

Human Amniotic Membrane: Healing Beyond Boundaries - A Narrative Review of Its Regenerative Potential and Clinical Applications in Endodontics

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Abstract

Human amniotic membrane (HAM) is a biologically active placental tissue with considerable potential in regenerative medicine. Its unique composition of extracellular matrix proteins, growth factors, cytokines, and antimicrobial molecules provides structural and biological support for tissue healing.¹ In endodontics, these properties have generated interest in pulp preservation, regenerative endodontic procedures, and periapical tissue regeneration.^{1,2} HAM can function as a biological scaffold, barrier, and source of signaling molecules that promote cellular attachment, proliferation, and tissue repair.^{1,2} Clinical reports have described its application in pulpotomy, regenerative endodontic procedures, and endodontic surgery.³ Although encouraging healing responses have been reported, predictable root maturation and restoration of pulp sensibility have not been consistently demonstrated.³ Therefore, HAM represents a promising biological material for endodontic regeneration; however, further well-controlled clinical studies are required before its routine clinical application can be recommended.

Keywords: Human amniotic membrane; HAM; regenerative endodontics; pulpotomy; revascularization; root end surgery; tissue regeneration; biological scaffold.

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Introduction

Endodontics has progressively evolved from conventional removal and replacement of diseased pulp tissue toward biologically oriented approaches that aim to preserve or regenerate dental tissues. Regenerative endodontics particularly focuses on restoring the biological functions of the pulp-dentin complex and promoting continued development of immature teeth.¹

Human amniotic membrane (HAM), also known as amnion, is the innermost membrane of the placenta. It surrounds the amniotic cavity and provides protection and support to the developing foetus. The membrane consists of an epithelial layer, basement membrane and avascular stromal matrix. Its extracellular matrix contains collagens (types I, III, IV and V), fibronectin, laminins,

particularly laminin-5 and laminin-10, and proteoglycans. Various growth factors and cytokines, including epidermal growth factor (EGF), transforming growth factor- β (TGF- β), fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and interleukins (ILs), contribute to its diverse biological and regenerative activities.²

The biological characteristics of HAM have resulted in its use in wound healing, ophthalmology, periodontal regeneration and oral surgery. Its application in endodontics is comparatively recent, with emerging evidence highlighting its increasing relevance due to its ability to function as both a physical scaffold and an active biological material that promotes tissue healing and regeneration.^{2,3}

HAM possesses anti-inflammatory, antimicrobial, anti-adhesive and wound-healing properties. Amniotic epithelial cells release several immunomodulatory substances that help regulate immune responses and reduce excessive inflammation. These characteristics provide a rationale

for its application in pulpotomy, regenerative endodontic procedures and guided tissue regeneration during endodontic surgery.⁴

The use of HAM in dentistry has attracted increasing interest because of its biological compatibility, extracellular matrix composition and potential to support tissue regeneration. Its ability to provide a favourable microenvironment for cellular attachment, proliferation and differentiation has made it particularly relevant to regenerative procedures.⁵

In endodontics, early clinical reports have explored HAM in pulpotomy, regenerative endodontic procedures and root-end surgery, while experimental studies have investigated its potential as a scaffold for dental pulp regeneration.⁶

The purpose of this narrative review is to discuss the biological properties, regenerative potential and clinical applications of HAM in endodontics, with emphasis on available clinical and experimental evidence.

1. Structure and Biological Properties of HAM

HAM is a thin, semitransparent and avascular biological membrane. It contains a basement membrane and underlying stromal matrix. The extracellular matrix is composed mainly of collagen and other proteins that provide structural support for cellular attachment and migration.³

The basement membrane contains collagen, fibronectin, laminin and proteoglycans. These components provide an extracellular environment that can support cell adhesion, proliferation and differentiation.³

One of the important properties of human amniotic membrane (HAM) is its anti-inflammatory activity. Amniotic epithelial cells secrete several immunomodulatory substances, such as transforming growth factor- β (TGF- β), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1Ra), prostaglandin E₂ (PGE₂), and HLA-G, which help regulate immune responses and reduce excessive inflammation.³ These properties may be particularly beneficial in endodontics because persistent inflammation can interfere with tissue regeneration.

