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Future of Bovine Amniotic Membrane: Bovine Membrane Application on Wound Healing, Surgery and Prospect of Use for Urethral Reconstruction

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Abstract

This chapter describes how bovine amniotic membrane could be indicated for wound healing, especially in complex surgery such as urethral reconstruction. Chemical studies have assessed both histologically and immunohistochemically that bovine amniotic membrane creates scaffold for wound healing. Whereas, clinical studies have shown that bovine amniotic membrane property could be substituted for wound dressing hence improving skin or mucosal integrity. Bovine membrane has been known to be used for many specialties such as ocular surgery, neurosurgery, maxillofacial and orthopedic surgery. This chapter includes such studies and shows the usage possibility of bovine amniotic membrane for other complex defect as shown in urethral reconstruction.

Keywords: bovine amniotic membrane, scaffold, urethral reconstruction, wound healing

1. Introduction

Fetal development in mammals is a complex pathway which occurs after prenatal embryonic development [1]. Fetal development system is achieved by meticulous interactions consisting in umbilical cord, amniotic fluid and placenta. Fetal membrane is part of the system, composed of two layers: an outer layer (chorion), which contacts maternal cells and an inner layer (amniotic membrane; AM). Fetal membrane holds important role in fetal development, essential for protection, breathing, nutrition and excretion. Amniotic membrane or amnion is a thin membrane on the inner side of the placenta forming a sac that completely surrounds the embryo/fetus and delimits the amniotic cavity, which contains amniotic fluid [2, 3]. AM holds important metabolic roles by transporting water, other soluble materials and the production of bio-active factors, including vasoactive peptides, growth factors and cytokines. AM also provides the fetus with protection against dessication and environment of suspension, thus promoting embryonal growth. This function is mainly attributed by tractional resistance

mainly related to the condensed layer of interstitial collagen type I, II and elastin [2, 4]. Toda et al. mentions that amnion also holds pluripotent differentiation ability with low immunogenicity and anti-inflammation property [5]. These properties creates opportunities and interest on the use of amniotic membrane for regenerative medicine.

The usage for human amnion as a surgical material for skin substitute was first suggested and reported by Davis in 1910. Sabella in 1913 performed the first human trial for skin grafting [6–8]. Bovine amnion first use for absorbable insulating material and suture material was described by Johnson in 1937. Bovine amniotic membrane (bAM) and bovine allantoic membrane was suggested as biological dressing [9]. A study by Rao stated that further preliminary experimental study using bovine amnion xenograft was suggested by Silveti et al. in 1957 [6]. Since then, there are many basic and clinical researches regarding usage of bovine amnion on regenerative medicine.

Bovine amnion was worth noting, due to its wide surface area compared with human amnion (6000–7500 sq. cm vs. 1600 sq. cm) and similar histological appearance. Both amnion characteristic is marked with single layer of cuboid epithelium [6]. Consideration should be taken for the AM harvest timing due to morphological change during gestational period. Morphological change of bovine amniotic membrane has been observed between 40 and 230 days of gestation. The epithelium changes from a single layer of flattened, squamous cell containing conspicuous cytoplasmic organelles into single or multi-layered cuboidal cells with numerous microvilli. This epithelium is further supported by a basement membrane rich in collagen. The extracellular matrix is infiltrated with fibroblasts, mesenchymal stromal cells, and tissue macrophages (“Hoffbauer cells”) [10].

Proteomic profile of bovine amniotic membrane is proved rich in proteins and signaling pathways. An analysis study by da Silva in 2021 identified 2105 proteins with an interactive network of 1271 nodes (proteins) and 8757 edges (interactions), some of which are known to be present in healing pathway. Notable proteins such as albumin, actin, collagen, fibronectin, histone, protein s100, vimentin, tubulin are abundant in its composition [11, 12]. Epithelial cell in amniotic membrane also secretes several growth factors and cytokines such as epidermal growth factor, vascular endothelial growth factor, keratinocyte growth factor, basic fibroblast growth factor, transforming growth factors alpha and beta (TGF- α and TGF- β), interleukin-8 (IL-8), angiogenin, dipeptidyl peptidase IV (DPPIV/CD26), serine protease inhibitor (serpin) E1, also known as type 1 plasminogen activator inhibitor (PAI-1), and insulin-like growth factors [3, 13–16].

