Original Article

Application of Freshly Collected Amniotic Membrane & Amniotic Fluid Dressing on Chronic Non Healing Ulcers Patients — A Hospital Based Experience from Kolkata

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Abstract

Background : The management of Chronic non healing ulcers possess great challenges because of the high prevalence, refractory nature, high rate of amputation, disfigurement, loss of wages & economic burden[1]. The placenta which was discarded in pits or incinerated so called trash is converted to gold here. Use of freshly collected & screened amniotic fluid and amniotic membrane as biological dressing in treatment of chronic non healing ulcer (the ulcer which doesn't heal completely within 3 months) is potentially beneficial & free of cost.

Material and Methods : Application of autologus skin graft, Aqua cell Ag+ (Silver) dressing, Hyperbaric oxygen therapy, Bioengineered skin, Negative pressure wound therapy, Amnio-Patch, Chorionic mesenchyme, Epi cell cultured Epidermis, Micronised dried Amniotic membrane, Amnion Cytokine extract, 3D Scaffold fabricated with other materials, Decellularised Amniotic membrane are very costly.

Conclusion : Most of these commercial products doesn't have any viable or no cells at all[2].

Key words : Amniotic fluid, Amniotic membrane, Biological Dressing, Non-healing ulcer.

Indian Studies on the epidemiology of chronic wounds estimated the prevalence at 4.5 per 1000 population.

Diabetic foot ulcers occur in up to 15% of all diabetic patients and are a leading cause of non-traumatic amputation worldwide.

The prevalence of vascular ulcer in the US is estimated at 5,00,000 to 6,00,000 and increased with age[3,4].

Focus of Study :

To understand and reveal benefit and potentiality of term intact freshly collected amniotic fluid and amniotic membrane to treat chronic and non healing ulcers in human patients.

Most Important Features of Freshly Collected Amniotic Fluid (AF) & Amniotic Membrane (AM) :

The Amniotic membrane is composed of 2 layers, an epithelial layer, containing 2 epithelial cell types of cuboid or columnar shape capped Amniotic Epithelial Cells (AECs) and a stromal matrix, avascularised layer containing amniotic mesenchymal stromal cells.

Amniotic membrane induces signalling pathways that are involved in cell migration and proliferation. Amniotic membrane contains Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Insulin Like Growth Factor IGF), Epidermal Growth Factor (EGF), Basic Fibroblast GF, Transforming Growth Factor (TGF) alpha and beta, Keratinocyte Growth Factor (KGF), Hepatocyte Growth Factor (HGF) & Nerve GF. They have been identified in freshly collected amniotic tissue. Amniotic membrane has antibacterial property mediated by human betadefensins (HB D1-3) and elafin and Secretory Leukocyte Protease Inhibitor (SLPI) & LL37[5], a cathelicidin family, has anti-biofilm properties-combating chronic infections.

Editor's Comment :

- Now we live in Stem Cell age. This novel cell therapy by freshly collected amniotic fluid and amniotic membrane will benefit mankind at large in future both autologous and allogenic, in different areas of tissue repair & in wound healing, in repair of nerves, heart lesions, muscles, organ damage and skin lesions.
- It is potentially beneficial and free of cost.

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Human Amniotic Mesenchymal Stromal Cell (hAMSc) expresses low levels of classical MHC-I molecules found in and do not express MHC-II molecules on their surface, making them suitable for regenerative medicine.

Amniotic fluid rich in :

Amniotic Fluid contain quite a good population of progenitor and stem cells with high telomere content which have unique property to trans differentiate into different lineages and participate in the process of tissue regeneration depending on the provided niche to them[6].

Amniotic Fluid rich in nutrients, growth factors, hyaluronic acid, mesenchymal stem cells essential for non-healing ulcers and wounds[7].

Amniotic Fluids have antibacterial properties also by lysozyme, 7S immunolobulin and lgA.

It has been reported by[8] that only 2 ml AF can provide upto 20,000 cells of which 80% has been stated to be viable.

These cells proliferate rapidly with doubling time 30-36 hours and do not require supportive feeder layers[9].

Pluripotency of AF derived stem cells have been reported by[10] as first evidence. These distinct subpopulations of proliferating cells (0.1-0.5%) expresses pluripotent markers Oct 4 (Octamer binding transcription factor 4).

