The Role of Allogenic Amniotic Membrane in Burn Treatment

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Amniotic membrane (AM) has been used in burns for nearly 100 years. The purpose of this article is to give a comprehensive review of the English literature published in the last two decades (1987–2007) to present the current state of this therapy form. Three medical databases (PubMed, Medline, The Cochrane Library) and specific burn journals were electronically screened for relevant articles using carefully selected retrieval strategies and keywords (AM, amnion grafts, burns, wound dressing, amnion banking). Bibliographies of relevant articles were analyzed for additional pertinent publications. After exclusion of articles which referred to the use of AM in reconstructive and ophthalmologic surgery, the inquiry yielded 31 relevant articles in English language dealing with AM and burns. There was no publication fulfilling the criteria of evidence level I, 6 articles had evidence level II, 10 had evidence level III, 6 had evidence level IV, and 9 were merely narrative (level V). The review testifies to—in view of good tissue practice—heightened use of processed AM in burns, especially in the last decade. Randomized clinical trials favored the use of amnion in burns in the first place for promotion of wound healing and in the second place for its comfortable and less dressing changes. Antimicrobial effects, pain relief, reduction of fluid, and scar formation were demonstrated additionally. (J Burn Care Res 2008;29:907–916)

Long before the amnion was introduced for therapeutic use, amniomancy, a method of divination was practiced in many cultures. Reading the caul, being remnants of the amnion sometimes covering the head of newborns, served the diviner for predicting the future. Infants born with a caul, so-called caulbearers, as legend has it that Alexander the Great was one of those, were regarded to be lucky in life and divined to greatness. Other cultures considered such persons to be immune to witchcraft and drowning. As a result of this, cauls were valuable talismans among sailors. In contrast to those preferable properties of amniotic membrane (AM) ancient cultures of Northern and Eastern Europe bound caulbearers to vampirism after death.

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This article reviews the history of human amniotic membrane (HAM) in reconstructive surgery and presents a detailed summary about current tissue preservation techniques. These techniques are introduced in matters of safety and cell viability. Moreover, the proposed beneficial effects and indications of HAM in reconstructive surgery are displayed. Level of evidence of the mentioned publications was evaluated by using a modified version of the Antes classification[1] (Table 1).

Medical History

The early years did not differentiate whether amnion or chorion or both were used, although each membrane comprises a separate entity. William Thornton, a student at Johns Hopkins Hospital was apparently the first one suggesting that fetal membranes, amnion and chorion, might be valuable for wound healing. The first case series about skin transplantation with those membranes in 550 patients followed in 1910 published by Thornton's teacher, Dr. Staige Davis.[2] Subsequent to this report burned and ulcerated surfaces were treated with unseparated amnion and chorion as skin substitutes.[3,4] After a time lag of two decades when fetal membranes seemed not to have been used at all, Brindeau[5] and Burger[6] applied fresh

Table 1. Quality of studies regarding the level of medical evidence

I	Systematic review of randomized controlled trials, meta-analysis
II	Randomized controlled trial
III	Experimental study without randomization (case–control study)
IV	Non-experimental study (case series/case report)
V	Narrative review, expert opinion

Modified after Antes G. [Evidence-based medicine]. Internist (Berl) 1998; 39:899–908.

amniochorionic tissue for the construction of artificial vaginas. In 1940, Chao and coworkers used chemically processed "dry" AM alone and called the membrane now "amnioplastin." "Amnioplastin" was initially recommended as dural substitute to avoid meningocerebral adhesions and subsequent epilepsia after craniotomy. Despite miscellaneous use for abdominal adhesiolysis, neurolysis, and tenolysis, the "amnioplastin" era ended within the forties probably after a warning because of severe adhesions and subsequent seizures when applied as dural substitute.[7,8] In the meanwhile, deRötth[9] and Sorsby and Symons[10] performed landmark procedures in ophthalmology by using both fetal membranes (de Rötth) and "amnioplastin" (Sorsby and Symons) for ocusurface reconstruction. After half a century of "silence" AM was rediscovered again by Batle and Perdomo[11] for HAM transplantation, which opened up one of the most expanding fields in recent ophthalmology. The 1950s and 1960s were signed by the work of Troensegaard-Hansen,[12,13] who applied boiled AM alone to chronic, nonhealing leg ulcers and achieved remarkable results. Human and animal experiments were carried out in the seventies to clarify the immunological response and antimicrobial properties of xenografted, allografted, and autografted unseparated amniochorionic tissue, but also of HAM alone.[14–17] Especially the importance of the AM-epithelial side above and stromal side down for "graft take" was investigated.[18–21] The last decades brought up several reports on different processed AM preventing abdominal and urogenital adhesions and its use in repairing tendons.[22–28] A multiplicity of recent publications deal with AM as wound dressing and point out the possibility to establish AM tissue banks.[29–32]