HAM also exhibits antimicrobial activity. Components such as β defensins, cystatin E, elafin and secretory leukocyte proteinase inhibitor contribute to innate antimicrobial defense.³

Another important characteristic is its ability to act as an active biological scaffold. The membrane contains growth factors and extracellular matrix molecules that can influence cellular activity and tissue repair.^{3,7}

Therefore, the biological activity of HAM is not limited to a passive barrier effect. It can simultaneously provide structural support, modulate inflammation, contribute to antimicrobial defense and provide biological signals for regeneration.^{3,7}

2. HAM as a Regenerative Scaffold

A regenerative scaffold should provide a suitable three dimensional environment for cellular attachment, migration, proliferation and differentiation. HAM possesses several characteristics that fulfill these requirements.^{7,8}

The collagen rich extracellular matrix provides structural support for cells, while fibronectin and laminin contribute to cell adhesion. Growth factors present within the membrane can further influence cellular activity.⁷

Bakhtiar et al. evaluated a HAM extracellular matrix scaffold for dental pulp regeneration using in-vitro and in vivo models. Their findings demonstrated the potential of HAM derived extracellular matrix as a scaffold for dental pulp tissue regeneration.⁷

Bang et al. investigated amnion chorion and collagen membranes as matrices during regenerative endodontic procedures and evaluated their influence on the regenerative microenvironment. These findings support the importance of scaffold selection in regenerative endodontics.⁸

HAM may also influence the behavior of cells derived from the apical papilla. The membrane can provide a favorable environment for cellular activity and may support osteogenic differentiation, which could contribute to periapical tissue regeneration.^{7,8}

3. Forms and Clinical Preparation

HAM can be prepared and preserved as fresh (4°C), frozen (-80°C), freeze-dried (20–25°C), or cryopreserved (-80°C to -196°C) preparations.³ The choice depends on the clinical application and preservation requirements.

Freeze dried HAM provides advantages in storage and handling. It can be rehydrated before clinical application. In the clinical cases reported by Johri et al., freeze dried HAM was activated with normal saline before placement during pulpotomy and regenerative endodontic treatment.²

Fresh HAM was used during root end surgery

as a guided tissue regeneration membrane. The difference in membrane preparation demonstrates that HAM can be adapted according to the intended clinical application.^{2,3}

Appropriate processing and sterilization are essential for safe clinical use. However, processing methods may alter the biological properties of the membrane and therefore require standardization.³

4. Clinical Applications of HAM in Endodontics

4.1 HAM in Pulpotomy

Pulpotomy aims to preserve the vitality of the remaining radicular pulp following removal of inflamed coronal pulp tissue. Successful healing requires control of inflammation and microbial contamination. The anti-inflammatory and antimicrobial properties of HAM make it a potential biological material for pulpotomy.^{2,3}

Johri et al. reported the use of freeze dried HAM in a permanent mandibular molar diagnosed with symptomatic irreversible pulpitis. Following removal of the coronal pulp and control of bleeding, activated HAM was placed over the pulp stumps in a 2–3 mm layer and covered with glass ionomer cement. At follow up, the patient remained asymptomatic. At 12 months, the tooth demonstrated a positive sensibility response comparable with control teeth, while radiographs showed a healthy peri-radicular region.²

The favorable outcome may be related to the anti-inflammatory and antimicrobial properties of HAM. However, because this was a single case, the result cannot establish the effectiveness of HAM as a routine pulpotomy material.²

Figure 1: Pulpotomy with human amniotic membrane (HAM), (a) Preoperative clinical picture of #46, (b) Root canal orifices after bleeding control, (c) Activated HAM placement over the pulp stumps, (d) Glass ionomer placement over HAM, (e) Resin-based composite restoration, 1 week postoperatively, (f) Preoperative radiograph, (g) Immediate postoperative radiograph, (h) 1-month follow-up, (i) 6-month follow-up, (j) 12-month follow-up.²

4.2 HAM in Regenerative Endodontics

Regenerative endodontics is one of the most promising applications of HAM. In immature teeth with necrotic pulp, the objective is to eliminate infection and ideally promote continued root development and restoration of the pulp-dentin complex.¹

Suresh et al. reported successful regenerative endodontic treatment of a non-vital immature

permanent central incisor using HAM as a novel scaffold. The report provided early clinical evidence supporting the use of HAM in regenerative endodontics.²

