

# Method of Obtaining and Preparation of Fresh Human Amniotic Membrane for Clinical Use

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Mohammad Ashraf Ganatra, Khalid M. Durrani ( Department of Plastic, Reconstructive Surgery and Burns, Dow Medical College and Civil Hospital, Karachi. )

## Abstract

Amniotic membrane was obtained from 36 mothers seronegative for hepatitis B surface antigen and syphilis, undergoing caesarean section, Membrane was separated from placenta and was washed first with saline and then saline solution containing penicillin. The processed membrane was found to be sterile and useable for upto one week. Of 36 placentas obtained, 33 were utilized In 22 patients, with no history of penicillin allergy, as biological dressing in acute burns. Each patient received three applications of membrane one every other day, over a period of six days. This method of obtaining amniotic membrane was simple and more practical for maintaining the biological effectiveness of membrane, as shown by quantitative reduction of bacterial counts in burn wounds (JPMA 46:126,1996).

## Introduction

Human amniotic membrane as a biologic dressing has been credited with reduction of pain<sup>1</sup>, as an effective vapor barrier<sup>2</sup>, bacteriostatic<sup>3</sup> and as a promoter of wound healing<sup>4</sup>. Amniotic membrane is a thin, tough, transparent membrane. It is about 10-15 micrometer thick. The tensile strength of whole membrane varies between 0.05 to 0.4 kg/cm<sup>2</sup>, with an average of 0.205 kg/cm<sup>2</sup><sup>5</sup>. It is made up of two parts, the inner amniotic membrane and the outer chorion. Chorionic side of the membrane is rougher and mucinous. Amnion can easily be peeled off from chorion laeve and placenta as far as the umbilical cord. Once separated, the amnion is found to be smooth and shining and much tougher and more elastic and easier to clean than the thicker chorion which does not strip easily from the placenta<sup>6</sup>. The chorion, although thicker, is more easily torn because it is much less elastic.

Amniotic membrane has been used in a variety of clinical situations, but the details of how to obtain and prepare the amniotic membrane for clinical use have not been described adequately<sup>17-15</sup>. This paper reports a detailed methodology for selection of donors and processing of the amniotic membrane.

## Materials and Methods

The requirements for procurement of fresh amniotic membrane included a sterile jar containing one litre normal saline, sterile pair of scissors, plain thumb forceps, pair of gloves, mackintosh, measuring scale and sterile petri dishes.

Criteria used for selection and processing of donors and the membranes included: 1) No history of jaundice, premature rupture of membranes, endometritis, malaria, toxemias and venereal disease; 2) Donors to be sero-negative for hepatitis B surface antigen and syphilis; 3) Only membranes of caesarean sections were obtained; 4) Meconium stained membranes were rejected.

Sterile jar containing one liter of normal saline was provided in the obstetric operation theatre of the hospital. Placentas from women undergoing caesarean section were procured at the time of delivery and placed in the jar of saline. If not procured at actual delivery, the placenta was kept in a sterile container in the main compartment of the theatre refrigerator and was transferred within two hours into

the jar containing normal saline. The placenta was then processed immediately for separation of amniotic membrane. If the placenta could not be processed immediately, it was kept in a refrigerator at 4°C and was processed within 24 hours.

After wearing sterile gloves, the placenta was taken out of the jar. The membrane stripped from the placenta was spread on the back of a pan or a kidney tray and rinsed with normal saline using 4"x4" moist gauze squares and several washes with normal saline, all blood, mucus and debris were removed. The membrane was then rinsed with a solution containing 200,000 units of crystalline penicillin per 100 ml of normal saline<sup>16</sup> and placed in a petri dish containing the same solution. The petri dish sealed with a tape all round and marked with date of obtaining the membrane was stored in a refrigerator at 4°C. The membrane was used within a week. Part left at the end of this period was discarded.

From every specimen, a small piece of membrane was put into test tube containing 5 ml trypticose soya broth (TSB) and incubated for 24 hours. If found to be contaminated that membrane was not included in the study.

**Method of application of membrane** Under Ketamine anesthesia, after washing burn wound with normal saline and solution of cetamide and Chlorhexidine (Savion), tangential excision of about 10cmx10cm area was done. Biopsy was taken from the wound for quantitative bacterial count and fresh amniotic membrane applied with its chorionic side towards the wound. This was covered with a layer of wet gauze, dry gauze, cotton and bandage. Membrane was changed on alternate days. i.e. 2nd, 4th and 6th day. Biopsies were taken for culture on all three days and for histology only on 6th day.

The biopsy taken for quantitative bacteriology was put directly into sterilized pre-weighed homogenizer bag containing 1 ml normal saline. This bag was reweighed. Weight of tissue obtained and bag was homogenized in homogenizer (Stomacher Lab blender- 80). Four-fold dilutions were made of homogenate specimen. From each dilution tube 0.01 ml was then inoculated on blood agar plates, incubated for 24 hours and number of colonies counted. Colony count per gram of tissue was obtained by the following formula:

$CFU/gm \text{ of tissue} = C \times D \times l - W \times 0.01$  where C is total number of CFU, D is dilution factor, W is weight of tissue, l is volume of normal saline and 0.01 is volume of inoculum.

