

Amniotic membrane grafting – Step by step

Human amniotic membrane grafting (AMG) is an adjuvant treatment used across surgical specialties. It is also a rich source of stem cells for culture in translational medicine. Amniotic membrane grafts are prepared from the placenta and can be used as a dressing in dermatological conditions, such as epidermolysis bullosa, chronic non-healing ulcers, burns, pemphigus, and toxic epidermal necrolysis. Although easily available with inexpensive preparation and storage techniques, it is underutilised due to a lack of experience in many centres. This pearl attempts to teach the use of AMG step-by-step, with images.

Properties of the human amniotic membrane

The human placenta is composed of the inner amnion and outer chorion. The amnion is 20 to 50 microns thick and has an inner stroma, basement membrane, and outer cuboidal epithelium. The stroma is composed of an exterior spongy layer, an inner compact layer, and a middle fibroblastic layer. AMG expresses anti-inflammatory, anti-microbial, and anti-fibrotic factors with a lack of immunogenicity and promotes epithelial growth.¹ Limitations include possibility of transmission of infection and lack of tensile strength.

Preparation

The placenta is obtained after elective caesarean section from mothers who have been tested negative for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies and syphilis. Placenta delivered by normal vaginal delivery and obtained from labour rooms are not preferred as there are higher chances of bacterial contamination, leading to infection and graft failure.²

Following steps are involved:

- 1: The placenta is collected in a sterile tray under aseptic precautions [Figure 1a].
- 2: Blunt dissection is used to separate the amniotic membrane from the chorion [Figure 1b].
- 3: The blood clots are removed from AMG by rinsing it with normal saline [Figure 1c].
- 4: The second rinsing is done with 500 mL of balanced salt solution (BSS) (available in ophthalmology) containing 100 µg/mL of neomycin and 40 µg/mL gentamicin (0.5 mL of a 40 mg/mL vial). Literature recommends the use of 500 mL BSS

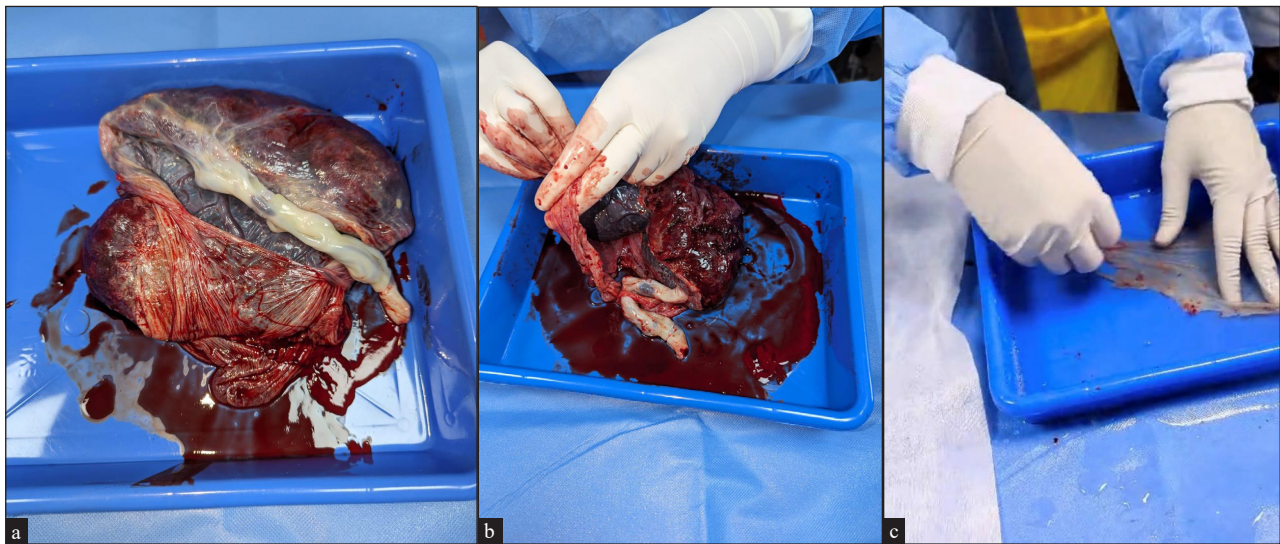


Figure 1a-c: Steps of harvesting the amniotic membrane

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containing 100 µg/mL of neomycin, 50 µg/mL of penicillin, 50 µg/mL of streptomycin, and 2.5 µg/mL of amphotericin B [Figure 2a].

