

# Placental Tissues- From Reproductive to Regenerative Biology

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**Abstract:** *The past decade has witnessed breakthrough advancements in the field of Regenerative medicine. With advancements in the knowledge and fabrication of tissue engineered constructs, complete regeneration of soft and hard tissues remains a farfetched goal. Stem cell therapy has emerged as one of the predictable; powerful tool to generate biological substitutes and regenerate damaged tissue with high proliferability, differentiability and function. Lately, much of the attention has been redirected to the placental tissues which are an abundant and reliable source of these multipotent stem cells. Various in vitro experimental studies and clinical trials have documented the role of amnion membrane in wound healing, transplantation and regenerative surgeries owing to its unparalleled biological and mechanical properties. However little research is done regarding the chorion membranes. This review highlights the potential of these fetal membranes as novel tissue engineered biomaterial.*

**Keywords:** placental tissues, regeneration, stem cells, amnion, chorion

## Placental tissues- From reproductive to regenerative biology

### 1. Introduction

The placenta is the life support system for the fetus. The study of human placenta has been intriguing the researchers, owing to its clinical and scientific importance. Placenta, till now was the subject of discussion only for its important role in development of the growing embryo by facilitating gas and nutrient exchange between the mother and fetus. However, lately much investigation has been directed towards the placental tissues as easily accessible and reliable source of stem cells. The fetal portion of the placenta is composed of the placental disk, the amniotic and chorionic membranes. The amnion is the innermost of the two human fetal membranes and, as such, is in contact with the contents of the amniotic sac, namely the amniotic fluid, the fetus and the umbilical cord. The chorionic membrane, which is attached to the outer surface of the amniotic membrane, separates the amnion from the decidua and the maternal uterus. Current research in the field of tissue engineering and regenerative medicine has suggested the potential role of 'human amniotic and chorionic mesenchymal stromal cells' (hAMSC) in mediating each phase of the wound-healing process: inflammatory, proliferative, and remodeling. This review attempts to provide an insight into the regenerative

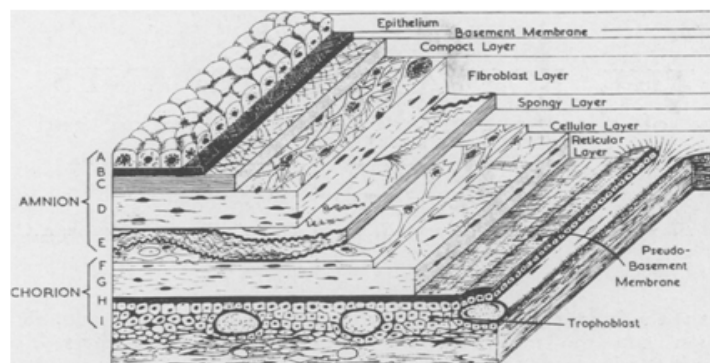
aspect of these placental tissues and especially their potential applications in the field of dentistry.

### 2. Amniotic and chorion membrane- as potential source of stem cells

#### 2.1 Amnion membrane

It is well accepted now that human amnion is not merely an epithelial lining for the uterine contents, but that it is a complicated tissue constructed histologically of several different layers. It is normally 0.02 to 0.5 cm in thickness and consists of five layers. These are, from within outwards (1):

1. Epithelium.
2. Basement Membrane.
3. Compact Layer.
4. Fibroblast Layer.
5. Spongy Layer.



Composite diagram to illustrate the appearances of the layers of reflected amnion and chorion when examined by both membrane preparations and routine cut sections.

Volume 3 Issue 11, November 2014

[www.ijsr.net](http://www.ijsr.net)

