#### **AMNIOTIC MEMBRANE**

#### **SCIENTIFIC PAPER REVIEW**

**APRIL 2017** 

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### Tissue Engineered Human Amniotic Membrane Application in Mouse Ovarian Follicular Culture.

Motamed M1,2, Sadr Z1, Valojerdi MR1,3, Moini A4,5, Oryan S2, Totonchi M6, Ebrahimi B1, Maroufizadeh S7, Taghiabadi E8, Fathi R9.

**Author information** 

#### **Abstract**

Since folliculogenesis requires a powerful cell-matrix interaction, natural scaffolds seem to be needed for follicular culture. Human amniotic membrane (HAM) offers promise as a support of in vitro ovarian follicular culture. HAM was decellularized with trypsin and EDTA. DNA and histology assays were performed to determine the elimination rate of genomic components. Cytobiocompatibility of decellular AM (DAM) was verified by the cell viability (MTT) test. The small parts of intact amniotic membrane (IAM) and DAM were coated on the bottom of 96-well and each well was filled with 150 µL of base medium. Mouse primary-secondary (PS) follicles were separated to three groups: 1culture in base medium (Control), 2-culture on IAM and 3-culture on DAM. Follicular size, morphology, viability, estradiol production and genes expression were evaluated and IAM group showed better growth and development in follicle culture. The viability rate and estradiol production in both experimental groups were statistically higher than the Control. Gdf9, Bmp15 and Cx37 were found to have higher expression levels in IAM group. Also, maximum apoptotic and survival indexes were determined in Control and IAM groups, respectively. Finally, IAM provides a better protective environment for mouse PS follicular culture that can reduce apoptosis level.

<u>J AAPOS.</u> 2017 Apr 24. pii: S1091-8531(17)30309-9. doi: 10.1016/j.jaapos.2017.04.002. [Epub ahead of print]

### Amniotic membrane transplants in the pediatric population.

Ahmad MS1, Frank GS2, Hink EM2, Palestine AG2, Gregory DG2, McCourt EA2. **Author information** 

#### **Abstract**

#### **PURPOSE:**

To investigate the indications for and results of amniotic membrane transplantation (AMT) for the treatment of ocular disease in pediatric patients at a single institution.

#### **METHODS:**

The medical records of patients <18 years of age who underwent AMT for ocular disease between January 1, 2003, and September 1, 2015, were reviewed retrospectively. Patients were determined to have reached a clinical endpoint if there was resolution of the ocular condition being treated after AMT placement, no additional surgery required for treatment of the ocular condition, and no active disease at most recent follow-up.

#### **RESULTS:**

A total of 48 records were reviewed. Of these, 32 patients (67%) received AMT for treatment of ocular disease related to Stevens Johnson syndrome (SJS), 29 (94%) of whom reached the clinical endpoint. The remaining 16 patients (33%) underwent AMT for indications other than SJS, including difficult-to-treat corneal epithelial defects and ulcers, conjunctival reconstruction, and scarring after strabismus surgery. Of these, 80% reached the clinical endpoint. There were no adverse effects related to AMT in either group.

#### **CONCLUSIONS:**

In our series, AMT was used successfully and without complications used in the treatment of various ocular conditions in children.

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## Amniotic membrane use for management of corneal limbal stem cell deficiency.

Sabater AL<sub>1</sub>, Perez VL. Author information

#### Abstract

#### **PURPOSE OF REVIEW:**

The current article reviews the most recent surgical techniques for management of corneal limbal stem cell deficiency (LSCD) using amniotic membrane tissue. **RECENT FINDINGS**:

Early successes with amniotic membrane transplantation (AMT) for the treatment of ocular surface disorders have encouraged clinicians to investigate new applications. The use of AMT as a temporary patch in emergency cases in which LSCD may develop has considerably improved the prognosis of these patients. Amniotic membrane does not have stem cells of its own, but it supports regeneration of limbal epithelial stem cells (LESCs). Similarly, the combination of AMT with classic surgical techniques has enhanced the surgical success rates in most case series. Furthermore, based on its advantageous properties as a cell carrier, new applications to support in-vivo and ex-vivo cell expansion have been reported recently.

