

Production of Amnion Grafts for Wound Covering Using local Honey as a Preservative Agent

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Abstract

Amniotic membranes obtained from placentae of healthy mother donors screened seronegative to HIV, HBV, and HCV viruses were processed using air drying method.

The membranes were impregnated in honey and preserved in room temperature. The initial bioburden (microbial count) from ten amniotic membrane (control) were estimated before preserved in honey, and detection tests for staph., pseudomonas, an aerobic, coliforms were also conducted. After 41 month preservation, sections (0.6 μm) from ten amniotic membrane preserved in honey were prepared and stained with H&E stain and microscopically examined to evaluate the effect of honey on the histological structure of the amniotic membrane.

Use of amniotic membrane

- Human amniotic membrane HAM is used as temporary dressing cover, for the treatment of burns, It can be considered a valid biological skin substitute because it assures a good temporary covering of burn wounds and donor sites without inducing immunological response, yet, no rejection phenomena have so far been reported [Magliacani G., (1990)].

Introduction

Why Human amniotic membrane is a very competitive as a temporary biologic dressing in the treatment of burns?

- **Non costly, (economic graft)**
- **It facilitated wound healing**
- **It is generally superior to either cadaver skin allograft or pigskin xenograft (especially in Muslim societies).**
- **Wounds unresponsive to usual therapeutic measures responded to membrane application**

Clinical features of A.M.

Amnion has many clinical favorable properties than the availability and low cost processing

- **It serves specific functions:**
- Reduction of bacterial contamination and prevention of further contamination. It is bacteriosatatic
- Reduction of fluid, protein, heat and energy loss.
- Reduction of pain.
- Promotion of healing.
- Protection of underlying structures.
- Psychologic improvement in the patient.
- Suppresses inflammation, inhibits scarring

Advantages of A.m. in burn treatment

In burn treatment in particular, HAM is preferred to other conventional dressing materials due to some important features i.e.

- rapid epithelialization of the burn.
- adheres well to the recipient bed. (transparent)
- reduces pain. (reduces use of medications, anesthesia)
- works as a barrier from contamination from surrounded environment. especially in burn hospitals (problems of nosocomial infections, pseudomonas and MRSA resistant bacteria)

Selected cases treated with Air Dried A.M.: BPS Hospital- Tripoli-Libya 2003



Selected cases treated with Air Dried

A.M.: BPS Hospital- Tripoli-Libya 2003



Why we thought in honey to preserve our amnions?

- The unavailability of radiation sterilization sources due high cost or because of technical problems and the high cost of maintenance, or low energy source(due to several half lives), is one of the reasons for using honey impregnated amnion grafts as an alternative to the gamma sterilized ones for treatment of burns.
- Irradiation facilities not available anywhere in developing countries (mainly in one city).
- The honey usage could achieve two goals at once, preservation and extension of the amnion grafts shelf life and accelerating wound healing at the time of application.

- The continuous surgeon demand on amnion to meet the increased number of burned patients.

Honey has a distinguished antibacterial and wound healing properties and would act synergetically with A. M. to accelerate wound healing.

Big quantities could be preserved in honey for long time without damaging physical and biomechanical properties.

Even if decided to radiation sterilize A.M. preserved in honey, the 25kGy dose is generally accepted for the sterilization of both honey and amniotic membrane.

Processing Procedures

carried out at the BTRC tissue bank, Tripoli-Libya

❑ the donor mother should be healthy and meets the inclusion criteria of tissue donation (standards).

❑ one placenta is one batch (don't mix or pool together)

❑ Washing under clean tap water.

❑ Peeling off amniotic membrane from rest of the placenta.



Processing

Washing:

- ✓ Under tap water until the blood is totally cleaned.
- ✓ Then the membrane is transferred to glass bottles for further shaking.



