/or growth factors and cytokines present in the tissue.[24,81] Only

a few commercial placental allografts are known to contain living endogenous cells.[7] One such product is ReNu, a combination of cryopreserved, viable amniotic fluid and a devitalized, micronized amniotic membrane.[71] However, the low number of MSCs in amniotic fluid suggests that the observed effects are likely mediated by matrix proteins and growth factors and possibly by AECs, which is the main cell type in amniotic fluid.[29]

The mechanism of action of placental cells and MSCs remains to be determined. One hypothesis is that MSCs are "medicinal signaling cells," and the paracrine effect is the main mechanism linked to therapeutic effects of MSCs rather than their differentiation into a desired cell type required for the replacement of lost cells and tissue repair.[10]

The literature describing the use of placenta-derived cells or placental allografts for orthopaedic sports medicine is limited and heterogeneous. However, encouraging safety results and positive outcomes described in the reviewed literature suggest continuing to explore the use of placenta-derived tissue allografts and cells for tendon, ligament, and cartilage repair. Investments in level I evidence are required to prove the benefits of placental therapies and to elucidate the mechanism of action of these therapeutics.

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