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REVIEW ARTICLE

Amniotic membrane & its structure, features and uses in dentistry – a brief review

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Abstract

Amnion allograft has been used in the field of medicine for its exceptional wound-modulating properties. However, in the field of dentistry, only a limited number of reports have explored its potential in healing of oral wounds. The amnion is a membrane building the amniotic sac that surrounds and protects an embryo. The amniotic membrane is a tissue of fetal origin and is composed of three major layers: A single epithelial layer, a thick basement membrane and an avascular mesenchyme. It contains no nerves, muscles or lymphatics and can be easily separated from the underlying chorion. The fetal membrane was first used for the transplantation of skin in 1910. With improvements in the processing and storage technologies, amniotic membrane has found application in various fields of medicine, including management of burns; reconstruction of the oral cavity, bladder and vagina; tympanoplasty; arthroplasty and so on. Recently, this multipurpose tissue has found application in the field of dentistry. Because of its inherent wound-modulating properties, amnion allograft may be used to enhance periodontal wound healing and enable tissue regeneration. Here is a review article highlighting various properties and uses of the amniotic membrane & its advantages in various dental field.

INTRODUCTION

Regenerative medicine is a new field primarily based on the concept of transplanting exogenous or stimulating endogenous stem cells to generate biological substitutes and improve tissue functions. Recently, amnion derived cells have been reported to have multipotent differentiation ability, and these cells have attracted attention as a novel cell source for cell transplantation therapy.[1] Historically, natural amniotic membranes have been successfully used for wound and reconstructive purposes since the early 20th century. The first reported use of fetal membrane as skin substitute was by Davis in 1910. In 1913, Salbella presented the first clinical report of successful use of amniotic membrane in the treatment of skin ulcerations.[2,3] Amniotic membrane allografts as a wound allograft material have a number of beneficial properties inherent in their makeup. The material provides a natural scaffold for wound healing and contains various important growth factors and biological macromolecules important in wound healing[4]. Specifically, the amniotic membrane (AM) has gained importance because of its ability to reduce scarring and inflammation; enhance wound healing; and serve as a scaffold for cell proliferation and differentiation as a result of its antimicrobial properties. AM is an excellent candidate to use as a native scaffold for tissue engineering.[5]

Unique properties of the material include the lack of immunologic markers, conferring an "immune privileged" status on the allograft; antibacterial properties; and the ability to reduce pain on application. Recently, techniques have been developed to dehydrate the material while preserving many of these wound-healing attributes,

to produce a temperature-stable allograft. Over the past 5 years, application of this material has been extended to include wounds of other areas, including diabetic neurovascular ulcers, venous stasis ulcers, postoperative or posttraumatic chronic wounds, and postsurgical wound dehiscence. Future research will continue to yield more information on the unique properties of the amniotic membrane allograft.[4]

AMNIOTIC MEMBRANE STRUCTURE AND FUNCTION

Amniotic membranes develop from extra-embryonic tissue and consist of a fetal component (the chorionic plate) and a maternal component (the deciduas). These two parts are held together by the chorionic villi and connect the cytotrophoblastic shell of the chorionic sac to the decidua basalis. The amniochorionic membrane forms the outer limits of the sac that encloses the fetus, while the innermost layer of the sac is the AM.[5] it is about 10-15 micrometer thick which consists of two fetal membranes; the inner amniotic membrane and the outer chorion. The innermost layer, nearest to the fetus, is called the amniotic epithelium and consists of a single layer of cells uniformly arranged on the basement membrane. [5, 6]

AM have two types of cells with different embryological origins: amnion epithelial cells derived from embryonic ectoderm and amnion mesenchymal cells from embryonic mesoderm. At ultrastructural level AM is a thin, tough, transparent, avascular composite membrane composed of three major layers: a single epithelial layer, a thick basement membrane, and an avascular mesenchyme consisting mainly of collagen. AM contains no blood vessels or nerves; instead, the nutrients it requires are supplied directly by diffusion out of the amniotic fluid and/or from the underlining decidua. The amniotic epithelial cell layer is a single layer of flat, cuboidal and columnar cells that are in direct contact with the amniotic fluid. It is from this layer that amniotic MSC (AMSC) are isolated and stored to be used for regenerating tissues. The amniotic mesoderm layer consists of macrophages and fibroblast-like mesenchymal cells. The basement membrane of the amnion is very similar to the basement membrane found in the other parts of the body like the conjunctiva or gingiva.[6]

