The Clinical Applications of Human Amnion in Plastic Surgery

Article *in* Journal of Plastic Reconstructive & Aesthetic Surgery · May 2014

DOI: 10.1016/j.bjps.2014.01.031

3 authors, including:

Neil Fairbairn
NHS Greater Glasgow and Clyde
20 PUBLICATIONS **135** CITATIONS
SEE PROFILE

Robert W. Redmond
Harvard University
167 PUBLICATIONS **4,793** CITATIONS
SEE PROFILE

Journal of Plastic, Reconstructive & Aesthetic Surgery (2014) 67, 662–675

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

REVIEW

The clinical applications of human amnion in plastic surgery

N.G. Fairbairn*, M.A. Randolph, R.W. Redmond

Division of Plastic Surgery, Massachusetts General Hospital, Harvard Medical School, 15 Parkman Street, WAC 453, Boston, MA 02114, USA

Received 29 May 2013; accepted 23 January 2014

KEYWORDS
Human amnion;
Plastic surgery;
Biological dressing

Summary Since the early 1900s, human amnion has been applied to a wide variety of clinical scenarios including burns, chronic ulcers, dural defects, intra-abdominal adhesions, peritoneal reconstruction, genital reconstruction, hip arthroplasty, tendon repair, nerve repair, microvascular reconstruction, corneal repair, intra-oral reconstruction and reconstruction of the nasal lining and tympanic membrane. Amnion epithelial and mesenchymal cells have been shown to contain a variety of regulatory mediators that result in the promotion of cellular proliferation, differentiation and epithelialisation and the inhibition of fibrosis, immune rejection, inflammation and bacterial invasion. The full repertoire of biological factors that these cells synthesise, store and release and the mechanisms by which these factors exert their beneficial effects are only now being fully appreciated. Although many commercially available biological and synthetic alternatives to amnion exist, ethical, religious, and financial constraints may limit the widespread utilisation of these products. Amnion is widely available, economical and is easy to manipulate, process and store. Although many clinical applications are of historical interest only, amnion offers an alternative source of multi-potent or pluripotent stem cells and therefore may yet have a great deal to offer the plastic surgery and regenerative medicine community. It is the purpose of this article to review the clinical applications of human amnion relevant to plastic surgery.

Introduction and history

Prior to the realisation of its medical and surgical applications, human amnion was the focus of myth and superstition. Being born with the fetal membranes or "caul" intact was considered extremely lucky. Children were gifted with life-long happiness, the ability to see spirits, and protection from death by arms and drowning. The magical powers of

* Corresponding author. Division of Plastic Surgery, Massachusetts General Hospital, Harvard Medical School, 78 Winter Street, Cambridge, Boston, MA 02141, USA. Tel.: +1 617 515 2701.
E-mail address: NGF174@hotmail.com (N.G. Fairbairn).

1748-6815/$ - see front matter
http://dx.doi.org/10.1016/j.bjps.2014.01.031

the caul were not confined to the original bearer and could be transferred by inheritance or legitimate sale. As a result, the trade of caul amulets became extremely popular, particularly between seafaring men during the 1800s at the time of the Napoleonic War.[1]

In 1910, Davis reported on early experience with fetal membranes in skin transplantation.[2] Over the last century, the beneficial effects of amnion have been applied to burns, chronic vascular and diabetic ulcers, dural defects, intra-abdominal adhesions, peritoneal reconstruction, genital reconstruction, hip arthroplasty, tendon repair, nerve repair, microvascular grafts, corneal repair, intra-oral reconstruction and reconstruction of the nasal lining and tympanic membrane. More recently amnion has been shown to be a viable source of stem cells with a potentially exciting future in tissue engineering and regenerative medicine. Although many of these roles are of historical interest only, an awareness of this history is an important pre-requisite for future development and innovation. It is the purpose of this article to review past and present applications of human amnion relevant to plastic surgery and how it may contribute to our future.

Anatomy and physiology

Amnion forms during the transition of the morula into the blastocyst at approximately 7-days following fertilisation.[3] Amnion is between 0.02 and 0.05 mm thick and consists of five distinct layers: (1) epithelium, (2) basement membrane, (3) compact layer, (4) fibroblast layer, (5) spongy layer (see Figure 1). The innermost epithelium consists of a single layer of cells in direct contact with amniotic fluid. Microvilli at the apical surface of these cells play an important role in amniotic fluid homeostasis.

The basement membrane border of the cells contains blunt projections that inter-digitate with similar processes in the basement membrane, forming a densely adherent bond. The basement membrane is a thin layer composed of reticular fibers. The compact, fibroblast and spongy layers are referred to as the amniotic mesenchyme and originate from the primary extra-embryonic mesoderm of the blastocyst. The mesenchyme contains collagen I—VII and non-collagenous proteins such as elastin, laminin, fibronectin and vitronectin. The compact layer is composed of a dense network of fibers and is almost entirely free from cells. Abundant type I, II and III collagen and elastin within this layer endow amnion with tensile strength and elasticity.[4] These properties help protect the fetus from mechanical stress and desiccation. The fibroblast layer is the thickest layer and is composed of a loose fibroblast network within a matrix of reticulin. The outermost spongy layer represents the transitional layer between amnion and chorion and is composed of bundles of reticulin within a background of mucin. The two layers are loosely adherent, allowing a degree of gliding during gestation and easy separation by blunt dissection during harvest.[5]

In spite of being devoid of vascularity, nerves, muscles and lymphatics, amnion is highly metabolically active.[5] Oxygen and nutrients are obtained by diffusion from amniotic fluid and chorionic vasculature. The epithelial layer is a source of prostaglandins, particularly prostaglandin-E2, and is thought to play an important role in the initiation and maintenance of uterine contractions.[6] The epithelium also contains human chorionic gonadotrophin receptors that regulate prostaglandin production and activity. Epithelial cells manufacture multiple vasoactive peptides, growth factors, cytokines and extracellular matrix (ECM) proteins.[5] These biological factors may reside in the epithelium or may be transported and accumulated in the mesenchyme where they act as a reservoir from which the amnion exerts its therapeutic effects following transplantation.

Mechanism of therapeutic effect

As a barrier and analgesic

The application of amnion to a wound bed prevents desiccation and excessive fluid loss and provides an analgesic effect by protecting exposed nerve ends from the environment.

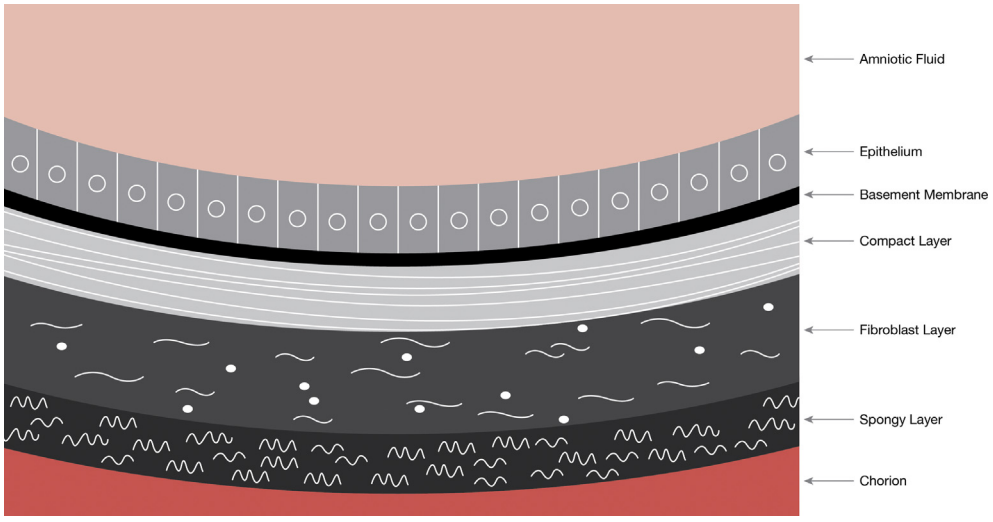

Figure 1 Schematic of amnion structure.