2. Bovine amniotic membrane role in regenerative medicine and wound healing

Regenerative medicine is a branch of medicine concerned with developing therapies that regenerate or replace injured, diseased, or defective cells, tissues, or organs to restore or establish function and structure [17]. Regenerative medicine has become an integrated part in medicine and surgery. It has brought potential therapy possibilities with the aim to restore and improve the function of the damaged tissue or organ [18, 19]. There are many approaches to regenerative medicine, including stem cell, tissue engineering, organ transplantation and biomaterials. Biomaterials are either synthetic or natural material that are used for medical purpose or in contact with biological system. Biomaterials in regenerative medicine are intended to facilitate repair mechanism of wound. Usage of biomaterials in wound healing is applied on biological dressing. Biological dressing prevents evaporative water loss, heat loss, protein and electrolyte loss, and contamination and also permit debridement and develop granulation and epithelialization of wound bed [20].

Due to its structure as fetal ectoderm, amnion is considered as a physiological biological dressing [2, 3]. Amnion is favored due to optimal barrier against bacterial colonization and prevention of water loss [21]. Several studies on burn patients showed rapid epithelization of wound bed with minimal graft loss [21–23].

Ideally, the graft should be harvested from the same species (allograft). However, human amniotic membrane harvesting has several limitations. Fresh amnion is proposed to carry several infectious diseases such as hepatitis, AIDS, syphilis and tuberculosis [22]. There are also several difference for each amnion harvested from the same donor. The thickness is different at various site of membrane. The thickness could vary from 0.02 to 0.5 mm. This pattern also relates to its transparency and translucency of the membrane. Multiple attempts from the harvest also creates different thickness from donor harvested near the placenta and another distant from it [24, 25]. Differences acquired from different donors are suggested to be contributed from racial variation, duration of gestation, parity, gravidity, labor term and trial of labor before caesarian section. These factors influence prostaglandin and pro-inflammatory cytokines when transplanted to the recipient [24]. Difficulties in finding donor, long time storage issue also limits the supply [26]. Freeze dried human AM is proposed to be the solution for storage issue. Thomson and Parks in between 1979 and 1980 had studied the preparation of human amnion using sodium hypochlorite 0.025% solution and stored it at -80° Celcius. There was no negative impact on its clinical benefits [23]. However, legal and religious issues still limit the supply of human amniotic membrane. Bovine amniotic membrane was proposed to help these shortcomings, providing stable supply.

Wound healing is characterized by sequential phases of inflammation, proliferation and remodeling [26, 27]. This process is mediated by many cytokines, growth hormones, and other mediators. Several numbers of studies were conducted resulting that bovine amniotic membrane may exert its therapeutic effects from inflammation to scarring process. Amniotic membrane contains proteinase inhibitors which inhibits migration of polymorphonuclear leucocyte cell to wound bed [28]. Polymoprhonuclear cell is one type of neutrophils that is extremely active on wound healing. Activated neutrophils produce several protease which help killing and degrading microbes. Neutropil-activated protease also breaks down extra-cellular matrix that in turn can debride the wound and facilitate cell migration. Nevertheless, excessive amount of neutrophils has negative impact on wound healing, by causing further tissue damage and inflammation [29]. A study by Kim et al. showed less polymoprhonuclear cell infiltration on amnion-membrane covered group thus promoting rapid healing and inhibiting proteolytic damage [30]. Another study by Shimura et al. proved that amniotic membrane attracts and traps inflammatory cells such as monocyte/macrophage, CD4(+) T cell and CD8(+) T cell which are responsible for cell apoptosis [31].

