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Potential use of bovine amniotic membrane in periodontal tissue engineering: A literature review

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Abstract

The goal of periodontal treatment is to reduce inflammation and regenerate the defects. As the structure of periodontium is composed of four types of different tissue (cementum, alveolar bone periodontal ligament, and gingiva), the regeneration should allow different cell proliferation in the separated spaces. However, it was difficult to achieve. Need a barrier membrane for epithelial exclusion to promote the healing of periodontal tissues in such a way that the original structure and function are preserved instead of repairing with junctional epithelium. During the occlusive period, the cells including cementoblast, osteoblast, osteoclast, and mesenchymal cells from PDL are activated to rebuild their missing tissues. The human amniotic membrane (HAM) has been used as a barrier membrane in furcation defects, intrabony defects, and gingival recession coverage. The HAM is associated with several problems, such as difficulty in finding donors, so that insufficient to provide mass production for this material. The use of HAM has also led to legal and religious concerns because they must be extracted from the human body. Therefore, the use of Bovine Amniotic Membrane (BAM) as an alternative might address the above limitations, making mass production possible without the ethical concerns of using human material.

Keywords: Bovine Amniotic Membrane; Stem cell; Tissue engineering; Periodontal regeneration

1. Introduction

Periodontitis is an infectious disease characterized by periodontal tissue disease, attachment loss, and alveolar bone destruction. Periodontal tissue damage in periodontitis begins with the accumulation of plaque containing pathogenic bacteria and toxins. The interaction between plaque bacteria and the product as well as the response of the host cell body triggers an inflammatory response that can cause damage to all components of the periodontal tissue, namely the gingiva, periodontal ligament, cementum, and alveolar bone.¹

Periodontitis treatment is aimed at achieving periodontal tissue regeneration, namely the formation of cementum and new alveolar bone, functional attachment of periodontal ligament fibers, and normal gingiva.^{2,3} In the early stages of periodontal tissue destruction, regeneration can be carried out by periodontal tissue. However, with the expansion and increasing severity of the periodontal breakdown, adequate regeneration can only be achieved through periodontal treatment.³

Various regenerative and reconstructive periodontal surgical techniques have been introduced to achieve treatment results in the form of periodontal regeneration, both with or without graft material. Several studies propose tissue engineering techniques to achieve regeneration of damaged periodontal tissues. Tissue engineering techniques

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combine 3 main components to form a network, namely cells, natural or synthetic scaffolds, and specific signal molecules.^{4,5}

In tissue engineering techniques, the amniotic membrane is a good scaffold because it has a fairly strong and stable attachment between the cell and the amniotic membrane. A good scaffold is a structure that can provide opportunities for cells to attach, proliferate and differentiate, this is influenced by the characteristics of a scaffold, including surface topography, microstructure, chemical, and mechanical properties.^{6,7}

The use of the amniotic membrane from the human placenta has properties that can accelerate epithelialization, anti-inflammatory, increase angiogenesis, anti-bacterial, anti-fibrotic properties, and do not trigger excessive innate immunity.^{8,9} The drawback of the amniotic membrane of the human placenta is the risk of spreading infectious diseases such as hepatitis, syphilis, tuberculosis, and HIV/AIDS. In addition, the resulting membrane preparation is relatively thin so it requires a technique that is quite difficult to use.⁸ The limited number of placenta donors, ethical, legal, and religious/religious issues cause the amnion membranes from human placentas to not be produced in large quantities.⁹ So we need an alternative material that can function well as a scaffold.

An alternative material that can be used is the bovine amnion membrane (BAM). This membrane has the same characteristics as the human amniotic membrane (HAM) and even has several advantages. BAM can provide a larger and thicker membrane surface area and the availability of more materials.⁹ With the freeze-dried method of sterilization and Gamma-ray irradiation, the amniotic membrane of the bovine placenta is technically easier to use, soft and flexible with hydration, can be sutured, can be stored at room temperature, and maintains sterility.^{10,11}

The use of BAM has been investigated both *in vitro* and *in vivo* in experimental animals. A study by Park et al 2008, described the potential of the bovine placental amniotic membrane in skin burns to promote tissue regeneration.¹⁰ However, its use in dentistry, especially in the field of periodontal, has not been widely studied. These limitations underlie the authors to write an article about the potential of using BAM as an alternative to HAM and is expected to be used as a basis for further research in dentistry.