Combined effect of Freshly collected AF and AM :

The beneficial effects of mesenchymal stem cells of AM & AF, on wound healing through paracrine interactions[11], secrets soluble factors to exert immune modulation, promote angiogensis, decrease wound inflammation, wound ECM remodelling (various components such as fibronectin, collagen and vitronectin provide structural support for the invading capillaries), MSC secret cytokine to promote dermal fibroblast proliferation, antiapoptotic and enhance regeneration of proper skin structure and function.

AM and AF have been used as sources of mesenchymal stem cells for transplantation. Both lineages show adipogenic, osteogenic and chondrogenic differentiation in vitro. They have been applied extensively as a wound dressing, cutaneous and corneal wound healing, as vectors for delivery of genes[12] in nerve regeneration[13] among many others.

Materials and Methods

Study Technique:

(1) Informed consent from patients to treat non healing wound were obtained, including the donor mothers for collecting the amniotic fluid and the amniotic membrane.

(2) Wound was first thoroughly irrigated with normal saline to remove debris, foreign particles, and other contaminants.

(3) Simple wound debridement, done as per demand of that particular ulcer. Again the ulcer is thoroughly washed with normal saline.

(4) Amniotic fluid is applied over wound & around the wound injected subcutaneously.

(5) Amniotic side is applied in superficial ulcers where epithelialisation is needed; chorionic side is applied where vascularisation is needed; amniotic or chorionic membrane is cut as per wound size and shape and wound bed is covered up to skin margin. Plain Vaseline tulle applied over amniotic membrane, then sterile gauzes are applied over it and bandaged with sterile bandage.

(6) Follow up is scheduled on 8th, Day.

Eligible mother was counselled before and informed consent obtained from her for donation of amniotic fluid & placenta.

Recipients were taken informed consent and were screened for HIV-I & II. Hepatitis “B” & “C”, Venereal Disease Research Laboratory Test (VDRL), toxoplasmosis, herpes infection.

Clinical History was taken meticulously.

Types of wounds, size, depth, pain score, exudation were evaluated.

Investigations : Previous investigations were noted and written.

***Recent investigations* —** Complete Blood Count (CBC), C-reactive Protein (CRP), Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), TFT, KFT, LFT, Lipid Profile, HbA1C (if requires) CXR, Electro Cardio Gram (ECG), Echocardiography, USG whole abdomen, Doppler Study (if requires). CT/MRI Scan / HP studies with MVD count, Examination of Pus from wound swab were sent for culture and sensitivity.

Ankle Brachial Pressure index ABPI $\geq$ 0.8 will be included[14].

Co-morbidities any & their evaluation and treatment of underline causes .

Micro Vessel Density Count – Quantitative assay :

Angiogenesis is one of the important pillar of tissue healing apart from Extra Cellular Matrix (ECM) remodelling & re-epithelialization.

Biopsy sample were taken for histopathology study to assess the rate of granulation tissue formation that predicts the quantitative assay of capillary remodelling / angiogenesis by micro vessel density count.

Microvessel Density Count (Fig 1) :

Patient : AM 46 years, Male.

Diabetic Leg Ulcer – healed within 12 weeks (Fig 2).

Patient : MM , Age 55 years, Female , Diabetic Foot Ulcer healed within 12 weeks (Fig 3).

Pictorial presentation of MVD count

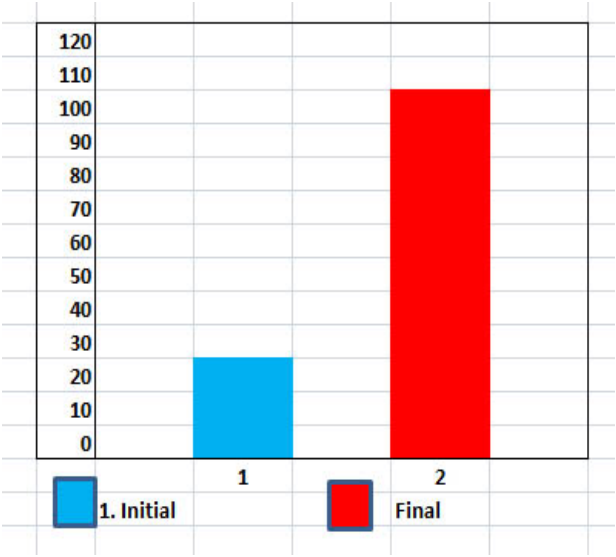


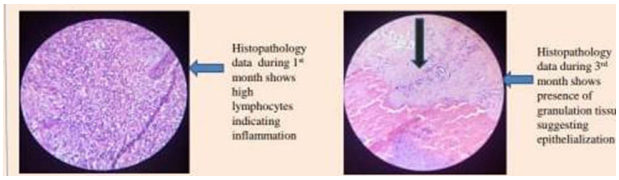
Fig 1 — Histogram showing the mean MVD count comparing histopathology of the junctional biopsy tissue counter stained with Hematoxylin –Eosin at initial and final phase of the study. Results obtained from counts of five randomly selected sites on the slide