Bovine amniotic membrane also contributes on proliferation phase of wound healing. Abundant growth factors found on its membrane are responsible for the stimulation of proliferation [32]. Epidermal growth factor for instance works during proliferation phase (from day 4 postoperative) and promotes wound healing by increasing the rate of epidermal proliferation and also accelerating wound contraction level related to myofibroblast proliferation and collagen deposition [33]. Insulin growth factor, combined with Platelet derived growth factor and fibroblast growth factor increases the proliferation of fibroblast [34]. Fibroblast is crucial on wound proliferation by creating extracellular matrix and collagen structure for wound bed, as well as contracting the wound. Once fibroblast has migrated into the matrix, it changes its morphology and synthesize granulation tissue components [35]. amniotic membrane also has anti-bacterial peptides which makes it ideal for cell proliferation. Expression of inflammatory molecule suggested that amniotic

membrane is one part of the barrier to progression of infection [36, 37]. Bovine amniotic membrane collagen formation is also similar to human amnion according to its viability, diffusion formation and degradation [38].

Proliferation phase is also characterized by angiogenesis, granulation and epithelization. Each of these steps could also be influenced by amnion application. Matrix metalloproteinase (MMP) is one of growth factor expressed on amniotic membrane. It has been implicated in invasive cellular growth [3, 39]. A study by Jeong et al. showed that increased expression of membrane-type matrix metalloproteinase enhanced the activation of MMP-2 and invasion and migration of endothelial cells which affect the induction of capillary tube formation [40]. Amnion cells secreted substantial amount of angiogenic factors including HGF, IGF-1, VEGF, EGF, HB-EGF and bFGF [41]. However, there are some studies about in vitro anti-angiogenic effects of amniotic membrane. Faraj et al. found that AM conditioned medium reduced proliferation and angiogenesis. This result was proposed to be induced by thrombospondin and tissue inhibitors of metalloproteinase (TIMP 1) and 2 [42–44].

Amniotic membrane also promotes granulation and epithelization. Analysis by Piscatelli et al. from in vitro model showed that wound contraction in fetus was influenced by combination of pro-contraction transforming growth factor- β 1 and anti-contraction epidermal growth factor [45]. Rapid epithelization was histologically confirmed on bovine amniotic membrane-treated wound with thicker collagen bundles. Keratinocyte migration was also observed on wound bed whereas immunohistochemistry staining for angiogenesis and fibroblast were consistent for proliferation phase [26]. The connective tissue of amniotic membrane contains laminin, fibronectin and collagen, which are the main components of basal membrane. A case by Martinez et al. showed that spontaneous epithelization on epidermolysis bullosa was completed in seven days [46].

Other notable clinical effects of bovine amniotic membrane observed are anti scarring, fluid permeability control and tensile strength property. Anti scarring is promoted by anti-inflammation effect of AM and hyaluronic acid found on its membrane [47, 48]. Fluid control on wound healing is essential. Extravasation of fluid in wound contributes in creating wound exudate. The exudate is a marker of the chronic state of injury. Exudate also creates an environment favorable for bacterial proliferation [49]. Amniotic membrane structure remains intact after sterilization. Clinical study by Rejzek et al. has reported heat, fluid and electrolyte loss prevention by amniotic membrane. Oxygen permeability in amniotic membrane was demonstrated by Yoshita et al., all contributing to accelerated healing process [50, 51].

3. Application of bovine amniotic membrane in surgery

Coradetti et al. demonstrated that mesenchymal stem cells could be derive from bovine amnion. Both amnion and amniotic fluid are capable of differentiating into ectodermal and mesodermal lineages. This study further showed the capability of osteogenic, chondrogenic, adipogenic, and neurogenic stem cell usage for bovine amniotic membrane [52]. Thus, bovine amniotic membrane could be used for many surgical applications.

4. Ophthalmic surgery

The first use of amniotic membrane for ophthalmology was documented by de Roth back in 1940. The conjunctiva defect caused by symblepharon was treated with fetal amniotic membrane. The graft was fixed to the tendon of rectus muscle

and was taken in all cases. The study showed that AM has transformation property toward conjunctiva tissue [53]. Since then, there have been many applications for ophthalmic disease ranging from ocular burn, corneal defects, retinal problems, strabismus, and neoplasia. However, most of them used human amnion [54–60].

