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A Review Paper

Application of Amniotic Tissue in Orthopedic Surgery

Nathanael Heckmann, MD, Richard Auran, BS, and Raffy Mirzayan, MD

Abstract

The use of amniotic tissue in orthopedic surgery has increased in recent years. While more studies are needed to fully understand the therapeutic potential and define the appropriate applications of these tissues, basic science and clinical studies are available that indicate promising results. This review will highlight these studies as they relate to the musculoskeletal system.

The amniotic membrane is a multilayer tissue forming the innermost layer of the amniotic sac that surrounds the developing fetus. It is comprised of 5 layers, from the inside out: a single layer of epithelial cells, a thick basement membrane, a compact layer, a fibroblast layer, and a spongy layer that abuts the surrounding chorion (**Figure 1**).[1] The amniotic membrane serves several functions, including synthesis of growth factors and cytokines, regulation of pH, transport of water and solutes, and provision of a permeable barrier to amniotic macromolecules.[2]

Amniotic epithelial cells are derived from the pluripotent epiblast at approximately day 8 of gestation. This is well before gastrulation occurs at days 15 to 17, considered the "tipping point" when pluripotent cells differentiate into ectoderm, mesoderm, and endoderm.[3] These cells express Oct-4 and Nanog, 2 molecular markers that are indicative of pluripotency.[3] Two cell types have been identified in amniotic tissues that possess stem cell-like characteristics: human amniotic epithelial cells and human amniotic mesenchymal stromal cells.[4] Both of these cell types have demonstrated the ability to differentiate into various cell lineages, includ-

