Amniotic Membrane in the Treatment of Leg Ulcers

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The treatments of wounds is as old as humanity. The initial wound treatment is associated with empirical knowledge, unfounded conclusions, potions and ointments, magical practices and mysticism.

- Topical use of plants or extracts as poultices and humidifiers of open wounds, as well as for systemic action, by ingestion.
- The technique of suturing has a prehistoric origin, having occurred between the primitive peoples of South America, parts of Africa and India, using the claws of giant soldier ants. It is interesting to note that this was occurring on different continents long before international communication was even possible.

The medical community is always pushing for innovation. In the last 20 years, there is a huge amount of new interventions, dressings, technology.

Similarly, clinical recommendations for the prevention of pressure ulcers have also been researched in search of advances over the years.

- Hughes, 1899: Clean and dry the skin (wash at least twice a day), the smoothness of the bedding, in severe cases associate the use of mattress or water cushions.
- Galloway, 1899: Pressure reduction, movement, skin cleansing, application of “talcum powder”, rub the skin with alcohol.
- Paget, 1873: Use of water beds, repositioning and firming of the skin with alcohol (whiskey in Scotland and brandy in England).

Among leg ulcers, the general prevalence of types is that 60-80% are venous, 10-25% are arterial and 10-15% fit both etiologies.

The gold standard of care is currently compressive therapy. The objectives of compressive therapy are:

- Reduce blood pressure in the superficial venous system
- Reduce caliber of superficial and deep veins, favoring venous return
- Reduce edema by forcing fluid into the deep venous system and lymphatic system
- Boost the effect of the muscular pump

- Reduce the symptomatology of venous dysfunction as the sensation of weight and pain
- Improve/decrease valvular incompetence

Still, the team didn't always have success with that and become frustrated. Looking for better results, they tried amniotic membrane.

What is amniotic membrane?

This is the membrane that surrounds a developing fetus. It is derived from fetal tissue. The fetal membrane is composed of two main layers: the chorion, which is the outer layer of the placenta coming in contact with the maternal cells, and the amniotic membrane (AM), the innermost layer that is in close contact with the fetus, and is separated from it only by amniotic fluid. Amnion is a translucent structure adjacent to amniotic fluid. Chorion is an opaque membrane attached to the decidua.

In the human species the amniotic membrane appears seven to eight days after conception. The human amniotic membrane is a translucent membrane composed of two layers of simple epithelium with columnar cells firmly adhered to an innermost layer called the mesenchyme layer containing a large amount of:

- Collagen type I, III, IV, V
- Laminin
- Thick basal layer
- Avascular matrix

Properties of amniotic membrane:

- Relief of pain-wound healing encouraging epithelialization
- Protection of wound contamination
- Decrease of Inflammatory reaction
- Reduction of healing Time
- Reduction of water, protein, electrolytes loss
- Improvement of the patients comfort
- No change of the primary wound covering is required

These properties mean that this membrane also serves as an anti-inflammatory agent and an analgesic, promoting regenerating and healing.

Clinical application historically (of amniotic membrane)

- First used by Davis in 1910 in skin transplant (Davis, 1910)
- 1913 used in Burn treatments
- 1940 documented in ophthalmic application (Roth, 1940)
- 1946 reported its use in acute ocular burns (Sorsby, 1946)
- 1995 reintroduced usage in 1995 (Kim & Tseng, 1995)
- Actually after laser resurfacing

It is also used in treatment of lower limb varicose veins, ulcer, Steven-Johnson syndrome, and Parkinson syndrome, etc. Especially in the field of ophthalmology, it is applied to various treatments of damaged cornea.

In the field of ophthalmology, amniotic membrane has proved successful in the treatment of eye surface pathologies such as:

- Corneal perforation
- Neurotrophic ulcers
- Conjunctiva neoplasia
- Chemical and thermal burns

Materials and Methods used in a descriptive study through a series of cases carried out over the period of February 2016 through October 2016:

The study was performed at the Angiology and Vascular Surgery Service of the Hospital Center of Sao Joao, Portugal.

The selection of the sample of this study was for convenience, e users enrolled in the refractory avascular consultation, place of work of the researchers, which allowed a greater robustness in the collection of data and a permanent monitoring of all the evaluations.

In this study, inclusion criteria were:

- Injuries ranging from 5-25 square centimeters
- Lower variation of the area to 30% in the last month
- Refractory to the best medical treatment
- Duration of more than 3 months

Exclusion criteria were:

- ITB <0.9
- Active ulcer infection
- Exposure of deep tissue, eg. bone
- Severe lower limb myopathy / sick disability
- Severe decompensated systemic disease

Objectives of the studies (5 studies were carried out):

- The primary objective of our study was to evaluate the healing rate with AM.
- The secondary objectives were the evaluation of the effect on different healing parameters, pain and the effects on ulcer-related symptoms.

