Processing and Use of Radiation Sterilized Human Amniotic Membrane Allografts as Biological Dressings of Wounds in Children: A Review

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Abstract

Radiation sterilized human amniotic membrane allografts are used as biological dressing of wounds in rehabilitative surgery. The amniotic membrane is used in many different clinical situations such as: heat burn, chemical burns, diabetic wound/diabetic foot ulcer, leprotic ulcer, abdominal wall reconstruction, pterigium removal site, peripheral corneal ulcer, in the management of pressure sore, etc. Non-viable lyophilized/oven-dried radiation sterilized amniotic membrane allografts could be processed for utilization as temporary biological dressing of wounds. The allografts used clinically should not be the carriers of germs or a source of infection. Human chorio-amniotic membranes collected for processing as tissue allografts to be used as biological dressings were reported to be contaminated with microorganisms such as species of Staphylococcus, Micrococcus, Bacillus and Pseudomonas. However, oven dried (40°C) or freeze dried (-50°C) human amniotic membranes were found to be sterilized by irradiation with the dose of 25 kiloGray (kGy) of gamma radiation. Careful screening and selection of tissue donors, proper processing and gamma radiation sterilization of human amniotic membranes minimize the risk of disease transmission to recipients through allografts.

Keywords: Human amniotic membrane, gamma radiation, sterilization.

Introduction

Davis et al in 1910 and Sabella et al in 1913 reported the clinical use of foetal membranes for transplantation. The application of human amniotic membranes has been increased, in time, to many areas of surgical treatments. The amniotic membranes have been used in burns, in the treatment of venous ulcers, as a physiological wound dressing, for leg ulcers, otolaryngologic head and neck surgery, pelvic surgery, etc. Human amniotic membrane grafts are used preferably as temporary biological dressings because of its unique characteristics and properties. No immunogenic rejection phenomenon does occur after the application of amniotic membrane grafts on the wound. Healing effect of the foetal membranes when used for treating different surgical and skin defects are thought to be either due to their content

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of some antibacterial, angiogenic or other biochemical factors, or to the biomechanical characteristics of the foetal membranes as such.\textsuperscript{18}

The amniotic membrane allografts prevent local inflammatory response\textsuperscript{19} and its antimicrobial properties reduce risk of post-operative infection.\textsuperscript{20}

The transplanted membrane may perform as a structural barrier to impede proliferation of fibrous tissue and thus decrease scarring.\textsuperscript{21,22}

**Microbial contamination of human tissue allografts**

The risk of infectious disease transmission with human tissue allografts is a major concern in tissue banking practice. Microorganisms can be introduced into the grafts during tissue collection, processing and storage, but even if all these procedures are done under aseptic conditions, the possibility of bacterial and viral disease transmission of donor origin cannot be excluded.\textsuperscript{23} In order to minimize the risk of disease transmission through allografts, several steps should be undertaken by tissue banks, including careful donor-screening, proper tissue processing and sterilization of tissue allografts.\textsuperscript{23}

Islam et al\textsuperscript{24} and Begum et al\textsuperscript{25} reported bacterial isolates obtained from human amniotic membranes and one of the bacterial isolates was reported to be highly radio-resistant. Chakraborty et al (2012)\textsuperscript{26} and Chakraborty et al (2015)\textsuperscript{27} reported association of microorganisms with human chorio-amniotic membranes. Chakraborty et al\textsuperscript{26} isolated bacterial contaminants from all the studied 34 post-delivery chorio-amniotic membranes collected from labor rooms of hospitals and the contaminants were identified as *Staphylococcus aureus*, *S. cohnii*, *S. epidermidis*, *S. sciuri*, *Micrococcus roseus*, *Bacillus* sp., *B. megaterium*, *Pseudomonas cepacia* and *P. diminuta*.

