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The Use of Decellularized Human Placenta in Full-Thickness Wound Repair and Periarticular Soft Tissue Reconstruction

An Update on Regenerative Healing

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KEYWORDS

- Amniotic membrane • Extracellular matrix • Growth factors • Placenta
- Regenerative healing

KEY POINTS

- The intrinsic properties of connective tissue matrix enhance the healing process by providing a scaffold for cell attachment, allowing the release of endogenous growth factors and cytokines.
- Micronized placenta has numerous applications in the foot and ankle, including the treatment of deep tunneling wounds and augmentation of various soft tissue and bone procedures.
- Although basic science and clinical studies show promising results, additional research is needed to better understand the applications and therapeutic benefits of amniotic tissue in regenerative medicine.

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INTRODUCTION

Injuries of the foot and ankle are a common source of pain and disability. They are especially challenging when the healing process is prolonged or incomplete. Consequently, investigators have begun searching for alternative treatment strategies. With advances in tissue engineering, decellularized placenta has been suggested as a means to achieve more rapid and complete healing.

SCIENCE AND APPLICATION
Structure

Decellularization is the process whereby cells are removed from soft tissue scaffolds to eliminate antigens that can cause an increased inflammatory process or a rejection response. It has been well described that controlling inflammation is the cornerstone to minimizing fibrosis and scar tissue formation. In order for the body to enter a pathway of *regenerative* healing that is defined as healing with minimal to no scar, 3 key events must occur. First, cells must proliferate within the soft tissue scaffold. Second, vascularization of the soft tissue scaffold must occur. Finally, the scaffold must transition into the host tissue in a functional form. Decellularized, allogenic soft tissue scaffolds may, when processed and handled correctly, provide an ideal platform for regenerative healing to occur. In 2003, Hopper and colleagues[1] first described the use of a decellularized human placenta as a vascular scaffold for large tissue engineered implants. The human placenta is a complex organ that facilitates a symbiotic relationship between mother and fetus. The placenta is rich in growth factors and, equally as important, contains a rich amount of extracellular matrix (ECM) components.[2] Since then, an increased understanding of tissue healing and regeneration has allowed scaffolds like decellularized placenta to be used in areas such as wound healing and soft tissue reconstruction.

Function

The way in which cells interact with the ECM often dictates the healing response within injured tissue. Decellularized human placental connective tissue matrix (CTM) (Interfyl, Alliqua BioMedical, Yardley, PA) contains key extracellular proteins important for cell adhesion, such as fibronectin and laminin. Pashuck and colleagues[3] demonstrated that human dermal fibroblasts, human epidermal keratinocytes, and human dermal microvascular endothelial cells adhere and proliferate on CTM. When these cells proliferate and adhere to the CTM, the stimulated cells release growth factors, such as vascular endothelial growth factor and fibroblast growth factor.[3] These same bench top data demonstrated that placental CTM support the migration of endothelial cells, prompt endothelial cell attachment, and, finally, promote the formation of endothelial tube formation.[3] Pashuck and colleagues[3] further hypothesized that CTM may act as a scaffold that can replace abnormal ECM in damaged tissue that allows endogenous cells to accelerate healing and regeneration.[3] Healing and regeneration occur through suppression of the M1 macrophage and increased expression of the M2 macrophage. The M2 macrophage is responsible for tissue remodeling. Improved and accelerated tissue remodeling coupled with endothelial tube formation and cellular migration provides an opportunity for placental CTM to be a pathway for regenerative healing.

Wound Care

Complex, nonhealing wounds present a significant challenge for foot and ankle surgeons. The environment of nonhealing wounds is wrought with deficits in the ECM,

elevated concentrations of proinflammatory cytokines and metalloproteinases, and decreased growth factor activity.[4,5] Consequently, many complex wounds fail to respond to standard treatment modalities. Chronic inflammation within the wound supports uncontrolled proinflammatory macrophages. The combination of an abnormal ECM and excess M1 macrophages does not support an environment for healing and ultimately needs to be interrupted for closure to occur.

The aforementioned bench top data presented by Pashuck and colleagues[3] demonstrate that placental CTM may be a scaffold that can help stop the uncontrolled cycle of inflammation and abnormal ECM. By providing a stable environment for cell attachment, endothelial cell formation, and ultimately the release of endogenous growth factors, CTM establishes a healthy cascade for regeneration and ultimately epithelialization.[3]

The surgical technique for the application of CTM to full-thickness wounds is very similar to the application of other allograft products. The key to successful application is aggressive debridement of the wound to eliminate all bioburden. Once all nonviable fibrinous tissue is removed, the scaffold is placed on the wound in an evenly distributed fashion. Alternative, the surgeon can use the flowable scaffold through a syringe or a particulate form out of a vial.