1. Amniotic membrane was collected in surgical intervention (cesarean section) which was authorized by the patients.
2. The membrane underwent serological tests for Hepatitis B, C, HIV1 and 2 HTLV1 and 2 and Syphilis, plus a PCR screening of HIV, HCV and HBV.
3. It was then packed into sterile containers, labeled and transported (along with the mothers' blood samples and documents) at consistent temperature through the use of dry ice.

Data collection:

- The data collection was performed, by direct observation, performed by clinicians of the service, based on the clinical process in order to obtain the necessary information for the participant's eligibility in the study.
- Evaluations occurred up to a maximum of 3 in 3 days, up to a maximum of 13 times which corresponds to a maximum of 40 days.

Data collection during treatment:

We performed the evaluation of the wounds with photographic record and healing evolution in all the treatments performed in which it was registered in the clinical process of the participant.

The amniotic membranes were prepared through a consistent process involving a sterile table, a cup with 2L of saline heated to 40 degrees C, a cup with 2L of saline at room temperature tongs without teeth, a cup of 500ml of saline at room temperature and a straight Mayo scissors.

Process of membrane application:

1. In a first stage the wound is cleansed to receive the amniotic membrane. This cleaning is performed with saline solution (0.9% NaCl).
2. Subsequently a sterile field is placed and the membrane is applied with the tweezers and should be well adhered to the wound.

Success: The team obtained an efficacy rate of 60% (3 cases), which had, on average, 5 treatments, with an average duration of 18.6 days.

The researchers tried to use 2 different things to measure the evolution of healing

PUSH and Resvech.

Clinical History for Patient A:

- Mrs. ACSC, 78-year-old female, obese, hypertensive with depression and varicose ulcer on the outside of the left lower limb, for 8 months.
- The usual medication consists of: Atarax, Lyrica, Tromalyte Sertalina. Internated on March 3, 2016 in the vascular surgery service due to worsening of the ulcer and marked pain.
- Starts dressing by negative pressure and antibiotic therapy with ceftazidime until 01/04/2016.
- After bacteriological examination revealed the presence of pseudomonas and therefore was suspended then -Imipenem + Amikacin although the ulcer showed signs of granulation with intense exudate.
- The Arm Ankle Pressure Index was evaluated, which was 0.98. The respective healing remains slow, thus opting for the amniotic membrane treatment method.

Patient A's response to treatment:

05/29/2016 – The lesion presented as dimensions 4.5 cm in length and 3.3 cm in width, in a total of 14.85 square centimeters.

02/06/2016 – The dressing was opened and we saw a clean ulcer, viable membrane on about 90% of ulcer surface.

05/06/2016 – Clean ulcer with amniotic membrane viable on 80% of ulcer surface.

08/06/2016 – Ulcer membrane with good implantation, clean and of reduced size.

11/06/2016 – The lesion healed and without vestiges of amniotic membrane.

Clinical History for Patient B:

- Ms. MLP, female, 71 years old, was referred from a USF, due to venous insufficiency, history of hypertension and dyslipidemia.
- In 2004, she underwent varicose vein surgery. The first consultation of vascular surgery in the Hospital Center S. Joao on 03/13/2016.
- It presents an infected ulcer on the medial aspect of the left lower limb. Begins antibiotic therapy on this day and dressing treatment in this hospital unit.
- However, he did a Doppler study that revealed permeability and competence of the deep venous system. Incompetence of the large left saphenous vein, bilaterally insufficient venous tract, superficial venous thrombosis.
- At the consultation on 09/04/2016, the ulcer no longer showed signs of infection and in light of this fact, the amniotic membrane was applied in the ambulatory.

Patient B's response to treatment:

05/29/2016 – The lesion presented as dimensions 14 cm in length and 5.2 in width, in a total of 72.8 square centimeters.

01/06/2016 – (Third day after application of the amniotic membrane) The lesion is clean and viable on about 90% of the surface of the ulcer. The lesions maintain almost the same dimensions

For these cases, the team tried to use negative pressure before trying this method, but it didn't work. They learned that the bigger the wound, the less likely it was to help (the AM) but they have concluded that more studies are needed.

Reduction of pain:

Even with the PIs that didn't heal completely, the team DID reduce pain for the patient with AM, consistently.

Conclusions:

From the results obtained, we verified that this treatment does not increase the risk of adverse reactions, which is in agreement with other studies. (Mermet, et all, 2007)

In our study, the maximum healing time was 21 days. In the studies of Alsina-Gilbert & Pedregosa-Fauste of 2012 and Osman & Elbadawy of 2016, we verified that the variability of the sample is superior to ours in healing.

The tested treatment did reduce pain in all the patients regardless of the success of healing.

However, our sample is scarce and as such it is not possible to state that this treatment may be an option to take into account in our National Health System.

Limitations and Disadvantages:

The major disadvantage of amniotic membrane is difficult storage and limited availability which makes wider application of it difficult.

Therefore, the specimens are cryopreserved, glycerol preserved or lyophilized and irradiated for storage.

It also prevents evaporative loss of moisture and infection as well as reduces pain.