**Sterilization of tissue allografts with ionizing radiation**

Radiation sterilization of medical materials on a commercial scale was applied for the first time in the middle of the 1950s with less than 10% of medical products sterilized by ionizing radiation in the 1970s, and nearly 50% at the period of report by Dziedzic-Gociawska et al\textsuperscript{23} with an increasing tendency. The International Atomic Energy Agency (IAEA) in Vienna promotes and supports the use of ionizing radiation for sterilization purposes. IAEA has elaborated and issued many guidelines and standards applicable for radiation sterilization.\textsuperscript{28} The Agency's promotion and support resulted in the development of national programmes on tissue banking and application of ionizing radiation for the sterilization of tissue allografts. Tissue banks were created in many countries of the Asia Pacific region under the auspices of IAEA.\textsuperscript{23}

The killing effect of radiation may be due to direct effect or indirect effect. The direct action of radiation involves the interaction between ionizing radiation and critical biological molecules, which results in excitation, lesion and scission of polymeric structure such as DNA.\textsuperscript{29} The indirect effect of radiation is due to biochemical changes within the organism. The interaction of ionizing radiation with water molecules within living cells leads to the production of short-lived free radicals and peroxy radicals. These radiolytic products interact with biological molecules including DNA; hence inactivating the reproduction process. This indirect effect of radiation normally occurs as an important part of the total chain of reactions of ionizing radiation.\textsuperscript{30,31} The indirect effect is more prominent and very much influenced by environmental factors including oxygen, water content, medium, temperature and chemicals present during irradiation.\textsuperscript{32-34} Chakraborty et al\textsuperscript{35} reported the influence of irradiation medium and initial viable cell numbers on the gamma radiation sterilization of the bacterial isolates recovered from human amnion membranes and bones. The sterilization efficacy of ionizing radiation lies in its good penetrability inside the matter and its high killing efficiency of microorganisms.\textsuperscript{23} Ionizing radiation is effective in the inactivation of microorganisms in the bulk of any material without associated problems of heat exchange, pressure differences or hindrances by diffusion barriers.\textsuperscript{36} Chakraborty et al\textsuperscript{36} studied the effect of gamma radiation on the sterility quality of tissue allografts and found that human amniotic membrane allografts irradiated with a dose of 25 kiloGray (kGy) of gamma radiation were sterilized.

Transmission of hepatitis C virus (HCV) by non-sterilized cadaveric tissue allografts has been described, while allografts irradiated with a dose of 17 kGy did not evoke infections of the graft recipients.\textsuperscript{37} There is no data on the sensitivity of hepatitis B virus (HBV) to ionizing radiation;
however, the virus is known to be resistant to heat sterilization.\textsuperscript{23}

Most of the studies on the effectiveness of ionizing radiation to inactivate viruses were done on the inactivation of human immunodeficiency viruses (HIV), particularly in blood plasma. The dose required to eliminate all active HIV-1 from the particular tissues depends on the amount of virus originally present (initial contamination).\textsuperscript{23} It has been postulated that the dose of irradiation needed to reduce the viral load by $1\log_{10}$ is 4 kGy\textsuperscript{38} or even 5.6 kGy\textsuperscript{39}. Fideler et al\textsuperscript{40}, using polymerase chain reaction (PCR), found that in order to inactivate HIV in fresh frozen bone-patellar ligament allografts, a dose in the range of 30-40 kGy was required. The sensitivity of HIV to ionizing radiation is reported to depend on the temperature during irradiation. The inactivation of the virus titre of 5 to 6 $\log_{10}$ was achieved at doses of 50 to 100 kGy in frozen (-80°C) plasmas and at the dose of 25 kGy at 15°C.\textsuperscript{41} Dziedzic-Goclaw ska et al\textsuperscript{23} reported that prions, responsible for Creutzfeldt-Jacob disease (CJD) in humans, are extremely resistant to most of the chemical and physical sterilizing/disinfecting agents including ionizing radiations.