2. Function and structure of amnion membrane

The amniotic membrane is the deepest layer of the placenta which is capable of regenerating the epidermis and the formation of granulation tissue in the wound, as well as controlling infection and exudation in the wound. Due to this beneficial effect, the amniotic membrane is superior to allograft in maintaining temperature stability and preventing dehydration of the wound surface as well as acting as a barrier against bacterial contamination.^{10,11}

The use of amniotic membrane itself has been widely used since 100 years ago when Davis in 1910 first used the human amniotic membrane as a wound dressing for open wounds on the skin. Since then, the use of amniotic membranes has continued to be developed in various fields. The development of the use of amniotic membranes is growing rapidly because it has advantages such as reducing the formation of scar tissue, reducing inflammation, and accelerating wound healing. The amniotic membrane also acts as a scaffold for cell differentiation and proliferation with its superior antibacterial properties.¹²

The placental membrane is derived from extraembryonic tissue. This membrane consists of several layers; the amnion layer and chorion layer, which are separated by a jelly-like layer, as shown in Figure 1. The amnion layer is also covered with a layer of epithelial cells. Both amnion and chorion, consist of a basement membrane and a stromal layer.¹³ The amniotic membrane is rich in collagen and has many growth factors and immune-suppressing cytokines, such as interleukin-4, interleukin-10.^{14,15}

The amniotic membrane consists of 3 parts, namely an epithelial layer, a basement membrane, and an avascular stroma. The epithelial layer consists of a single layer of cuboidal cells and plays a role in secretory and transport functions. The epithelial layer is attached to the basement membrane. The basement membrane of the amniotic membrane consists of collagen types I, II, IV, and V.¹²

The main fibrous framework of the amniotic membrane is found in the stroma, which forms a compact layer. The fibroblast layer hangs mesenchymal cells and plays a role in the secretion of collagen, especially collagen types I and III which form bonds to maintain membrane integrity. The layer that separates the amnion and chorion is known as the spongy layer or zone which is composed of cross-linked type III collagen. The shape is spongy because it contains a lot of glycoproteins and proteoglycans/proteoglikan.^{7, 12}

In table 1, we can show the differences between HAM and BAM. The HAM has a normal thickness ranging from 0.02-0.5mm which is composed of 6-8 layers of cells. The average surface area of the amniotic membrane is about 1600cm². The amniotic membrane is resistant to proteolytic factors because it contains interstitial collagen. Elastin found in the amnion plays a role in the elasticity of the amniotic membrane.¹² The modulus of elasticity of the amniotic membrane ranges from 2.29 to 3.6 MPa.⁷ The amniotic membrane with a freeze-dried process has a suturing retention strength of about 481 mN, a tensile strength of 6.9mm. In vitro, the biodegradation of the amniotic membrane is about 35% in the first week and 90% after 2 weeks.¹⁶

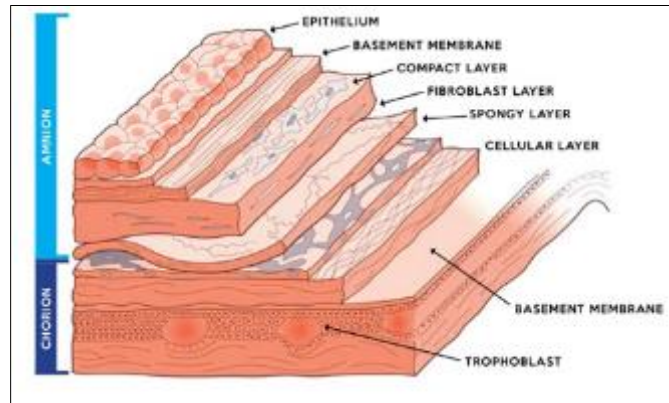


Figure 1 Amniotic membrane and chorion membrane layers.¹⁵

The amniotic membrane in cattle has a different structure from the human amniotic membrane. The BAM has a cylindrical shape, while the HAM is spherical. The thickness of the BAM is 15m, while that of the human amnion is 10m. Histologically, the BAM and the HAM consist of a single layer of cuboidal epithelium, but the surface area of the BAM is 6,000-7,500cm². The surface area is 4-5 times larger than the average surface area of the HAM, which is 1,600cm².^{10,17} Therefore, the use of BAM as an alternative material for HAM allows for mass production so that the needs will be met. The spread of infectious diseases in humans can also be minimized. In addition, ethical, legal and belief/religious issues can also be minimized by using BAM.