Plantar Fasciitis

The underlying pathologic processes of plantar fasciitis are poorly understood, though most histologic studies report degenerative changes at the plantar fascia enthesis. The most common pathologic features are deterioration of collagen fibers, increased secretion of ground substance proteins, focal areas of fibroblast proliferation, and increased vascularity.[6,7] Thus, plantar fasciitis seems to be more of a degenerative process than an isolated inflammatory process.

Plantar fasciitis is widely considered a self-limited condition[8] with spontaneous resolution of symptoms occurring in approximately 80% of patients within 12 months.[9] Initial conservative management includes a variety of therapies, including activity modification, icing, nonsteroidal antiinflammatory drugs, orthotics, splinting, and physical therapy. Approximately 10% of patients will fail to improve with conservative care, and chronic heel pain greatly impacts quality of life for many patients before resolution.[10] For patients who fail conservative treatment, other modalities have been described in the literature, including extracorporeal shock wave therapy, platelet-rich plasma injections, corticosteroid injections, and surgical interventions.

A localized corticosteroid injection has generally been considered an effective, relatively long-term solution and, therefore, a mainstay of nonsurgical treatment.[11,12] Despite their widespread use, the risks associated with corticosteroid injections include fat pad atrophy and plantar fascia rupture.[13] The degenerative pathophysiology of plantar fasciitis also calls into question the use of corticosteroids for long-term relief.

In contrast, human amniotic tissues are well known for their healing characteristics. Studies have shown that the biomechanical properties of amniotic membranes promote soft tissue healing by enhancing regenerative stages while limiting scarring and inflammation.[14,15] In a study by Zelen and colleagues,[16] patients with refractory plantar fascial pain showed a statically significant improvement in American Orthopedic Foot and Ankle Society scores over an 8-week period with micronized dehydrated amniotic/chorionic membrane allograft heel injections. More recently, Hanselman and colleagues[17] demonstrated that the use of cryopreserved human amniotic membrane was as safe and efficacious as corticosteroids when injected into patients with plantar heel pain. **Fig. 1** shows a plantar fascia injection with decellularized human placental CTM (Interfyl, Alliqua Biomedical).

Fig. 1. Plantar fascia injection with Interfyl.

Tendons

Tendon healing occurs in a series of stages.[18] Initially a fibroblastic splint is formed. During this time, the tendon is in its weakest state. During the second stage, there is an increase in paratenon vascularity and collagen proliferation. During stage 3, approximately 3 full weeks from the start of the healing process, the collagen fibers align longitudinally. During this time, the tendon shows a moderate increase in strength and controlled passive range of motion can be initiated. The fourth and final phase begins at 4 weeks, whereby the tendon has increased strength and can tolerate active mobilization.[18]

Use of amniotic membrane for tendon repair has the potential to decrease inflammation and tissue adhesion early in the tendon healing process. In animal models, investigators have confirmed the ability of amniotic cells to increase the number of proliferating tendon reparative cells,[18] improve tendon strength,[16,19] decrease adhesion formation,[19,20] and differentiate into tendonlike material.[19,21] Despite these encouraging results, there is a paucity of clinical evidence.

In a sheep model, amniotic epithelial cells were applied to calcaneal tendon defects and demonstrated the presence of amniotic cells up to 1 month after implantation.[20] Furthermore, the study showed that amniotic cells have the ability to increase the number of proliferating tendon reparative cells, which are important for collagen products and, in turn, tendon healing.[20] Similar results were found by Kueckelhaus and colleagues[19] who transected and primarily repaired the Achilles tendon in 2 groups of rats. One group received a saline injection, whereas the other received an injection of amnion-derived cellular cytokine solution (ACCS) in carboxy-methyl cellulose

(CMC) gel. The treatment group that received the ACCS in CMC had improved breaking strength, tensile strength, and yield strength early in the reparative process.[19] This finding is important, as increased healing earlier on in the process will allow for earlier rehabilitation of the involved tendon and may lead to improved functional outcomes.

In 2009, Ozböulük and colleagues[21] presented the results in rabbit models comparing 3 treatment groups. Each group underwent division of the flexor fibularis tendon with repair using a modified Kessler technique. One group underwent tendon repair (control group), one group underwent tendon repair with amniotic membrane application (treatment group 1), and the final group underwent tendon repair with application of periosteal autograft (treatment group 2).[21] Biomechanically, the group treated with tendon repair and application of periosteal autograft showed the greatest strength. Adhesion formation in both the amniotic membrane group and the periosteal autograft group were found to yield greater outcomes than the control group.[21] Although this study was conducted using a rabbit model,[21] it suggests that the application of an amniotic membrane in a tendon repair model can decrease adhesion formation and, presumably, postoperative pain.