As a non-immunogenic material

Several investigators have concluded that amniotic epithelial and mesenchymal cells lack HLA class A, B, DR and co-stimulatory molecules CD-40, CD-80 and CD-86.[7] In contrast, others have shown the presence of class-1 and class-1b antigens in epithelial cells, mesenchymal cells and fibroblasts.[8] Radiobiological studies suggest that although amnion cells retain the ability to synthesise HLA, they do not express HLA-A, B, C or DR antigens of β-2 microglobulin on the cell surface.[9] Mesenchymal stromal cells may inhibit the maturation of peripheral blood monocytes into antigen-presenting dendritic cells.

As a promoter of epithelialisation and an inhibitor of fibrosis and scar

Amniotic epithelial and mesenchymal cells contain epidermal growth factor (EGF), keratinocyte growth factor (KGF), keratinocyte growth factor receptor (KGFR), hepatocyte growth factor (HGF), and hepatocyte growth factor receptor (HGFR). These growth factors are responsible for proliferation, migration and differentiation of epithelial cells and the promotion of epithelialisation.[10] Basic fibroblast growth factor (bFGF), and transforming growth factor (TGF) -β1, β2, β3 have also been demonstrated in amnion cells. bFGF is a pro-angiogenic factor and plays a role in the formation of granulation tissue through the proliferation of fibroblasts. The TGF-β family is responsible for the synthesis and deposition of ECM proteins and the regulation and transformation of fibroblasts into myofibroblasts.[11] Mesenchymal hyaluronic acid may inhibit TGF-β and the generation of excessive fibrosis and scar.[10] This may explain the beneficial effect amnion has on scar formation and why fetal wound healing is essentially scarless.

As an anti-inflammatory and anti-bacterial

Amniotic epithelial cells contain interleukin 10 (IL-10) that down-regulates the expression of Th1 cytokines, major histocompatibility complex (MHC) class II antigens and co-stimulatory molecules on macrophages.[12] IL-10 also enhances B-cell survival, proliferation and antibody production and has been shown to inhibit the production of pro-inflammatory cytokines such as interferon-γ, IL-2, IL-3, tumour necrosis factor-α (TNF-α), and granulocyte macrophage colony stimulating factor (GM-CSF). Other anti-inflammatory mediators such as IL-1 receptor antagonist and tissue inhibitors of metalloproteinase-1, 2, 3, 4 (TIMPs) have also been found in amniotic cells.

Amniotic fluid contains lysozymes and immunoglobulins.[13] In vitro experiments confirm reduced viability of group-A and group-B Streptococcus, *Staphylococcus aureus and Staphylococcus saprophyticus* in the presence of amnion.[14] Amnion has also been shown to produce human-beta-3-defensin. These antimicrobial peptides are implicated in the resistance of epithelial surfaces to microbial colonisation and have been shown to be upregulated in inflamed amnion.[15] Amnion epithelial cells can be induced to express intercellular adhesion molecule-1 (ICAM-1) by pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and IL-1β.[16] ICAM-1 has a role in the attraction and adhesion of leukocytes and may also have a role in signal transduction in pro-inflammatory pathways resulting in the recruitment of inflammatory mediators such as macrophages and granulocytes.[17]

As a regulator of angiogenesis

The angiogenic influence of amnion is uncertain. The presence of platelet derived growth factor (PDGF) and vascular endothelial derived growth factor (VEGF) are suggestive of a pro-angiogenic role.[18] bFGF may have an even greater pro-angiogenic influence than PDGF and VEGF. However, a large amount of ophthalmological research contends that it is the ability of amnion to suppress angiogenesis that renders it useful in corneal healing. The expression of tissue inhibitors of metalloproteinase (TIMP-1, 2, 3, 4), thromboplastin-1 and endostatin in amniotic cells supports these claims.[12]

Amnion collection and processing

Elective cesarean section donors undergo rigorous serological screening for human immunodeficiency virus-1/2, Hepatitis B, Hepatitis C, human T-cell lymphotrophic virus, syphilis, cytomegalovirus, and tuberculosis.[19] Following delivery, amnion is separated from the placenta by blunt dissection (see Figure 2). Once gross contaminants are removed, amnion is usually de-epithelialised to limit immunogenicity, sterilised to reduce risks of disease transmission, and preserved to improve longevity and convenience for storage. Improvements in processing have focused on preserving membrane architecture and growth factor content in order to optimise therapeutic effect.

De-epithelialisation can be performed by mechanical scraping or exposure to chemicals.[19] It is uncertain how these protocols affect the levels of growth factors and ECM proteins. Koizumi et al. showed that, although amnion denuded of its epithelium contained EGF, TGF-α, KGF, HGF, bFGF, TGF-β1, and TGF-β2, protein levels were reduced in comparison to samples with intact epithelium.[10] Whether this is clinically significant is uncertain. Neurotransmitters, neurotrophic factors and neuropeptides are concentrated in the epithelium and therefore amnion with intact epithelium may be

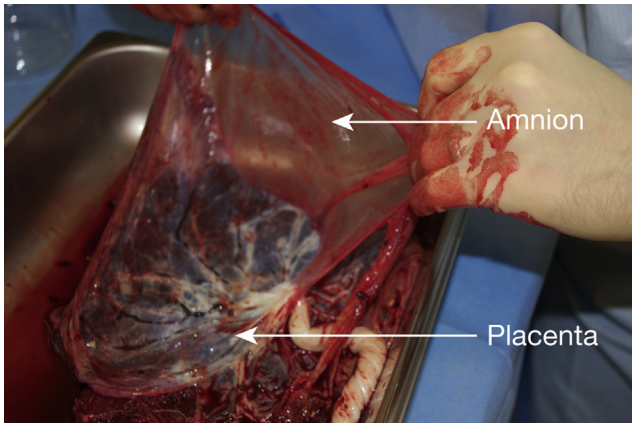

Figure 2 Amnion being bluntly dissected from human placenta.

superior when applied to neural injury.[20] In contrast, denuded amnion results in superior cell adhesion, migration and proliferation and therefore may be preferable when applied to acute and chronic wounds.[21] As the majority of clinical applications concern wound healing, the use of denuded amnion has greater representation in the literature.