#### **SUMMARY:**

LSCD constitutes a general indication for AMT. Based on the clinical scenario, AMT may be performed alone to support regeneration of LESCs, in combination with other surgical techniques, or even supporting the in-vivo or ex-vivo expansion of LESCs.

<u>Placenta.</u> 2017 Apr 12. pii: S0143-4004(17)30230-8. doi: 10.1016/j.placenta.2017.04.008. [Epub ahead of print]

#### Fetal and perinatal stem cells in cardiac regeneration: Moving forward to the paracrine era.

Balbi C1, Bollini S2.

**Author information** 

#### **Abstract**

Cardiovascular disease (CD) is a major burden for Western society. Regenerative medicine has provided encouraging results, yet it has not addressed the focal defects causing CD and mainly related to the inefficient repair programme of the heart. In this scenario, stem cells have been broadly investigated and their paracrine effect proposed as a possible working strategy to boost endogenous mechanisms of repair and regeneration from within the cardiac tissue. The scientific community is now focusing on identifying the most effective stem cell secretome, as the whole of bioactive factors and extracellular vesicles secreted by stem cells and endowed with regenerative potential. Indeed, the adult stem cell-paracrine potential for cardiac regeneration have been widely analyzed with positive outcome. Nevertheless, low yield, invasive sampling and controversial self-renewal may limit adult stem cell application. On the contrary, fetal and perinatal stem cells, which can be easily isolated from leftover sample via prenatal screening during gestation or as clinical waste material after birth, can offer an ideal alternative. These broadly multipotent immature progenitors share features with both adult and embryonic stem cells, show high self-renewal, but they are not tumorigenic neither cause any ethical concern. While fetal and perinatal stem cells demonstrated to improve cardiac function when injected in the injured heart, the comprehensive characterization of their secretome for future applications is still at its infancy. In this review, we will discuss the paracrine potential of the fetal and perinatal stem cell secretome to provide cardiac repair and resurge the dormant mechanisms of cardiac regeneration for future therapy.

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#### **KEYWORDS:**

Amniotic fluid; Amniotic membrane; Cardiac regeneration; Cardio-protection; Conditioned medium; Extracellular vesicle; Mesenchymal stem cells; Paracrine effect; Placenta; Umbilical cord; Wharton's Jelly

<u>Placenta.</u> 2017 Apr 10. pii: S0143-4004(17)30227-8. doi: 10.1016/j.placenta.2017.04.005. [Epub ahead of print]

### Amniotic membrane application for the healing of chronic wounds and ulcers.

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**Author information** 

#### **Abstract**

Wound healing usually follows a predictable sequence and prognosis of events. Its evolutionary process is the result of a complicated interaction between patient-related factors, the wound, the treatment used and the skills and knowledge of the professionals who treat them. Only through a meticulous initial assessment of the wound is it possible to identify the factors that contribute to its complexity. The challenge for professionals will be to implement efficient therapies at the right time and in the most cost-efficient way in order to reduce associated problems, treat the symptoms and expectations of the patients and achieve adequate wound healing whenever possible. This is particularly evident in big chronic wounds with considerable tissue loss, which become senescent in the process of inflammation or proliferation losing the ability to epithelialize. Generally, chronic wounds do not respond to current treatments, therefore they need special interventions. AM is a tissue of particular interest as a biological dressing and it has well-documented reepithelialization effects which are in part related to its capacity to synthesize and release biological active factors. Our studies have demonstrated that amniotic membrane (AM) is able to induce epithelialization in chronic wounds that were unable to epithelialize. AM induces several signaling pathways that are involved in cell migration and/or proliferation. Additionally, AM is able to selectively antagonize the antiproliferative effect of transforming growth factor-ß (TGF-β) by modifying the genetic program that TGF-β induces on keratinocytes. The combined effect of AM on keratinocytes, promoting cell proliferation/migration and antagonizing the effect of TGF-β is the perfect combination, allowing chronic wounds to move out of their non-healing state and progress into epithelialization. Copyright © 2017 Elsevier Ltd. All rights reserved.

#### **KEYWORDS:**

Amniotic membrane; Cell migration; Chronic wounds; Negative pressure therapy; TGF-β; Wound healing

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# Therapeutic effects of human amnion-derived mesenchymal stem cell transplantation and conditioned medium enema in rats with trinitrobenzene sulfonic acid-induced colitis.