The piece of biopsy obtained on 6th day was immediately placed in a bottle containing 10% formaline, sealed and processed in the routine manner for histopathological studies of H & E stained paraffin sections to see the vascular growth.

## Results

Thirty six placentas were obtained from obstetrics operation theatre of Dow Medical College and Civil Hospital, Karachi. Three were found contaminated and were therefore, not included in the study. Remaining 33 placentas provided usable membranes. Two pieces of 10x10 cm were cut from each membrane and stored separately at 4°C in petri dishes in the refrigerator for up to 1 week.

A total of 22 patients of burns received fresh amniotic membrane on their wounds. Fifteen were males and 7 females (age range 15 to 33 years, mean 23.7). Burn extent averaged  $20\% \pm 1.26 \text{ SEM}$  of total body surface area.

The membrane was applied as a biological dressing on day of admission and was changed on 2nd and 4th day. Thus each patient received three applications. Bacteriological studies of the wound were undertaken as described above. There was decline in bacterial count of the burn wound which was evident from day four of application and was significant by day six (Table).

**Table. Bacterial counts per gram tissue.  
Study bacterial counts (Log Scale).**

No	Days			
	0	2	4	6
F1	00.0	5.21	5.95	-
F2	6.17	5.73	7.30	8.43
F3	6.01	7.21	5.85	6.51
F4	6.76	7.70	4.96	6.61
F5	6.92	6.64	7.06	5.69
F6	6.18	6.06	5.69	8.23
F7	7.02	6.67	5.75	7.09
F8	5.02	6.21	8.90	6.65
F9	5.77	6.60	6.28	7.37
F10	6.77	5.80	LAMA	-
F11	5.32	5.71	6.74	6.71
F12	5.75	7.31	7.07	4.99
F13	6.29	8.87	8.09	7.89
F14	9.19	10.08	8.80	5.46
F15	9.49	9.13	8.98	8.00
F16	6.06	4.80	7.07	6.30
F17	5.49	5.54	5.97	LAMA
F18	6.17	7.17	5.44	LAMA
F19	6.25	7.10	8.84	7.67
F20	7.00	8.33	8.87	6.46
F21	5.46	6.61	6.33	5.89
F22	5.53	7.53	6.20	6.32
Mean	6.13	6.91	6.95	6.79
SEM	±0.373	±0.283	±0.28	±0.23

Out of 22 cases, histological studies were done in 12 cases to see vascular ingrowth into the membrane. No evidence of vascular ingrowth or capillary budding was noted<sup>17</sup>.

## Discussion

In the preparation of human amniotic membrane two precautionary aspects are important: first, avoidance of transmission of disease or infection and secondly, ridding the prospective graft of unneeded tissue layers and detritus so that the proper layer may adhere to the recipient bed. Amniotic layer and chorionic layer are easily separable but we do not separate them. Some workers, including us, believe that use of amniotic layer along with chorionic layer prevents the desiccation of amniotic layer and affords better protection<sup>18</sup>. The use of fresh human amniotic membrane has become negligible in developed countries because of fear of transmission of Acquired Immuno Deficiency Syndrome (AIDS) and availability of expensive synthetic and biological skin substitutes. In developing countries with a high incidence of household burns and very busy obstetrics wards, amniotic membrane as a wound dressing offers an attractive alternative. Pakistan is also fortunate in as much as AIDS is not too prevalent as yet<sup>19</sup>. We continue to harvest and use amniotic membrane in selected critical life threatening situations. Bacterial contamination was seen in 11% of our membranes which is comparable with other series<sup>20</sup>. Different authors have used different methods of washing and sterilizing the membrane. Troensegaard-Hansen<sup>21,22</sup> used the amnion after cleansing with normal saline and then boiling. Dino<sup>23</sup> and Robson and Krizek<sup>24</sup> after washing the membrane with saline gave it a single rinse of 0.025% sodium hypochloride (NaHOCl). Bose<sup>25</sup> used Daikin's solution and Rao<sup>26</sup> sodium bicarbonate to wash the membrane. Different solutions used to maintain amnion after washing include 1% Kanamycin sulfate or neomycin sulfate solution<sup>27</sup>, 0.9% saline to which polymyxin ampicillin, gentamycin and amphotericin B were added<sup>28</sup>, povidine iodine<sup>29</sup> and tissue culture medium<sup>30,31</sup>,

The method reported here was very similar to that described by Dino et al<sup>16</sup>. Use of chemicals was avoided in order to preserve the inherent properties of amniotic membrane as they might alter its biological effectiveness especially the antibacterial property. In conclusion, the method of preparation and preservation of amniotic membrane for use as a biological dressing in acute burns described here has been found to be simple, safe and cost effective.

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