Developed in the late 1980s, cryopreservation in glycerol is the most widely used preservation technique. Antibacterials and anti-fungals are often added before freezing at −80 °C. Cells are devitalised although not sterilised.[23] Viable bacteria and viruses can be present following several months of storage.[24] The effect on biological properties is uncertain. Thomasen et al. reported no detrimental impact on sterility, histological integrity or the availability of biological mediators. Amnion cryopreserved in 50% glycerol/DMEM at −80° for 1-month contained EGF, TGF-α, KGF, HGF, bFGF, TGF-β1, -β2, β3, KGFR and HGFR.[22] Cryopreservation requires expensive equipment that may be unavailable for some institutions, particularly in developing nations.

Lyophilisation is an alternative technique allowing storage of amnion at room temperature, obviating the requirement for deep freeze facilities and increasing surgeon convenience. Lyophilised membranes are commonly sterilised with gamma irradiation. Concerns exist regarding detrimental changes to membrane architecture and growth factor levels. Nakamura et al. reported no significant difference in tensile properties, tissue structure or ECM composition between lyophilised, gamma-irradiated and cryopreserved membrane.[25] Lim et al. showed that lyophilisation reduced the levels of several growth factors and ECM proteins although there was no appreciable difference in clinical performance when compared with cryopreserved samples.[26] Other methods of preservation and sterilisation exist although these are less well accepted.

The variation in processing within the literature makes it difficult to draw definitive conclusions on the optimal method. Variation also exists amongst commercially available products (Table 1). Independent of processing technique, several donor specific factors can influence the biochemical composition of amniotic membrane. Lopez-Valladares et al. showed that in fresh, cryopreserved and lyophilised amnion, levels of bFGF, HGF, KGF and TGF-β1 were significantly lower in those membranes of greater chronological and gestational age.[27] Velez et al. found significant differences in cytokine profiles between African Americans and Caucasians.[28] Membrane architecture and growth factor profile can also vary depending on what area of amnion a specimen originates from.[29] As a result, standardisation of collection and processing may be essential if consistent therapeutic results are to be achieved. If consistent relationships between donor variables and biochemical profile exist, it may become possible to select certain varieties of amnion for specific clinical situations.

Applications relevant to plastic surgery

Broadly speaking, amnion has been applied as an alternative biological dressing or has in some way augmented reconstruction. Table 2 provides examples of

Table 1 Commercially available human amnion products.

Manufacturer	Product	Membrane thickness	Indications for use	Processing technique
MiMedx (Marietta, Georgia)	AmnioFix membrane	50—100 microns	Dural reconstruction, spinal surgical barrier	Proprietary Purion process (dehydration and sterilization)
	AmnioFix Wrap	50—100 microns	Tendon and soft tissue inflammatory conditions	As above
	AmnioFix Injectable	N/A	Nerve and tendon repair	As above
	EpiFix	50—100 microns	Chronic and acute partial and full thickness wounds	As above
Bio-Tissue Inc (Miami, Florida)	Prokera corneal bandage	50—100 microns	Corneal erosion, infectious and inflammatory keratitis, herpes, superficial epithelial defects	Proprietary CryoTek process (cryopreservation)
	AmnioGraft	50—100 microns	Chemical burns, Pterygium, corneal defects, leaking glaucoma blebs, Stevens-Johnson syndrome, Strabismus	As above
	AmnioGuard	300—400 microns	Coverage of glaucoma drainage devices	As above
AcelaGraft Cellular Therapeutics (Cedar Knolls, New Jersey)	AcelaGraft	50—100 microns	General wound dressing and ophthalmic wounds	Deoxycholic acid, gel drying, electron beam irradiation

Table 2 Summary of evidence on the applications of human amnion relevant to plastic surgery.

Clinical Scenario	Author	Study design	Application	Amnion Prep	Summary of outcomes
Biological dressing					
Burns	Lin et al., 1985[30]	OCC ($n = 11$)	Overlay on autograft	Fresh	Amnion compared to conventional dressings. Good adherence, not rejected by patient, reduced pain, infection, bleeding, number of dressing changes and time to healing in amnion group
	Subrahmanyan, 1995[31]	CS ($n = 22$)	Overlay on micro-skin grafts	Fresh	Epithelialisation within 7—10 days in 16 patients. Superior wound healing due to occlusive, growth promoting effect of amnion
	Sawhney, 1989[32]	OCC ($n = 90$)	PT burns	Processed	Amnion vs conventional silver dressings. Superficial, mid-dermal and full thickness burns. Amnion reduced wound exudate, expedited epithelialisation and reduced granulation tissue and scar formation in all groups. In mid-dermal burns, amnion degraded and required regular replacement. Amnion applied to FT burns once eschar separated
	Ramakrishnan et al., 1997[33]	OCC ($n = 350$)	PT burns	Processed	Amnion compared to conventional dressings. Amnion had superior adherence, porosity allowing egress of exudate, transparency allowing wound monitoring, reduction in pain, healing times demands on nursing staff and cost
	Branski et al., 2007[34]	P-RCT ($n = 102$)	PT burns	Processed	Amnion vs topical antimicrobials. Significantly less dressing changes with amnion. Time to healing, length of stay and incidence of hypertrophic scarring were not significantly different between groups
	Singh et al., 2007[35]	OCC ($n = 50$)	PT burns	Processed	Gamma irradiated amnion compared with glycerol preserved amnion. Radiation sterilised amnion easier to apply that glycerol sterilized. No significant difference in time to healing, infection, scarring
	Fraser et al., 2009[36]	Animal ($n = 21$)	PT burns	Processed	Symmetrical lower limb deep dermal burns. Amnion vs paraffin gauze. Histopathological and immunohistochemical analysis showed significantly reduced scar tissue formation in amnion group.
	Mostaque et al., 2011[37]	P-RCT ($n = 102$)	PT burns	Processed	Paediatric burns. Amnion vs silver sulfadiazine dressings. Amnion resulted in: significantly reduced mean hospital stay, dressings changes, mean time to epithelialisation, reduced pain, increased mobility. Patient and surgeon

					satisfaction high.
	Adly et al., 2010[38]	P-RCT ($n = 46$)	PT + FT burns	Processed	Amniotic membrane group compared to Tegaderm dressings. Amnion resulted in significantly faster healing, lower rates of infection, lower pain scores and lower levels of electrolyte and albumin loss.
	Mohammadi et al., 2013[39]	P-RCT ($n = 38$)	Symmetric upper and lower limb burns	Fresh	Right limb autograft + amnion overlay vs left limb autograft + conventional dressing. Graft success assessed after 21 days. Mean graft take in right limbs was significantly higher than left (90% vs 67%)
Acute wounds	Seashore et al., 1975[40]	CS ($n = 16$)	Omphalocele and gastroschiasis	Fresh	Fresh amnion compared to porcine xenograft and silastic sheeting; mean time to healing 55 days; amnion superior due to ready availability, reduced bacterial counts, rapid epithelialisation
	Tekin et al., 2007[41]	CS ($n =$ UNK)	Coverage of exposed viscera	Fresh	Amnion applied as a cover in place of Bogota bag every 48 h; reduction in serosal erosions and adhesions between bowel loops; visceral and abdominal wall oedema reduced
Chronic wounds	Troensegaard-Hansen et al., 1950[42]	CS ($n = 7$)	Chronic leg ulcers	Processed	Amnion used in case patients compared with 1 control treated with conventional dressings. Chronic ulceration duration 4—15 years. All amnion patients healed within 10 weeks. No healing in control patient. No wound breakdown during follow-up
	Faulk et al., 1980[18]	CS ($n = 15$)	Chronic leg ulcers	Fresh	Amnion vs regular dressings. Samples for histology + immunohistochemistry before and after 5-days amnion. New vessel formation + granulation tissue superior in amnion group.
	Ward et al., 1984[43]	CS ($n = 28$)	Chronic leg ulcers	Fresh	Amnion applied for 5-days after which ulcer was autografted. 50% recurrence at 1-year (defined as ulceration >1 cm)
	Ward et al. 1989[44]	CS ($n = 27$)	Chronic leg ulcers	Various	Healing compared amongst groups treated with fresh, frozen, tissue cultured maintained or lyophilized amnion. No statistically significant difference between groups. Lyophilised judged to be easiest to use and store
	Singh et al., 2004[45]	CS ($n = 50$)	Chronic leg ulcers	Processed	Successful pain relief and healing of ulcers of varying aetiologies
	Gajiwala et al., 2003[46]	CS ($n = 8$)	Pressure sore	Processed	Superficial sores treated with lyophilized, irradiated human amnion. Easy to handle and apply, analgesic, reduction in exudate, accelerated epithelialisation. Complete healing with single application

