Human Amniotic Membrane–Derived Products in Sports Medicine: Basic Science, Early Results, and Potential Clinical Applications

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Published online November 19, 2015 in advance of the print journal.

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What is This?

Basic Science Update

Human Amniotic Membrane–Derived Products in Sports Medicine

Basic Science, Early Results, and Potential Clinical Applications

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Background: Amniotic membrane (AM)–derived products have been successfully used in ophthalmology, plastic surgery, and wound care, but little is known about their potential applications in orthopaedic sports medicine.

Purpose: To provide an updated review of the basic science and preclinical and clinical data supporting the use of AM-derived products and to review their current applications in sports medicine.

Study Design: Systematic review.

Methods: A systematic search of the literature was conducted using the Medline, EMBASE, and Cochrane databases. The search term *amniotic membrane* was used alone and in conjunction with *stem cell, orthopaedic, tissue engineering, scaffold*, and *sports medicine*.

Results: The search identified 6870 articles, 80 of which, after screening of the titles and abstracts, were considered relevant to this study. Fifty-five articles described the anatomy, basic science, and nonorthopaedic applications of AM-derived products. Twenty-five articles described preclinical and clinical trials of AM-derived products for orthopaedic sports medicine. Because the level of evidence obtained from this search was not adequate for systematic review or meta-analysis, a current concepts review on the anatomy, physiology, and clinical uses of AM-derived products is presented.

Conclusion: Amniotic membranes have many promising applications in sports medicine. They are a source of pluripotent cells, highly organized collagen, antifibrotic and anti-inflammatory cytokines, immunomodulators, and matrix proteins. These properties may make it beneficial when applied as tissue engineering scaffolds, improving tissue organization in healing, and treatment of the arthritic joint. The current body of evidence in sports medicine is heavily biased toward in vitro and animal studies, with little to no human clinical data. Nonetheless, 14 companies or distributors offer commercial AM products. The preparation and formulation of these products alter their biological and mechanical properties, and a thorough understanding of these differences will help guide the use of AM-derived products in sports medicine research.

Keywords: amniotic membrane; stem cell; regenerative medicine; scarless healing

ANATOMY AND FUNCTION OF HUMAN FETAL MEMBRANES

The fetal membranes are a complex structure that plays a critical role in fetal development. The membranes are composed of 2 layers: the outer chorion that is in contact with maternal tissue and the thin (0.02-0.5 mm[61]) inner amniotic membrane (AM) that completely surrounds the embryo and delimits the amniotic cavity.[49] The AM forms from the trophoblast—the first group of cells to differentiate from the fertilized egg, which forms the outer layer of the early blastocyst.[39] The AM is a translucent structure with no innervation or lymph nodes.[61] As a result, it is entirely dependent on diffusion for nutrition.

Structure

The AM consists of 3 histological layers: the epithelial layer, a thick basement membrane, and avascular mesenchymal tissue (Figure 1).[61] The innermost epithelial layer contains a single layer of cuboidal epithelial cells with many microvilli that play an active role in secretory and cellular transport functions.[39] The underlying basement membrane (BM) is the thickest BM of all human tissues.[49] Below the basement membrane is the avascular mesenchymal tissue, which can be divided into compact, fibroblast, and intermediate layers.[49] Type I and III collagens produced in the fibroblast layer create parallel bundles that give most of the structural integrity to the AM.[49] Type V and VI collagens create cross-links between this network of parallel bundles and the basement membrane.[49] The intermediate layer, also known as the spongy layer, has an abundant content of proteoglycans and glycoproteins with a nonfibrillar network of type III collagen, which is only loosely connected to the adjacent chorion.[49] This allows the AM to be easily separated from the chorion by blunt dissection at all stages of development.
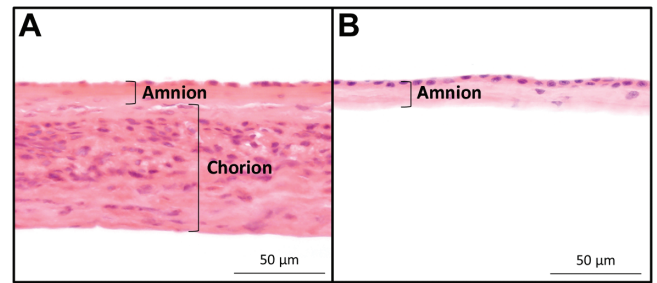
Function

The AM is a metabolically active tissue that has the following functions: water and soluble material transport for the fetus, synthesis of growth factors, vasoactive peptides and cytokines, and regulating amniotic fluid pH.[39] Amniotic epithelial cells have been shown to produce transforming growth factor–β (TGF-β), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor–α (TGF-α), keratinocyte growth factor, and hepatocyte growth factor.[26] In addition, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), noggin, and activin have also been identified in the AM.[28,63]

AMNIOTIC MEMBRANES AS A SCAFFOLD FOR COMPLEX TISSUE REGENERATION

AM was first used as a biologic scaffold in 1910 for the treatment of a skin defect.[49] Since then, it has gained popularity as a biologic dressing and/or scaffold for tissue regeneration. As for all tissue regeneration scaffolds, great attention must be given to how AM is harvested, processed, and stored, as this can affect its mechanical and biological properties.