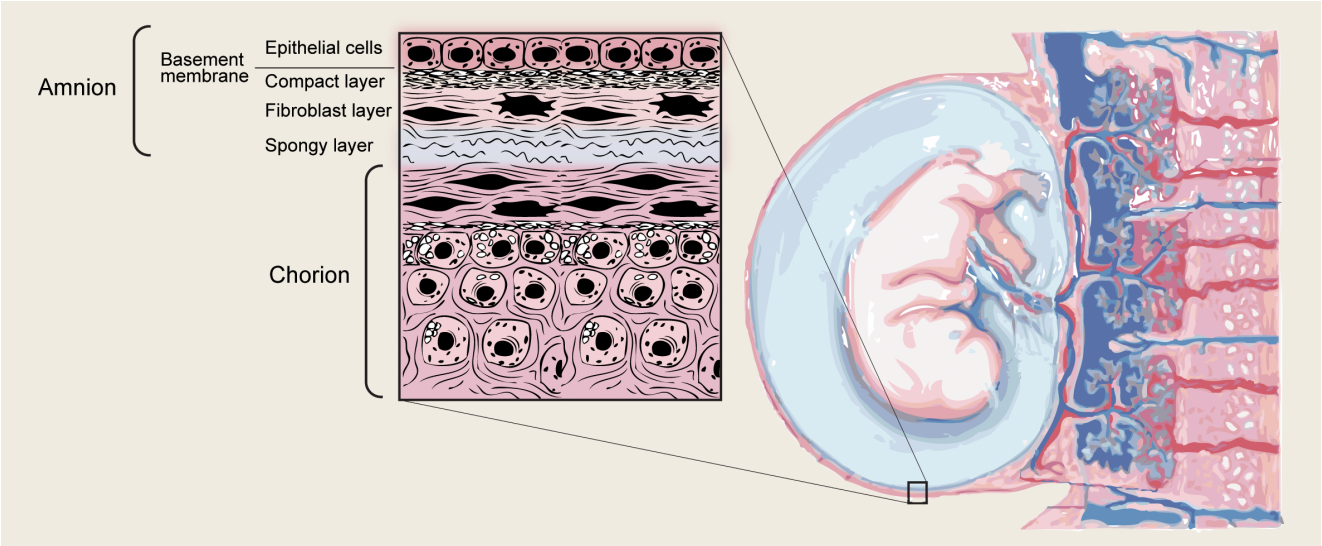

Figure 1. Anatomy of the amniotic membrane

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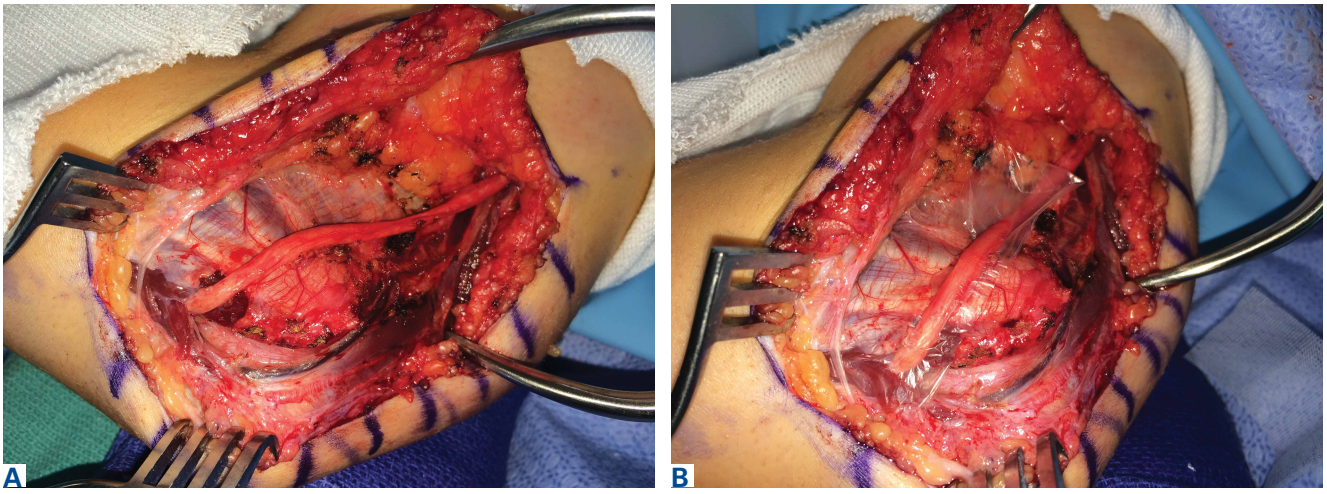

Figure 2. (A) Ulnar nerve transposition/decompression. (B) Dehydrated, cross-linked amniotic membrane placed around the nerve to prevent adhesions.

ing endothelial cells, adipocytes, myogenic cells, neurogenic cells, chondrocytes, tenocytes, and osteogenic cells.[5-7] These previously reported findings indicate that amniotic cells and tissue have the capability to generate mesenchymal tissues.

FDA Classification and Available Forms

The US Food and Drug Administration (FDA) classifies amnion as an allograft tissue under Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) 361. To meet criteria, the tissue needs to be minimally manipulated. It is to be for homologous use and cannot be combined with other cells or tissues. There can be no systemic effect or dependence on the metabolic activity of living cells to achieve its primary function. The tissue has to have a localized effect in vivo. Therefore, amnion allograft tissue can be commercialized, provided it is not marketed as a stem cell product or to contain viable cells.

Amniotic tissue is commercially available in several forms. These include fresh-frozen injectable amniotic liquid that may contain viable amniotic cells and/or particulated amniotic membrane, a micronized freeze-dried (lyophilized) particulate powder that is directly applied to a wound or resuspended for injection, and a cross-linked dehydrated membrane acting as an adhesion barrier (**Figure 2**).