Extracellular Matrix

Extracellular matrix materials form the structural components of the architecture of the membrane and contain a variety of specialized proteins including fibronectin, proteoglycans, glycosaminoglycan, laminin, and other similar materials. The basal lamina contains a large amount of proteoglycans like heparin sulfate that is one of the major proteoglycans in the gingiva. The spongy layer on the stromal portion of the amnion has an abundance of hydrated proteoglycans and glycoproteins that form a non fibrillar network along with collagen.[7] AECs secrete collagen type III and IV and non collagenous glycoproteins like laminin, nidogen, fibronectin and vitronectin within the basement membrane that serve as adhesion ligands transmitting signals and interacting at cell surface receptors.[8] Laminin-5 being the most prevalent in the amnion membrane helps in cellular adhesion of gingival cells, invasive growth of fibroblasts and angiogenesis in the early phases of wound healing.[5,6]

The amniotic cells are connected to each other by numerous desmosomes and tight junctions occluding the lateral intercellular spaces with limited paracellular transport.[9] The amniotic epithelium has a specialized arrangement of intracellular cytoskeletal filaments such as actin, α-actinin, spectrin, ezrin, cytokeratins, vimentin, and desmoplakin indicating their role in the structural integrity and modulation of cell shape of the healing tissue. The presence of integrin a6/b4 as the main ligands in the basement membrane which participates in the construction of the hemidesmosome like structure favours the adhesion and anchoring of ESCs to the healing wound.[6] Immunohistochemically, collagen III, collagen IV & laminin are expressed in the basement membrane of amniotic membrane. AM contains many growth factors and exhibit anti inflammatory and anti bacterial properties and has been reported to reduce scarring.[10]

The matrix of human AM contains abundant growth factors like keratinocyte growth factor (KGF), basic-fibroblast growth factor (b-FGF), transforming growth factor-beta (TGF-β), nidogen growth factor (NGF), and epidermal derived growth factor (EDGF) which promote periodontal regeneration. These growth factors provide a natural healing environment, accelerate healing and mimic the stem cell niche for ex vivo growth. Various stem cell markers such as octamer-binding transcription factor (OCT) - 4, hepatocytes nuclear factor-3β (HNF-3β), nestin and nanog which are specific stem cells markers are also found in the amnion membrane. The stem cell markers like epidermal marker CA125 and general epithelial markers such as cytokeratins and vimentin are present in large amount in the amniotic epithelial cells. Moreover, human amniotic mesenchymal cells are positive for CD44 and desmin, CK19/ vimentin, indicating coexpression of both epithelial and mesenchymal cell markers. These MSCs have the ability to accelerate the inflammatory phase towards the proliferative phase which is critical for treating chronic wounds like periodontitis.[6]

Cellular material includes the epithelial lining of the amnion facing the infant but also pleuripotent stem cells important in regenerating new cellular materials within the membrane lining. Epithelial stem cells, in particular,

have also been isolated from the epithelial layer of the amniotic membrane. Fibroblasts are also present and provide lining and strengthening of tissues. The epithelial cells are also biologically active in the healing process through various receptors on the cell surface.[4] The role of hematogenous, mesenchymal, and closely located stem cells is also affected by the interactions among various components of the membrane.