Proteins expressed on bovine amniotic membrane showed to be abundant in human cornea: including keratocan, decorin, lumican, TGF- β -induced protein ig-h3, and albumin. These proteins are responsible for corneal healing pathways. Numerous signaling pathway responsible for corneal healing are also revealed in the membrane. Selected pathways include integrin signaling pathway, Cytoskeletal regulation by Rho GTPase, Ubiquitin proteasome pathway, WNT signaling pathway, epidermal growth factor (EGF) receptor signaling pathway [11]. Corneal healing was demonstrated on canine corneal erosion with significantly higher proliferation [61].

5. Plastic and reconstructive surgery

Bovine amnion function as biological dressing holds great potential in reconstructive surgery. There has been several clinical studies including bovine amnion membrane for burn patients [62–64]. Rao published a study in 1981 regarding the use of bovine amnion on burn and pressure ulcer patients. The study revealed biostatic ability to control infection and faster granulation process [6]. Another study by Zhu et al. showed significant difference of burn wound treated with bovine amnion compared with vaseline gauze dressing as control. The different results studied were healing time, infection ratio and residual burn wound [62]. Bovine amnion as burn biological dressing was also proven having similar efficacy as human amnion. A study by Park et al. displayed similar histological grading, epithelization rate and infection rate [22].

Ablative laser used for removing superficial skin has notable adverse effect even though its efficacy is better than non-ablative laser. Adverse effect such as postoperative erythema is caused by complete elimination of epidermis and upper dermal layer [65]. Bovine amnion membrane was found clinically effective in reducing erythema due to its anti-inflammatory mechanism [26].

6. Head and neck surgery

Amnion is also applied in head and neck surgery. Although, some of them used human amnion. One of such use was documented as the use of amniotic membrane compared with collagen membrane, in a study conducted by Munoyath consisted of twenty patients with facial soft tissue injury with whom all patients had either single or multiple soft tissue loss all over the face. The size of the wounds ranged from 7 mm \times 10 mm to 80 mm \times 150 mm and depth of the wound ranged from 2 to 5 mm. the results showed pain score of greater than 3 was observed in 50% of the patients in Amnion group and in 80% of the patients in Collagen group. The average time for appearance of healthy granulation tissue over the wounds that were treated with Amnion dressing was 5 to 9 days and for Collagen group was 7 to 12 days. Though vascularity was not compromised in both the groups, the height of the wound at 3-month follow up showed a clinically significant difference (100% patients in AM group had flat wound whereas only 80% showed normal wound height); though statistically not significant [66].

In recent years, research of amniotic membrane in head and neck surgery, especially bovine amniotic membrane, has increased. The use mostly relates to wound dressing. Further research could evaluate the use of bovine amniotic membrane

in head and neck surgery in the years to come. The amniotic membrane compared with collagen membrane, with both materials combined with deproteinized bovine bone mineral was compared in clinical trials by Kim for the treatment of periodontal inflammations. Both the use of amniotic membrane and collagen membrane combined with deproteinized bovine bone mineral improves the condition of periodontium. The amnion did not cause a significant gum recession. Another use of amniotic membrane for oral cavity problem was studied for temporomandibular joint ankylosis, which is a serious condition, mainly due to injuries responsible for the reduction of mandible functionality [67, 68].

A bovine amniotic membrane study for facial abrasions was done in a Korea, comparing the use Amnisite BA_{tm} with foam and gel dressings. The study demonstrated all patients were well healed completely after appliance of dried bovine amniotic membrane or foam dressing without any complication. However healing time for patients treated with dried bovine amniotic membrane was significantly shorter and no significant difference between the two groups regarding treatment costs, scar formation, skin elasticity or moisture content was noted. This study demonstrate the potential practical clinical use of bovine amniotic membrane as a facial soft tissue trauma as one of potential dressing [69].