Processing

Then, amnion is transferred to glass bottle containing sterile saline solution



Processing

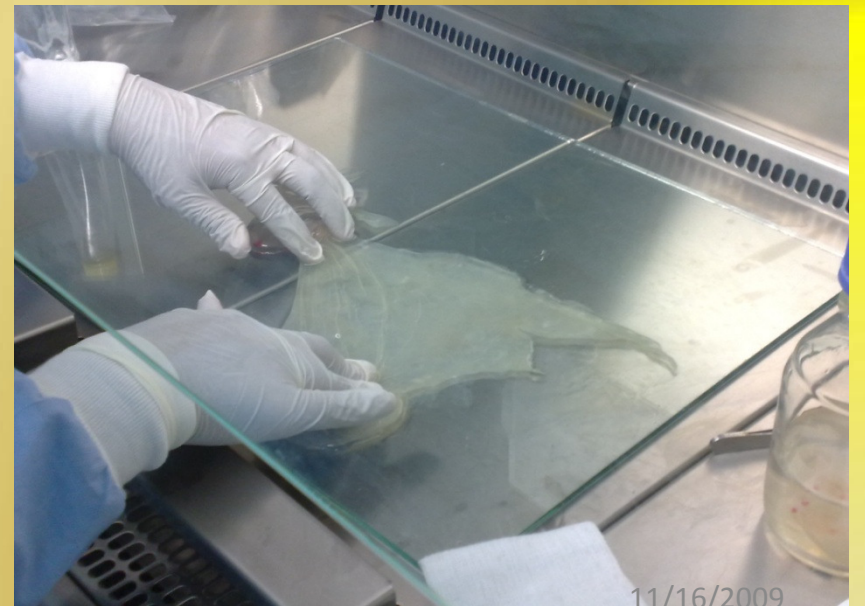
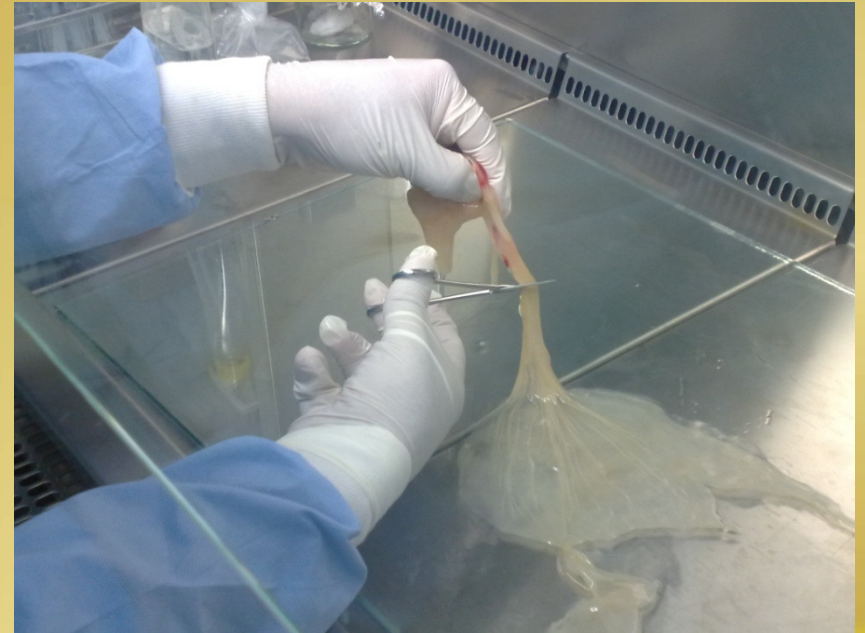
- Washing in a sterile saline solution and shaking several times to remove blood and clots.
- Washing in 70% alcohol for 15 min. followed by 2 washs in sterile dist. Water.