AM is harvested from volunteer donors who undergo rigorous screening for human immunodeficiency virus (HIV) 1 and 2, hepatitis B, hepatitis C, human T-cell lymphotrophic virus, syphilis, cytomegalovirus (CMV), and tuberculosis.[26] The fetal membranes can be harvested after vaginal or cesarean delivery; however, both commonly undergo terminal radiation sterilization. The amnion is then separated from the placenta by blunt dissection.

The first distinction is between fresh and preserved AM. Fresh AM is extensively washed in phosphate-buffered saline containing penicillin and streptomycin[54] and stored at 4°C.[1,6,44,54,56,58,62] There are several concerns with fresh AM transplantation, including the risk of disease transmission

Figure 1. Histological structure of amniotic membrane grafts. Hematoxylin and eosin stains of (A) dehydrated amnion/chorion and (B) amnion-only grafts. The amnion has a single layer of epithelial cells, under which lies a thick basement membrane and an acellular and avascular layer of mesenchymal tissue.

and the need for immediate or near-immediate transplantation.[54] The reported infection rate with fresh AM transplantation is 8%, and gram-positive organisms are the most common pathogens.[22,40] The main reason for this high infection rate is that AM grafts may be transplanted during the incubation period of an infectious pathogen, and seroconversion may not occur until after AM implantation. As a result, several preservation techniques have been developed, including cryopreservation and freeze-drying (lyophilization).[33,47] Cryopreservation involves long-term storage of AM grafts at –80°C with a cryoprotectant such as glycerol or dimethyl sulfoxide (DMSO).[47] Freeze-drying, also known as lyophilization, removes the water from AM tissue by freezing the material, then reducing the ambient pressure to allow the frozen water to convert directly from the solid to the gas phase in a process known as sublimation.[47]

These methods all have some degree of influence on the mechanical and biological properties of AM grafts.[33] An excellent study has compared the effects of cryopreservation and lyophilization on AM grafts.[47] Cryopreserved and lyophilized AM both have lower maximal loads to failure and elongation to failure than fresh AM.[47] In addition, lyophilized AM grafts are thinner and more fragile than fresh and cryopreserved AM.[47] However, all graft types show similar suture retention properties.[47] At the microscopic level, graft preservation led to thinning or even disruption of the compact layer with decreased levels of collagens I and III, fibronectin, and laminin.[47] Nonetheless, the basement membrane was well preserved with cryopreservation and lyophilization. Integrity of the basement membrane may be crucial for cell adhesion when using AM as a scaffold in tissue regeneration or engineering.[47] Interestingly, lyophilized AM allowed for greater host cell adhesion and viability than fresh or cryopreserved AM, perhaps because there is less competition between native AM epithelial cells and donor cells.[47]

INTRINSIC PROPERTIES OF ACELLULAR AMNIOTIC MEMBRANE GRAFTS

Some benefits of AM are maintained even after the cellular component is no longer viable. This has been evaluated,

TABLE 1
Intrinsic Properties of Preserved
Amniotic Membrane Grafts

Provide a matrix for cellular migration and proliferation
Enhance the wound-healing process
Nonimmunogenic
Reduce inflammation
Reduce scar formation
Antibacterial
Reduce pain at application site
Natural biologic barrier

although by testing grafts that have been cryopreserved or lyophilized. This is due, in part, to the preservation of both growth factors and the structure of the extracellular matrix.[27] The acellular membrane allows for cell adhesion and proliferation,[27] leading to more organized wound healing rather than scar formation.[12] While much of the excitement about the use of AM in sports medicine centers on its role as a source of pluripotent cells or as a scaffold for mesenchymal tissue engineering, most of the current uses of AM use the intrinsic properties of preserved grafts.[12] A summary of these properties is shown in Table 1.