**Selection of tissue donors**

Tissues could be procured from both living and cadaveric donors. Tissues from living donors mainly fall into two categories, namely amniotic membranes and bones obtained after surgical procedures. Tissues from cadaveric donors (e.g., skin, bone, cartilage, tendon etc.) are obtained under sterile or non-sterile conditions.\textsuperscript{42} The main goal of the tissue bank is to provide safe and high quality tissue grafts to the patients. Therefore, each potential donor must be evaluated through a careful review of the donor’s medical and sexual history, physical examination and laboratory screening. Generally, the ideal donor is a young, healthy adult under 55 years of age.\textsuperscript{42} Donor selection is based on a review of social and medical history, physical examination, autopsy results (if performed) and blood tests. Donors are excluded if any elements of past social, sexual and medical history indicate the risk of HIV (human immunodeficiency virus), hepatitis B and C, or jaundice of unknown etiology, infectious diseases including tuberculosis, malignant diseases, presence or suspicion of neurodegenerative symptoms including dementia, the use of pituitary derived hormones, systemic connective tissue diseases (e.g., lupus erythematosus, rheumatoid arthritis), chronic steroid treatment, exposure to toxic substances and exposure to irradiation at the site of tissue donation.\textsuperscript{23}

Serological as well as microbiological examinations are needed to fully screen donors. The minimum testing accepted universally includes VDRL (Venereal Disease Research Laboratory test)/RPR (rapid plasma reagin), hepatitis B surface antigen, hepatitis C antibodies, HIV antibody and microbiological (culture) testing of small donor tissue samples or swabs. Other tests can be done selectively when indicated such as: CMV (cytomegalovirus) test, hepatitis B core antibody, histopathological examination, antigen testing for HIV using antibody or polymerase chain reaction (PCR) and HTLV (Human T-lymphotrophic virus) I and II.\textsuperscript{42}

**Collection and preservation of tissues**

Tissues are collected aseptically in airtight sterile containers and transported to the tissue processing laboratory in an insulated container that can keep the tissues at a temperature of 4°C for at least 12 hours (h). Normally ice slabs or dry ice are placed in the container to keep the temperature low. Chorio-amniotic membranes collected after delivery should be kept completely immersed in sterile normal saline in a plastic container and stored in a refrigerator (4°C) for not more than 24 h.\textsuperscript{42}

**Processing of radiation sterilized human amniotic membranes for use in rehabilitative surgery**

The procedure of processing of radiation sterilized human amnion membrane allografts for use in rehabilitative surgery is described elsewhere.\textsuperscript{30,43} Post-delivery human chorio-amniotic membranes from clinically suitable donors are collected aseptically in sterile normal saline and temporarily preserved in the freezer. The frozen tissues are first thawed and then the amnion is separated aseptically from the chorion using sterile surgical instruments. The amniotic membranes are washed several times with sterile normal saline using an electric orbital platform shaker to remove blood and tissue debris. The membranes are then aseptically cut into pieces, stretched on surgical gauge and mounted on surface sterilized (70% ethanol) plastic frames and dried overnight at 40°C temperature in an electric controlled oven. The dried membranes are then cut under a laminar air flow into pieces and each piece
of amniotic membrane is then packaged in triple layer with polyethylene packages, labeled, vacuum sealed, and then irradiated with gamma radiation at the dose of 25 kiloGray (kGy). The irradiated membranes are preserved at refrigerating temperature. The sterility of the irradiated membranes is determined by sterility testing using microbiological culture media, and then the sterile membranes are supplied to hospitals/clinics for use in rehabilitative surgery.26,43

Effect of processing and radiation sterilization on the biomechanical properties of tissue allografts

Chakraborty et al (2007)44 studied the effect of oven drying (40°C), freeze drying (-50 °C) and gamma irradiation (25kGy) on the tensile strength of human amnion membranes. When the tensile strength of the amnion membranes was measured without rehydration of dried samples, it was found to be increased at different stages of processing including oven dried (7.93% increase), freeze dried (6.38% increase), oven dried-gamma irradiated (7.57% increase) and freeze dried-gamma irradiated (6.97% increase) as compared to that of the controls. Rehydration of dried and irradiated membranes resulted in a decrease in tensile strength. After rehydration in normal saline for 5 minutes, the retention of tensile strength by oven dried-irradiated and freeze dried-irradiated amnion membranes was respectively about 70% and 61% of that of controls.