Safety

Amniotic tissue has been used for over 100 years in burn, ophthalmology, and chronic wound patients with favorable outcomes and no adverse effects reported in the literature. Unlike embryonic stem cells, which may be tumorigenic,[8] amniotic cells do not possess any known tumorigenicity.[9] In one study, 50 immunodeficient mice were injected with 1 to 2 million amniotic epithelial cells and observed for a maximum of 516 days with no tumorigenicity observed in any of the animals.[10] In another study, amniotic epithelial cells were implanted into the forearms of healthy volunteers and no immunologic response was observed in any of the recipients.[11] Furthermore, viable amniotic cells were recovered via biopsy 7 weeks following transplantation, demonstrating viability of the transplanted cells.[11] The lack of tumorigenicity and immunologic response in hosts is due in part to the fact that amniotic cells do not express human leukocyte antigen class II antigens and only express class I antigens in small amounts.[3]

Advantages of Amnion Tissue

Amniotic tissue is readily available, as it is often discarded after childbirth. The use of this tissue poses no added risk to the fetus or mother, eliminating the ethical concerns associated with obtaining embryonic stem cells. Amniotic tissue is comprised of an extracellular matrix, which acts as a natural scaffold for cellular attachment and structural support for cells as well as collagen types I, III, IV, V, and VI, hyaluronic acid, and a host of growth factors.[12] In addition, it possesses antimicrobial properties, including beta-defensins.[13]

Amniotic tissue has been shown to exert an anti-inflammatory effect by inhibiting the inflammatory cascade. Specifically, it has been shown to inhibit cytokines such as tumor necrosis factor-alpha in the presence of dendritic cells,[14] as well as inhibiting transforming growth factor-beta, interleukin-8, and fibroblast proliferation.[15] These

findings indicate that amniotic tissue has the ability to dampen the "cytokine storm" that occurs after an injury in an adult, which would lead to beneficial impacts on healing and scar formation in patients.[16]

Basic Science and Animal Studies

Several studies have demonstrated promising outcomes for orthopedic applications in vitro. A comparison of osteogenic potential found that amniotic fluid-derived cells were able to produce approximately 5 times more mineralized matrix than bone marrow-derived mesenchymal stem cells.[17] More recently, Si and colleagues[18] compared the osteogenic potential of human amniotic epithelial cells, amniotic cells, and human bone marrow-derived mesenchymal stem cells. They found that all 3 cell lines were osteogenic, though the amniotic epithelial cells had better immunomodulatory properties and marginally less osteogenic potential than the other 2 cell types. Furthermore, several in vivo animal studies have demonstrated the ability of human amniotic cells to stimulate bone growth in rats,[19,20] rabbits,[21] and sheep.[22]

Amniotic tissue also possesses potential for chondrogenesis. Cryopreserved human amniotic membrane cells used for in vitro human osteoarthritis tissue scaffolds did not differentiate in culture, and they integrated and repaired damaged articular cartilage.[23] Various in vitro[24,25] and animal in vivo[26,27] studies have reported similar supportive findings. Kunisaki and colleagues[28] used sheep amniotic fluid mesenchymal stem cells to reconstruct lamb tracheal cartilage in utero, concluding that cells obtained from the amniotic fluid possess chondrogenic capabilities. Further in utero lamb studies of cartilage artificial defects, given 7 days to settle before adding a hypocellular matrix as a scaffold, showed chondrocyte density and cell architecture was restored at the defect site after 28 days without the formation of an inflammatory response or scar tissue.[29]

Amniotic tissue has had similar success in tendon repair studies in vivo.[9,30,31] Barboni and colleagues[32] implanted amniotic epithelial cells (AECs) into artificially created sheep Achilles tendon defects in situ, inducing superior structural and mechanical recovery in the defects at a faster rate compared to controls not receiving AECs. Healing via AECs started at the healthy tissue around the borders of the defect and progressed centrally, suggesting recruitment of native progenitor cells to the lesion.[32] Kueckelhaus and colleagues[33] investigated the role of amnion-derived cellular cytokine solution in the healing of transections of rat Achilles tendons, reporting improved mechanical properties of healing tendons at early time points compared to controls. Beredjiklian and colleagues[34] compared the healing of transected extensor tendons of pregnant ewes and of their fetus in utero, reporting a reparative form of healing with scar formation in adult subjects and regenerative form of healing without scar formation or inflammation in fetal subjects.