AMNIOTIC MEMBRANES AS A SOURCE OF STEM CELLS

Amniotic membranes have revealed themselves as an excellent source of pluripotent cells.[25] The major advantage of AM-derived stem cells over fetal stem cells is the relative lack of ethical concerns over their harvest. Amniotic membrane–derived cells are gathered from the placenta of volunteers after childbirth—tissue that would otherwise be discarded. A key concern when using any pluripotent cell population for clinical applications is the risk of tumorigenesis. As many as 50 cases of amniotic stem cell transplantation have been reported in the treatment of lysosomal storage diseases, with no reports of tumor formation.[41] In animal models, AM-derived stem cells have failed to show malignant behavior, even in immunocompromised hosts.[43] In addition, studies have confirmed the genetic stability of cultured AM-derived stem cells.[41]

Amniotic Membrane Stem Cell Types

When discussing AM as a source of stem cells, it is important to distinguish between amniotic epithelial cells (AECs) and amniotic mesenchymal stromal cells (AMSCs).[25] AECs are maternal-derived cells that have the stereotypical markers of epithelial cells such as cytokeratin. They also express stem cell markers such as OCT-4, SOX-2, Lefty-A, FGF-4, REX-1, TDGF-1, crypto-1, and Nanog[41] and have the ability to differentiate into all 3 germ layers.[42] AMSCs, in contrast, are derived from the embryonic mesoderm and also have the ability to differentiate into mature cell lineages from the mesoderm, endoderm, and ectoderm.[74] AECs are easily removed from AM by using trypsin enzymatic digestion.[25] Isolation of AMSCs requires a 2-step process in which AECs

are first removed with trypsin, and AMSCs are released from their surrounding matrix by collagenase and DNAse treatments.[25] Both AECs and AMSCs can differentiate into classic mesodermal lineages for orthopaedic tissue engineering, including myocytes, osteocytes, and chondrocytes.[18,65]

Immunomodulatory Effects

During gestation, the maternal immune system develops a tolerance for the semi-allogeneic fetus, a process that is at least in part regulated at the level of the AM.[15] A full review of these mechanisms is beyond the scope of this article, as it involves interactions between the fetus, mother, placenta, and amnion. We focus rather on the intrinsic immunomodulatory properties of AM. These are related at least in part to the depletion or absence of major histocompatibility antigens (human leukocyte antigen [HLA] classes A, B, DR) on the surface of AM-derived stem cells.[38] Extensive research into the immunomodulatory properties of AM-derived stem cells has been performed and has shown effects on both the innate and adaptive immune systems.[19] In addition, anti-inflammatory cytokines contained within the AM matrix contribute to its privileged immune status.

The effect of AM-derived stem cells on the innate immune system is largely modulated by their ability to prevent the differentiation of monocytes into dendritic cells—a critical cell type for antigen presentation.[37] Several putative soluble factors involved in this process are CCL2, CXCL8, or interleukin (IL)–6.[19] In addition, AM-derived stem cells are essentially immune to lysis by natural killer (NK) cells, important effector cells of the innate immune system.[19] This immunity is thought to be mediated by migration inhibitory factor, a potent inhibitor of NK cell-mediate lysis.[19]

AM-derived stem cells also have well-documented interactions with the adaptive immune system. When grown in coculture with AM-derived stem cells, T cells show decreased proliferation in response to polyclonal antigens.[19] This inhibitory effect is thought to be mediated by cell-to-cell pathways, in contrast to the effect of AM-derived stem cells on the innate immune system.[68] This inhibition is dependent on the state of T-cell activation. Indeed, naive and memory T cells are highly sensitive to the effects of AM-derived stem cells, while activated T cells are not.[2] The production of cytokines by T cells during a mixed lymphocyte reaction is also affected by co-culture with AM-derived stem cells. There are significant increases in IL-2, IL-4, IL-7, and IL-15 while levels of IL-17 and interferon–γ (IFN-γ) are downregulated.[21] TGF-β and IL-10 are also thought to play a critical role in the immunosuppressive abilities of AM-derived stem cells.[5]

ANTIMICROBIAL AND ANTIVIRAL EFFECTS

AM has well-documented antimicrobial effects. It has antibacterial efficacy against gram-positive cocci, including *Streptococci* and *Staphylococcus aureus*, as well as gram-negative bacilli, including *Escherichia coli* and *Pseudomonas aeruginosa*.[23,24] These effects can be attributed to the production of antimicrobial molecules by AM cells, including bactricidin,

β-lysin, transferrin, and 7S immunoglobulin.[13] Other secreted factors that may have antimicrobial properties are elafin, leukocyte proteinase inhibitor, human β3 defensin, and cystatin E.[26,55] However, some investigators believe that the antimicrobial properties of AM are only related to its properties as a barrier against microbial inoculation.[38]