(*continued on next page*)

Table 2 (*continued*)

Clinical Scenario	Author	Study design	Application	Amnion Prep	Summary of outcomes
	Insausti et al. 2010[47]	CS ($n = 2$)	Large post-traumatic wounds	Processed	Amnion applied to large, deep wounds. Accelerated epithelialisation. Up-regulation of c-Jun expression and modification of keratinocyte migration
Reconstruction					
Dura	Tomita et al., 2012[48]	CS ($n = 10$)	Skull base	Processed	No CSF leakage or adverse outcome directly related to amnion were observed
	Hasegawa et al., 2004[49]	CR ($n = 1$)	Myelomeningocele	Fresh autograft	Autologous onlay graft; no rejection; prevention of infection following wound dehiscence; graft epithelialised; absence of excessive scar tissue formation; rapid, water-tight solution; neurotrophic factors from amnion promoted neural healing
	De Weerd et al., 2013[50]	CR ($n = 1$)	Myelomeningocele	Fresh, autograft	No rejection; absence of excessive scar tissue formation; graft epithelialised; rapid, water-tight solution; neurotrophic factors from amnion promoted neural healing
Oral cavity	Lawson, 1985[52]	CS ($n = 12$)	Pectoralis major flap oral mucosal lining	Fresh	Amnion provided scaffolding function. Flaps formed granulation tissue and epithelialised twice as fast untreated flaps. Wound contracture reduced.
	Samandari et al., 2004[55]	CS ($n = 7$)	Mandibular vestibuloplasty	Fresh	No infection or graft rejection. Amnion present for 3 weeks and led to rapid granulation tissue formation, mucosalisation and maintenance of post-operative buccal vestibular height. Prosthodontic surgery possible at 1-month
	Kothari et al., 2012[56]	CS ($n = 10$)	Mandibular vestibuloplasty	Processed	No infection or graft rejection. Amnion present for 3 weeks and led to rapid granulation tissue formation, mucosalisation and maintenance of post-operative buccal vestibular height. Prosthodontic surgery possible at 1-month
Genitalia	Tancer et al., 1979[53]	CS ($n = 4$)	Vaginal reconstruction	Fresh	Amnion applied over obturator; no rejection or infective complications; epithelialisation complete in all 4 cases by 8 weeks
	Ashworth et al., 1986[54]	CS ($n = 15$)	Vaginal reconstruction	Fresh	No rejection; purulent discharge between obturator changes although no overt infection; excellent results in partial or complete vaginal agenesis reconstruction; improvement in vaginal strictures; epithelialisation by 4 weeks
Flap and microvascular	Ozkaya et al., 2012[57]	Animal ($n = 32$)	Random pattern skin flaps	Fresh	Amnion applied to undersurface of flaps; greater survival of treated flaps. Significant reduction in polymorphonuclear leukocyte number; significant increase in capillary

					proliferation and density
	Gray et al., 1987[58]	Animal (n = 120)	Vascular interpositional grafts	Processed	Grafts soft, pliable; no collapse of graft walls; transparent wall helped to prevent back wall suturing; easier to suture than alternative synthetic (PTFE) grafts; No rejection. Patency after 3 months comparable to other synthetic grafts but inferior to control autogenous vein grafts
Tendon and nerve	Ozboluk et al., 2010[59]	Animal (n = 42)	Flexor tendon repair	Fresh	Adhesion formation reduced in amnion treated group compared with untreated control group after 6 weeks follow-up
	Meng et al.[60]	Animal (n = 36)	Nerve wrap	Processed	Significant improvements in functional recovery and nerve histomorphometric outcomes at early time points; no significant difference after 12 weeks; significantly less perineural scar tissue formation in amnion group
	Henry et al., 2009[61]	Animal (n = 24)	Nerve wrap	Processed	Photochemical sealing of amnion wrap to neurorrhaphy site resulted in significant improvement of electrophysiological and histomorphometric outcomes and reduction in axonal escape
	O'Neill et al., 2009[62]	Animal (n = 48)	Nerve wrap	Processed	Photochemical sealing of amnion wrap to neurorrhaphy site showed significantly improved functional and histomorphometric outcomes and reduction in extraneural adhesions
	Mohammad et al., 2000[63]	Animal (n = 66)	Nerve conduit	Processed	Amnion conduits vs silicone conduits vs standard autograft over 1 cm defect. Regeneration through amnion conduit comparable to autograft and superior to silicone conduit. Functional recovery statistically better at early time points in amnion group. Amnion degraded by 4-months.
	O'Neill et al., 2009[64]	Animal (n = 24)	Nerve conduit	Processed	Amnion conduits secured with either photochemical tissue bonding (PTB) or suture and compared to autograft. Functional outcomes, muscle mass retention and histomorphometry in amnion conduit + PTB comparable to autograft

PT = partial thickness; FT = full thickness; OCC = observational case controlled trial; CS = case series; CR = case report; P-RCT = prospective randomised controlled trial; UNK = unknown.

experimental and clinical evidence supporting therapeutic benefit according to these categories. In spite of the large evidence base, there is a paucity of well-designed, randomised controlled trials testing amnion performance against gold standard alternatives. A list of ongoing human clinical trials, as listed by ClinicalTrials.gov, is provided in Table 3. The following discussion makes reference to this evidence and aims to provide a more cohesive understanding. A summary of the advantages and disadvantages of amnion according to application are listed in Table 4. The applications amnion has for tissue engineering and regenerative medicine is also discussed.