7. Neurosurgery

In the field of neurosurgery, autologously harvested amniotic membrane has been used to repair duramater defects in myelomeningocele. Although the human cranial neurosurgery applications of amniotic membrane have not been thoroughly investigated, an in vivo rat cranial surgery model demonstrated that human xenograft amniotic membrane was efficacious and had an adequate safety profile. Eichberg put forward their retrospective pilot study about the use of allograft amniotic membrane for the augmentation of dural repair in craniotomies. The reported rates of postoperative CSF leaks differ among studies and craniotomy locations; leaks may prevailed as many as 4–17% craniotomy for posterior fossa lesions. In 122 craniotomies, including 18 craniotomies for posterior fossa lesions, none were complicated by postoperative CSF leaks. These results suggest that amniotic does not contribute to the increased risk of CSF leaks. Further, the interpretation of the data is complicated by the fact that the patients in the study received a sheet of bovine collagen dural substitute layered on top of the dehydrated amnion membrane; thus, the outcomes may be due to both materials. While this retrospective pilot study does not prove the superiority of dehydrated amnion over other dural adjuncts, or the efficacy of use, they demonstrate that it has an adequate safety profile with no complications directly related to its use in closures for craniotomies. They also report very low CSF leak rates and infection rates, particularly in craniotomies for infratentorial lesions [70–72].

8. Pediatric urology: the prospect of urethral reconstruction using bovine amniotic membrane

In the field of urology or pediatric surgery, uroepithelial reconstruction for several pathology is challenging. There is no synthetic material that is considered ideal as a substitute for the urethra and there is no research that firmly determine a good synthetic material to replace urethral defects. Strictures still found after the transplantation of acellular scaffold was also reported [73]. Therefore, the use of cell-seeded scaffold is proposed to be a better material used in urethral reconstruction. Bovine amniotic membrane has unique properties including anti-adhesive effects, bacteriostatic

properties, wound protection, pain reduction, and epithelialization effects. Another characteristic of amniotic membrane is the lack of immunogenicity [26].

Amniotic membrane also has some advantage compared to other allographs, such as bladder mucosa, buccal mucosa, and also appendix tissue. First of all, amnion harvesting does not need extra surgery unlike other organ. Postoperative care is simpler with shorter hospital time. However, there is technical difficulty in handling the membrane. Due to the thinness of the amniotic membrane and the release of the amniotic layer to the chorion, there is a higher risk of perforation or tear or separation of the two layers during surgery [74].

Bovine amniotic membrane has been studied mostly on animal studies with promising effects. One experimental study for urinary bladder reconstruction by Bakhtiari in 2000 was using fresh and formalin based bovine amniotic membrane for canine. Urinary bladder and urethra share similar histology. Both are consisting of epithelium on the lumen surrounded by rich collagen connective tissue and muscle layer. Both are responsible for maintaining structural integrity of the organ and transporting or expelling the urine [75]. The surgical procedure was conducted by 5 cm resection from the cranial bladder. Postoperative graft using two types of bovine amniotic membrane was observed. Graft site observations on postoperative days 30 and 60 showed adhesion at the graft site (100%), the graft floating within the bladder lumen (40%), good graft adhesion to the bladder, and no evidence of leakage or fistulation. Histopathological examination revealed regeneration of uroepithelial tissue and smooth muscle at the graft site. In addition, congestion, edema, and inflammatory cell infiltration were also seen in two cases. According to this study, despite complications such as infection, release of amnion from the bladder, “less than normal” distension and adhesions of the bladder at the graft site, it can be argued that fresh and preserved bovine amnion acts as a scaffold for the repair of canine bladder defects. The regeneration of the urothelium and the presence of microscopic smooth muscle and the important complications that may occur from enterocystoplasty can encourage the use of bovine amniotic membrane for bladder reconstruction. However, long-term studies are still needed to assess other clinical and laboratory findings before measuring for clinical use [76].

In another study, Shakeri demonstrated the ability of the human amniotic membrane to induce epithelialization in experimental study on rabbits by reconstructing the urethra using the human amniotic membrane. The evaluation was conducted after 30 days post-operative. The result showed re-epithelialization of urethral without inflammation and tissue loss. The author also concluded that amniotic membrane is an inexpensive, easy, and biodegradable graft with very little antigen effect which seems to be the ideal solution for urethroplasty [74].