REGULATORY EFFECTS OF AMNIOTIC MEMBRANES ON ANGIOGENESIS

Angiogenesis, also known as neovascularization, involves the formation of capillaries from preexisting microvessels.[48] Angiogenesis is critical to normal wound and fracture healing.[48] In contrast, excessive, pathologic angiogenesis is involved in tumoral processes as well as inflammatory arthropathies and chronic inflammatory conditions.[48] The effects of AM grafts and AM-derived cells on angiogenesis are poorly understood.[36] Most AM-derived cells express thrombospondin-1 as well as metalloproteinase inhibitors TIMP (tissue inhibitors of metalloproteinases)–1, -2, -3, and -4, all of which are known antiangiogenic factors.[17] In addition, these proteins can be found in denuded AM without cells. In contrast, other studies have shown that AM-derived cells express proangiogenic factors such as vascular endothelial growth factor (VEGF), IL-8, angiogenin, IFN-γ, IL-6, bFGF, EGF, and platelet-derived growth factor (PDGF). In an elegant animal study, it has been shown that the epithelial component of AM is an inhibitor of angiogenesis, while the mesenchymal component increases angiogenesis.[48] This provides a unifying theory for the seemingly contradictory data from earlier studies. It is clear that depending on the intended use for AM grafts or AM-derived cells, the epithelial or mesenchymal subunits may be best suited based on their role in neovascularization.[48]

ANTISCARRING EFFECTS OF AMNIOTIC MEMBRANES

One of the purported advantages of AM grafts is their ability to promote scar-free healing.[39] TGF-β is a soluble factor that is known to induce fibrotic responses through activation of fibroblasts. AM, in turn, inhibits the expression of TGF-β, reducing scar formation.[39] Hyaluronic acid in the mesenchymal portion of AM is thought to be the primary inhibitor of TGF-β.[11] In addition, amniotic mesenchymal and epithelial cells contain growth factors involved in epithelialization and wound healing such as EGF, keratinocyte growth factor (KGF), keratinocyte growth factor receptor (KGFR), hepatocyte growth factor (HGF), and hepatocyte growth factor receptor (HGFR).[26]

COMMERCIALLY AVAILABLE HUMAN AMNIOTIC MEMBRANE–DERIVED PRODUCTS

As of February 2015, there were 14 companies or distributors that provide commercially available AM-derived products (Table 2; see also the online Appendix, available at http://ajsm.sagepub.com/supplemental). Many use proprietary processing methods, and most others have not made their processing methods public. Given the known effects of AM processing on its mechanical, biological, and cellular properties, clinicians and investigators are encouraged to communicate with vendors about their tissue processing before using an AM product. A variety of formulations are available, including sheets, dehydrated sheets, tubes, liquids, and powders (Table 2). To date, little to no research has been done on the effect of AM formulation on intended clinical use, and one cannot assume equivalency of the different formulations.

CURRENT CLINICAL USES OF HUMAN AMNIOTIC MEMBRANE–DERIVED PRODUCTS

One common use of AM is in ophthalmology[38] as a treatment of corneal surface injury, as a reconstructive scaffold after resection of ocular surface lesions, and as a promoter of limbal stem cell regeneration.[38] AM has also been widely applied by plastic surgeons and wound specialists. Its use in wound care is now a US$100 million market as it has been successfully used in humans as a biological dressing for burns as well as acute and chronic wounds.[11] Furthermore, it has been shown to reduce adhesion formation after flexor tendon repair and has been used as a nerve wrap or conduit.[11] Podiatrists and orthopaedic foot and ankle surgeons have demonstrated the efficacy of AM in accelerating and improving healing of diabetic foot ulcers and postoperative infected or nonhealing wounds.[8,57,59,64,66,69,71-73]

CURRENT INVESTIGATIVE USES OF HUMAN AMNIOTIC MEMBRANE–DERIVED PRODUCTS IN SPORTS MEDICINE

The use of AM products in sports medicine is far less documented and to date is entirely experimental. A summary of the in vivo and in vitro studies pertaining to the use of AM products in sports medicine is shown in Table 3. The primary indications being explored are cartilage restoration,[‡] ligament and tendon healing,[§] nonoperative treatment of knee osteoarthritis,[67] and plantar fasciitis.[16,70]

The use of AM products for cartilage restoration has gathered momentum in recent years. Since 2007, ten studies have been published investigating this approach. Four of these studies used animal models[14,20,35,65] (rat or sheep) while the others were cell culture studies.[10,29,34,50,60,75] Three major strategies have been employed in these early studies: (1) using AM as a scaffold for bone marrow–derived mesenchymal stromal cell (MSCs), (2) using AM as a scaffold for delivery of chondrocytes, and (3) inducing AM-derived pluripotent cells toward a chondrogenic phenotype.[∥] The cell culture studies provide robust evidence that in vitro, AM-derived pluripotent cells (both amniotic epithelial cells and AMSCs) can be directed toward a chondrogenic fate using bone morphogenetic protein (BMP)–2,

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‡References 10, 14, 20, 29, 34, 35, 50, 60, 65, 75.

§References 3, 4, 7, 9, 30-32, 45, 46, 51-53.

∥References 10, 14, 20, 29, 34, 35, 50, 60, 65, 75.