Biological wound dressing

Burns

The history of amnion use for the management of burns is extensive. The use of amnion for corneal burns and other ophthalmological epithelial defects is commonplace and has led to the development of several commercially available products (Table 1). Membranes have been used as overlay following standard autografting and microskin grafting and also in place of conventional dressings following superficial and mid-dermal burns, including cadaveric allograft and porcine xenograft.[30–39]

Acute wounds

Amnion has been used as an alternative temporary biological dressing to protect exposed viscera in cases of congenital abdominal wall defects such as omphalocele and gastroschiasis and also full thickness defects secondary to major trauma, infection or oncological resection.[40] Amnion provides an alternative to the Bogota bag and can form part of a staged abdominal wall reconstruction with or without negative pressure therapy (NPT).[41]

Chronic wounds

Chronic wounds represent a major financial burden on healthcare services worldwide. Multiple studies have reported superior wound healing following the application of amnion to chronic leg ulcers of varying aetiology[18,42–45] Areas of pressure necrosis have also been treated although the suitability of amnion in these complex and often extensive wounds is most likely limited to only the most early and superficial cases.[46] Amnion has also been applied to areas of stalled healing following large traumatic soft tissue loss in patients unfit for complex reconstruction.[47] In each of these situations, amnion can be applied in conjunction with NPT.

Reconstruction

Dural repair

Amnion has been used to reconstruct dural defects in the skull base and in cases of myelomeningocele.[48–50] Water tight closure in these situations is essential in order to prevent CSF leak and potentially life threatening infection. Although synthetic materials are available in these situations, autologous solutions are preferred. In congenital anomalies such as myelomeningocele, amnion can be applied as an autograft immediately or as a delayed procedure following storage. When soft tissue defects are large, amnion can form part of a layered closure under loco-regional or free tissue transfer.[49,50] Amnion may

Table 3 Summary of registered clinical trials using human amnion.

Description	Design	Trial identifier	Institution	Status
The treatment of partial thickness burns: treated amnion versus currently in use topical medication	Phase 2/3 RCT	NCT00674999	University of Texas, Galveston	Recruiting
Evaluation of the cryopreserved amniotic membranes in the care of resistant vascular ulcers	Phase 2 single group interventional	NCT00820274	University Hospital, Limoges Etablissement Français du Sang	Recruiting
An Evaluation of the Effect of the AmnioFix™ Amniotic Membrane Allograft on Scar Tissue and Adhesions in Patients Undergoing Posterior Instrumentation Removal	Phase 2/3 Observational case controlled	NCT01357187	UNKNOWN	Not yet recruiting
The role of AMT (amniotic membrane transplantation) in treating epithelial defects and symbelpharon, preventing corneal opacification, decreasing pain, improving visual acuity and treating acute chemical burns	Phase 2/3 RCT	NCT00370812	Shaheed Beheshti Medical University	Recruiting

RCT = Randomised controlled trial.

Table 4 Advantages and disadvantages of human amnion for different clinical application.

Amniotic membrane for clinical application		
Application	Advantages	Disadvantages
General		
	Abundant supply; no donor morbidity; inexpensive; easy processing and storage; off-the-shelf availability; high tensile strength; non-immunogenic; anti-bacterial, anti-inflammatory, anti-fibrotic, regulator of angiogenesis; reduced social, cultural, religious obstacles compared with allograft and xenograft products	Donor screening; risk of disease transmission; optimal processing method uncertain; problematic handling and suturing; variable biological properties depending on sample location, donor and gestational age and race; proteolytic degradation
Biological dressing		
Burns	Readily adherent; transparent allowing wound monitoring; reduction in exudate and infection; accelerated epithelialisation; analgesic; reduction in dressing changes, analgesia requirements, demand on nursing staff; reduced cost; reduced scarring	Handling and suturing may be difficult; Membrane architecture and growth factor content varies with location, gestational age, donor age and race; Degradation may require reapplication; no adherence in full thickness burns
Acute wounds	As above; allows temporary coverage of exposed abdominal viscera; autograft possible in omphalocele and gastroschiasis;	As above
Chronic wounds	Conformable to deep, irregular wounds; permeable allowing egress of exudates; reduced requirement for autografting, time to autografting and graft failure; reduced scarring; use with NPT	As above
Reconstructive		
Dural repair	Water tight barrier; neurotrophic factors support neural tissue; autografting possible (myelomeningocele)	As above
Oral cavity and vaginal vault	Rapid epithelialisation removing need for autograft; bone coverage possible	As above
Flap and microvascular	Angiogenic effect; inhibits neutrophils and free radicals; allows manufacture of vascular grafts; amnion scaffold permits growth factor and stem cell seeding	As above; degradation may interfere with vascular graft success
Nerve and tendon	Wrap reduces scar tissue/adhesions; prevents leakage of growth factors; provides neurotrophic support; allows manufacture of conduits; amnion scaffold permits growth factor and stem cell seeding	As above; empty nerve conduits limited to short distances; degradation may interfere with conduit support

support underlying neurological tissue through the production of neurotrophic factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and brain natriuretic peptide (BNP).[14,51]

Mucosal lining

Amnion has been used to expedite epithelialisation of muscle flaps following intra-oral and vaginal vault reconstruction.[52–54] In the absence of a cutaneous paddle, these surfaces can be reconstructed with split and full thickness skin grafts, buccal mucosa grafts, intestinal mucosa, peritoneum or commercially available collagen based products. Skin grafts are perhaps the most widely practiced technique although they are associated with complications such as donor site wound, colour and texture mismatches, dryness, desquamation, hair growth, poor mobility and contracture. Commercial products are expensive and require complex processing that can reduce their clinical efficacy. Amnion has also been used to successfully cover bone following excision of gingival leukoplakia and vestibuloplasty.[55,56]

Flap and microvascular

Partial or complete flap necrosis is a dreaded complication when performing tissue transfer. Amnion applied to the undersurface of random pattern skin flaps has been shown to significantly increase capillary proliferation, reduce infiltrating neutrophils and improve flap survival.[57] Local factors liberated by amnion may reduce leukocyte activation and free radical formation, limiting endothelial injury, thrombosis and flap necrosis.

The survival of free tissue transfer relies on successful microvascular anastomosis. In major trauma or complex elective reconstruction, this can require interpositional grafting. Autogenous vein remains the gold standard graft material although the associated donor morbidity is unsavoury. Alternative biological or synthetic materials with equivalent patency and functional outcomes are desirable. Amnion has been rolled into interpositional grafts and, in a rat model, resulted in re-endothelialisation at equal time points compared with vein autografts.[58]

Tendon and nerve

Successful functional recovery, particularly in the hand and upper limb, is dependent on accurate reconstruction of tendons and nerves. Wrapping tenorrhaphy and neurorrhaphy sites with amnion can reduce adhesions and improve functional recovery.[59,60] Reducing suture burden at the repair site can also reduce scar tissue formation. Amnion wraps have been sealed around neurorrhaphy sites with a novel, sutureless, photochemical tissue bonding technique, resulting in improved functional and histological outcomes.[61,62] Amnion scaffolds may act as a reservoir of neurotrophic factors. Sealing the regenerative milieu may prevent the elution of these and other endogenous neuroregenerative factors into the surrounding tissue.

As with vessel injury, loss of nerve tissue may require bridging techniques. Autogenous nerve grafts remain the gold standard technique although in situations of major trauma, demand for autogenous material can exceed supply. Processed allografts and biological and synthetic nerve conduits are options although all are associated with limitations. Amnion provides an alternative material for the construction of nerve conduits.[63,64] As with any hollow conduit, applications remain limited to short deficits.