Anatomy

AM is the innermost of the fetal membranes lining the amniotic cavity. With a thickness of 0.02 to 0.5 mm the human amnion consists of five layers. A single cell layer which rests on its basement membrane is in contact with the amniotic fluid. The underlying connective tissue attaching the basement membrane comprises of another three layers, namely a compact layer, the fibroblast layer, and the spongy layer which in turn is connected with the cellular layer of the chorion (Figure 1). The amnion is translucent tissue devoid of any vasculature.[33,34]

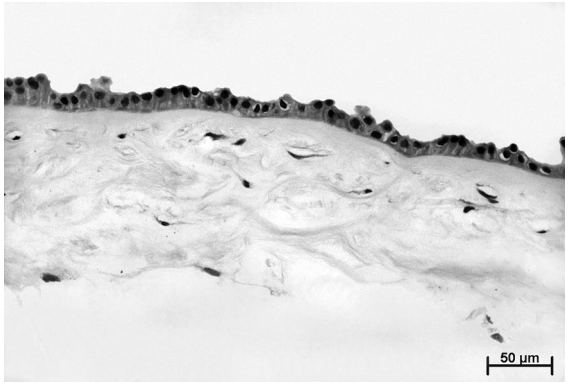

Figure 1. Human amniotic membrane after glycerol/dulbeccos modified eagle medium cryopreservation. A single layer of epithelial cells is attached to a thick basement membrane and an avascular stromal matrix. Hematoxylin and eosin stain; ×200.

Maternal Donors and Amnion Harvest

The Food and Drug Administration (FDA) published an interim final rule on human cells, tissues, and cellular and tissue-based products (HCT/P) in the Federal Register of May 25, 2005. They suggested that any individual intending to cryopreserve his/her HCT/P has to be tested 7 to 10 days before cryopreservation or within a short period after cryopreservation. The eligibility determination for donors of HCT/P final rule 1271.75 recommends that all donors have to be screened for HIV, hepatitis B and C, *Treponema pallidum*, and human transmissible spongiform encephalopathy, including Creutzfeld-Jacob disease. Some authors suggest additional testing on *Toxoplasma gondii*, cytomegaly virus and exclude persons with a history of malignoma, premature rupture membranes, endometritis, and meconium ileus as well as recipients of human growth factor preparations or dura mater transplants from donation.[31,32,35,36] Current protocols for amnion membrane harvest recommend exclusively tissue which is obtained after caesarian sections under sterile conditions. To ensure highest quality of amniotic tissue informed consent has to be given by the pregnant donors and those donors have to be tested before donation and 6 months after.[35,36] The FDA final rules for HCT/P mentioned above advise a quarantine period of 6 months for any HCT/P from di-

rected and anonymous donors, including anonymous donors whose identity might be disclosed.

Current Techniques for Preparation and Preservation

Although fresh AM is frequently applied,[37] the potential risk of disease transmission is advocated. To overcome doubts about infectiousness and fulfill transplantation laws of many countries quite a number of preserving techniques have been developed over the years. Long-time storage avoids the possibility that the donor may be in the "window period" of infection. Hence, even if serological test are inconspiciuous, it is advisable to repeat testing after 6 months. AM can be stored until both samples are negative. The most common procurements for tissue banking are illustrated in the following sections.[38]

Cryopreservation and Glycerol Preservation. After having reintroduced AM for ocular surface reconstruction in the 1990s Lee and Tseng[39] suggested cryopreservation of the fetal membranes which would retain its properties and would render the amniotic epithelial cells nonviable and thus nonimmunogenic. Initially, the placenta is cleansed from blood clots with an antimicrobial solution before AM and chorions are separated (Figure 2). The AM is then apposed onto a nitrocellulose paper with the epithelial side up. The sheets are placed in sterile vials containing dulbeccos modified eagle medium and glycerol (86%) in a ratio of 1:1. For storage these vials are frozen at −80°C.[38,39] This procedure was recommended as a preservation method by the FDA.[38] Other groups proposed an alternative glycerol storage technique. After cleansing and separation, AMs are placed in 85% glycerol for 24 hours at room temperature and afterwards stored in another bottle with 85% glycerol at 4°C. This method does not need specialized equipment, is very simple, and causes low costs. In contrast to other methods, glycerol storage allows long-term preservation up to 5 years.[32,40]

Figure 2. Preparation of the amniotic membrane. Separation of amnion and chorion under the lamellar flow hood.