The amniotic membrane (AM) has two types of cells with different embryological origins: amnion epithelial cells derived from embryonic ectoderm and amnion mesenchymal cells from embryonic mesoderm. 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. There are no nerves, muscles, or lymphatics in the amniotic membrane. (2, 3) The amniotic mesoderm layer consists of macrophages and fibroblast-like mesenchymal cells. These human amniotic epithelial (HAE) cells and human amniotic mesenchymal cells (HAM cells) express pluripotency and are potent stem cells reservoirs. (3) Amniotic epithelial cells (AEC) secrete collagen type III and IV and non collagenous glycoproteins like laminins, nidogen, fibronectin and vitronectin within the basement membrane that serve as adhesion ligands transmitting signals and interacting at cell surface receptors. (4) Native human amnion/chorion membranes contain an array of growth factors, which play critical roles in regulating tissue development and growth in utero. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), transforming growth factors alpha and beta (TGF $\alpha$ ,  $\beta$ ), nerve growth factor (NGF), and hepatocyte growth factor (HGF) are some of the growth factors that have been identified in fresh and preserved amniotic membrane tissues. (5) Also, it has been shown that although AECs are pluripotent, these cells do not form teratomas when transplanted into the testes of SCID mice. (6) Few other factors making amnion membrane as a suitable alternative in the field of tissue engineering are (7):

- 1) AECs are immunologically inert and hence reduced risk of rejection or immune reaction upon transplantation.
- 2) These cells can proliferate without needing a second cell type serving as a feeder layer. AECs create their own feeder layer with some cells spreading out at the bottom of the culture dish.
- 3) It has been reported that an average yield of AEC is more than 100 million AECs per amnion collected. With attention to the robust proliferation of these cells in the presence of certain growth factors such as EGF, large numbers of stem cells could potentially be available using the amniotic membrane as a source. (8)
- 4) Bone marrow mesenchymal stem cell and progenitor cells unlike AM mesenchymal cells show decreasing proliferative potential with increasing age.
- 5) The stem cell markers like epidermal marker CA125, octamer-binding transcription factor (OCT) - 4, hepatocytes nuclear factor-3 $\beta$  (HNF-3 $\beta$ ), nestin and nanog which are specific stem cells markers membrane and general epithelial markers such as cytokeratins and vimentin are present in large amount in the amniotic epithelial cells. (9,10)

## 2.2 Chorion membrane

The non-placental chorion consists of four layers. These are, from within outward: Cellular Layer, Reticular Layer, Pseudo-basement Membrane and Trophoblast. The reticular layer contacts the spongy layer of the amnion and forms a majority of chorion's thickness. The reticular network is

composed of collagens I, III, IV, V, and VI. The basement membrane anchors the trophoblasts to the reticular layer with collagen IV, fibronectin, and laminin. The trophoblast layer is the deepest layer, consisting of 2–10 layers of trophoblasts which contact with the deciduas. (11) The chorionic villi of human placenta are a rich source of mesenchymal stem cells (PMSCs), also known as human chorionic mesenchymal stromal cells. The Brescia Symposium has decided to call this type of cells from the placenta as "chorionic stromal mesenchymal cells", not stem cells as these cells have genetic and behavioral characteristics of both multipotent and adult stem cells. (12) It has been suggested in a study that these mesenchymal stem cells (PMSCs) have a pericyte-like phenotype. The vascular niche of placenta harbors a pool of PMSCs that can give rise to committed progenitors for tissue maintenance and repair, and that PMSCs contribute to vessel maturation and stabilization. These cells have the capacity for forming clones (i.e. clonogenicity) and their capability to differentiate into adipocytes, chondrocytes and osteocytes. (13) PMSCs have been propagated for 100 doublings without any decrease in telomere length and activity. These cells were positive for embryonic stem cell markers like OCT-4, NANOG, SSEA-3, AND TRA-1. An ex vivo perfused human lung preparation injured by *Escherichia coli* endotoxin and in vitro human lung epithelial culture demonstrated that these cells have the capacity to facilitate repair of injured epithelium. (14) The karyotyping analysis has shown chorionic stem cells maintain chromosomal stability after serial passage, demonstrating chorion as a promising source of cell for future therapy. (15) HLA-ABC cluster, in one study has confirmed the absence of the HLADR $\alpha$  transcript from chorionic mesenchymal cells in all the tested cases, while class I HLA molecules are highly expressed. HLA expression provides information that might help explain the immunological mechanisms of tolerance between the maternal organism and fetal structures. (16) In addition, the chorionic villi contains abundant growth factors like insulin growth factor, Heparin-binding EGF-like growth factor (HBEGF), Vascular endothelial growth factor (VEGF), Transforming growth factor- $\alpha$  (TGF- $\alpha$ ) and these factors work as endothelial cell mitogens and are required for cell signaling and prevent apoptosis of cells. (17, 18)

