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UP-TO-DATE USE OF AMNION IN SURGERY

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Abstract: *This review article presents the properties, modalities of preservation and therapeutic applications of human amniotic membranes, as revealed by numerous data from literature. Amniotic membranes have a series of qualities: wound protection, pain decreasing, infection prevention, good adherence to wound bed, promotion of epithelization, low antigenicity, cost effectiveness and quite easily availability. The main clinical utilization has been for burns treatment, as temporary biological dressings. Other therapeutic applications have been for wounds coverage and as mucosal substitutes in different surgical specialties. Further studies are necessary to establish the best therapeutic indications and modalities of application.*

Key words: *human amnion, burns, temporary biological dressing.*

1. Introduction

The temporary biological dressing of extensive burns or open wounds has been an important resource in the wound care armamentarium and has generally proven to be a life-saving therapeutic measure [15]. In the extensive deep burns, with insufficient skin reserves to obtain an efficient skin covering, the use of temporary skin substitutes allow the organism to regenerate its own epithelial reserves, to decrease the loss of fluids, proteins and evaporative heat from the wounds, to protect the wounds from the environment and to prevent subsequent bacterial colonization [15], [27].

Temporary skin covering can be achieved by natural skin substitutes such as human skin allografts, pig skin xenografts and human amniotic membranes, and also by synthetic and semisynthetic skin substitutes, such as bilayered synthetic structures and composite materials based on collagen (*Biobrane*) [27]. Fresh human cadaver skin allograft remains the most valuable temporary skin substitute, which cannot be replaced presently with any synthetic, semisynthetic or culture material [15], [27]. However, legal issues regarding organ donation in some countries and the cost of procuring, preparing and storing allografts (skin banks) may limit their extensive use in burns' treatment. In contrast, the use of human amnion represents a cost-effective alternative of treating burns, especially in developing countries, where the high incidence of burn injuries is further complicated by financial constraints [10], [19].

2. Objectives

This article has intended to achieve an analyses and synthesis of the data from literature regarding the utilization of

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human amniotic membranes for the wounds treatment, and also to provide a series of conclusions and recommendations on their use in practice.

3. Materials and methods

A lot of observations, studies and results are available in the literature regarding the use of human amniotic membranes for the treatment of wounds and burns. These data are available from databases, such as Medline, from journals, such as Burns and Annals of Burns and Fire Disasters, from online wound care resources and from specialized books. This article has performed comprehensive analyses of the utilization of human amniotic membranes, by selecting the most reliable and representative studies, without applying any restrictions regarding the source or date of publication.

4. Results and Discussions

The human amniotic membranes have been used for centuries as a biological wound dressing. In China and Japan, amnion is considered a potent medication for the treatment of many diseases and is believed to have „magical strength of youth" [6]. In Western medicine, amniotic membranes have been used since the beginning of the last century [2], [6], [15], [24]. In 1910, Davis introduced the use of human fetal membranes in skin transplantation, and 3 years later Sabella described it for burned and ulcerated skin surfaces [6], [15]. The aim of these early attempts was to achieve durable wound coverage and, although the organism ultimately rejected the amniotic membranes, lack of infections and alleviation of pain were noted [15]. In 1952, Douglas reported the use of amniotic membranes to temporarily cover burn wounds [6], [15]. Since then, human amniotic membranes have achieved good results as temporary biological dressings and have been mostly used for the treatment of partial-thickness burns [2], [3], [6], [10], [14], [17], [19].

Amniotic membrane or amnion is a thin semitransparent tissue forming the innermost layer of the placenta. It has an avascular stroma, a monolayer epithelium and a thick continuous basement membrane with collagen types IV and V and laminin, and contains several proteinase inhibitors [4]. Amnion has an inner layer of cuboidal or flatteened epithelial cells that are continuous with the ectoderm of the embryo and an outer surface covered with mesenchymal connective tissue. The use of amniotic membranes offers the advantage that thicker intact membrane can be handled more easily and remains on the wound longer than amnion by itself [15].