Table 1 Characteristics of Human Amniotic Membrane (HAM) and Bovine Amniotic Membrane (BAM).^{10,17}

Characteristics	HAM	BAM
Surface area (cm ²)	1.600	6.000 – 7.500
Shape	round	cylinder
Thickness (µm)	10	15
Histological picture	A layer of cuboidal epithelium	A layer of cuboidal epithelium

2.1. Antiviral and antimicrobial

The method of making freeze-dried sterilization and Gamma-ray irradiation was able to eliminate or reduce the virus in the amniotic membrane of cattle. Park et al (2008) in their research carried out the inactivation of the virus in BAM using several stages, namely 70% ETOH, 0.05% sodium hypochlorite, and Gamma irradiation. This procedure can inactivate several pathogenic viruses in cattle such as herpes virus, diarrhea virus, parvovirus, and influenza virus in cattle, making it safe to use.¹⁰ With this process, the BAM is also easier to use, soft and flexible with hydration, can be sutured, can be stored at room temperature and sterility is maintained.^{10,17}

The amniotic membrane can produce β -defensin which is dominated by β 3-defensin. The amniotic membrane can block the invasion of microorganisms into the wound area when used as a barrier.^{12,18} Research by Park et al (2008) in vitro proved that HAM and BAM did not penetrate microorganisms. Both membranes were planted on agar culture media Tryptone Soybean Agar (TSA) and treated with *Staphylococcus aureus* and *Escherichia coli* bacteria. Observations were made after incubation at 37°C for 72 hours. Negative results for *S. aureus* and *E. coli* bacteria occurred in the amniotic membranes of humans and bovine.¹⁰ However, studies on bacteria that cause periodontal disease such as

Porphyromonas gingivalis, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* on the BAM have not been studied.

2.2. Anti-inflammatory

The stromal matrix of the amnion membrane was able to suppress the production of inflammatory cytokines such as IL-1 α , IL-1 β , TNF- α through IL-1ra and was able to increase the production of anti-inflammatory cytokines such as IL-4 and IL-10. The stromal matrix also contains matrix metalloproteinase (MMP) inhibitors such as TIMP 1,2,3 and 4.^{12,19} Experimental studies in experimental animals showed minimal inflammation in the wound area covered by BAM and HAM compared to the wound area covered by polyurethane foam dressing material.¹⁰

2.3. Anti fibrosis

The amniotic membrane can reduce the risk of fibrosis by downregulating TGF- β and its receptor expression on fibroblasts. The amniotic membrane triggers tissue reconstruction more than the formation of scar tissue.¹²

Experimental research by Park et al. (2008) showed the presence of dry blood clots in the experimental animals' wounds, the BAM group looked thinner than the control group on the third day. After the seventh day, tissue epithelialization became active and blood clots gradually disappeared in the BAM group, but in the control group, there was thickening of dry blood clots to the thickness of the dermis layer.¹⁰ This shows a better tissue repair effect on the use of BAM.

2.4. Protein and DNA content

The results of the SDS-PAGE test on the HAM in the research of Siswanto et al. (2013) showed a protein molecular weight of 70 kDa. The same test was performed on the BAM. The results showed that the molecular weight of the protein in the BAM was 70 kDa.²⁰

Research conducted by Park et al. (2008) showed that the average BAM DNA content was 6.89 \pm 0.53 μ g/mg. The DNA level in the BAM is equivalent to the DNA level in the HAM in the late embryonic phase, which is around 35-40 weeks of gestation.¹⁰ So that, the protein molecular weight and DNA content in the results of these studies can be used as the basis for the potential of bovine amniotic membranes as an alternative material for human amniotic membranes in their use as regeneration materials and tissue engineering.