Clinical uses and effectiveness of radiation sterilized amniotic membranes

Human amniotic membrane is used in many different clinical situations such as: superficial burns,17, 45 mixed burns,46 leprotic ulcer,47,48 chemical and thermal burns,49 deep corneal ulcers,50 in the management of pressure sore51 etc. It is reported to reduce pain, decrease electrolyte, fluid and protein loss, and promote epithelialization.9 It is also reported to prevent local inflammatory response and decrease corneal haze.19 Bari et al52 reported a prospective study of 40 patients with deep and deep dermal burns in which radiation sterilized (25 kGy) human amniotic membranes were used. Nessa et al43 reported the use of oven dried (40°C) and gamma radiation sterilized (25kGy) human amniotic membranes in the treatment of 168 patients with heat burn, acid burn, diabetic wound/diabetic foot ulcer, leprotic ulcer, abdominal wall reconstruction, chemical (acid) burn (corneal surface Rt), chemical (lime) burn, pterygium removal site, peripheral corneal ulcer, and orbit reconstruction in hospitals/clinics in 2008 in Bangladesh. For utilization as temporary biological dressing of wounds, non-viable lyophilized/oven-dried radiation sterilized amniotic membrane grafts could be processed.17

Use of amniotic membrane allografts in the treatment of burns in children

Khan et al17 reported treatments of children with superficial burns using radiation sterilized amniotic membranes as biological dressing. The authors mention that the amnion grafts were very easily applied to the wounds and the patients did not feel pain during repeated dressings. No side effect was also noticed after the application of grafts and all the patients healed satisfactorily without any post operative complication. Chakraborty et al46 reported management of mixed (superficial and deep) burn in children with allogeneic amnion and autogeneic skin grafting. Shahid et al50 reported the use of radiation sterilized amnion membrane as local burn wound coverage and demonstrate that amniotic membrane is a suitable and effective biological dressing in reducing patient’s morbidity when compared with non-biological medicated tulle (sofratulle). Ullah et al54 reported the use of radiation sterilized amniotic membranes in the treatment of a total of 370 patients (mean age 2.76 years) with superficial partial thickness burn. The authors54 mention that amnion dressing suppresses bacteria in the wound as well as reduces infection. Amnions have good adherent characteristics, which reduces infection as well as reduction of oozing of plasma from the wound.54

Burn injuries are one of the most devastating physical and psychological injuries that a person can suffer.55 From 1981 to 1990 it was the second most common surgical problem in paediatric age group in the department of pediatric surgery, Dhaka Shishu Hospital.56 Ullah et al54 reported the use of radiation sterilized amniotic membranes in the treatment of a total of 370 patients with partial thickness burn. The authors mention that amnion dressing suppresses bacteria in the wound as well as reduces infection. Amnions have good adherent characteristics, which reduces infection and also reduce oozing of plasma from the wound. It has a role on burnt pain reduction, frequency of dressing change, rate of healing, cost, and duration of hospital
stay. Ullah et al\textsuperscript{54} conclude that amniotic membrane is one of the effective biological skin substitutes used in burn wounds, with efficacy of low bacterial counts, has advantages of reducing loss of protein, electrolytes and fluids, decreasing the risk of infection, minimizing pain, accelerated wound healing and good handling properties. It is readily available and does not present immunological problem and allergy response. It is cost effective and very helpful for developing countries.

**Conclusion**

The facility of preservation of oven dried and radiation sterilized human amniotic membranes in a refrigerator, makes it possible to build up a large stock of allografts for use as biological dressing of wounds. Processing and preservation of radiation sterilized tissue allografts in a tissue bank can ensure ready availability of tissue allografts for safe clinical use.

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