**AMNIONIC MEMBRANE ALLOGRAFTS, A VIABLE SOURCE OF MESENCHYMAL STEM CELLS FOR REGENERATIVE NON SURGICAL TREATMENT OF SPORTS INJURIES**

The cure for some chronic sports injuries can be elusive. The most challenging aspect is returning to sports without chronic pain or a permanent injury. The expanding field of regenerative medicine has offered a new solution: amnion cell injections. Recently published randomized and case studies reported improvement with amnion membrane injection (\*). The medical literature also abounds with evidence of successful treatment with platelet-rich plasma (PRP) injections.

Unlike PRP, amnion does not require the patient's own blood.  Several advantages to the amniotic membrane allograft over PRP are salient. Theoretically, they both deliver a "regenerative punch" to the injured tissues by concentrating growth factors calling into action the body's own adult stem cells to repair the damage by regrowing the nascent tissue cells instead of healing with scar tissue. Platelet-derived growth factors from PRP are less effective because adult stem cells are already significantly differentiated and unable to regrow into progenitor cell lines. To improve these odds, leukocyte-rich supernatant can be extracted from the patient, but requires a much larger volume (about a pint) of blood exfusion.

Placenta has been used for a century as a source of human donor tissue because of the regenerative properties provided by mesenchymal stem cells and lack of host rejection(\*\*). Mesenchymal stem/progenitor cells (MSCs) are present in the fetus (blood, liver, BM, and kidney) and sparsely present in the adult human body (bone marrow, kidney, lung, liver.) They possess osteogenic and adipogenic differentiation potentials under appropriate conditions. Research shows that placenta-derived cells have multilineage differentiation potential similar to MSCs in terms of morphology, cell-surface antigen expression, and gene expression patterns. The placenta is therefore a useful source of MSCs. (1)

 Properly processed, amniotic membrane preserves the complete spectrum of human growth factors. Some products actually contain living mesenchymal stem cells.  Amnion injection stimulates a much more physiologic cascade of chemotaxis, inflammatory leukocyte infiltrate and remodeling by modulation of tissue colony aggregates that similarly simulate the genesis of embryonic tissue cell lines in the fetus.  Amnion cells are also immune-privileged. Therefore, host vs. graft rejection has not been reported in the naïve host.  The reason for this immune characteristic is the fetally-derived and maternal blood cells formed by the villous trophoblastic barrier, where the syncytiotrophoblast surface permanently floats in maternal blood. Further contact is made by some extravillous cytotrophoblast cells, either located at villous tips, in so-called cell islands, or the endovascular trophoblast population within the uteroplacental spiral arteries. The third contact zone or the junctional zone within the decidua where the invading extravillous trophoblast cells, encounter all maternal tissue leukocytes, which are mainly NK cells, macrophages and T cells.  The junctional zone extends at the edge of the placenta to the amnio-chorionic membranes where the chorionic laeve trophoblast has intimate contact with decidua tissue which, even in healthy pregnancies, fetal and maternal lymphoid cells transgress the trophoblastic barrier previously thought to be completely impermeable. This contact between fetal foreign cells to the foreign immune system is the crucible for non-antigenic status of the placental cells with respect to antigens of the Major Histocompatibility complex. The role of the highly polymorphic classical class I molecules HLA -A, -B, -C, which are expressed on almost all somatic cells, is the induction of a specific immune response by presenting peptide antigens to T -lymphocytes. In contrast, the non-classical HLA class I molecules HLA-G and HLA-E are thought to be involved in the induction of immune tolerance by acting as ligands for inhibitory receptors present on NK cells and macrophages. (2)