The amniotic membranes meet most of the qualities of an ideal skin substitute, which are as follows [25], [27]:

- able to prevent water loss
- firm adhesion to the receptor site
- able to withstand shear forces
- able to resist infection
- cost-effective
- widely available
- long shelf life and easy to store
- lack of antigenicity
- flexible thickness
- durable with long term wound stability
- conformable to irregular surfaces
- easily secured and applied
- lack of toxicity
- pain decreasing.

Amniotic membrane has shown a series of unique properties including wound protection, pain reduction, bacteriostatic properties, infection prevention, good adherence to wound bed and promotion of re-epithelization [3], [6], [10], [24].

Another useful property of amniotic membrane is the lack of immunogenicity

[7], [24]. It is flexible and conformable to irregular wound surfaces, easily moldable and removable, cost effective and quite easily available [6]. However, contrary to human skin allograft, it has a fragile structure and is technically more difficult to handle [25].

In the past, amnion was generally used fresh, but frozen, dried, lyophilized, gamma irradiated or glycerol preserved preparations have also been made up. For clinical use, amnion must be easily stored long-term, free of contamination and without biodegrading [15]. Studies have shown that amnion can be maintained in proper condition for up to 6 weeks if stored aseptically at 4^0C in 0.5% silver nitrate solution [10]. Cryopreservation of amnion in liquid nitrogen at -70 to -90^0C allows it to be stored for over 6 months, but in this case the material must be thawed before use. Glycerol serves the basic mainstay for tissue preservation; glycerolization of skin tissue was first implemented by Basile in 1982 to preserve porcine skin for long periods at low cost. Glycerol dehydrates tissue by physically replacing most of the intracellular water but does not change the cells' ionic concentration, thus protecting cell integrity. Moreover, microbiological studies have demonstrated that concentrated glycerol is slow but effective antibacterial agent. When this conservation process is used, amnion retains its structural properties and is comparable to fresh amnion in its effectiveness as a biological dressing [15].

The major advantages of 85% glycerol preservation have been shown to be [20]:

- Simple and low cost
- Ease of preservation and reconstitution
- Preservation of the morphological structure of the cells in the skin grafts/amnion [12], [21].
- Low antigenicity as the cells are non-viable [12].
- Antibacterial and antiviral properties of glycerol, both extracellular and intracellular, and no danger of transmission of bacterial or viral diseases [16].
- Glycerol preserved amnion is as effective as fresh amnion in terms of adherence, pain release, structural maintenance, decreasing bacterial load in wounds and healing activity.

As for other biological dressing, the main concern for amnion use is the transmission of infectious diseases. In order to avoid any risk of infectious contamination from vaginal deliveries, human amniotic membranes are recommended to be obtained according to the guidelines of tissues harvesting [6], after informed consent, only from caesarean deliveries of booked patients selected by following criteria [20]:

- Without medical history for potential risk factors such as cancer, infectious and venereal diseases;
- Without history of endometritis, premature rupture of membranes or meconium ileus;
- Serologic tested negative for HBV, HCV, HIV and Syphilis, preferably at the time of delivery and 60–90 days after delivery, during a scheduled outpatient appointment;
- Rejection of meconium stained amnion.

Washings with antibiotic solutions, lyophilization, sterilization with 60Co gamma radiation, or long-term glycerol preservation have all been used to free amniotic membranes from bacterial and fungal contamination. Amnion used as a biologic dressing has been proven to be impervious to micro-organisms and is usually free from toxic material. However, disease transmission remains a possibility as with any biological material. Human cytomegalovirus (CMV), known to replicate in vitro in human fibroblastic cells, was found to replicate in epithelial human amnion (HA) cells as well and may constitute a real risk whenever amnion is used as a biologic dressing. This is more of

a theoretic concern than a real threat. In a study performed on severely burned immunosuppressed patients, 52% became infected with either herpes simplex virus (HSV) or CMVor both, with no significant association of such infections with mortality. In another retrospective survey of serum for viral antibodies in pediatric burn patients, in 33% of the children CMV infection developed, in 25% herpes simplex infection, and in 17% adenovirus infection. In all of the most severely burned children CMV infections developed, and both primary and reactivation infections were observed. With these facts, any potential risk of amniotic membranes or any other biologic material transmitting CMV or herpes simplex infection becomes irrelevant. However, a real concern for infection still remains [20], [25].