Freeze-Drying (Lyophilisation) and Gamma-Irradiation. This widespread method was supported by a multinational project initiated by the International Atomic Energy Agency. A quality management system with guidelines for retrieving, processing, and distribution donor tissue was established. Gajiwala and Gajiwala described this technique extensively. AM was pasteurized at 60°C after cleansing, treated with 70% ethanol and finally freeze-dried to remove 95% of the moisture. Terminally, the packed and sealed AM was sterilized by exposure of 25 kGy gamma-radiation in a Cobalt 60 Gamma chamber unit or at an ISO-certified radiation plant.[41] The freeze-dried and gamma-sterilized tissue was stored at room temperature and protected from light up to 6 months. Gomes et al[42] modificated this conservation method by sterilization of the AM with ethylene oxide instead of gamma-radiation.

Peracetic Acid/Ethanol Sterilization. An alternative method which primarily was used for bone tissue sterilization was presented by Pruss et al[31] AM was sterilized with peracetic acid 2% under addition of ethanol 96%. To remove the air bubbles of the peracetic reaction the procedure was carried out under negative pressure of 200 mbar and permanent agitation of the jar for 4 hours.[43]

Silver Impregnation. To increase the antimicrobial properties of HAM silver nitrate impregnation was suggested. After cleansing HAM with sterile saline it is rinsed with distilled water. Subsequent silver binding is limited by removal of the chloride under this procedure. Silver nitrate impregnation is performed by placement of HAM in light-proof bottles for 2 hours containing 0.5% silver nitrate.[44] Singh et al reintroduced silver coating of HAM in a recent publication. Processing is widely similar to that of air-dried irradiated HAM. Before membranes are sterilized by gamma-radiation at 25 kGy a novel method was applied to deposit silver by in situ reduction onto the membrane.[45] A randomized in vitro study exhibited a 3-log reduction of bacteria by silver-impregnated HAM compared with uncoated HAM.[45]

Impact of Preservation Technique on Cell Viability, Antimicrobial Properties, and Growth Factor Content. Hennerbichler et al[46] assessed the viability of AM under various storage conditions in a randomized controlled trial. Different preservation processes in use for HAM were assessed with trypan blue staining and a cell proliferation and cytotoxicity assay. Fresh amniotic tissue saved as positive control

with an ascribed 100% viability. Within 28 days of storage above 0°C viability was diminished to 15 to 35% of the original level. Freezing over a time of 3 weeks reduced the viability to 13 to 18% of the base level, regardless of the cryo-temperature or the cryo-medium.[46] Glycerol storage of HAM resulted in immediate cell death. These findings agreed with the investigations of Kruse et al,[46,47] who compared the cell viability of fresh and glycerol-cryopreserved amnion of four placentas by trypan blue stain in a randomized trial. Rama et al[48] used 10% dimethyl sulfoxide instead of glycerol for cryopreservation and achieved a retention of cell viability of approximately 40% in its preparation technique. Hence, glycerol-cryopreserved (−80°C) and glycerol-preserved HAM (+4°C) were considered to function primarily as matrix, and therefore viability was not appreciated to be essential for the biological effectiveness.[40,47] The microbial permeability and sterility of air-dried radiation processed preparations was investigated by Singh et al. Membranes which were stored under different temperature and humidity conditions were compared. To exclude experimental errors different processed tissue samples derived from the same donor were observed in a randomized controlled trial. In the result, neither the antimicrobial impermeability of the membranes nor the sterility was affected by different environmental conditions.[49] Branski et al investigated the influence of processing methods and irradiation on growth factor content of HAM in a controlled randomized study. HAM of group 1 was only washed in saline, group 2 additionally in hypochlorite solution, group 3 also received antimicrobials for 3 days, and the last group was deepithelialised by trypsin. All the membranes were glycerol-stored at −80°C and half of the samples of each group were gamma-irradiated. The content of six growth factors in HAM was detected by enzyme-linked immunosorbent assay and found to be significantly lower in the fully processed group 4 than in fresh HAM. Transforming growth factor (TGF)-α levels remained unchanged in all samples, whereas radiation only reduced concentration of fibroblast growth factors (FGF-1 and FGF-2) in the saline-hypochlorite washed group. As a result gamma-irradiated fresh HAM was recommended as an ideal preparation.[50]