5: The third rinsing is done with BSS.

6: The graft is then placed on a nitrocellulose paper with the epithelial surface up [Figure 2b].

7: A sample of AMG should be sent for culture on a blood agar plate during collection from the OT, to rule out any bacterial contamination. The sample must be plated in OT to prevent external contamination during AMG processing and transportation.

8: The membrane is cut into appropriate graft sizes according to the recipient area.

9: The recipient area is cleaned with normal saline to remove any discharge or crusting, if present [Figure 2c].

10: The AMG is placed over the recipient area in a single layer, with the epithelial surface facing outward and the stromal surface facing the wound [Figure 3a].

11: The AMG is covered with non-adhesive dressings, such as paraffin tulle or Urgotul [Figure 3b].

The AMG is left in situ and usually gets incorporated into the wound within a week [Figure 3c]. The procedure is repeated every week.

Tips:

A freshly harvested amniotic membrane can be stored using various methods for future use. Few are

- i) Membrane dipped in 85% glycerol can be stored at 2°C - 8°C for 2 months.
- ii) The membrane is cut into small pieces and stored in sterile vials containing Dulbecco's modified Eagle's medium and glycerol at a ratio of 1:1 (volume/volume). The vials are frozen at -80°C.
- iii) Cryopreservation, freeze-drying of the membrane, air drying, heat drying, and gamma irradiation are other methods to preserve the amniotic membrane, which may not be possible in resource-poor settings.³

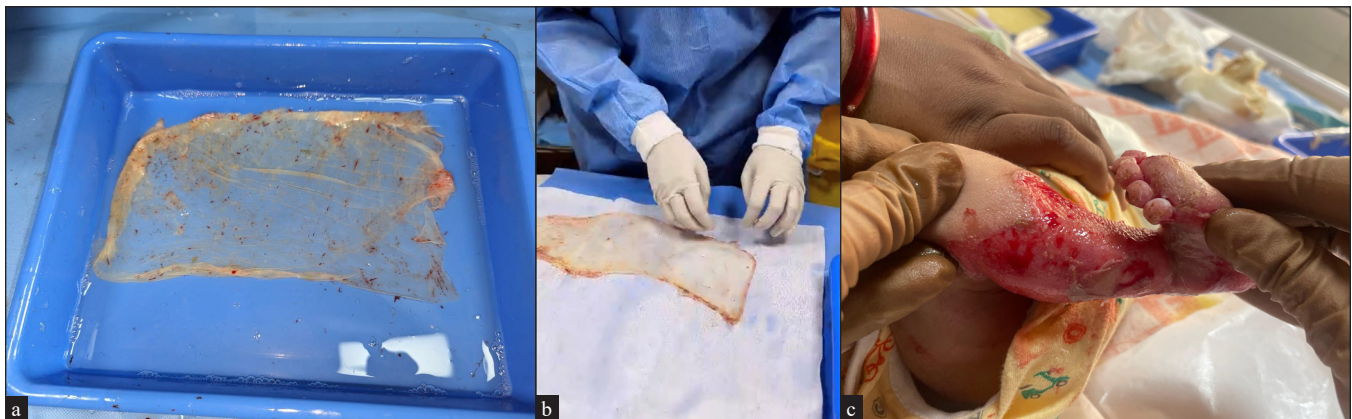


Figure 2a-c: Steps of preparing the amniotic membrane and the recipient site.



Figure 3a-c: Amniotic membrane dressing of the lesion.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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References

1. Malhotra C, Jain AK. Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. *World J Transplant* 2014;4:111-21.
2. Al-Yousuf N, Alsetri H, Farid E, George SM. Amniotic membrane transplantation an experience of a locally prepared tissue. *TRRM* 2022;Volume 14:7-19.
3. Hofmann N, Rennekampff HO, Salz AK, Börgel M. Preparation of human amniotic membrane for transplantation in different application areas. *Front Transplant* 2023;2:1152068.