TYPES OF AMNIOTIC MEMBRANE

Frozen membrane

Amniotic membrane is frozen by passing through liquid nitrogen at -196°F. Cooling preserves the membrane for an indefinite time, produces bacteriologically pure and immunologically almost inert material. Cryopreservation with dimethyl sulphoxide (DMSO) at -80°C allows retention cells in the AM at approximately 50% for several months. The several angiogenic growth factors and cytokines are removed during cryopreservation of the AM. However, if the AM is cryopreserved in 50% glycerol, the viability of AECs is lost. It has been noted that storage of the AM in glycerol at 4°C results in immediate cell death.[27]

Freeze dried irradiated (lyophilized) [2,28]

In this process, membrane, after obtained from placenta is freeze dried at -60°C under vacuum (atmospheric pressure 102) for 48 hours. It is then irradiated with 2.5 mega rads (25 K Gray) in a batch type cobalt-60 irradiator. By the method of freeze drying there is sublimation of liquid moisture of membrane to gaseous state without having undergone the intermediate solid stage. This method helps the membrane to maintain its original size and shape with minimum cell rupture. The freeze dried membrane can be readied for use by soaking it in normal saline for 1 minute.

FEATURES OF AMNIOTIC MEMBRANE

Immunogenicity of amniotic membrane

Amniotic membrane has low or no antigenicity. Amniotic membrane when used as an allograft in peritoneal cavity or buried under skin has shown long term survival with no evidence of any immune reaction. Robson, Samburg and Krizekts preferred to use the unseparated amniochorionic membrane with the chorion placed against the wound in deep second degree skin injuries. Although vascularization and vigorous "rejection" develops from the mesenchymal side of the chorion, both the procedures seem to be less intense when the epithelial cells are placed on the wound.[2] The difference in immunology of amnion and chorion is related to the presence of fragments of maternal decidua on the chorion. The native AECs express the non-polymorphic, non-classical human leukocyte antigen (HLA-G) but lack the polymorphic antigens HLA-A, B (Class IA) and HLA-DR (Class II) on their surfaces.[11] The class I antigen is seen in almost all cells of the amniotic membrane unlike the class II antigen which is only present in some fibroblasts. AECs do not express HLA-A, -B, -D, and -DR antigens on the cell surface, but express HLA-G on their surfaces, suggesting that acute rejection would not occur after transplantation.[12,13]

The immune barrier is due to lack of expression of co-stimulatory cell surface molecules such as CD80 and CD8 mostly in the human. They are actively suppressive of T cell, dendritic cell and B cell function that down-modulate exuberant inflammation. AECs may be immunologically inert with reduced risk of rejection or immune reaction upon transplantation.[6]

Anti inflammatory and anti microbial property

AM reduces inflammation. The AM stromal matrix markedly suppresses the expression of the potent pro-inflammatory cytokines, IL-1α and IL-1β. Matrix Metalloproteases (MMPs) are expressed by infiltrating polymorphonuclear cells and macrophages. Hyaluronic acid is a high molecular-weight glycosaminoglycan that exists in large quantities in the AM and acts as a ligand for CD44, which is expressed on inflammatory cells and plays an important role in adhesion of inflammatory cells, including lymphocytes, to the AM stroma.[2, 6]

The β-defensins are a major group of anti-microbial peptides that are expressed at mucosal surfaces by epithelial cells and leukocytes, and are an integral part of the innate immune system. The innate immune system has evolved to eliminate microorganisms upon entry into the tissues, creating antigens necessary to produce an adaptive immune response. ACEs also have the ability to produce β- defensins. The β3-defensin is the predominant defensin in the amniotic epithelial cells. The two low-molecular-mass elastase inhibitors, secretory leukocyte proteinase inhibitor (SLPI) and elafin are expressed in the AM. In addition to their anti-inflammatory properties, elafin and SLPI both have antimicrobial actions and act as components of the innate immune system to protect related surfaces

from infection. AM with both lactoferrin and interleukin-1 receptor antagonists make the AM both anti-microbial and anti-inflammatory. Lactoferrin is a globular multifunctional protein, which has both anti-microbial and anti-inflammatory effects, by serving as an antioxidant and an iron chelator in tissues. Lactoferrin suppresses the production of interleukin-6 in the amniotic fluid during amniotic infection. The interleukin-1 receptor antagonist is a potent inhibitor of interleukin-1 and thus will suppress the inflammation mediated by interleukin-1.[6]