Processing

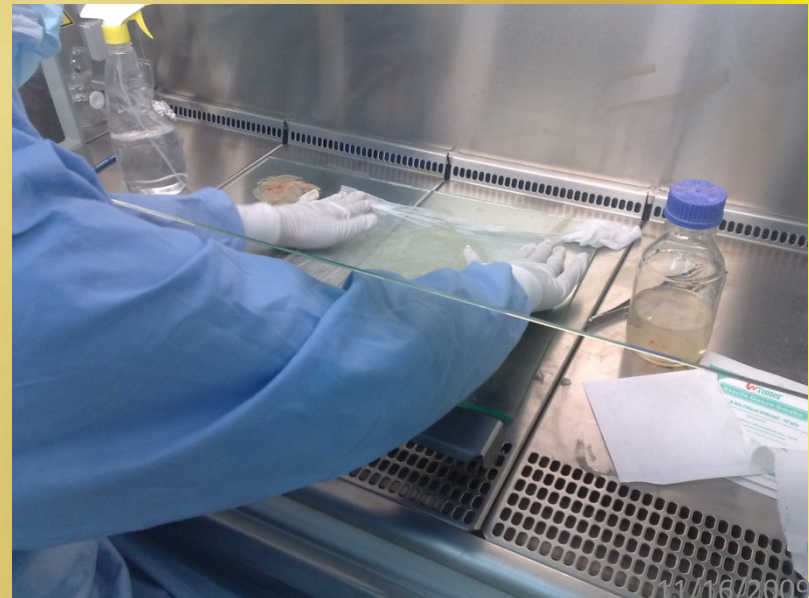
☐ Removing of un wanted tissues.

☐ Cleaning and cutting in appropriate sizes



Processing

- ❑ Stretching over a sterile gauze.
- ❑ Fixing on a special frame for drying.



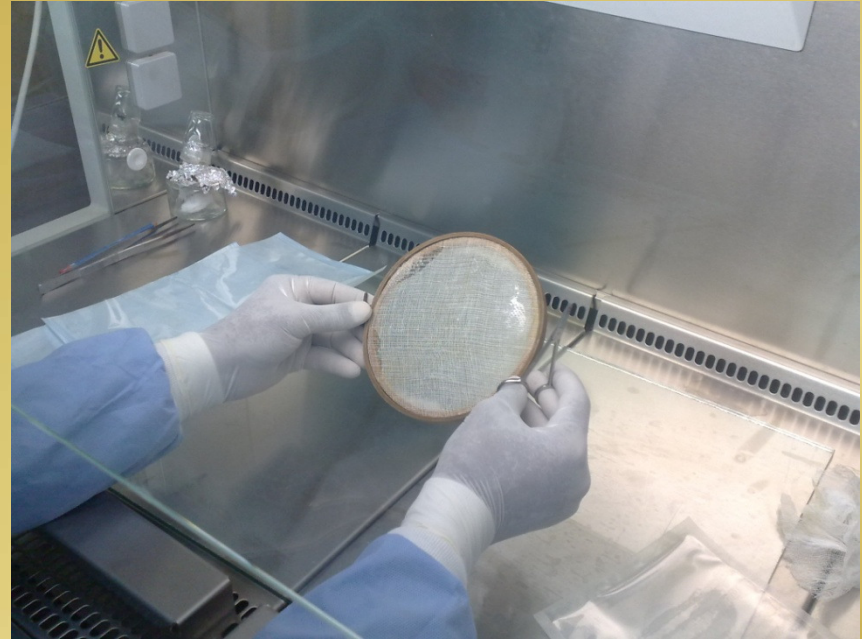
Processing

Conducted in
septic conditions
under a clean
disinfected
Laminar air flow
safety cabinet, to
minimize the
bacterial
contamination



Processing

Cutting and packaging.
After 18-24h. drying in
a sterile air, the
membranes stretched
over gauze are cut
aseptically into
appropriate sizes and
packed in polyethylene
packs,