TABLE 2
Commercially Available Amniotic Membrane Allograft Products in Orthopaedics[a]

Product (Company)	Composition	Growth Factors	Processing	Storage	Shelf Life	Available Configurations	Proposed Effects and Uses
Amniofix (MiMedx)	Composite amniotic tissue membrane	EGF, KGF, HA, IL-6	PURION process[b]	Ambient conditions	5 y	• Sheet/membrane • Particulate • Wrap	• Protects the collagen matrix and its natural properties • Reduces scar formation • Reduces inflammation in the surgical site • Enhances soft tissue healing area • Acts as a barrier to reduce the amount of scar tissue formation • Use in surgical, soft tissue, tendon, and nerve applications
AmnioFLEX (SurgiLogix)	Cryopreserved amnion and amniotic fluid	PDGF-AA, PDGF-BB, bFGF, TGF-βl, EGF, FGF, VEGF	NL ("aseptic processing" via cryo-preservation)	Refrigerator/ freezer	NL	• Liquid	• Localized soft tissue covering • Provides a structural matrix for use in filling soft tissue defects, inflammation, and injury • Presence of viable cells may provide ancillary clinical benefits by enhancing the body's natural regenerative process • Proposed uses to address areas of inflammation and pain, fill soft tissue defects, and address tendon and ligament injuries, cartilage injuries, muscle injuries, fascial injuries, injured nerves, tendinosis, osteoarthritis, or joint tendinitis
Epifix (MiMedx)	dHACM allograft (single layer of epithelial cells, basement membrane, and an avascular connective tissue matrix)	EGF, KGF, HA, IL-6	PURION process	Ambient conditions	5 y	• Sheet/membrane • Particulate	• Preserves and delivers multiple ECM proteins, growth factors, cytokines, and specialty proteins • Regenerates soft tissue • Acute and chronic wound care • Enhances healing • Reduces inflammation • Reduces scar tissue formation
Neox (Amniox Medical Inc)	Cryopreserved human AM/UC	EGF, KGF, HA, IL-6	CRYOTEK process[c]	Refrigerator/ freezer	3 mo at 1°C to 10°C; 1 y at −49°C to 0°C; 2 y at −85°C to −50°C	• Sheet/membrane • Particulate (Neox Flo)	• Modulate inflammation • Wound covering for dermal ulcers and defects
Clarix (Amniox Medical Inc)	Cryopreserved human AM/UC	EGF, KGF, HA, IL-6	CRYOTEK process	Refrigerator/ freezer	3 mo at 1°C to 10°C; 1 y at −49°C to 0°C; 2 y at −85°C to −50°C	• Sheet/membrane • Particulate (Clarix Flo)	• Use as a surgical covering, wrap, or barrier • Modulates a local wound environment to simulate fetal scarless healing
Flograft, Flograft FREEDOM (Applied Biologics LLC)	Amnion and amniotic fluid	NL	NL ("aseptic processing" via cryo-preservation)	Refrigerator/ freezer	NL	• Liquid	• Soft tissue defect filler • Soft tissue trauma • Tendinitis • Tendinosis • Chronic wounds • Localized inflammation • Joint pain • Strains, partial tears (muscles, ligaments, tendons)
XWRAP HYDRO PLUS (Applied Biologics LLC)	Chorion-free, human amnion allograft	NL	NL	Ambient conditions	1 y	• Sheet/membrane	• Soft tissue covering to reduce or minimize scar tissue formation near or on the dura or surgical sites • Carpal tunnel • Nerve wrap • Rotator cuff • Tendon repair • Placed beneath plate/hardware for difficult fractures
BIoDRestore (BioD LLC)	Amniotic membrane	IL-1 receptor antagonist, TIMP, PDGF, VEGF, EGF, FGF, TGF-β	NL	−65°C or cooler	NL	• Liquid	• Soft tissue injuries • Tendinitis • Plantar fasciitis • Inflamed nerves • Muscle tears • Repetitive motion injuries
NuCel (NuTech)	Amnion and amniotic fluid	NL	NL	NL	NL	• Liquid	• Provides an enhanced environment for tissue growth, repair, and healing • Offers antiscarring and anti-inflammatory properties

[a]AM/UC, amniotic membrane and umbilical cord; bFGF, basic fibroblast growth factor; dHACM, dehydrated human amnion/chorion membrane; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; HA, hyaluronic acid; IL, interleukin; KGF, keratinocyte growth factor; NL, not listed; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor–β; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

[b]The PURION process of cleaning, dehydration, and sterilization safely and carefully separates placental tissues, cleans and reassembles layers, and subsequently dehydrates the tissue to preserve the key elements associated with healing. The amniotic membrane scaffolding is protected while blood components are removed, so an intact extracellular matrix is left behind. No chemical cross-linking or decellularization occurs during the process.

[c]The CRYOTEK process uses cryopreservation (deep freezing) to maintain the innate biological and structural integrity of the natural tissue while maintaining its natural hydrated state.