## 2.3 Other Properties of amnion and chorion membrane

1. Antimicrobial and anti inflammatory: The placenta is a highly selective mechanical and immunological barrier against the dissemination of infectious agents by hematogenous routes or by the ascending route from the vagina. These defenses serve to limit both the degree and frequency of bacterial colonization within the placenta. (19) The failure of both microbial pathogens and bacterial LPS to cross the placental membranes may be attributed in part to the antimicrobial and endotoxin-neutralizing potential of histone H2A and H2B proteins coating the epithelial surface of the placenta. (20) The beta 3-defensin is the predominant defensin in the amniotic epithelium. In addition, 2 low-molecular-mass elastase inhibitors, secretory leukocyte proteinase inhibitor (SLPI) and elafin, are also expressed in the Amniotic membrane. (21,22) The AM stromal matrix markedly suppresses the expression of the potent pro-inflammatory cytokines, IL-1 $\alpha$  and IL-1 $\beta$  and also contains

natural inhibitors of MMPs. (23) Treatment of the AM with both lactoferrin and interleukin-1 receptor antagonists make the AM both anti-microbial and anti-inflammatory.(24) . In a study, Chorionic membranes were found to have an inhibitory effect on Hemolytic streptococcus group A, Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter calcoaceticus and Lactobacillus species.(25) Similar antimicrobial profile of chorion and amnion against several bacterial and fungal pathogens has been reported, although it was suggested that amnion has maximum activity. (26)

2. Revascularization and angiogenesis: Human amniotic membranes have been successfully used to treat chronic cutaneous wounds.(27,28) dHACM is a dehydrated human allograft comprised of laminated amnion and chorion membranes derived from the placenta. Treatment with dHACM allografts has also demonstrated improved healing in patients with a variety of additional wound types for which traditional therapies were ineffective, including venous leg ulcers, crush injury, arterial insufficiency, immunological skin disease/scleroderma, and snake bite.(29) In another study, RT-PCR shows the expression of pro-angiogenic factors such as Tie2, Ang1, VEGF, VEGFR, vWF, KDR and Flt4 in native AMMSCs. The results demonstrated that MSCs from an avascular amnion have a natural ability to initiate endothelialization and angiogenesis *in vitro*, suggesting an inherent propensity for promoting angiogenesis and could be an ideal choice in wound healing, stroke and ischemic diseases that require rapid vascularization and tissue restoration.(30) Chorionic villi has high vascular system characterized by maturation of luminized vessels from primitive haemangioblastic cords, and margination to a situation of peripherally located vessels.the increase number of peripheral vessels is required for the normal development mechanisms.(31). In another study, PURION® Processed and dehydrated human amnion/chorion membrane (dHACM) allograft has shown to contain a multitude of pro-angiogenic growth factors including PDGF-AA, PDGF-BB, PlGF, granulocyte colony-stimulating factor (GCSF), and VEGF, among others. It has also been demonstrated that dHACM grafts contain angiogenic growth factors retaining biological activity, promote amplification of angiogenic cues by inducing endothelial cell proliferation and migration and by upregulating production of endogenous angiogenic growth factors by endothelial cells; and support the formation of blood vessels *in vivo*. dHACM grafts are a promising wound care therapy with the potential to promote revascularization and tissue healing within poorly vascularized, non-healing wounds.(32)

3. Promotion of Epithelialization: Fetal membranes have been used for the management of skin burn, superficial wounds and for skin transplantation. Lately, the area of use has expanded to these fetal membranes being used as a graft or dressing in the reconstruction of the oral cavity, bladder, and vagina; tympanoplasty; arthroplasty and so forth. Amniotic membrane serves as a basement membrane which facilitates epithelial cell migration, reinforces adhesion of basal epithelial cells, promotes epithelial differentiation and prevents epithelial apoptosis.(33,34) Laminin isoforms,

present in the basement membrane, facilitate adhesion and expansion of corneal epithelial cells. The ability of the basement membrane of the amnion to support expansion of progenitor cells can explain application of AMT for treatment of partial limbal stem cell deficiency.(35) Good biocompatibility and mechanical properties like permeability, stability, elasticity, flexibility, plasticity, and resorbability also makes it a promising scaffolding material in tissue engineering as in cell adhesion and the potential for delivery of biomodulatory agents such as growth factors and genetic materials.(36,37)