Properties of the Amniotic Membrane

Promoter of Epithelization. Many authors emphasized AM in numerous indications for accelerating epithelization as pointed out later. Growth factor and growth factor receptor expression of cryopreserved AMs stored at −80°C for 1 month were examined by Koizumi et al. Reverse-transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay identified significant mRNA expression and protein concentrations of the epidermal, the keratinocyte, the hepatocyte, the basic fibroblast growth factors and the TGFs (EGF, KGF, HGF, bFGF, TGF-α, and TGF-β-1, -2, -3) as well as the growth factor receptors keratinocyte growth factor receptor and hepatocyte growth factor receptor in stromal and epithelial amnion regions. Those factors were regarded as significant for epithelial regrowth.[51]

Immunogenicity. Initially no human leukocyte antigen (HLA-A, -B, or -DR) was detected in cultured AM and it was believed to be nonimmunogenic. Subsequent case series showed no rejection of fresh AM after implanting it into subcutaneous pockets of seven volunteers.[14,52] Further studies by various groups showed class 1 antigen and comanifestation of class 1a (HLA-A, -B, -C, or -DR) and class 1b (HLA-A, -B, -C, or -DR) antigens in epithelial cells, in mesenchymal cells and in fibroblasts of HAM.[53–56] AM was considered to be immune-privileged tissue despite expression of 1a and 1b antigens and containing immunomodulatory factors that class include HLA-G and Fas ligand.[53] Using the preparation method suggested by Lee and Tseng[47] the amniotic cells are not viable, and therefore immunogenicity is of no consequence.

Analgesic Properties. AM dressing stopped the contact to the environment of lesions-like burns by covering the exposed nerve endings. Several authors discussed this topic as reason for immediate pain relief after AM coverage.[57–59]

Protection From Protein/Fluid Loss. The adherence of AM on wound beds within 8 to 16 hours is likely to be the factor which helped to prevent infection and limits the loss of fluid mainly in burns.[58,60] Salisbury et al conducted a prospective randomized study to investigate evaporation of biological dressings on granulating wounds. Within 24 hours after coverage cadaver allograft was twice as effective as sheet porcine xenograft and five times as affective as meshed porcine xenograft and fresh fetal membranes (undissected amnion and chorion).[61]

Antimicrobial Activity. Robson and Krizek[16,17] investigated the antibacterial activity of saline-stored fetal membranes (amnion and chorion together) in several randomized controlled animal trials. In a burn model with 50 rats the wounds were inoculated with *Pseudomonas aeruginosa* and several dressings were applied. Ninety-six hours later the fetal membrane group showed a significantly decreased bacterial count than animals dressed with human skin or left undressed. A second burn infection experiment in rats evaluated fetal membrane dressing equal to au-

tograft, but superior to allografted and xenografted skin.[16,17] Contradictory effects were observed by Salisbury et al, who conducted a randomized trial with different coverage on human burn wounds. AM was found to be as effective as cadaver skin, but not superior to xenogenous porcine grafts.[62] Heiligenhaus et al induced corneal ulcers in BALB/c mice with HSV1 in a randomized trial. After 14 days, 16 mice were covered with HAM and received subsequent tarsoraphy, whereas another 16 mice received tarsoraphy alone as controls. Compared with the controls HAM mice showed at days 2 and 7 postoperatively reduced stromal inflammation and ulceration.[63] Talmi et al placed fresh HAM alone, fresh undissected HAM and chorion and a synthetic polyurethane-based membrane on discs which were inoculated with five types of bacteria. After this randomized trial all membranes displayed an antimicrobial effect in vitro. However, these results were more likely contributed to the close adherence of the membranes to the wound surface than to the content of antimicrobial factors.[64] The innate immune system of amnion cell lines and amniotic tissue of five healthy patients and eight patients with chorioamnionitis were investigated by RT-PCR and immunohistochemistry.[65] A basal human-β-3-defensin expression as well as expression after stimulation to microbial wall constituents was detected in amnion cells. In addition, the participation of amnion in innate immunity was confirmed by immunohistochemistry with human-β-3-defensin antibodies in healthy tissue samples and verification of a significant overexpression by semiquantitative score in inflamed tissue samples.[65] Hao et al proved the existence of anti-inflammatory proteins in mesenchymal and epithelial amniotic cells derived from amniotic tissue of healthy women. Interleukin (IL)-1ra, structurally similar to IL-1β, and to a lesser extent IL-10, both anti-inflammatory cytokines, were both detected by RT-PCR and were contributed to the observed properties of HAM.[66]