Joseph et al. described a regenerative endodontic procedure in a traumatized immature tooth using HAM. The report further demonstrated the feasibility of using amniotic membrane as a biological scaffold in immature permanent teeth.³ Nagaveni et al. reported revascularization of a non-vital immature permanent tooth using HAM. Their findings added further clinical support for the application of HAM in regenerative endodontics.⁴

Johri et al. used HAM as a scaffold in an immature maxillary lateral incisor with pulp necrosis and apical periodontitis. Following disinfection, activated HAM was placed into the apical portion of the canal and MTA was used coronally. The patient remained asymptomatic and the periapical lesion resolved. However, no increase in root length or width was observed during the 24-month follow-up, and pulp sensibility remained negative.⁵

This finding emphasizes that periapical healing does not necessarily indicate complete pulp-dentin regeneration. Although the primary clinical objective of resolution of symptoms and periapical pathology was achieved, continued root maturation was not demonstrated.⁵

Figure 2: Revascularization with human amniotic membrane (HAM) in #12. (a) Access with round bur under rubber dam isolation, (b) Circumferential filing with ISO 25K file, (c) Placement of activated HAM with the help of sterile tweezers, (d) Compaction of HAM with hand plugger, (e) Preoperative radiograph, (f) Immediate postoperative radiograph showing coronal placement of MTA, (g) 6-month postoperative radiograph, (h) 12-month postoperative radiograph, (i) 24-month postoperative radiograph.²

4.3 Experimental Evidence for Pulp Regeneration

Clinical observations have been supported by experimental research.

Bakhtiar et al. evaluated a HAM extracellular matrix scaffold for dental pulp regeneration **in vitro** and **in vivo**. The findings demonstrated that the membrane-derived extracellular matrix has potential as a scaffold for dental pulp tissue engineering.⁶

Bang et al. evaluated amnion-chorion and collagen membranes and demonstrated that scaffold composition can influence the regenerative microenvironment.⁷

Kim et al. investigated human-derived amnion-chorion membrane as a bioactive scaffold for regenerative endodontic therapy in mature teeth using an animal model. This study provided experimental evidence for extending amniotic membrane-based

approaches beyond immature teeth.⁸

Together, these studies support the biological plausibility of HAM as a regenerative scaffold, although the translation of experimental findings into predictable clinical regeneration remains a challenge.^{7,8,9}

4.4 HAM in Endodontic Surgery

Endodontic surgery frequently produces osseous defects that may benefit from guided tissue regeneration. A membrane can act as a barrier against unwanted soft tissue invasion while providing a favorable environment for bone regeneration.⁹

Uppada et al. reported a combination of hydroxyapatite, platelet-rich fibrin and amnion membrane in regenerative periapical endodontic surgery. The combination demonstrated potential for management of periapical defects.⁹

Johri et al. used fresh HAM as a guided tissue regeneration membrane during root-end surgery in a large periapical lesion. Following root-end preparation and MTA placement, hydroxyapatite was placed into the bony defect and HAM was positioned over the defect. At follow-up, significant bone formation was observed, followed by complete radiographic healing of the osseous defect.²

The proposed functions of HAM in this application include:

- **Scaffold:** provides a framework for tissue formation.
- **Active scaffold:** releases growth factors and other biological mediators.
- **Barrier:** prevents undesirable connective tissue migration into the defect.^{9,10}

Johri et al. also investigated HAM compared with platelet-rich fibrin membrane for bone healing after endodontic surgery in a randomized controlled study, providing stronger clinical evidence for its potential role in postoperative bone regeneration.¹⁰

Figure 3: Clinical pictures of root-end surgery with human amniotic membrane (HAM) placement. (a) Preoperative three-dimensional cone-beam computed tomography images showing lesion involvement of #21 and #22, (b) Raised mucoperiosteal flap, (g) Bony crypt after removal of granulation tissue and necrosed bone, (h) Hydroxyapatite graft placement inside the bone crypt, (i) Intraoperative picture showing retrograde root-end cavity (red arrow), (j) Clinical picture showing placement of HAM over the lesion, (k) Immediate postoperative radiograph, (l) 7-month follow-up radiograph, (m) 12-month follow-up radiograph, (n) 18-month follow-up radiograph.²

5. Mechanisms of HAM Mediated Healing

The regenerative potential of HAM can be explained through several complementary mechanisms.^{3,7}

Scaffold effect

The extracellular matrix provides a physical

framework for cell attachment, migration and proliferation.^{3,7}

Anti-inflammatory effect

HAM contains immunomodulatory mediators that can reduce excessive inflammation and create a favorable environment for tissue repair.³