2.5. Epithelialization

The use of BAM in the wound healing process showed the same epithelialization effectiveness when compared to HAM. On day fourteenth, epithelialization was almost complete in the BAM group.¹⁰ This shows a better tissue repair effect when using BAM as a tissue regeneration material and the effect is the same as using HAM.

Other studies also support good epithelialization in the use of BAM. The research of Siswanto et al. (2013) proved that the presentation of epithelialization on the 3rd, 6th, and 13th days, in rat skin wounds covered with amniotic membranes reached 100%.²⁰ In addition, it was stated that the Freeze-dried bovine amniotic membrane was non-toxic to fibroblast cells with 99.69% viability of fibroblast cells.^{15,20}

2.6. Creating and storage process

The process of making amniotic membranes can be done by various methods. Several studies discuss the effectiveness of various methods of making amniotic membranes as summarized in table 2.

Table 2 Various ways of making and storing BAM can be used and have been investigated.

No	Kinds of Manufacturing and Storage	Process	Advantage	Disadvantage	Research
1	Fresh membrane	Cleaning the membrane from the stoma, blood, and meconium with irrigation and washing	Fast processing Can be used immediately Protein does not dissolve during processing	cannot be saved >24 hours the possibility of transmission of	10,21

		using sterile 0.9% saline. Disinfection using 0.025% sodium hypochlorite solution. Covered with sterile gauze and stored in a sterile place at 4°C	Provides good treatment results	infection is greater than other methods	
2	Dried membrane	The amniotic membrane was dried at room temperature then put into sterile vacuum plastic and hot vacuumed using a vacuum (safety pressure 1,650 mbar) to seal the membrane)	The use gives results that are as effective as fresh membranes Can be stored for 9 months at room temperature Can retain cell components better than cryopreserved	Before use, it needs to be soaked in sterile saline for \pm 1 minute	17,22
3	Frozen membrane	Freezing -20°C in liquid nitrogen	Can be stored for 6 weeks at -20°C Storage media available in hospitals and research laboratories	Requires special storage media and the price is relatively expensive	23
4	Stabilized membrane	Glutaraldehyde fixation Glycerine 99% solution	Has the same effect as a fresh membrane Can be stored for up to 48 months Relatively cheap manufacturing costs	Requires a sterile glass container used as a storage area so that it breaks easily and requires a larger storage space than dried and freeze-dried membranes	24
5	Cryopreserved membrane	Freezing -80°C in liquid nitrogen	Can maintain the histology and biochemical amnion membrane Can be stored for 6 months at a temperature of -80°C	Expensive cost Requires special storage media and the price is relatively expensive (only available in research laboratories) There are soluble proteins and albumin when compared to fresh and dried membranes	21–23, 25,26
6	Freeze-dried irradiated membrane	Freeze-dried at -60°C under vacuum 102 for 48 hours. Gamma-ray irradiation, then dried using a lyophilizer	Can maintain normal size and shape of membranes with minimum cell damage	Before use need to be soaked in sterile saline for \pm 2 minutes	15,20

2.7. The role of the amnion membrane in periodontal tissue engineering

The use of amniotic membranes, especially those from the human placenta, has been widely used for 100 years ago when Davis in 1910 first used the human amniotic membrane as a wound dressing for open wounds on the skin. Since then, the use of the amniotic membrane has continued to be developed in various fields.²⁷ The use of the amniotic membrane then developed rapidly because of its ability to regenerate tissue well. In addition, the amniotic membrane also has many advantages including reducing scar tissue formation, antimicrobial effect, anti-inflammatory effect,

accelerating wound healing, biocompatible, good cell adhesion, and has favorable mechanical properties (permeable, stable, elastic, flexible, and resorbable).^{10,15,28}

Amniotic membranes in dentistry, especially in the field of periodontology, have been widely used as wound dressings and Guided Tissue Regeneration (GTR) for tissue regeneration. The use of HAM as wound dressings has been widely applied to humans as an open root closure therapy.^{18,29,30} The HAM has also been extensively studied and used in humans as GTR.