Tissue engineering and regenerative medicine

Amnion as a scaffold

Biological scaffolds require the presence of extracellular matrix proteins such as collagen, laminin and fibronectin. Adhesion molecules specific to these proteins facilitate cell adhesion, transmembrane receptor activation and intracellular signalling cascades that regulate cell migration, proliferation, differentiation and apoptosis.[65] Ideal scaffolds are biocompatible, mechanically stable, flexible, resorbable at a rate consistent with repair and allow the incorporation of growth factors and genetic materials.[66] Amnion basement membrane contains collagen III, IV and other glycoproteins such as laminin and fibronectin. Amnion scaffolds have been used to cultivate epithelial cells in vitro before in vivo transplantation. This has been used to reconstruct corneal surfaces following chemical burns, limbal stem cell deficiency and other related pathology.[67] Amnion scaffolds seeded with human keratinocytes have generated living skin equivalents and have been successfully transplanted into an animal model.[68] Denuded amnion has been used as a carrier matrix for chondrocytes and cartilage regeneration.[69] Amnion seeded with human umbilical vein endothelial cells and human vascular smooth muscle cells has been rolled into a cell dense, mechanically stable, multi-layered blood vessel conduit.[70] Although growth factor levels in denuded amnion may be reduced, several studies have suggested scaffolding function is more effective in the absence of epithelium. Due to the interference of hemidesmosome formation, amniotic epithelium may hinder uniform cell expansion.[71]

Amnion as an alternative source of stem cells

The use of pluripotent embryonic stem cells (ESCs) is hindered by ethical controversy. Mesenchymal stem cells (MSCs) are a less controversial, non-embryonic source of multipotent cells. Bone marrow mesenchymal stem cells (BM-MSCs) are perhaps the gold standard adult multipotent cell. However, due to the invasive and painful nature of harvest, alternatives such as adipose derived mesenchymal stem cells (AD-MSCs) have become popular. Adipose tissue is abundant, readily accessible with low morbidity, provides cell numbers and stem cell fractions that greatly exceed that of BM-MSCs, and have superior proliferation capacity and differentiation potential in vitro.[72] Adipose derived stem cells can also be induced into pluripotent cells. These cells are reprogrammed into pluripotency by inducing the expression of transcription factors characteristic of undifferentiated embryonic stem cells.[73]

Several limitations of AD-MSCs exist. Cell populations are not homogenous. Considerable variations in phenotype, proliferative capacity and differentiation potential exist between and within individuals. Proliferative capacity and differentiation potential may decrease with donor age, a characteristic shared by all adult derived MSCs.[74] The secretion of tumour promoting factors such as IL-6 and the pro-angiogenic effect of these cells have also raised concerns regarding malignant transformation.[75] With regards to induced pluripotency, the persistence of source cell epigenetic memory may render these cells unstable and unpredictable.[76]

Amnion has advantages over all adult derived MSCs. Amnion supply is unlimited and is arguably more convenient to obtain than adipose tissue. Total cell number and stem cell fraction from amnion is thought to greatly exceed both BM-MSCs and AD-MSCs.[72] In addition to amnion, placental tissue provides chorionic membrane, chorionic villi, maternal decidua, umbilical cord, umbilical cord blood and Whartons jelly. These provide additional MSCs and also embryonic populations such as endothelial and haematopoietic stem cells.[77] Proliferative capacity and differentiation potential of amnion derived cells is thought to exceed that of adipose tissue. Derivatives from all three germ layers such as adipogenic, osteogenic, chondrogenic, hepatic, pancreatic, cardiac, vascular and neural cells have been cultured and shown to possess reparative and functional capabilities. Placental cells of fetal origin (amnion, chorion, chrionic villi) may have greater differentiation potential than those of maternal origin (decidua).[78] Fetal origins may also prevent age related reductions in proliferative and differentiation potential characteristic of adult cells. Due to a maximum gestational age of 9—10 months, it is also likely that amnion provides a population of cells that have accumulated less genetic damage than adult sources.

It is currently uncertain whether amnion cells are truly pluripotent or whether multiple sub-populations of mutlipotent stem cells exist. The existence of multiple subpopulations is potentially problematic. Not unlike growth factor level, the proliferative and differentiation characteristics of these cells may vary according to membrane location, gestational and donor age, race and processing technique. In addition, different methods of culture, isolation and expansion may artificially select certain subpopulations and obscure true biological activity. Pluripotency is supported by the identification in amniotic cells of multiple molecular markers typically found on embryonic stem cells, such as octamer-4 (OCT-4), NANOG, sex-determining Y-box-2 (SOX-2), Lefty-A, FGF-4, REX-1 and

Table 5 Comparison between amnion, adipose tissue and bone marrow as alternative sources of mesenchymal stem cells.

Variable	Tissue		
	Amnion	Adipose	Bone marrow
Invasiveness of procurement	−	+	+ +
Ethical issues	−	−	−
Availability of tissue	+ + +	+ +	+
Stem cell fraction (% of total cells)	5–50	1–5	0.01–0.05
Proliferation capacity in vitro	+ + +	+ +	+
Senescence with passage	+	+ +	+ + +
Differentiation potential	+ + +	+ +	+
Reduction in differentiation potential with donor age	−	+	+
Age and environmental acquired DNA damage	−	+	+
Cryogenic storage of cells for future use following birth	+	−	−

teratocarcinoma derived growth factor-1 (TDGF-1).[79] OCT-4 is responsible for the maintenance of pluripotency and it has been shown that the level of this marker decreases with increasing cellular differentiation. Embryonic stem cells are derived from the inner cell mass of the blastocyst, which in turn gives rise to the epiblast. The epiblast, from which the amnion is derived, gives rise to all 3 germ cell layers. It is therefore possible that amniotic cells retain epiblastic pluripotency. In addition, gastrulation plays an important role in the differentiation and determination of cell fate. Amnion forms prior to this phase and it is therefore possible that these cells are pluripotent.[80] Table 5 provides a comparison of the salient characteristics of bone marrow, adipose tissue and amnion as sources of stem cells.

Conclusion

Human amnion provides the plastic surgeon with an incredibly versatile material. It is economical, widely available, easy to harvest and store and has no ethical constraints. Amnion contains a plethora of biological mediators and is a well-established alternative wound dressing. It is biocompatible, highly conformable, thin, and yet retains considerable tensile strength. Amnion can mechanically support and improve survival of transferred tissue and, through the manufacture of vessel and nerve conduits, may also directly contribute to neurovascular reconstruction. Amnion has provided a vehicle for the development of a novel photochemical tissue bonding technique that has proved efficacious for the sutureless repair of skin, tendon, nerve and vessel. Amnion may prove useful as a biological scaffold for tissue engineering and is emerging as an alternative source of multipotent and even pluripotent stem cells. After more than a century of clinical use, the application of human amnion in plastic and reconstructive surgery continues to evolve.

Conflict of interest

None.

Funding

None.