Anti-microbial effect

Antimicrobial peptides and proteins present in human amniotic membrane (HAM) contribute to protection against microbial invasion. Important components include β -defensins, lactoferrin, lysozyme, SLPI, elafin, and LL-37, which exhibit broad-spectrum antimicrobial activity. They act mainly by disrupting microbial membranes, degrading bacterial cell walls, sequestering iron, and modulating local immune responses.³

Growth-factor activity

HAM contains various biological mediators, including growth factors such as EGF, FGF, VEGF, TGF- β , and HGF, as well as cytokines and extracellular matrix proteins, which can influence cell proliferation, angiogenesis, migration, and tissue regeneration.^{3,7}

Barrier effect

When used in endodontic surgery, HAM can separate the surgical defect from surrounding connective tissues and potentially guide regeneration.^{9,10,11}

Thus, HAM functions as both a physical scaffold and biologically active regenerative material.^{3,7}

6. Advantages of HAM

The principal advantages of HAM in endodontics include:

- Excellent biological compatibility.
- Anti-inflammatory properties.
- Antimicrobial activity.
- Presence of extracellular matrix proteins.
- Growth-factor release.
- Support for cellular attachment and proliferation.
- Barrier function.
- Potential osteogenic and regenerative activity.
- Availability in different forms.
- Potential application in both regenerative endodontics and endodontic surgery.^{2, 4-10}

Its ability to combine several biological functions within a single material represents an important advantage over conventional passive barriers.

7. Limitations

Despite its promising biological characteristics, HAM has several limitations.

The most important limitation is the limited amount of high level clinical evidence. Much

of the available endodontic literature consists of case reports, case series and experimental studies.^{2,4} The clinical outcomes are also not always predictable. In the case reported by Johri et al. periapical healing occurred without continued root maturation or recovery of pulp sensibility.²

Another concern is variation in processing and preservation. Fresh, freeze dried and cryopreserved membranes may demonstrate different biological properties.³

Patient acceptance can also influence clinical use because HAM is derived from placental tissue. Johri et al. reported that some potential participants were uncomfortable with the use of placental membrane.²

Standardization of tissue collection, processing, sterilization, storage and clinical handling is therefore essential.³

8. Future Perspectives

The future of HAM in endodontics lies in developing more predictable regenerative strategies. Potential areas include:

- HAM combined with stem cells for regenerative endodontic treatment.
- HAM combined with growth factors for pulp–dentin regeneration.
- Decellularized HAM extracellular matrix scaffolds for pulp tissue regeneration.
- Amnion–chorion composite scaffolds for periodontal tissue regeneration.
- Application in mature teeth for regenerative endodontic treatment.
- Pulp–dentin complex regeneration for pulpal tissue regeneration.
- Periapical bone regeneration for periapical lesion repair.
- Advanced tissue engineering approaches for complete pulp–dentin regeneration.¹²

Future studies should also determine the ideal membrane form, thickness, processing method, placement technique, and clinical indication.

Most importantly, large randomized clinical trials with long-term follow-up are required to establish whether the promising results observed in early clinical studies can be consistently reproduced.

9. Conclusion

Human amniotic membrane represents a promising biological material for regenerative and reparative endodontics. Its unique combination of extracellular matrix components, growth factors, anti-inflammatory mediators, antimicrobial molecules and barrier properties allows it to function beyond a conventional passive membrane.^{3,7}

Clinical evidence has demonstrated its potential in pulpotomy, regenerative endodontics and

endodontic surgery.^{2,9,10} Suresh et al., Joseph et al. and Nagaveni et al. reported favorable clinical outcomes using HAM as a scaffold in immature teeth.^{4,6} Bakhtiar et al. provided in vitro and in vivo evidence supporting HAM extracellular matrix for dental pulp regeneration.⁷ Johri et al. demonstrated applications in pulpotomy, regenerative endodontics and root end surgery.²

However, the evidence remains preliminary. Healing of periapical tissues can occur without predictable root maturation or restoration of pulp sensibility.² Therefore, HAM should currently be considered a promising regenerative biomaterial rather than an established routine endodontic material.

With further advances in tissue engineering, biomaterials and regenerative medicine, HAM may extend the boundaries of conventional endodontic healing toward more biologically driven regeneration.

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