GTR is a reconstructive surgical technique with the principle of using a membrane to prevent epithelial migration along the cementum pocket wall and maintain space for stabilizing blood clots. This method is based on the theory that the periodontal ligament and perivascular cells have the potential for regeneration on the root surface. The GTR consists of the placement of various types of barriers (membranes) to cover the bone and the periodontal ligament, thereby temporarily separating them from the gingival epithelium and connective tissue. Separating the epithelium and gingival connective tissue from the root surface during the postoperative healing phase not only prevents the migration of the epithelium into the wound but also increases the repopulation of the area by cells of the periodontal ligament and bone.² Although the BAM is capable of equivalent to that of the HAM, even though it has several advantages, its development and use in periodontal tissue regeneration is still very little. Along with its development, the BAM was developed not only as a tissue regeneration material but as a tissue engineering material. In tissue engineering, the BAM acts as a scaffold for cell differentiation and proliferation (Figure 2) due to its good cell adhesion ability, easy transfer of biomodulatory agents such as growth factors and genetic materials.^{10,15} In addition, the amniotic membrane is a stem cell reservoir.^{11,12}

Tissue engineering (TE) is defined as the development of biological substitutes for tissue to restore, maintain, or enhance tissue function. In its application, it takes the principles and methods of good engineering and science.^{7,32} Scaffolds were developed to support stem cells during TE, promoting their differentiation and proliferation during their formation into new tissues. Therefore, the design and selection of the biomaterials used for the scaffold are important in TE. During TE, seeding of stem cells onto the scaffold is the first step in three-dimensional tissue culture and plays an important role in determining tissue development.^{32,33}

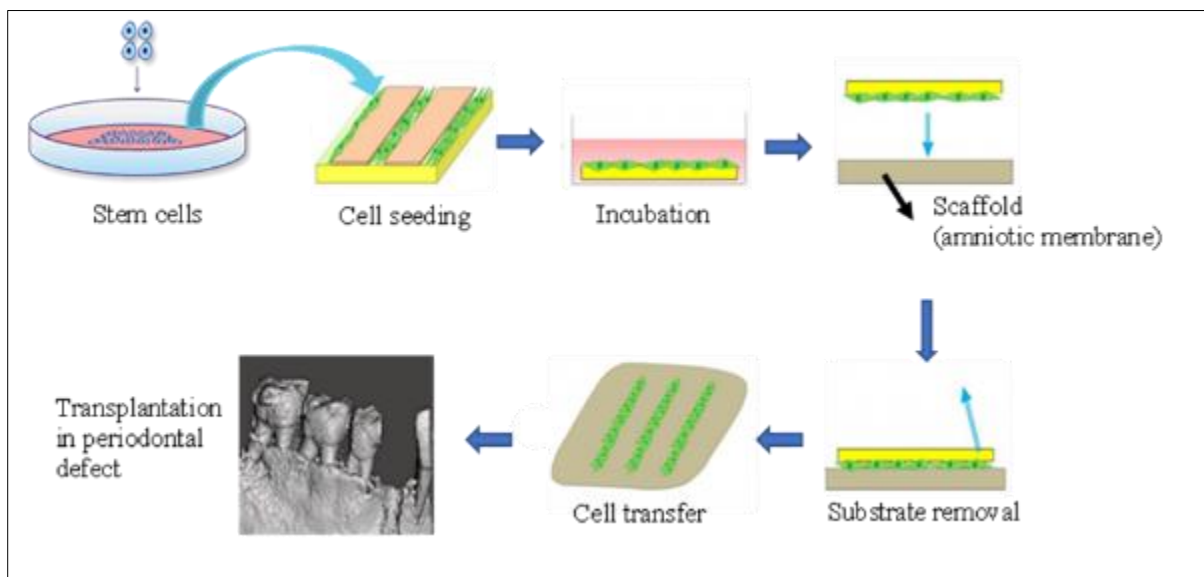


Figure 2 Schematic of cell transplantation with cell transfer technology. Cells are transferred to the surface of the amnion using a pattern or cell layer transfer medium. The cell-transferred amnion is trimmed and transferred to the tissue that needs transplantation.