(Anti-)Angiogenicity. The effects of AM on vessel development were discussed controversial. Faulk et al covered chronic leg ulcers in a case series of 15 patients with AM which had been kept in tissue culture medium. Five days after coverage biopsies from the ulcer bed were taken and a significant vessel proliferation was identified by histological examinations and immunohistochemistry.[67] Another case series assessed the blood flow to intraoral lyophilized AM grafts. Using the 133Xe technique the graft regions showed increased vascularisation compared with preoperative normal mucosa.[68] However, ophthalmologists accentuate the use of AM for its antiangiogenic qualities on corneal surfaces. Hao et al studied fresh and glycerol-cryopreserved HAM as well as amniotic mesenchymal and epithelial amniotic cells for antiangiogenic factors. RT-PCR evaluated mRNA expression of tissue inhibitors of metalloproteases (TIMP-1, -2, -3, -4), thrombospondin-1, and endostatin in amniotic cells which could be corrobated by immunohistochemical analysis of fresh and cryopreserved HAM.[66]

Amnion in Burns

Sabella[4] treated a burn patient with AMs for the first time on June 6, 1912. Since then coverage with AM is a common, well-known technique in burns. The appearance of the human immunodeficiency virus and Creutzfeld-Jakob disease changed handling with human tissue and tissue-based products substantially. The potential risk of disease transmission prompted several authors to discuss the continued use of amnion.[69] Thus, the current review concentrates on the last 20 years of "amnion in burns," especially in light of the preservation techniques which evolved during that time. Most of the recent articles focused upon the role of AM in corneal and conjunctival burns, which is clearly not the goal of this article.

Three medical databases (PubMed, Medline, The Cochrane Library) were screened for relevant articles from 1987 to 2007. Bibliographies of relevant articles which were detected by the keywords "AM," "amnion graft," "burns," "wound dressing," and "amnion banking" were analyzed for additional pertinent publications. Supplemental search based on these keywords was conducted in burn journals as *Journal of Burn Care and Rehabilitation*, now *Journal of Burn Care and Research*, *Burns*, and *Annals of Burns and Fire Disasters*, formerly known as *Annals of the Mediterranean Burns Club*. After exclusion of articles which dealt with the use of AM first and foremost in ophthalmology or other fields out of our range of interest, the inquiry yielded 31 relevant articles in English language. In concordance with the scientific levels of evidence determined in Table 1 no publication fulfilled the criteria of level I, but 6 articles those of level II, 10 of level III, 6 of level IV, and 9 were merely narrative (level V). Twenty-eight of 31 articles included the treatment of partial-thickness burns as central theme (Table 2).

Matthews et al suggested categorization of AM in burns to management of donor sites, full-thickness burns, and partial-thickness burns.[70]

Donor Sites. Burn wounds are usually covered with split-thickness skin grafts, which cause donor site wounds on their part. Coverage of these donor sites with HAM or both fetal membranes was mentioned in several publications before 1987.[57,71,72] This clinical practice was continued by a couple of surgeons

Table 2. Use of amnion in second-degree burns. Review of the cited literature since 1987