TABLE 3
Basic Science and Clinical Studies on the Use of AM-Derived Products in Sports Medicine

Authors (Year)	Journal	Study Design	Amniotic Product Type	Study Subjects	Results
Cartilage regeneration					
Liu et al[35] (2014)	*Genet Mol Res*	Animal model (rabbit)	Control vs human acellular AM vs human acellular AM loaded with bMSCs	24 rabbits	AM loaded with bMSCs resulted in greater cartilage fill and improved Wakitani cartilage scores compared with control and AM alone in femoral condyle defects
Lindenmair et al[34] (2014)	*Cell Tissue Bank*	Cell culture	Fresh living human AM	NA	Fresh living human AM grown in chondrogenic conditions accumulated GAGs and collagen II, expressed chondrogenic markers
Garcia et al[14] (2014)	*Curr Stem Cell Res Ther*	Animal model (sheep)	Control vs fresh AM vs cryopreserved AM vs cryopreserved AM loaded with bMSCs	12 sheep	All treatments resulted in improved ICRS and O'Driscoll scores compared with control in femoral condyle defects; no difference between groups
Nogami et al[50] (2012)	*Transplantation*	Cell culture	Human amniotic MSCs	NA	Human amniotic MSCs can be induced to a chondrocyte phenotype with chondrogenic medium and BMP-2
Zhou et al[75] (2011)	*Int Orthop*	Cell culture	Human amniotic epithelial cells	NA	Human amniotic epithelial cells expressed chondrocyte markers (SOX-9, collagen II and X, aggrecan, CEP-68) when grown in the presence of BMP-7
Tan et al[60] (2011)	*Cell Tissue Bank*	Cell culture	Air-dried AM vs freeze-dried AM in culture with bMSCs	NA	bMSCs adhered to dried AM and when grown in chondrogenic medium led to increased GAG production
Krishnamurthy et al[29] (2011)	*J Biomed Mater Res A*	Cell culture	Air-dried human AM vs freeze-dried human AM vs control seeded with rabbit chondrocytes	NA	Chondrocytes adhered to both freeze-dried and air-dried AM, and cell proliferation and GAG production were increased in both AM groups compared with monolayer culture on plastic
Diaz-Prado et al[10] (2010)	*Cell Tissue Bank*	Cell culture	Freeze-dried human AM seeded with human chondrocytes	NA	Chondrocytes grew on the stromal side of AM, not on the epithelial side; AM seeded with chondrocytes maintained a chondrocyte phenotype in an OA cartilage tissue culture system, and the membrane integrated with surrounding cartilage
Wei et al[65] (2009)	*Cloning Stem Cells*	Cell culture and animal model (rat)	Human amniotic MSCs	NA	Amniotic MSCs expressed more chondrocyte markers than did amniotic epithelial cells; BMP-2 can induce a chondrocyte phenotype in amniotic MSCs in vitro and in vivo; collagen I gels loaded with amniotic MSCs and BMP-2 can fill cartilage defects in rat femoral condyles
Jin et al[20] (2007)	*Tissue Eng*	Cell culture and animal model (rabbit)	Fresh vs denuded human AM seeded with rabbit chondrocytes	12 rabbits	Chondrocytes preferentially grew and expressed collagen II on the stromal side of denuded AM; denuded AM seeded with chondrocytes regenerated hyaline cartilage in femoral condyles
Tendon and ligament healing					
Kueckelhaus et al[30] (2014)	*Eplasty*	Animal model (rat)	Amnion-derived cellular cytokine solution (supernatant of amniotic MSCs in culture) vs control	NA	Amnion-derived cellular cytokine solution improved the mechanical properties of Achilles tendon repairs and increased collagen production and cross-linking at the repair site
Philip et al[53] (2013)	*Eplasty*	Animal model (rat)	Amniotic MSCs vs amnion-derived cellular cytokine solution vs control	NA	Amniotic MSCs improved the Young modulus and ultimate failure strength of Achilles tendon repairs compared with amnion-derived cellular cytokine solution and control
Muttini et al[46] (2013)	*Res Vet Sci*	Animal model (horse)	Ovine amniotic epithelial cells	12 horses	Ovine amniotic epithelial cells integrated at the healing site of chronic exertional tendinopathy and supported healing by production of collagen I
Lange-Consiglio et al[32] (2013)	*Cytotherapy*	Animal model (horse)	Horse amniotic MSCs vs bone marrow–derived MSCs	95 horses	Amniotic MSCs resulted in decreased reinjury rates compared with bMSCs when injected into sport-induced superficial digital flexor tendon lesions
Lange-Consiglio et al[31] (2013)	*Stem Cells Dev*	Animal model (horse)	Horse amniotic MSCs conditioned medium vs control	13 horses	Conditioned medium reduced reinjury rate after spontaneous tendon and ligament injuries
Barboni et al[4] (2012)	*Cell Transplant*	Animal model (sheep)	Ovine amniotic epithelial cells vs control	NA	Ovine amniotic epithelial cells injected into Achilles tendon defects resulted in improved mechanical properties, ECM remodeling, and collagen maturation compared with controls