11/16/2009

Processing

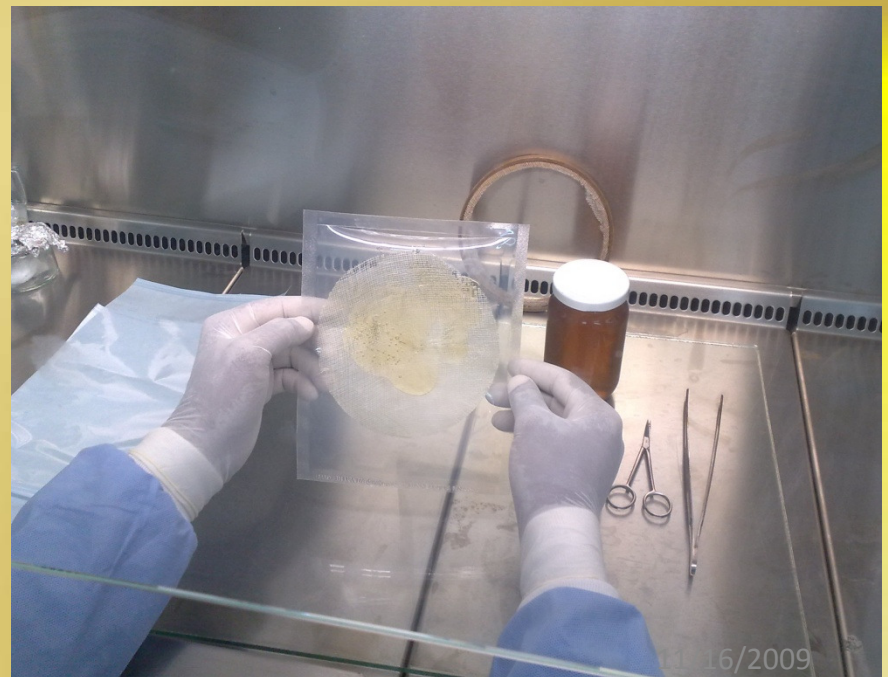
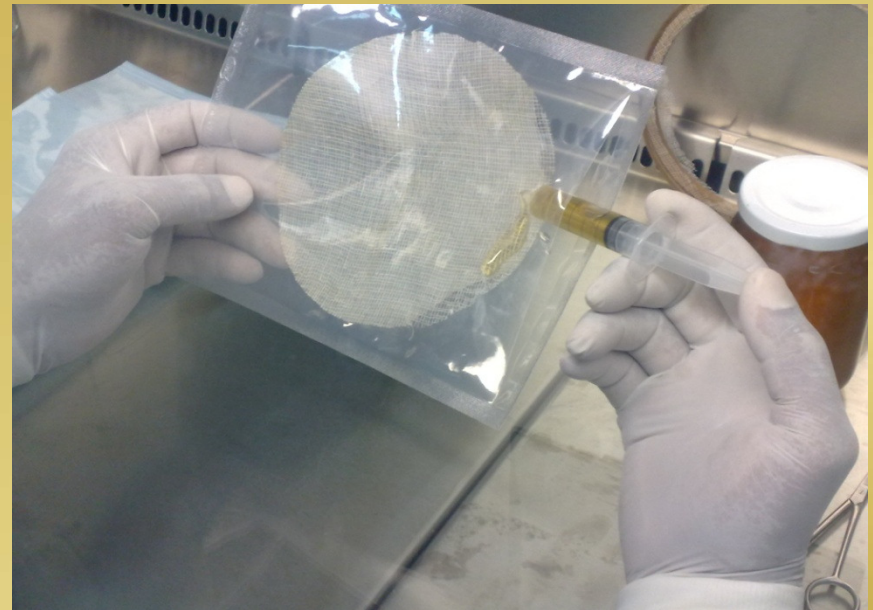
A label is put for every pack of the processing batch involving all the information required.



Addition of Honey

- pasteurized honey is added aseptically to membrane graft.
- the amount is about 20-30ml for each pack
- Local honey known as (rabiee) which is multifloral honey, is added aseptically to the dried membrane until the membrane is completely covered the amount is about 20-30 ml.

Sterile honey is not available at our local market, so we used the non sterile , before use honey is a pasteurized at 50 degrees Celsius for 3 hours.



Packaging

Amniotic
membrane graft is
heat sealed,
double packed,
labeled and heat
sealed again.



Results

1. Microbiological Quality Control (Bioburden Estimation)

Basing on ISO 11737-1/1995

Microbiological quality control (bioburden estimation basing on ISO 11737- 1/1995)

- Counts before honey treatment
- Results shows no growth after 41 months period of storage at room temp.
- the original bioburden is even eliminated.