Author	No. Patients	Level of Evidence	Preparation Method	Assessed Properties/Outcome
Haberal et al[44]	745	IV	Fresh silver-incorporated	AM
Haberal et al[79]	62	III	Fresh silver-incorporated vs fresh	WH, AM, P
Kasi et al[84]	3	IV	Freeze-dried irradiated	WH, P, FF
Haberal et al[80]	125	V	Fresh silver-incorporated	
Lorusso et al[78]	11	II	Sterilized, frozen	WH, AM, FF, HO
Sawhney[77]	90	II	Fresh (saline-hypochlorite stored)	WH, AM, P, FF, CO, SC, DC
Ding and Han[85]		V	Fresh	WH
Dioguardi et al[92]		V		WH
Magliacani[91]		V	Fresh (liquide stored)	WH, DC
Alsbjorn[86]		V		WH, P, FF
Atanassov et al[74]	21	IV		WH
Ugar and Haberal[81]	52	II	Fresh silver-incorporated	WH, AM, P, DC
Subrahmanyam[58]	24	III	Fresh (saline washed)	WH, P, CO, SC
Hadjiiski and Anatassov[75]	20	III	Saline washed, ethyl-alcohol preserved	WH, AM, P, FF, DC
Ganatra and Durrani[37]	22	III	Fresh	AM, VA
Ramakrishnan and Jayaraman[87]	2/350	IV	Fresh (saline-hypochlorite stored)	WH, P, FF
Tyszkiewicz et al[35]		V	Deep-frozen irradiated	WH, AM, P, FF
Maral et al[40]	106 rats	II	Glycerol-preserved vs fresh	WH, AM
Keswani et al[69]		V		WH, P, FF
Sheridan and Moreno[88]		V		VA
Rejzek et al[89]		III	Glycerol-preserved/fresh	HI
Ganatra[93]		V	Various	WH, AM, P, FF
Ley-Chavez et al[59]	12	III	Air-dried irradiated	P, HO
Ravishanker et al[32]	71	III	Glycerol-preserved	WH, AM, P, CO, DC
Gajiwala and Gajiwala[90]	24	III	Freeze-dried irradiated	WH, FF, DC, VA
Gajiwala and Gajiwala[41]	35	III	Freeze-dried irradiated	WH, FF, DC, VA
Singh et al[82]	50	II	Air-dried irradiated vs glycerol-preserved	WH, AM, FF, DC
Branski et al[50]	53	II	Glycerol-freezed + topical antibiotics	WH, SC, DC

WH, time of wound healing; *AM*, antimicrobial effect; *P*, pain level; *FF*, fluid formation; *HO*, Length of hospital stay; *CO*, Contracture formation; *SC*, Scar formation; *DC*, no. dressing changes; *VA*, vascularisation; *HI*, histological observation.

although their reports lack a high level of evidence, which is set in brackets, respectively.

Talmi et al described numerous patients who underwent free skin grafting and conducted coverage of donor sites with fresh AM. After good adherence of AM to the wound bed marked pain relief was observed (IV).[73] Maral et al produced split-thickness skin wounds in rats. Those wounds were randomized (II) and subsequently covered with preserved skin, fresh amnion, glycerol-preserved amnion, or left uncovered as control. Preserved skin performed best with regard to wound adherence, epithelization, and leukocyte infiltration. Fresh and glycerol-preserved AMs were effective as well. When AM was adherent epithelization was completed by 7 days. Wounds that were unhealed at day 7 showed loose adherence of AM and increased inflammatory reaction similar to control wounds.[40] A Bulgarian group reported about their experiences (IV, V) in covering donor regions with AM. AM was immediately applied after grafting and haemostasis. Membranes were left in place until epithelization was completed which took 2 weeks. Donor wounds treated in this manner caused less pain, and infection was reduced in comparison with conventional clinical dressings.[74,75] Bari et al (IV) covered the split-skin donor area of five patients with gamma-irradiated AM. In contrast to the other studies, severe pain, probably due to dryness resulting from irritation of nerve endings, was noticed.[76]

Partial-Thickness Burns. The use of HAM in partial-thickness burns has frequently been published in the last two decades. Hence, the present review describes only those articles with evidence level II and III in greater detail. To avoid redundant information evidence levels of all articles regarding this topic are listed in Table 2. Sawhney divided up 90 patients with partial-thickness burns in 3 subgroups of 30 patients each: superficial, intermediate, and deep dermal burns. Half of the patients of each subgroup were treated either with