(continued)

TABLE 3
(continued)

Authors (Year)	Journal	Study Design	Amniotic Product Type	Study Subjects	Results
Barboni et al[3] (2012)	*PLoS One*	Cell culture	Ovine amniotic epithelial cells	NA	Amniotic epithelial cells cocultured with tenocytes adopted a tenocyte phenotype in vitro
Ozboluk et al[51] (2010)	*J Hand Surg Eur Vol*	Animal model (rabbit)	Fresh human AM	42 rabbits	Application of fresh human AM to flexor tendon repaired the incidence and severity of adhesions without compromising repair strength
Muttini et al[45] (2010)	*Vet Res Commun*	Animal model (sheep)	Ovine amniotic epithelial cells vs control	3 sheep	1 mo after injection of AECs into Achilles tendon defects, the AECs were still alive without adverse reaction; abundant ECM and collagen deposition in the defect were seen
Coban et al[7] (2009)	*Foot Ankle Surg*	Animal model (rat)	Human fresh AM vs amniotic fluid vs control	36 rats	Injection of fresh AM with or without amniotic fluid did not improve the histological appearance of healing Achilles tendons
Ozgenel[52] (2004)	*J Bone Joint Surg Br*	Animal model (chicken)	Human fresh AM $\pm$ HA vs control	144 tendons	Application of fresh human AM with HA reduced adhesion formation in flexor tendon repairs without compromising the biomechanical properties of the repaired tissues
Demirkan et al[9] (2002)	*Arch Orthop Trauma Surg*	Animal model (chicken)	Human fresh AM vs control	NA	Application of fresh human AM reduced adhesion formation in flexor tendon repairs without compromising the biomechanical properties of the repaired tissues
Knee osteoarthritis Willett et al[67] (2014)	*Arthritis Res Ther*	Animal model (rat)	Micronized dehydrated human AM vs control	NA	Micronized dehydrated human AM had no effect on intact cartilage; in an OA model, however, the micronized AM reduced the number and severity of chondral lesions and helped maintain higher proteoglycan levels
Plantar fasciitis Hanselman et al[16] (2015)	*Foot Ankle Int*	Human RCT	Cryopreserved human AM	23 patients	No difference between cryopreserved AM and corticosteroids for the treatment of plantar fasciitis; no adverse side effects from AM injection
Zelen et al[70] (2013)	*Foot Ankle Int*	Human RCT	Micronized dehydrated human AM	45 patients	AOFAS and pain scores improved with micronized AM compared with saline injections

AEC, amniotic epithelial cell; AM, amniotic membrane; AOFAS, American Orthopaedic Foot and Ankle Society; BMP, bone morphogenetic protein; bMSC, bone marrow stromal cells; ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronic acid; ICRS, International Cartilage Repair Society; MSC, mesenchymal stromal cell; NA, not available; OA, osteoarthritis; RCT, randomized controlled trial.

BMP-7, or chondrogenic medium.[10,29,34,50,60,75] Chondrogenic differentiation was confirmed by the expression of SOX-9, type II and type X collagen, and aggrecan.[14,20,35,65] In addition, it has been shown that AM is a suitable scaffold for bone marrow–derived MSC adhesion and chondrogenic differentiation,[35,60] as well as mature chondrocyte adhesion and proliferation.[10,20,29] All of these strategies led to filling of full-thickness chondral defects in animal models. However, these treatments have not been compared against other standard approaches such as microfracture, and to date, there are no human studies available. In summary, AM offers promise as an alternative to collagen I/III membrane scaffolds for 2-stage cartilage repair and as a source of pluripotent cells that does not require a morbid harvest (such as a bone marrow aspirate or a cartilage biopsy specimen).

Another promising field of investigation with wide applicability in sports medicine is the use of AM-derived products to enhance tendon and ligament healing. A single-cell culture study showed that AM-derived epithelial cells assume a tenocyte phenotype when cocultured with mature tenocytes.[3] The 11 other studies available investigated the use of various AM-derived products in animal models (horse, sheep, rabbit, chicken, and rat).[4,7,9,30-32,45,46,51-53] The studies in horses were veterinary clinical trials, rather than controlled laboratory experiments.[31,32,45] These 3 studies totaled approximately 100 horses and showed that reinjury rates were reduced with amniotic epithelial cells, AMSCs, and even conditioned medium extracted from AMSC cultures.[31,32,45] These products had better clinical outcomes than placebo and bone marrow–derived MSCs.[31,32,45] The remaining animal studies were controlled laboratory experiments using Achilles[4,7,30,45,53] and digital flexor tendons[9,51,52] as healing models. While an injection of fresh AM into a healing Achilles tendon did not improve the histologic appearance of the tendon, conditioned medium from AMSC cultures and, to a greater degree, the MSCs themselves increase collagen production and cross-linking in the healing tendon, resulting in improved biomechanical characteristics.[7,30,53] Fresh AM membranes were used for

a different purpose in flexor tendon healing models. In this scenario, the membranes were used for their ability to reduce adhesion formation, which they reliably did in rabbit and chicken models, without compromising the mechanical properties of the healing tendons.[9,51,52]