Adherence of amnion to the burn wound by eliminating its exposed status may itself lower bacterial count in the wound. The close adherence of the membrane to the wound is said to be via a fibrin and elastin. Furthermore, the amniotic membrane has a high thrombin activity which allows a very rapid and efficient attachment to living dermis or granulating tissue. This close adherence allows restoration of lymphatic integrity protects circulating phagocytes from exposure and allows removal of surface debris and bacteria. The initial neo-vascularization is held responsible for effective decrease in bacterial counts and promotes healing.[2]

Reduction of pain

Amnion membrane (AM) has the unique ability to reduce the pain during the surgical procedure as it diminishes inflammation and provides a better state of hydration that soothes the wound bed to promote faster healing.[14] The soft mucoid lining of amniotic membrane also protects the exposed nerve endings from external irritant that help to decrease pain sensation by preventing nerve stimuli. CAM also supported the growth of the epithelium and facilitates migration and reinforced adhesion.[6]

Increase vascularization or revascularization

The rapidity of in growth of epithelium from the borders of the wound in full thickness defects and rate of re-epithelization of partial thickness burns appear to be increased by the use of amniotic membrane. There is an enhanced induction of Vascular Endothelial Growth Factor (VEGF) both for VEGF receptors 1 and 2 by the cells of the AM[15]. The release of angiogenic factor like insulin derived growth factor (IGF) helps in the development of tissue engineered vascular grafts which are useful in revascularization of ischemic tissues, chronic ulcers, repair of bone and cartilage. The increase formation of blood capillary blood flow to the lyophilized amniotic membranes when used as graft material in vestibuloplasty has also shown rapid revascularization and promotes faster healing.[6,16]

Mechanical properties

The biomechanical property of the AM has been for the investigation of premature rupture of the foetal membrane (PROM). PROM is defined as rupture of chorio amnion prior to the onset of labour. The foetal membrane must bear the load of hydrostatic pressure from amniotic fluid during gestation. In addition to chronic load of during normal pregnancy, foetal membranes must also likely bear repetitive minor loads, such as Braxton-hicks contractions The AM is approximately 20% of the chorio amnion thickness at term, but dominates the mechanical responses of the bi-layer, with both stiffness and strength by an order of magnitude greater than the chorion layer. The AM demonstrates a mechanical response that is inherently time-dependent, described as "viscoelastic". Viscoelasticity is a critical property of scaffolding in a majority of tissues[10]. Preterm AM has a greater mechanical integrity than term AM; the stiffness of term AM is more reasonable for a majority of tissue engineering protocols. Elastin, which is detected in the foetal amnion, is proposed to provide the molecular basis for elasticity in the AM.

USE OF AMNIOTIC MEMBRANE IN DENTISTRY

AM is semi permeable membrane and is an immunotolerant structure. The AM fulfills the current mechanical concept of GTR which amends it with the modern concept of biological GTR. Biomechanical GTR proposed here in using amniotic membrane, not only maintains the structural and anatomical configuration of regenerated tissues, but also contribute to the enhancement of healing through reduction of post-operative scarring and subsequent loss of function and providing a rich source of stem cells. Amniotic membrane enhances gingival wound healing properties and reduces scarring. Excellent revascularization of the amniotic membrane is another favorable property. Amniotic membrane was potentially a good grafting material with very good wound coverage. It enhanced wound healing process, good post-operative function and esthetics without any complications. Human amniotic membrane could be one of the considered options in reconstruction of oral cavity defects as it ensures good reconstruction, post-operative function and esthetics.[18]