silver sulphadiazine cream or with fresh AMs. Observation criteria were pain, oozing, scar formation, and healing time. Dressing changes were painful in controls and comfortable in HAM patients. Oozing was reduced in HAM patients and hypertrophic scarring did not occur in HAM-treated patients with intermediate wounds, but in controls. Mean healing time was significantly faster in all groups with amnion coverage than in controls (superficial 9.3 vs 12.5 days, intermediate 15.7 vs 23.9 days, deep 27.5 vs 37.5 days).[77] Subrahmanyam described partial-thickness burns of 64 patients either treated with honey-impregnated gauze or fresh AM. Honey-impregnated gauze significantly decreased time of epithelization to 10 to 15 days compared with 16 to 30 days under AM dressing. Pain assessment evaluated 82.5% of the patients treated with honey-impregnated gauze having no or moderate pain, whereas only 54.1% of the AM patients suffered from moderate pain or remained painless. In addition, scar formation after 3 months was 8% in honey-gauze treatment and 16.6% after HAM treatment.[58] Lorusso et al analyzed sterilized, frozen AM compared with Biobrane® (Bertek Pharmaceuticals, Morgantown, WV) in second-degree superficial and deep burns of 11 patients. The two substitutes were applied to contiguous burned areas in the same patient and controlled after days 2 and 4. Dressings adhered completely in all cases except two and secretion was only apparent in one case. Wound swabs evaluated bacterial contamination in 3 of 22 wound surfaces after 4 days. In conclusion, both membranes showed efficacy in quality and wound healing, but amnion was estimated to be more competitive from the economic point of view.[78] Twelve pediatric patients having received air-dried, gamma-irradiated HAM after burns with 1 and 2 degrees were monitored by a pain scale (scale of 10). In 92% of all patients, pain was reduced dramatically after amnion application from level 8 to 9 to level 0 to 2. The remaining 8% reported about pain decrease from pain level 7 to 2.[59] In the 1980s, a Turkish group applied fresh, saline-washed in 40 patients and 0.5% silver nitrate incorporated HAM to 56 patients with partial and full-thickness burns. Wound cultures displayed generally less infection in silver nitrate incorporated AM (SNIAM)-treated persons, although *Pseudomonas aeruginosa* population was slightly less in wounds treated with AM alone.[44] Two additional case series of this team described 745 cases with burn injuries, and 125 cases suffering from electrical thermal injuries. By the means mentioned above, wounds of the patients were dressed with SNIAM as well. Results of wound cultures of 745 patients evaluated *Pseudomonas* alone in 40.4% and mixed bacteria in 25.6% of the cases. SNIAM was judged to be more effective when used alternately with local chemotherapy agents such as sulformylon and silvaden.[79,80] Another prospective, randomized study in 52 patients showed a reduced healing time of about 12 days after Water Jel® (Water-Jel Technologies, Carlstadt, NJ) and SNIAM treatment compared with nitrafurazone embedded gaze and Omiderm™ (Omniderm Ltd., Hollywood, FL). The clinical wound infection rate in those patients which were divided up in four treatment groups was lowest in the SNIAM group. Water Jel was found to be the most effective dressing material for pain relief in partial thickness burns, SNIAM and Omiderm caused no pain increase on application.[81] Later on the Haberal et al passed into use of glycerol-preserved HAM and studied the effectiveness of glycerol-preserved HAM in a randomized animal trial. Fresh HAM, preserved human skin and untreated wounds served as controls. Macroscopic and histological examination of full-thickness and partial-thickness wounds in rats showed no differences in graft adhesion after 2 days, but skin grafts developed less leukocyte infiltration compared with both HAM preparations. The bacterial counts of iatrogenic burn wounds were reduced about 100% within 96 hours after skin graft application, about 94% after fresh HAM, 88% after preserved HAM and 31% in untreated controls.[40] Bulgarian surgeons favored AM as dressing for superficial burns. They observed a reduction of fluid and protein loss, no painful dressing changes and development of a strong epithelium with good quality. Burn wound swabs of 20 patients presented bacterial colonization in 13 of 20 patients before AM treatment and in 7 of 20 patients during AM treatment.[75] Ravishanker et al treated 71 superficial and partial-thickness burn patients with glycerol-preserved HAM as well and compared those data to 73 patients with closed dressing. Initially full-thickness burn patients were included in the study, but the practice of HAM coverage was stopped after two wounds became infected. No infection was described in superficial burns 1 week after HAM application in contrast to 37 of 73 patients with closed dressing. A dramatic pain relief was noticed after AM application whereas 80% of the controls complained about pain. The authors did not present statistically confirmed data but described wound healing within 7 to 10 days in superficial and about 20 days in partial thickness burns. Further wound adherence, especially in superficial wounds, easy application, maintenance of a moist wound environment and the economic advantages are highlighted and attributed to HAM.[32] A recent publication compared air-dried irradiated

and glycerol-preserved HAM for its effectiveness in partial-thickness burns of 50 patients.[82] Half of the wound area of each patient was covered with glycerol and irradiated HAM. Both materials were followed up for several criteria. Application of irradiated tissue was much easier and comfortable, and therefore provoked less pain compared with glycerol-stored HAM. Fluid under the membranes developed in five patients with glycerol-preserved and four patients with irradiated HAM, whereas four patients with glycerol-preserved and three patients with irradiated HAM developed hypertrophic scars. No one suffered from restrictions in joint movement. Overall no differences in healing time were observed which was completed between 10 and 14 days in all wound samples.[82] Lack of prospective randomized trials on amnion as coverage in burns induced Branski et al to compare HAM with standard topical treatment in the management of pediatric second-degree burns. Fifty-three patients with burns in the face, head, and neck region received HAM with topical antibiotic cream and 49 patients received topical antibiotic cream alone. During follow-up patients with amnion dressing had significantly less membrane changes and time to total healing was faster (6 ± 2 days vs 8 ± 2 days), whereas scar formation, infection rate, and length of hospital stay did not differ between both treatment groups.[83]