#### 2.4 Uses of chorion and amniotic membranes in dentistry

The biological properties of amniotic and chorion membrane as antimicrobial, anti inflammatory, in promoting rapid vasculogenesis, epithelialization and above all an abundant source of stem cells has made these fetal tissues a suitable choice in the field of reconstructive and regenerative medicine. In the field of dentistry, these tissues find an application especially in Oral maxillofacial surgery and Periodontology. Antiinflammatory and antiscarring property of AM have shown decreased necrosis and rapid healing of ulcers with herpes simplex virus (HSV), varicella zoster virus–infected tissues, erythema multiforme major (Stevens-Johnson syndrome) and cervical necrotizing fasciitis.(38) HAM has been tried in the reconstruction of TMJ ankylosis as it prevents fibrosis and reankylosis when used as an interpositional material.(39) AM is even used as a carrier for local delivery of the various drugs like antibiotic netilmycin (NTM) and antiviral drugs like acyclovir (ACV) and trifluridine (40) Demineralized freeze dried bone allograft (DFDBA) and bovine derived xenogenic bone graft (BDX) [Bio-Oss] with amniotic membrane (AM) has been used as guided tissue regeneration (GTR) in the treatment of human periodontal Grade II buccal furcation defects. The clinical and radiographic parameters were recorded at baseline, 6 and 9 months. At 9 months after surgery, healing was uneventful. There was statistical significant gain in bone fill along with reduction in pocket depth and gain in clinical attachment levels. (41) Cryopreserved amniotic membrane (CAM) has been known to promote periodontal soft tissue healing and is also effective in helping cicatrization, wound healing, epithelialization, facilitated migration and reinforced adhesion.(42) Gurinsky demonstrated that the processed allograft amnion may provide an effective alternative to autograft tissue in the treatment of recession defects. (43) Similarly, many studies have reported the effectiveness of amnion membranes in root coverage and increase in thickness of gingival biotype. (44) A recent case report compares the effectiveness of amniotic membrane in comparison with Platelet-rich Fibrin (PrF) in bilaterally occurring multiple Millers class I recession. The clinical outcome of the surgical procedure accounted for 100% root coverage, an enhanced gingival biotype, with both the membranes. Furthermore, the results were stable even after seven months in the amniotic membrane-treated site.(45)

Chorion membrane, which is a rich source of various collagen and non-collagen proteins, such as laminin, fibronectin, and proteoglycans, has been used for root coverage and enhancement of thin gingival biotype to thick biotype.(46) Chorion membrane has extensive healing and

revascularization properties, and this has been proved in another study. The Chorion membrane covered by a modified coronally advanced flap, is a new approach that has shown promising results in terms of root coverage, increased width of keratinized tissue and thickness of the gingival biotype.(47) Chorion has also been assessed for its properties in treating periodontal infrabony pockets in severe periodontitis patients. Clinical parameters included gingival index (GI), plaque index (PI), pocket probing depth (PPD) and relative attachment level (RAL). Digital images were analysed for bone gain (BG) and density. Statistical significant differences were found at 12 months for GI, PI, PPD and RAL with increase in bone fill. (48)

Recently, second generation placental membrane- Bio-Xclude™ (Citagenix Inc.) has been developed from the amniotic sac that has anti inflammatory properties and known to promote cell migration. It is available in convenient sizes, which requires minimal trimming and can be folded into any shape to cover the exposed roots. This membrane also does not require any suturing.

### 3. Conclusion

It can be concluded that placental tissues hold great promise in the clinical setting. The existence of various sources of the mesenchymal stem cells in these tissues presents an opportunity for the development of new therapeutic strategies. The properties of amnion and chorion as

### 8. Contribution details

	<i>Study design</i>	<i>Manuscript preparation</i>	<i>Data collection</i>	<i>Revision of manuscript</i>
Dr.Swati Gupta	✓	✓	✓	✓
Dr.Richa Gupta		✓	✓	✓

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### Author Profile



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