Amniotic membrane could be used as potential source for stem cell. Ghionzoli and Chung showed that it could be differentiated into smooth muscle and urothelial cell, both which are becoming integral parts creating urethral tissue [77, 78]. Despite using human amniotic membrane, some preclinical studies have also explored urological applications. Iijima demonstrated that amniotic membrane could successfully be used for bladder augmentation in rats. Human amniotic membrane-augmented bladder revealed regeneration of urothelium, detrusor smooth muscle, and nerve fibers within 3 months post-operatively. Bladder capacity was also found to be normal within 4 weeks post-operatively [79].

Pusateri evaluated placental membrane grafts for urethral replacement in rabbit model. The procedure consisted of mobilization of urethra, dorsal urethrotomy and graft placement. Dorsal onlay urethroplasty was performed afterwards. Observation after 3 month showed urethral patency in all rabbits. On pathologic examination, urothelial cell replacement was observed in all rabbit without malignant transformation. Urothelium was intact and circumferentially normal in all sections of graft bed. On cystourethroscopy, there was no strictures, fistulas or masses reported [80].

Wang et al. looked further to this idea, namely using the collagen scaffolding of amniotic membrane as potential regenerative material in urethroplasty. The authors separated basement layer of amnion to retrieve denuded human amniotic scaffold. Rabbit urethral epithelial cell was inoculated on its surface and the response showed mild immune reaction with no rejection. This maximizes the biocompatibility of amniotic membrane making it potential biomaterial for urethral reconstruction [81]. Gunes took another approach by combining amniotic membrane and buccal mucosa for penile urethral reconstruction in rabbit model. Both membrane were obtained from rabbit. Gunes compared whether buccal mucosa, amniotic membrane or both might be useful in urethroplasty. The best result of epithelial transformation was shown in combined group after 8 weeks with no complication regarding fistula or dehiscence observed [82].

Hariastawa et al. compared both bovine and human amniotic membrane for reconstruction of urethral defect in rabbit animal model. His study aimed to discover the difference in mucosal integrity between both groups. Epithelium layer was formed in both group with no significant difference of postoperative mucosal grading on day 7, 14 and 28 postoperatively. Authors concluded that bovine amniotic membrane could be used as good, cheap alternative in urethral defect reconstruction [83]. In another study, Hariastawa used dried amniotic membrane scaffold with adipose derived-mesenchymal stem cell seeding for rabbit penile urethral reconstruction. Adipose cell was cultured from rabbit neck and mixed with fetal bovine serum. Viability of stem cell was tested before the surgical procedure. The urethral wall was cut transversely before the scaffold that had been seeded with stem cells was implanted as urethral graft. Urethroplasty was done afterwards. Post-operative clinical observation showed urethral integrity alongside the defect. Urethral specimen was harvested on day 28 post-operatively. The specimen was then observed using fluorescence microscope. Neovascularization and best epithelial transformation was seen in combined amniotic membrane and adipose-derived mesenchymal stem cell seeding group. The promising result showed that stem cell could be used as adjunct treatment for amniotic membrane application [84].

Although limited to human amniotic membrane, clinical researches have been accepted. The first clinical report regarding the use of human amniotic membrane for anterior urethral defect repair was reported by Razzaghi et al. This pilot study included patients with previous hypospadias repair [85]. Hypospadias repair remains a challenge due to many anatomical variation of the pathology, surgical techniques and comorbidities of patient. Complications vary from dehiscence, stenosis to fistula formation. Secondary or salvage procedure were often needed for failed primary repair [86, 87]. After reconstruction of neourethra and proper hemostasis from urethroplasty, the allograft was used to cover the suture lines. Observation between 7 to 18 months post-operatively showed no long term complications. Amniotic membrane graft was proposed as an applicable, low-cost, biodegradable cover for second hypospadias repair [85]. Oottamasathien et al. proposed that amniotic membrane could be used for reducing complication rate, particularly from high re-operation rate of hypospadias. The underlying premise is to provide a barrier layer with robust source of tissue, vascular growth factors and anti-inflammatory environment for soft tissue healing [88].

9. Summary

Potential future application of bovine amniotic membrane could be explored widely. Broad number of biological properties found in amniotic membrane described above presents future studies. Preclinical and clinical researches could be used for basic scaffold for other applications. Combination with other biomaterials would be considered further.

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