The main clinical utilization of amniotic membranes has been for burns treatment. The amniotic membranes have been used for more than a century as skin substitute for temporary burn coverage. Amniotic membranes can be used in two ways [11]:

- in toto (as a whole: amnion + chorion) on deep burns
- amnion alone (epithelium with basic membrane) on superficial burns.
- According to studies and clinical trials, they have a series of advantages [11], [20]:
- significant relief from pain
- easy and economic to prepare, sterilize and storage. The storage of glycerol preserved amnion requires only an ordinary refrigerator.
- histological structure similar to that of skin
- no allergic reactions
- promotion of healing, probably the best feature of the amniotic membranes. Wound healing in superficial burns has occurred completely within 7–10 days. In partial thickness burns, healing has taken longer, but was again complete within 20 days. As the wounds healed, the membranes would peel off spontaneously.
- supply and maintenance of local moist environment. The applied membranes dried on the burn wound and as they desiccated, they separated from the healed areas leaving behind a well healed soft and supple skin.
- rapid adherence of amnion to the burn wound and lack of neovascularization or immunological rejection are other advantages of this tissue compared to homografts or xenografts [15].
- useful for islamic, jewish or Indian populations, with cultural and religious barriers to the acceptance of homografts or xenografts (bovine and porcine skin).

The indications for the application of human amniotic membranes for burns and wounds treatment are as follows:

- superficial burns: facial burns, limited superficial burns, open treatment of patients with large burn areas [6], [7], [11]. A dry dressing is applied for 24 hours, which makes the amniotic membranes adhere to the wound surface. The following day, a control dressing is performed and if the membrane is already dry, the burn surface may remain open until complete epithelisation [11];

- after surgical debridement of partial thickness burns, until complete healing, which varies depending upon the extent and depth of the wound and the amount of exudates [4], [9]. Compared to patients with superficial burns, in whom amniotic membrane dressing change are seldom necessary, patient with deeper burns require more frequent changes, as the dressings tend to disintegrate, but the amniotic membranes keep the underlying tissue fresh and viable [11];

- temporary coverage of full thickness burns, preparing the wound bed for eventual necessary skin grafting [2], [4];

- cover for microskin grafts or an overlay of widely meshed autografts, promoting

early epithelization and rapid wound healing [2], [4], [18].

- immediately coverage of donor regions, after graft take and hemostasis. The amniotic membrane is left in place until complete epithelisation of the domor region surface [11].

- after dermabrasion [11]; the abrasive surface is covered with amniotic membrane, which detaches after healing.

- leg ulcers, non-healing wounds and pressure sores [5], [9].

Apart from being a skin substitute and a wound dressing, amnion has been used as a mucosal substitute, in different surgical specialties:

- ophthalmology, as a tissue bandage for cornea infections and sterile melts, and as a graft for ocular surface reconstruction, in various procedures [1], [26].
- maxillo-facial surgery, as a graft material for oral cavity or in lower anterior ridge vestibuloplasty [24]
- otorhinolaryngology [28]
- urology, for bladder and urethral reconstruction [8], [13].
- gynaecology, for vaginoplasty [22], [23].

5. Conclusions

The human amniotic membranes are easy to obtain, safe and cost effective biological materials for skin and mucosal substitution. The multiple qualities of amniotic membranes, ensuring quick, reliable and painless cure, make them useful tool in burn care armamentarium. They should be used with confidence in burns treatment, as an extremely economical alternative to other biologic or to synthetic skin substitutes, with limited availability and sometimes with poorer outcomes. Due to their properties and especially due to their low antigenicity, amniotic membranes have been also used as mucosal substitutes in different clinical situations. Further studies are necessary to establish the best therapeutic indications and modalities of application. Also, an amnion bank would be very useful for these objectives and for surgical practice.

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