A complete survey including all relevant articles on this issue is presented in Table 2.[32,35,37,40,41,44,58,59,69,74,75,77–93]

Full-Thickness Burns. Treatment of third-degree burns with AM had been challenging. Dino et al covered three cases of third-degree burns with saline- and hypochlorite-washed HAM. The membranes did not adhere to burned sites and easily pealed off after 24 to 48 hours, which induced the authors to give up this practice.[57] The required management when using HAM was much more difficult than dressing with allografts and frequent infection was noticed.[57,71,94] Beside these historical observations from the 1960s to the early 1980s, some current reports deal with HAM and third-degree burns. Evidence level of those studies is indicated by roman numerals. As mentioned above, Sawhney examined 30 patients with deep dermal burns and found dissolution of fresh AM when the burn wound was covered with dead tissue. If epithelial regeneration had already began, AM was suggested for epithelial protection (III).[77] Gajiwala and Gajiwala investigated in two studies the coverage of 24 and 35 patients with second- and third-degree burns with freeze-dried irradiated HAM, respectively. The exact number of third-degree patients remained unclear in this case control studies (III), but the authors recommended amnion only after removal of the eschar and not in infected wounds. If infection occurred, which was visible through the membrane, HAM was removed and fresh dressing was applied. The use of HAM was highlighted because of reduced exudation, reduced induration, more comfort, less pain, and good re-epithelization.[90] Subrahmanyam reported about 18 patients with full-thickness burns who received composite grafts from minced split-thickness skin poured over fresh amnion (III). After 5 to 7 days wounds were redressed with fresh HAM and appeared fully epithelized within 7 to 10 days. Five wounds did not heal or developed infections.[95] Bari et al treated deep dermal burns (III) either with irradiated AM (group A, 25 patients) or with meshed split-skin graft and AM coverage (group B, 15 patients) in a prospective study. Group A showed quick granulation tissue formation (average 8.7 days) and the number of patients with wound infections dropped down from 10 to 4 within 7 to 10 days. Group B showed delay in coalescence of the mesh-graft in all patients. Hypertrophic granulation tissue protruded through the meshed skin and prolongated healing.[76] Some authors reported about experiences (V) with amnion and chorion used in toto for coverage of deep burns, although this method remains doubtful in the light of disease transmission.[75,91,92]

CONCLUSIONS

This review underlines the versatile properties of human AM, which has been used since 1912 as fresh amnion in burn treatment. In the 1990s, amnion coverage has become popular again as a result of its efficacy in ocular surface reconstruction.

The key role for the revival of AM in reconstructive surgery played the development of long-time preservation techniques to overcome demurs on immunogenicity and infectiosity.

A systematic validation of amnion in burn treatment is limited by the poor evidence level of most of the relevant publications. As valuable source to gain reasonable information for clinical decisions the randomized clinical trials (6 of 31)[1] were preselected. All of them observed the effects of AM on time of wound epithelization. Whereas AM was superior to silver sulphadiazine cream, Omiderm, nitrafurazone-embedded gaze, or application of topical antibiotics alone; it was inferior compared with Water Jel and preserved human skin, whereas there was no difference between the use of Biobrane and frozen amnion. Four of six of the randomized controlled trials focused on the number and comfort of dressing changes when using AM compared with other burn wound coverage. Amnion was comparable with Omiderm, but superior to all remaining applica-

tions, and irradiated HAM seemed to be more comfortable than glycerol-preserved HAM. Antimicrobial effects were studied in five of six RCTs with heterogeneous results. HAM helped to reduce overall burn wound infection; however, preserved human skin was more effective. Biobrane had comparable effects with HAM, whereas treatment with membranes and topical applications mentioned above resulted in higher infection rates. Further beneficial effects of HAM in burns were demonstrated for pain relief, fluid loss and scar reduction and, especially in developing countries, low costs.

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