Demirkan and colleagues[22] reported similar findings with respect to adhesion formation in a chicken model. They reported on the use of amniotic membrane in hand surgery. Three groups were compared: tendon repair alone, tendon repair with tendon sheath repair, and tendon repair with application of an amniotic membrane. The adhesions in group 3 (application of an amniotic membrane) were significantly reduced. The investigators also noted that 3 months following repair, in group 3, there was no evidence of the amniotic membrane remained, indicating full incorporation and conversion of the cells into tendinous structure.[22] These findings are further supported by Barboni and colleagues[23] who extracted amniotic epithelial cells and coincubated those cells with explanted tendons or primary tenocytes of fetal or adult calcaneal tendon. In the coincubated group of amniotic cells with fetal-derived tendon or tenocytes, the investigators reported high differentiation of the amniotic cells into tendon-like material, exhibiting a highly organized structure.[23]

Figs. 2 and 3 illustrate the injection of CTM (Interfyl, Alliqua BioMedical) for tendon sheath repair and tendon repair, respectively. Fig. 4 shows closure of the tendon sheath.

Bone

Autogenous bone graft is the gold standard for the reconstruction of bone defects and is the preferred adjunctive tissue for arthrodesis procedures. Be that as it may, there is a limited supply of autogenous bone as well as a risk of donor site morbidity. As a result, bone graft substitutes have been developed. Examples include silicone, polymethyl methacrylate, hydroxyapatite, demineralized bone matrix, and tricalcium phosphate.[24–26] These materials help to augment boney procedures, while avoiding graft procurement.

Until recently, bone autograft was the only material available with osteogenic, osteo-inductive, and osteo-conductive properties. Advancement in bioengineering has introduced a means of biologically augmenting skeletal fractures and arthrodesis procedures.

Daniels and colleagues[27] studied the efficacy and outcomes of purified recombinant human platelet-derived growth factor BB homodimer combined with beta-tricalcium phosphate (rhPDGF-BB/β-TCP-collagen) in ankle and hindfoot arthrodesis. They showed complete fusion at 24 weeks in 84% of patients treated with rhPDGF-BB/β-TCP-collagen versus 65% of patients treated with autograft. Additionally, successful

Fig. 2. Tendon sheath injection with Interfyl.

Fig. 3. Injection of Interfyl onto a tendon repair.

Fig. 4. Tendon sheath closed after application of Interfyl.

clinical outcomes at 52 weeks were achieved in 91% of rhPDGF-BB/β-TCP-collagen–treated patients versus 78% of autograft patients.[27] Platelet-derived growth factor (PDGF) has been shown to stimulate blood vessels and play a key role in promoting tissue repair.[28] The angiogenic properties of PDGF, combined with strong mitogenic chemotactic effects on mesenchymal cells, play a central role in the early phases of the healing cascade.[28]

Starecki and colleagues[29] studied the healing potential of amniotic tissue augmentation in a rat model. They created a critical-sized femoral gap in 3 separate groups: no treatment specimens, specimens treated with commercially available bone graft, and, finally, specimens treated with bone graft and amniotic tissue. The study demonstrated statistically significant potential for amniotic membrane products to provide bridging of bone defects.[29]

In 2012, Zhong and colleagues[30] showed that placenta-derived mesenchymal stem cells (PDMSCs) have a promising advantage for bone tissue engineering. In their study, PDMSCs offered advantages in availability, biological activities, cell adhesion, immunogenicity, cytokine production, and osteoblastic differentiation.[30]

The surgical technique for applying placental CTM is very straightforward for the surgeon. Each joint to be fused is prepared by denuding the articular surface and subchondral plate using curettage, and the subchondral bone is fenestrated using a 0.062-mm Kirschner wire. Approximately 2.5 cm^3 of cancellous autogenous bone is harvested from either the ipsilateral calcaneal body or proximal tibia and mixed with 100 mg of allogeneic, decellularized, particulate human placental CTM. The combined 2.5 mL/100 mg mixture is placed in each joint to be fused. Appropriate hardware is placed around the fusion as the surgeons deems necessary.