The dehydrated amnion/chorion membrane allograft can also be micronized which allows it to be administered as a topical powder or mixed with saline to create an injectable solution or a topical gel. Use of amniotic membrane has recently increased clinically as an allograft material for chronic and acute wound care management, for scar tissue reduction, as a barrier membrane, and as a soft tissue regeneration graft.[19]AM is highly useful and effective as a culture substrate. The effectiveness of new amniotic membrane-based regenerative therapy

to oral health care through successful preparation of a cultivated oral mucosal epithelial cell sheet on AM and the establishment and clinical application of an auto transplantation technique for various types of oral mucosal defects in dental oral surgery. The graft of amniotic membrane is a viable and reliable method to cover the exposed periosteum as they serve as a good alternative to mucosal and skin grafts.[20] Amnion allograft might be a suitable alternative to connective tissue graft in procedures to cover denuded root surfaces and can reduce recession depth.[21,22] Recently, the AM-based cell-culture system to culture PDL derived cells for regenerative therapy for periodontal tissue. These cells are considered capable of proliferation and potentially maintaining their PDL-like properties even on AM.[23]

Use of the amniotic membrane has been successful for oronasal fistula repair and use of the multilayer technique and protective plate utilization prevent membrane ruptures.[24] when used in vestibuloplasty, these grafts of the amniotic membrane are viable and reliable for covering of the raw surface, as they prevent secondary contraction after vestibuloplasty and maintain the postoperative vestibular depth.[25] The amniotic membrane can be a favorable graft material for vestibuloplasty, promoting healing and preventing relapse. It is easily available and preserved and is a cost-effective material.[26]

PREPARATION OF AMNIOTIC MEMBRANE[2]

Fresh membrane is obtained from the placenta at the time of delivery, either vaginal or caesarian section. Robson and Krizekl rinsed the membrane in a 0.025% solution of sodium hypochlorite and stored at 4°C in sterile solution containing penicillin. They showed that membranes remained sterile up to 6 weeks. Dinno and associates performed cultures to study sterilization of amniotic membranes. Preservation with 1:40 dilution of sodium hypochlorite revealed no positive cultures until 30 days.

METHOD OF USE[2]

Before the membrane is applied, the wound should be prepared as for any dressing or for skin grafting. Surgical scrub with antiseptic and minimal debridement is followed by moist compression until oozing has stopped and the wound surface is reasonably dry. This procedure is preferably done in a clean sterile dressing room, observing all aseptic measures. No local or general anesthesia is required. Membrane is applied with rough (chorionic) surface next to the wound. Care is taken to ensure no trapping of air bubbles between membrane and wound by gentle pressing. Membrane is followed by a layer of anti-bacterial gauze (e.g. Soframycin tulle), some moist gauze, dry gauze, cotton and bandage. Dressing should be changed along with the membrane at least every 48 hours and preferably after every 24 hours. Dressing should be continued for 7-10 days or until wound appears clinically clean. Split skin grafting should be done after 7- I 0 days or when wound contains less than 10^5 organisms/gram of tissue.

Freeze dried irradiated membrane is also used as described above, but before application it is soaked in sterile saline for 1-2 minutes.

PRESERVATION OF AMNIOTIC MEMBRANE

Glycerol has been used as a cryoprotective agent for a long time. Due to its high osmotic pressure it extracts interstitial water from the amniotic membrane. In this method, 80% glycerol is used for drying the amniotic membrane which can thereafter be preserved at 4ºC for a long time, although it loses some of its biologic properties. This type of preserved amnion is used for dressing burn wounds.[29,30]

CONCLUSION

Human amniotic membrane is a uniquely suited material for use as an allograft in wound management. Used in its natural form, then later in preserved preparations, the material assists in the healing process through a number of properties such as physical, biochemical and molecular biological pathways to promote regenerative healing while simultaneously reducing scar formation.

The AM has many characteristics, which make it potentially suitable for use in TE. The epithelial layer of the AM includes cells that have similar characteristics to stem cells. The AM can act as a scaffold for TE. The ECM components of the basement membrane from the AM include collagen, fibronectin, laminin and other proteoglycans important for overlying cell growth. These ingredients are the ligands for integrin receptors, and hence, have a great role in cell adhesion during the cell seeding protocol. Other properties of the AM include anti inflammation, anti-fibrosis, anti-scarring, anti-microbial, low immunogenicity and reasonable mechanical property, which are all important for use in TE. To conclude, amnion from discarded placenta can be an interesting source of cells for regenerative medicine.

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