References

1. Richardson R. Copperfields caul. *Lancet* 2002;**359**:2209.
2. Davis JW. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Johns Hopkins Med J* 1910;**15**:307–96.
3. Trelford JD, Anderson DG, Hanson FW, Mendel V, Sawyer RH. Amnion autografts and allografts as a cover for skin defects in sheep. A preliminary report. *J Med* 1972;**3**:81–7.
4. Mamaede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ, Botelho MF. Amniotic membrane: from structure and functions to clinical applications. *Cell Tissue Res* 2012;**349**:447–58.
5. Bourne GL. The microscopic anatomy of the human amnion and chorion. *Am J Obstet Gynecol* 1960;**79**:1070–3.
6. Okazaki T, Casey ML, Okita JR, MacDonald PC, Johnston JM. Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. *Am J Obstet Gynecol* 1981;**139**:373–81.
7. Bilic G, Zeisberger SM, Mallik AS, Zimmermann R, Zisch AH. Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. *Cell Transpl* 2008;**17**:955–68.
8. Hammer A, Hutter H, Blaschitz A, et al. Amnion epithelial cells, in contrast to trophoblast cells, express all classical HLA class I molecules together with HLA-G. *Am J Reprod Immunol* 1997;**37**:161–71.
9. Adinolfi M, Akle CA, McColl I, et al. Expression of HLA antigens, beta 2-microglobulin and enzymes by human amniotic epithelial cells. *Nature* 1982;**295**:325–7.
10. Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res* 2000;**20**:173–7.
11. Sporn MB, Roberts AB, Wakersfield LM, de Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol* 1987;**105**:1039–45.
12. Hao Y, Ma DH, Hwang DG, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea* 2000;**19**:348–52.
13. Thadepalli H, Bach VT, Davidson EC. Antimicrobial effect of amniotic fluid. *Obstet Gynecol* 1978;**52**:198–204.
14. Kjaergaard N, Hein M, Hyttel L, et al. Antibacterial properties of human amnion and chorion in vitro. *Eur J Obstet Gynecol Reprod Biol* 2001;**94**:224–9.
15. Buhimschi IA, Jabr M, Buhimschi CS, et al. The novel antimicrobial peptide beta3-defensin is produced by the amnion: a possible role of the fetal membranes in innate immunity of the amniotic cavity. *Am J Obstet Gynecol* 2004;**191**:1678–87.
16. Marvin KW, Hansen WR, Miller HC, Eykholt RL, Mitchell MD. Amnion-derived cell express intercellular adhesion molecule-1: regulation by cytokines. *J Mol Endocrinol* 1999;**22**:193–205.

17. Etienne-Manneville S, Chaverot N, Strosberg AD, Couraud PO. ICAM-1-coupled signaling pathways in astrocytes converge to cyclic AMP response element-binding protein phosphorylation and TNF-alpha secretion. *J Immunol* 1999;**163**:668—74.
18. Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, Hsi BL. Human amnion as an adjunct in wound healing. *Lancet* 1980;**1**:1156—8.
19. Riau AK, Beurman RW, Lim LS, Mehta JS. Preservation, sterilization and de-epithelialisation of human amniotic membrane for use in ocular surface reconstruction. *Biomaterials* 2010;**31**:216—25.
20. Sakuragawa N, Elwan MA, Uchida S, Fujii T, Kawashima K. Non-neuronal neurotransmitters and neurotrophic factors in amniotic epithelial cells: expression and function in humans and monkey. *Jpn J Pharmacol* 2001;**85**:20—3.
21. Koizumi N, Rigby H, Fullwood NJ, et al. Comparison of intact and denuded amniotic membrane as a substrate for cell-suspension culture of human limbal epithelial cells. *Graefes Arch Clin Exp Ophthalmol* 2007;**245**:123—34.
22. Hennerbichler S, Reichi B, Pleiner D, Gabriel C, Eibl J, Redi H. The influence of various storage conditions on cell viability in amniotic membrane. *Cell Tissue Bank*; 2007:81—8.
23. Van Baare J, Cameron PU, Vardaxis N, et al. Comparison of glycerol preservation with cryopreservation methods on HIV-1 inactivation. *J Burn Care Rehabil* 1998;**19**:494—500.
24. Thomasen H, Pauklin M, Noelle B, et al. The effect of long term storage on the biological and histological properties of cryopreserved amniotic membrane. *Curr Eye Res* 2001;**36**:247—55.
25. Nakamura T, Yoshitani M, Rigby H, et al. Sterilized, freeze-dried amniotic membrane: a useful substrate for ocular surface reconstruction. *Invest Ophthalmol Vis Sci* 2004;**45**:93—9.
26. Lim LS, Poh RW, Riau AK, Beuerman RW, Tan D, Mehta JS. Biological and ultrastructural properties of acelagraft, a freeze dried γ-irradiated human amniotic membrane. *Arch Ophthalmol* 2010;**128**:1303—10.
27. Lopez-Valladares MJ, Teresa Rodriguez-Ares M, Tourino R, Gude F, Teresa Silva M, Couceiro J. Donor age and gestational age influence on growth factor levels in human amniotic membrane. *Acta Ophthalmol* 2010;**88**:e211—6.
28. Velez DR, Fortunato SJ, Morgan N, et al. Patterns of cytokine profiles differ with pregnancy outcome and ethnicity. *Hum Reprod* 2008;**23**:1902—9.
29. Gicquel JJ, Dua HS, Brodie A, et al. Epidermal growth factor variations in human amniotic membrane used for ex vivo tissue constructs. *Tissue Eng Part A* 2009;**15**:1919—27.
30. Lin SD, Lai CS, Hou MF, Yang CC. Amnion overlay meshed autograft. *Burns Incl Therm Inj* 1985;**11**:374—8.
31. Subrahmanyam M. Amniotic membrane as a cover for micro-skin grafts. *Br J Plast Surg* 1995;**48**:477—8.
32. Sawhney CP. Amniotic membrane as a biological dressing in the management of burns. *Burns* 1989;**15**:339—42.
33. Ramakrishnan KM, Jayaraman V. Management of partial thickness burn wounds by amniotic membrane: a cost-effective treatment in developing countries. *Burns* 1997;**23**:S33—6.
34. Branski LK, Herndon DN, Celis MM, et al. Amnion in the treatment of pediatric partial-thickness facial burns. *Burns* 2008;**34**:393—9.
35. Singh R, Purohit S, Chacharkar, et al. Microbiological safety and clinical efficacy of radiation sterilized amniotic membranes for treatment of second degree burns. *Burns* 2007;**33**:505—10.
36. Fraser JF, Cuttle L, Kempf M, et al. A randomised controlled trial of amniotic membrane in the treatment of standardised burn injury in the merino lamb. *Burns* 2009;**35**:998—1003.
37. Mostaque AK, Rahman KB. Comparisons of the effects of biological membrane (amnion) and silver sulfadiazine in the management of burn wounds in children. *J Burn Care Res* 2011;**32**:200—9.
38. Adly OA, Moghazy AM, Abbas AH, et al. Assessment of amniotic and polyurethane membrane dressings in the treatment of burns. *Burns* 2010;**36**:703—10.
39. Mohammadi AA, Seyed Jafari SM, Kiasat M, et al. Effect of fresh human amniotic membrane dressing on graft take in patients with chronic burn wounds compared with conventional methods. *Burns* 2013;**39**:349—53.
40. Seashore JH, MacNaughton RJ, Talbert JL. Treatment of gastroschisis and omphalocele with biological dressings. *J Pediatr Surg* 1975;**10**:9—17.
41. Tekin S, Tekin A, Kucukkartallar T, Cakir M, Kartal A. Use of chorioamniotic membrane instead of bogota bag in open abdomen: how i do it? *World J Gastroenterol* 2008;**14**:815—6.
42. Troensegaard-Hansen E. Amniotic grafts in chronic skin ulceration. *Lancet* 1950;**1**:859—60.
43. Ward DJ, Bennett JP. The long-term results of the use of human amnion in the treatment of leg ulcers. *Br J Plast Surg* 1984;**37**:191—3.
44. Ward DJ, Bennett JP, Burgos H, et al. The healing of chronic venous leg ulcers with prepared human amnion. *Br J Plast Surg* 1989;**42**:463—7.
45. Singh R, Chouhan US, Purohit S, et al. Radiation processed amniotic membranes in the treatment of non-healing ulcers of different aetiologies. *Cell Tissue Bank* 2004;**5**:129—34.
46. Gajiwala K, Lobo Gajiwala A. Use of banked tissue in plastic surgery. *Cell Tissue Bank* 2003;**4**:141—6.
47. Insausti CL, Alcaraz A, Garcia-Vizcaino, et al. Amniotic membrane induces epithelialization in massive posttraumatic wounds. *Wound Repair Regen* 2010;**18**:368—77.
48. Tomita T, Hayashi N, Okabe M, et al. New dried human amniotic membrane is useful as a substitute for dural repair after skull base surgery. *J Neurol Surg* 2012;**73**:302—7.
49. Hasegawa M, Fujisawa H, Hayashi Y, Yamashita J. Autologous amnion graft for repair of myelomeningocele: technical note and clinical implication. *J Clin Neurosci* 2004;**11**:408—11.
50. de Weerd L, Weum S, Sjavik K, Acharya G, Hennig RO. A new approach in the repair of a myelomeningocele using amnion and a sensate perforator flap. *J Plast Reconstr Aesthet Surg* 2013;**66**:860—3.
51. Itoh H, Sagawa N, Hasegawa M, et al. Brain natriuretic peptide is present in the human amniotic fluid and is secreted from amnion cells. *J Clin Endocrinol Metab* 1993;**76**:907—11.
52. Lawson VG. Oral cavity reconstruction using pectoralis major muscle and amnion. *Arch Otolaryngol* 1985;**111**:230—3.
53. Tancer ML, Katz M, Veridiano NP. Vaginal epithelialization with human amnion. *Obstet Gynecol* 1979;**54**:345—9.
54. Ashworth MF, Morton KE, Dewhurst J, Lilford RJ, Bates RG. Vaginoplasty using amnion. *Obstet Gynecol* 1986;**67**:443—6.
55. Samandari MH, Yaghmaei M, Ejlali M, Moshref M, Saffar AS. Use of amnion as a graft material in vestibuloplasty: a preliminary report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**97**:574—8.
56. Kothari CR, Goudar G, Hallur N, Sikkerimath B, Gudi S, Kothari MC. Use of amnion as a graft material in vestibuloplasty: a clinical study. *Br J Oral Maxillofac Surg* 2012;**50**:545—9.
57. Ozkaya O, Egemen O, Yesilada A, Sakiz D, Ugurlu K. The effect of non-preserved human amniotic membrane on the survival of ischaemic skin flaps in rats. *J Plast Reconstr Aesthet Surg* 2012;**65**:1700—5.
58. Gray KJ, Shenaq SM, Engelmann UH, Fishman IJ, Jeraj K, Spira M. Use of human amnion for microvascular interpositional grafts. *Plast Reconstr Surg* 1987;**79**:778—85.
59. Ozboluk S, Ozkan Y, Ozturk A, Gul N, Ozdemir RM, Yanik K. The effects of human amnion membrane and periosteal autograft on tendon healing: experimental study in rabbits. *J Hand Surg Eur Vol* 2010;**35**:262—8.