Stem cell attachment to the scaffold is largely influenced by the extracellular matrix component of the scaffold (ECM). The presence or absence of certain ECM molecules such as collagen, laminin, fibronectin, and vitronectin in any basement membrane has a major influence on the adhesion and growth of the overlying stem cells. In addition to enabling cells to adhere and migrate, ECM molecules also function as adhesion ligands, transmitting signals through their interactions at cell surface receptors. Cell adhesion plays a role in stimulating signals that regulate cell differentiation, cell cycle, cell migration, and cell survival. The ability of cell adhesion to substrates is an important

consideration in design and development. the more cells attached, the more the number of chemical bonds present on their surface. When epithelial and mesenchymal cells are implanted on the AM scaffold, they are highly interconnected and able to penetrate the porous structure of the amniotic membrane.^{11,28,32,33}

Stem cell attachment to the scaffold is influenced by internal cell factors, scaffold factors, and environmental factors. To form a suitable microenvironment for stem cells, stem cells will activate their internal signals to be able to bind and adapt to the scaffold. One of the common mechanisms of stem cells is the expression of integrins to aid their attachment to the scaffold. The amniotic membrane as a scaffold has laminin and fibronectin components that can bind to integrins expressed in stem cells (Figure 3). Laminin and fibronectin as the extracellular matrix will bind to integrin transmembrane proteins from stem cells, especially integrins class $\alpha 5\beta 1$, $\alpha 11\beta 3$ dan αv .^{34,35} The amniotic surface that has been transferred to stem cells is then trimmed and transferred to areas that require tissue engineering (Figure 2).³³

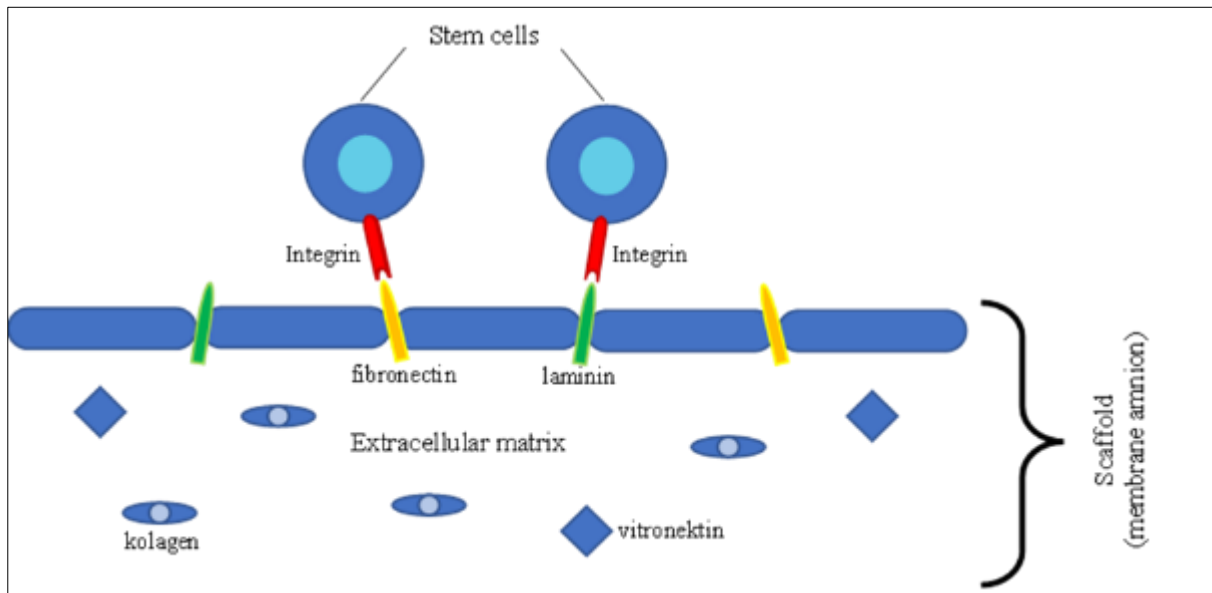


Figure 3 Laminin and fibronectin as extracellular matrix will bind to the integrin transmembrane protein of stem cells

3. Conclusion

Bovine Amniotic Membrane (BAM) has potential to be used as an alternative membrane in periodontal tissue engineering.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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