SUMMARY/DISCUSSION

During healing, the body may deposit excess fibrous collagen at the site of injury. Current measures for treating excess scarring and adhesions on traumatized tendons and chronic wounds include bovine collagen wraps, sheets of hyaluronic acid, hydroscopic polymer-based barriers (eg, polyethylene glycol), xenograft sheets, and numerous other agents to assist in healing. In published clinical studies, none of these approaches have been shown to consistently reduce the incidence of adhesions or scar formation following repair of the injured extremity. As described earlier, decellularized human placental CTM has been shown to support regenerative healing in the clinical setting[16,17,27] and through bench top data.[3] Regenerative healing includes cellular proliferation across the scaffold, endothelial penetration, and ultimately a transition to functional host tissue. This transition to functional tissue allows for an environment that has minimal scar tissue that closely resembles that of the preinjured state.

REFERENCES

1. Hopper RA, Woodhouse K, Semple JL. Acelluarization of human placenta with preservation of the basement membrane: a potential matrix for tissue engineering. Ann Plast Surg 2003;51:598–602. Available at. https://insights.ovid.com/pubmed?pmid=14646657.

2. Choi JS, Kim BS, Kim JD, et al. In vitro cartilage tissue engineering using adipose-derived extracellular matrix scaffolds seeded with adipose-derived stem cells. Tissue Eng A 2012;18:80–92. Available at. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3246412/.

3. Pashuck ET, Mao Y, Kim K, et al. A human placenta-derived decellularized connective tissue matrix (CTM) supports cellular functions involved in wound healing processes. Symposium on Advanced Wound Care. 2016.

4. Falanga V, Grinnel F, Gilchrest B, et al. Experimental approaches to chronic wounds. Wound Repair Regen 1995;3:132–40. Available at. http://onlinelibrary.wiley.com/doi/10.1046/j.1524-475X.1995.30205.x/abstract;jsessionid=8DDACCD771C11D82889D3C90F2C8DBD7.f02t03.

5. Foy Y, Li J, Kirsner R, et al. Analysis of fibroblast defects in extracellular matrix production in chronic wounds. J Am Acad Dermatol 2004;50:P168. Available at. http://www.jaad.org/article/S0190-9622(03)04002-7/pdf.

6. Lemont H, Ammirati K, Usen N. Plantar fasciitis: a degenerative process (fasciosis) without inflammation. J Am Podiatr Assoc 2003;93:234–7. Available at. http://www.drkristaarcher.com/wp-content/uploads/2012/09/plantarfasciitis.pdf.

7. Jarde O, Diebold P, Havet E, et al. Degenerative lesion of the plantar fascia: surgical treatment by fasciectomy and excision of the heel spur. A report on 38 cases. Acta Orthop Belg 2003;69:274–6. Available at. https://s3.amazonaws.com/academia.edu.documents/41953815/09-jarde-vernois-.pdf?AWSAccessKeyId=AKIAIWOWYYGZ2Y53UL3A&Expires=1502818495&Signature=JTh%2F9zzjxBPgAFPs9fIjKtL5NaQ%3D&response-content-disposition=inline%3B%20filename%3DDegenerative_lesions_of_the_plantar_fasc.pdf.

8. Buchbinder R. Clinical practice. Plantar fasciitis. New Engl J Med 2004;350:2159–66. Available at. http://www.nejm.org/doi/full/10.1056/NEJMcp032745.

9. Wolgin M, Cook C, Graham C, et al. Conservative treatment of plantar heel pain: long-term follow up. Foot Ankle Int 1994;15:97–102. Available at. http://journals.sagepub.com/doi/abs/10.1177/107110079401500303?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

10. Irving DB, Cook JL, Young MA, et al. Impact of chronic plantar heel pain on health-related quality of life. J Am Podiatr Med Assoc 2008;98:283–9. Available at. http://www.japmaonline.org/doi/abs/10.7547/0980283?code=pmas-site.

11. Genc H, Saracoglu M, Nacir B, et al. Long-term ultrasonographic follow-up of plantar fasciitis patients treated with steroid injection. Joint Bone Spine 2005;72:61–5. Available at. http://www.sciencedirect.com/science/article/pii/S1297319X04000752.

12. Porter MD, Shadbolt B. Intralesional corticosteroid injection versus extracorporeal shock wave therapy for plantar fasciopathy. Clin J Sport Med 2005;15:119–24. Available at. https://insights.ovid.com/pubmed?pmid=15867552.

13. Acevedo JI, Beskin JL. Complications of plantar fascia rupture associated with corticosteroid injection. Foot Ankle Int 1998;19:91–7. Available at. http://journals.sagepub.com/doi/abs/10.1177/107110079801900207?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

14. Niknejad H, Peirovi H, Jorjani M, et al. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater 2008;15:88–99. Available at. http://www.ecmjournal.org/papers/vol015/vol015a07.php.