60. Meng H, Li M, You F, Du J, Luo Z. Assessment of processed human amniotic membrane as a protective barrier in rat model of sciatic nerve injury. *Neurosci Lett* 2011;**496**:48—53.
61. Henry FP, Goyal NA, David WS, et al. Improving electrophysiologic and histologic outcomes by photochemically sealing amnion to the peripheral nerve repair site. *Surgery* 2009;**145**:313—21.
62. O'Neill AC, Randolph MA, Bujold KE, Kochevar IE, Redmond RW, Winograd JM. Photochemical sealing improves outcome following peripheral neurorraphy. *J Surg Res* 2009;**151**:33—9.
63. Mohammad J, Shenaq J, Rabinovsky E, Shenaq S. Modulation of peripheral nerve regeneration: a tissue engineering approach. The role of amnion tube nerve conduit across a 1 cm nerve gap. *Plast Reconstr Surg* 2000;**105**:660—6.
64. O'Neill AC, Randolph MA, Bujold KE, Kochevar IE, Redmond RW, Winograd JM. Preparation and integration of human amnion nerve conduits using a light activated technique. *Plast Reconstr Surg* 2009;**124**:428—37.
65. Alpin AE. Cell adhesion molecule regulation of nucleocytoplasmic trafficking. *FEBS Lett* 2003;**534**:11—4.
66. Yang S, Leong KF, Du Z, Chua CK. The design of scaffolds for use in tissue engineering. Part 1. Traditional factors. *Tissue Eng* 2001;**7**:679—89.
67. Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. *Ophthalmology* 2001;**108**:1569—74.
68. Yang L, Shirikata Y, Shudou M, et al. New skin equivalent model from de-epithelialised amnion membrane. *Cell Tissue Res* 2006;**326**:69—77.
69. Jin CZ, Park SR, Choi BH, Lee KY, Kang CK, Min BH. Human amniotic membrane as a delivery matrix for articular cartilage repair. *Tissue Eng* 2007;**13**:693—702.
70. Amensag S, McFetridge PS. Rolling the human amnion to engineer laminated vascular tissues. *Tiss Eng Part C Methods* 2012;**18**:903—12.
71. Burman S, Tejwani S, Vemuganti GK. Ophthalmic applications of preserved human amniotic membrane: a review of current indications. *Cell Tissue Bank* 2004;**5**:161—75.
72. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. *Stem Cells* 2005;**23**:1549—59.
73. Park IH, Zhao R, West JA. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;**451**:141—6.
74. Witkowska-Zimny M, Walenko K. Stem cells from adipose tissue. *Cell Mol Biol Lett* 2011;**16**:236—57.
75. Walter M, Liang S, Ghosh S, Hornsby PJ, Li R. Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. *Oncogene* 2009;**28**:2745—55.
76. Blasco MA, Serrano M, Fernandez-Capetillo O. Genomic instability in iPS: time for a break. *EMBO J* 2011;**30**:991—3.
77. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue derived MSC. *Cell Commun Signal* 2011;**9**:12.
78. De Coppi P, Bartsch G, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007;**25**:100—6.
79. Miki T. Amnion-derived stem cells: in quest of clinical applications. *Stem Cell Res Ther* 2011;**2**:25.
80. Enders AC, King BF. Formation and differentiation of extraembryonic mesoderm in the rhesus monkey. *Am J Anat* 1988;**181**:327—40.