Analysis and Results

Application of amniotic fluid and Amniotic Membrane are potential safety and effacious on non-healing ulcers has seen quick epithelialisation and decrease or absence of exudation in 95% of cases. Upon the course of treatment pain score dropped from 80% to 6%. Inflammation decreased to a great extent which was reflected by returning CRP count in its normal range in cases near to complete healing. Wound size decreased gradually and no uneventful circumstance are noted. Tapering of insulin dose and oral anti diabetic therapies were successful. At the end of the 12 weeks number of patients healed 92.59%.

In few cases hypo-pigmentation noted, which gradually returned to normal skin colour within six months follow-up.

Histopathological data –reduction in polymorpho-nuclear infiltration near to healing stage and presence of granualation tissue and new vessels.



Statistical Evaluation :

In this observational and prospective study, 27 patients were included with chronic non healing ulcers with different aetiology. In the control group patients were 21. AM & AF were applied every 8th day.

Friedman's ANOVA with multiple comparisons shows statistically significant ($p<0.001$). At the end of 12th week number of patient healed 25 ie, 92.59% and number of patient not healed 2 ie, 7.41%.

As per our study AM & AF dressing effective not only for reduction of ulcer dimension but also effective in respect to final outcome & healing.

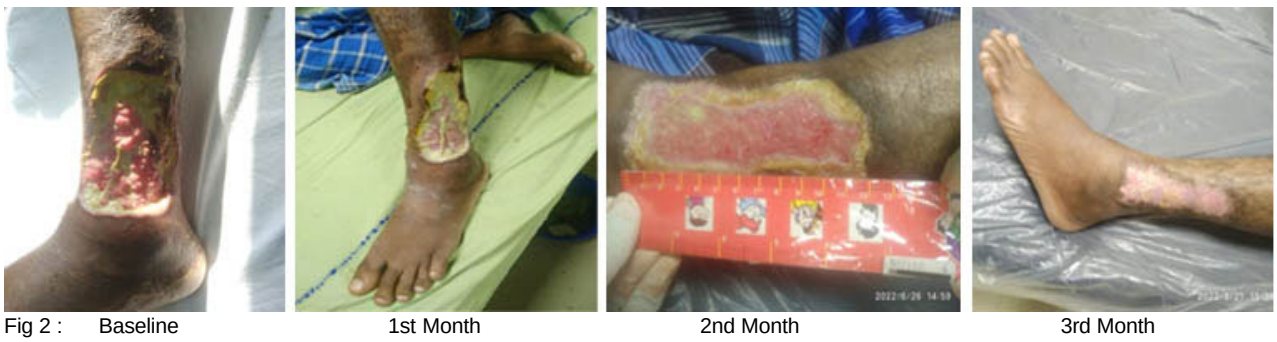


Fig 2 : Baseline 1st Month 2nd Month 3rd Month

Fig 3 : Baseline 1st Month 2nd Month 3rd Month

Conclusion

Freshly collected Amniotic fluid and Amniotic Membrane contains angiogenic[15,16] and pro-apoptotic factors and greatly promotes epithelialisation as it facilitates epithelial cell migration, reinforcement of basal cellular adhesion and encouragement of epithelial differentiation. Apart from that freshly collected Amniotic Membrane bathed in amniotic fluid also showed antimicrobial properties[17], due to presence of lysozyme, lactoferin, hyaluronic acid, cystacin-C, transforming GF beta 3, peroxidases, immunoglobulin and zinc peptide.

The human amniotic fluid contains amniocytes which are a large pool of self renewal cells, mainly fetal in nature. These amniocytes have an extremely complex molecular behaviour and express trophoblastic, ectodermal, endodermal and mesodermal cells, specific regulators. There are embryonic pluripotent like stem cells, which are distinct from both embryonic and induced pluripotent stemcells have the propensity to be reprogrammed into a primitive fully functional pluripotent stem cell.

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Conflict of Interest : None

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