15. Adzick NS, Lorenz HP. Cell, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg 1994;220:10–8. Available at. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1234281/.

16. Zelen CM, Poka A, Andrews J. Prospective, randomized, blinded, comparative study of injectable micronized dehydrated amniotic/chorionic membrane allograft for plantar fasciitis–a feasibility study. Foot Ankle Int 2013;34:1332–9. Available at. http://journals.sagepub.com/doi/abs/10.1177/1071100713502179?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

17. Hanselman AE, Tidwell JE, Santrock RD. Cryopreserved human amniotic membrane injection for plantar fasciitis: a randomized, controlled, double-blind pilot study. Foot Ankle Int 2014;36:151–8. Available at. http://journals.sagepub.com/doi/abs/10.1177/1071100714552824?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

18. Fitzgerald RH. Management of acute and chronic tendon injury. In: McGlamry's comprehensive textbook of foot and ankle surgery. 4th edition. Philadelphia: Lippincott Williams & Wilkins; 2014. p. 1549–80.

19. Kueckelhaus M, Philip J, Kamel RA, et al. Sustained release of amnion-derived cellular cytokine solution facilitates achilles tendon healing in rats. EPlasty 2014; 14:e29. Available at. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4124919/.

20. Muttini A, Mattioli M, Petrizzi L, et al. Experimental study on allografts of amniotic epithelial cells in calcaneal tendon lesions of sheep. Vet Res Commun 2010;34: 117–20. Available at. https://link.springer.com/article/10.1007%2Fs11259-010-9396-z.

21. Ozböulük S, Ozkan Y, Oztürk A, et al. The effects of human amniotic membrane and periosteal autograft on tendon healing: experimental study in rabbits. J Hand Surg Eur Vol 2010;35:262–8. Available at. http://journals.sagepub.com/doi/abs/10.1177/1753193409337961?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

22. Demirkan F, Colakoglu N, Herek O, et al. The use of amniotic membrane in flexor tendon repair: an experimental model. Arch Orthop Trauma Surg 2002;122:396–9. Available at. https://link.springer.com/article/10.1007%2Fs00402-002-0418-3.

23. Barboni B, Russo V, Curini V, et al. Achilles tendon regeneration can be improved by amniotic epithelial cell allotransplantation. Cell Transpl 2012;21:2377–95. Available at. http://journals.sagepub.com/doi/abs/10.3727/096368912X638892?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

24. Nauth A, McKee MD, Einhorn TA, et al. Managing bone defects. J Orthop Trauma 2011;25:462–6. Available at. https://insights.ovid.com/pubmed?pmid=21738065.

25. Murugan R, Ramakrishna S. Development of nanocomposites for bone grafting. Comp Sci Technol 2005;65:2385–406. Available at. http://opac.vimaru.edu.vn/edata/E-Journal/2005/Composite%20science%20and%20technology/Vol65is15-16/bai%2011.pdf.

26. Bucholz RW, Carlton A, Holmes RE. Hydroxyapatite and tricalcium phosphate bone graft substitutes. Orthop Clin North Am 1987;18:323–34. Available at. http://europepmc.org/abstract/med/3561978.

27. Daniels TR, Younger AS, Penner MJ, et al. Prospective randomized controlled trial of hindfoot and ankle fusions treated with rhPDGF-BB in combination with β-TCP-collagen matrix. Foot Ankle Int 2015;36:739–48. Available at. http://bcfootandankle.com/wp-content/uploads/2016/03/Injectible-PDGF.full_.pdf.

28. Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factors and its involvement in disease. Mayo Clin Proc 2006;81:1241–57. Available at. http://www.mayoclinicproceedings.org/article/S0025-6196(11)61237-8/fulltext.

29. Starecki M, Schwartz JA, Grande DA. Evaluation of amniotic-derived membrane biomaterial as an adjunct for repair of critical sized bone defects. Adv Orthop Surg 2014;2014:572586. Available at. http://downloads.hindawi.com/journals/aos/2014/572586.pdf.

30. Zhong ZN, Zhu SF, Yuan AD, et al. Potential of placenta-derived mesenchymal stem cells as seed cells for bone tissue engineering: preliminary study of osteoblastic differentiation and immunogenicity. Orthopedics 2012;35:779–88. Available at. https://www.healio.com/orthopedics/journals/ortho/2012-9-35-9/%7Bdbf57f5b-0b05-4296-b10f-fb5bd664f72a%7D/potential-of-placenta-derived-mesenchymal-stem-cells-as-seed-cells-for-bone-tissue-engineering-preliminary-study-of-osteoblastic-differentiation-and-immunogenicity.