Amniotic tissue has properties that prevent adhesion formation around tendons following injury and reconstruction.[35] Ozgenel[36] investigated the effects of hyaluronic acid and amniotic membrane alone and in combination on the presence of adhesions and the rate of healing following chicken flexor tendon repair. The study found amniotic membrane wrapped around the repaired tendon was superior in preventing adhesion formation. Kim and colleagues[37] report a similar reduction in fibrosis and adhesion following application of a human amniotic membrane wrap to rabbit ulnar neurorrhaphy sites.

This barrier function of amniotic tissue has also been investigated in the prevention of surgical scarring and peridural fibrosis in animal models following spinal discectomy. A study in canine models showed a reduction of scarring following the application of cross-linked amniotic membrane compared to freeze dried amniotic membrane.[38] Similar reductions in scarring in rat models with the application of freeze-dried amniotic membrane compared to negative controls have been reported.[39]

Human Studies

A randomized trial investigated the outcomes of prenatal vs postnatal repair of myelomeningocele in humans, finding a reduced need for implanted shunts and improved functional outcomes at 30 months of life in the prenatal intervention group compared to the postnatal group.[40] This study was concluded early due to the efficacy of prenatal surgery and the benefit of nervous system repair in utero in the presence of amniotic growth factors.

Vines and colleagues[41] performed a 6-patient feasibility study using amnion injections to treat symptomatic knee osteoarthritis. Each patient received a single intra-articular cryopreserved amniotic suspension allograft (ASA) injection and was followed for 1 year. No adverse outcomes were reported, with the only abnormal finding being a small increase in serum immunoglobulin G and immunoglobulin E levels. Intra-articular ASA injection was found to be safe, but a large-scale trial investi-

gating symptomatic relief was recommended.[41]

Most of the human studies using amnion pertain to foot and ankle surgery. Its use as a treatment for diabetic foot ulcers and recalcitrant plantar fasciitis was one of the early-recognized successes.[42-45] Zelen and colleagues[46] investigated the applications of injectable micronized dehydrated human amniotic/chorionic membrane as an alternative to surgical intervention in the treatment of refractory plantar fasciitis. This prospective, randomized trial with 45 patients showed significant improvement in plantar fasciitis symptoms at 8 weeks compared to controls (saline injections). A similar study compared the use of cryopreserved human amniotic membrane (c-hAM) injections to corticosteroid injections in plantar fasciitis patients.[47] The results indicated that c-hAM is safe and comparable to corticosteroids, with the authors noting that pain improvement was greatest in patients receiving 2 injections of c-hAM at 18 weeks.

Amniotic membrane-derived tissues are safe and non-tumorigenic, producing an abundance of growth factors that have shown promise as tissue scaffolds and as aids in the regeneration of human bone and soft tissues.

Tendon wrapping, in which the amniotic membrane is laid over a tendon repair, has been reported with success. Amniotic membrane is superior to collagen for tendon wrapping as it actively contributes to healing while minimizing adhesions, which collagen alone cannot do.[48] The membrane serves as a protective sheath around repaired tendons with anti-inflammatory, anti-adhesive, immunomodulatory, and antimicrobial benefits. A 124-patient study demonstrated the safety of using amnion in this manner, and the authors reported a decreased rate of complication compared to previously published data.[49] Another study of 14 patients undergoing foot and ankle surgery with tendon wrapping reported clinical improvement with reduced pain and greater functional outcomes postoperatively compared to preoperative measurements.[50]

Conclusion

Amniotic membrane-derived tissues are safe and non-tumorigenic, producing an abundance of growth factors that have shown promise as tissue scaffolds and as aids in the regeneration of human bone and soft tissues. Amnion applications in orthopedic surgery may be numerous, but development is ongoing. Given the vast array of in vitro and in vivo animal data supporting the benefits of amnion in tissue regeneration, orthopedic surgeons and researchers should place emphasis on conducting clinical studies to validate the safety and efficacy of amniotic cells in the treatment of orthopedic conditions.

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This paper will be judged for the Resident Writer's Award.