Am no.	PCA	PDA	Average count	Pseudomonas	An aerobic
1	1	2	1	-ve	-ve
2	26	210	118	-ve	-ve
3	2	18	10	-ve	-ve
4	2	73	37	-ve	-ve
5	1	35	18	-ve	-ve
6	1	3	2	-ve	-ve
7	2	2	2	-ve	-ve
8	3	7	5	-ve	-ve
9	4	3	3	-ve	-ve
10	2	3	3	-ve	-ve
Av.	4.4	35.6	20 c.f.u.	--	--

11/16/2009

Microbiological quality control- detection of pathogenic bacteria

Results showed that the average initial bioburden on non honey preserved (control) amniotic membranes was 20 c.f.u./graft.

- No bioburden on the ten grafts preserved in honey (41 month storage) which showed no growth and counts zero c.f.u./graft.
- In addition, bacterial spores, pseudomonas, staphylococcus aureus, an aerobic bacteria, coliforms were not detected on any of the ten honey impregnated amniotic grafts.

Results

HISTO-MORPHOLOGICAL EXAMINATION:

Results

- histomorphological examination shows that long preservation in honey has no effect on the cuboidal epithelia and basement membrane which are essential in the healing of wound.
- basement membrane is more important than amnion epithelial cells; as it is containing collagen type IV which is thought responsible for promoting epithelialization (Anna dziedzic-Goclawska (2000)).
- there is slight effect on the mesenchymal collagen fibroblast, which seems to shrink or lyses due to effect of honey preservation, or might be degraded by the action of the proteolytic enzymes found in honey or bacterial contaminants on amniotic membrane.
- However, for the acceleration of wound healing and granulation and re-epithelialization, amnion (epithelium & base membrane) is sufficiently used [dioguardi et. al 1990]. With epithelial side facing outwards, it is believed that epithelia and basement membrane would promote migration, attachment and spreading of the host cells which finally encourages.
- The amnion prime property of encouraging re-epithelialization that leads to fast wound healing is revealed also to its rich content of growth factors namely; Epidermal growth Factor (EGF), Transforming Growth Factor alpha (TGF- α), Insulin-like Growth Factor (IGFs), Vascular Endothelial Growth Factor (VEGF)
- interpretation of the shrinkage or disappear of the collagen layer is need to be clarifying further in another work.

Histo-morphological Examination:

Figure (1) shows section of air dried amniotic membrane **not preserved in honey,**

Cuboidal epithelia

Basement membrane

Collagen sheet



Histo-morphological Examination:

Figure (2) shows section of air dried amnion **preserved in honey** without any damage of the non viable layers (except the amniotic Collagen sheet) without signs of degradation during storage, this emphasizes that there is no change in the material property of the amniotic membranes preserved in honey.



Conclusion:

- ❑ Preservation of HAM in honey shows obvious reduction in the bioburden after a storage period of 41 months with no change in the histological characteristics of the amniotic membrane.
- ❑ The availability, and the clinical properties of both honey and amnion, especially those inhibits bacterial growth and promoting wound healing could be combined to enhance convenient application and reducing pain in patients suffering superficial, or deep burns, ulcers and open wounds.
- ❑ the antibacterial activity, viscosity and acidity of honey is thought to have a bactericidal effect on the microbial load of amnion and honey; further, the air drying of amnion in a sterile conditions to about 7% water content, might has also a bacteriostatic effect on the bacterial contaminants, the obvious reduction in microbial count might be due to this synergetic effect.

Conclusion cont.

- Amniotic membrane impregnated in honey is suitable graft especially for developing countries because of it is low cost, availability and it's clinical value.
- The used procedure (air drying method) is simple, the membranes could be maintained in room temperature for more than 3 years.
- Big quantities could be prepared and kept as a stock at room temp. for big accidents and disasters.

Recommendations and future studies:

- Samples of amnion for microbiological quality have to be taken interally to check the sterility of the banked amnion grafts.
- Animal trial and clinical studies on volunteer patients to evaluate the healing properties of the air dried HAM preserved in honey. And to assess and evaluate the granulation and epithelialization of the burn or wound.

Additional quality control tests such as biomechanical strength(tensile strength, stability), tests on experimental animals and volunteer patients, has to be completed.

- Compositional and chemical analysis of various types of local honeys (acidity, antibacterial, H_2O_2 , osmolarity effect and sugar and water content) is advised in future, to decide the best local honey for antibacterial, anti-inflammatory actions and clinical application.

Thank you for your attention