A single study has investigated the use of micronized dehydrated AM for intra-articular use in knee osteoarthritis.[67] In a rat osteoarthritis model, micronized AM reduced the number and severity of chondral lesions and maintained higher circulating proteoglycan levels.[67]

The highest level of clinical evidence supporting the use of AM in sports medicine is for its use in plantar fasciitis. Two randomized clinical trials have been performed in this setting. The first demonstrated that micronized AM resulted in improved functional outcome scores compared with saline injections.[70] The most recent study demonstrated that injection of cryopreserved human AM had similar functional outcomes as corticosteroid injections for plantar fasciitis, without any documented adverse effects.[16]

ONGOING CLINICAL TRIALS USING HUMAN AMNIOTIC MEMBRANE–DERIVED PRODUCTS

A query of www.clinicaltrials.gov indicates that 40 clinical trials investigating the use of AM are registered with the site. Of these, only 3 are in the field of orthopaedics. One study investigating the use of AM in reducing scar formation after total knee arthroplasty has completed enrollment, but the data are not yet available. The other study has not yet opened to enrollment and will assess the effects of AM on outcomes of zone II flexor tendon repairs. Finally, a study comparing injection of AM allograft with hyaluronic acid and placebo in the treatment of knee osteoarthritis will soon be open to patient enrollment. A study investigating the use of particulate AM injections for lateral epicondylitis was prematurely terminated by the supplier in March 2014.

REGULATORY AND ETHICAL ISSUES IN THE USE OF HUMAN AMNIOTIC MEMBRANE–DERIVED PRODUCTS

Fresh human AM falls under the supervision of the Center for Biologics Evaluation and Research (CBER) at the US Food and Drug Administration (FDA). Specifically, fresh AM falls under Section 361 as it is an intact human tissue. Similar "361 products" include ligaments, tendons, cartilage, and bone. All other variations of human AM products are categorized as Section 361 only if they satisfy the FDA's regulatory definitions. This decision is made based on the concept of "minimal manipulation"—meaning that anything more than minimal manipulation of AM categorizes it as a drug or biologic, which is the 351 pathway, requiring a more onerous and costly route to approval. Specifically, a Biologics License Application (BLA) and an Investigational New Drug (IND) application need to be completed.

Since 2000, the FDA has indicated its position on several questions that may help guide researchers and

companies intending to use human AM products. The first principle is that AMs seeded with allogeneic cells (including stem cells) are considered a biologic "351 product" and require a BLA and IND. However, dehydrated and decellularized AM is considered a "361 product" only as a wound covering. All other uses are considered nonhomologous. Finally, micronized cryopreserved AM products are more than minimally manipulated and have to undergo a BLA and IND for any intended clinical use.

On the basis of these guidelines, essentially all of the investigational uses of AM in sports medicine would be considered off-label. Early adopters using AM products therefore have an ethical obligation to disclose to patients when these products are being used off-label. In addition, all patients should be educated about the origin and preparation of AM products, given the potential for personal, religious, or political opposition to their use.

Finally, given the expense associated with the use of AM, the sports medicine community should focus on identifying the correct indications for its use and demonstrating clinical efficacy and cost-effectiveness through rigorous studies before encouraging its widespread use.

CONCLUSION

Human AM-derived products represent a wide variety of tissues and cell populations that show promise in the field of sports medicine. Broadly speaking, human AM can be used as a source of pluripotent cells, but the relative benefits and drawbacks of amniotic epithelial cells and AMSCs have yet to be elucidated. Growth factor–rich medium from pluripotent amniotic cell cultures also shows promise in enhancing tissue healing and regeneration and may find similar applications as platelet-rich plasma. Finally, AM can be used in fresh, cryopreserved, or lyophilized forms as a barrier against adhesion formation, a scaffold for musculoskeletal tissue engineering, or a carrier for biologics and therapeutic cell populations.

Amniotic membrane–derived products have the advantage of minimizing the ethical issues shared by embryonic stem cells while still having the promise of an easily attainable population of pluripotent cells and soluble factors that promote scarless, fetal-type healing. Nonetheless, the regulatory environment around AM products is complex and evolving, and translational applications should be carefully assessed before recommending their clinical application.

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