Tendons and ligaments are some of the strongest connective tissues of the body, and as such, are subject to common overuse injuries from cumulative microtrauma. Tendon cells, or tenocytes, are elongated fibroblast type cells. The cytoplasm is stretched between the collagen fibers of the tendon. They have a central cell nucleus with a prominent nucleolus. Tendon cells have a well-developed rough endoplasmic reticulum and they are responsible for synthesis and turnover of tendon fibers and ground substance. Tendon cells form a connecting epithelial layer between the muscle. Muscle cells are attached to the collagenous myotendinous space via hemidesmosomes. The myotendon space is then attached to the base of the tendon cells via basal hemidesmosomes, while apical hemidesmosomes, which sit atop microvilli, attach the tendon cells to a thin layer of collagen. These is in turn attach to organic fibers which insert into bone.  Tendon cells appear columnar and contain a large basal cell nucleus. The cytoplasm is filled with granular endoplasmic reticulum and sparse Golgi bodies. Dense bundles of microfilaments run the length of the cell connecting the basal to the apical hemidesmosomes.  More research is needed to explore the regulation of tenocyte stem cells and their response to MSCs or placental growth factors.

Treatment of sports injuries with amniotic membrane injection offers advantages over other invasive or surgical methods. Typically the patient will be evaluated with a thorough physical exam and different imaging modalities that may include radiographs, echo sonogram or musculoskeletal ultrasound, MRI or a combination of these. Once the problem is ascertained, treatment recommendations usually involve, rest, ice, compression and elevation (RICE), Custom orthoses, bracing, athletic tape strappings and physical therapy adjunctive modalities.

However, when rapid recovery is desired, amniotic injections offer a promising alternative. The product is selected by physician’s preference and experience with its use. Several products are available in the market. Many are cryopreserved; some contain “living cells”. Others are combinations of dehydrated ultramicronized amnion-chorion membranes. All products are manufactured from donated placentas delivered from planned caesarian- section births and are processed under FDA-approved patented processes that allow for antisepsis and minimal tissue manipulation in order to qualify for human tissue implantation. All manufacturers are global US-based prestigious biotechnology companies with reputable track records.

The injection procedure involves provisions for local anesthesia, preparation of the site with an antiseptic solution, reconstitution of the dry placental particulate or thawing of the prefilled cryopreserved syringe. The injection is then applied with minimal trauma to the patient and a light sterile bandage is applied. The practitioner may opt to immobilize the extremity for a short period and recommend icing and narcotic analgesics for pain control if necessary. The convalescence period is typically short from 3-5 days of rest with return to regular activity at the patient’s own tolerance.

Costs vary by product manufacturer and range from $450 to $650 plus the physician’s fee for handling the product and fee for injection and office supplies. These costs are not covered by insurance companies but that trend may be reversing given the much lower relative cost of this office-based treatment versus the cost of surgery, anesthesiologist and hospital operating suite.

The authors have experience with tendon injuries injections including a favorable outcome for a series of 35+ Achilles tendon injections by Dr. Alberto Abrebaya and successful injections by Dr. Ben Pearl to his patients and his own injured Achilles tendon. The plantar fascia, capsule and joint areas of the foot and ankle are other areas that show promise for amnion injections. The injections are usually successful as a singular treatment event but may be repeated as necessary in different anatomical areas without limitation until complete healing is achieved. The authors refrain from prescribing post-injection corticosteroids or NSAIDS to avoid disrupting the chemotaxis of pluripotential adult mesenchymal stem cells to the treatment site.

Clinical experience is building a strong base of case-evidence that allograft injection of amniotic membrane can further enhance successful nonsurgical treatment for Tendoachilles tendinosis, tendinopathies, plantar fasciitis, other soft tissue and bone and joint maladies.   Further study of the mechanism of action of amniotic membrane on the physiology of tendon healing will inevitably unveil useful information for enhancing the successful outcomes of these types of regenerative therapies for such limiting musculoskeletal ailments, which currently embody the “Achilles Heel” of non-surgical treatments for sports injuries.

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Human Placenta-Derived Cells Have Mesenchymal Stem/Progenitor Cell Potential

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