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**Author information** 

#### Abstract

Cell therapy with mesenchymal stem cells (MSCs) is expected to provide a new strategy for the treatment of inflammatory bowel disease (IBD). Large amounts of MSCs can be obtained from human amnion. Therefore, we investigated the effect of transplantation of human amnion-derived MSCs (hAMSCs) or enema of conditioned medium (CM) from hAMSCs into rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. In the first experiment, 10-week-old male Sprague-Dawley rats were intravenously injected with hAMSCs (1 × 106 cells) 3 h after rectal administration of TNBS (45 mg/kg). In the second experiment, rats with TNBS-induced colitis received CM by enema into the colon for 3 days. Colitis was investigated by endoscopy, histology, immunohistochemistry, and by measuring mRNA expression of inflammatory mediators. Administration of hAMSCs or CM enema significantly improved the endoscopic score. In addition, these two interventions resulted in significantly decreased infiltration of neutrophils and monocytes/macrophages and decreased expression levels of TNF-α, CXCL1, and CCL2. In conclusion, transplantation of hAMSCs and CM enema provided significant improvement in rats with TNBS-induced colitis. CM from hAMSCs and hAMSCs may be new strategies for the treatment of IBD. **KEYWORDS:** 

Mesenchymal stem cells; amnion; colitis; conditioned medium; trinitrobenzene sulfonic acid

Int Wound J. 2017 Mar 29. doi: 10.1111/iwj.12748. [Epub ahead of print]

## In vitro assessment of a novel, hypothermically stored amniotic membrane for use in a chronic wound environment.

McQuilling JP1, Vines JB1, Mowry KC1. **Author information** 

#### Abstract

Chronic wounds require extensive healing time and place patients at risk of infection and amputation. Recently, a fresh hypothermically stored amniotic membrane (HSAM) was developed and has subsequently shown promise in its ability to effectively heal chronic wounds. The purpose of this study is to investigate the mechanisms of action that contribute to wound-healing responses observed with HSAM. A proteomic analysis was conducted on HSAM, measuring 25 growth factors specific to wound healing within the grafts. The rate of release of these cytokines from HSAMs was also measured. To model the effect of these cytokines and their role in wound healing, proliferation and migration assays with human fibroblasts and keratinocytes were conducted. along with tube formation assays measuring angiogenesis using media conditioned from HSAM. Additionally, the cell-matrix interactions between fibroblasts and HSAM were investigated. Conditioned media from HSAM significantly increased both fibroblast and keratinocyte proliferation and migration and induced more robust tube formation in angiogenesis assays. Fibroblasts cultured on HSAMs were found to migrate into and deposit matrix molecules within the HSAM graft. These collective results suggest that HSAM positively affects various critical pathways in chronic wound healing, lending further support to promising qualitative results seen clinically and providing further validation for ongoing clinical trials.

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#### **KEYWORDS:**

Amnion; Chronic wound healing; Chronic wounds; Hypothermically stored amniotic membrane; Regenerative healing

J Surg Case Rep. 2016 Oct 22;2016(10):rjw174. doi: 10.1093/jscr/rjw174. eCollection 2016.

#### Utility of human amniotic membrane allograft in reepithelialization of the nasal tip.

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#### Abstract

Variations in skin thickness and contours pose significant challenges to reconstruction of the lower third of the nose. Human amniotic membrane allograft offers a potential alternative to tissue transfer in reconstruction of the lower third of the nose. We reviewed the procedure and photographs of a healthy 56-year-old male with a 22 × 18 mm lower third nasal defect involving full thickness skin and subcutaneous tissue. Following preparation for grafting, dehydrated human amniotic membrane was fashioned to the dimensions of the defect and applied. No further surgical intervention was provided for 3 months. Complete re-epithelialization of the nasal and adjacent defects was achieved with minimal scar formation. Human amniotic membrane allograft provides an efficacious and cosmetically acceptable alternative to local and regional tissue transfer.

<u>J Int Soc Prev Community Dent.</u> 2017 Jan-Feb;7(1):15-21. doi: 10.4103/jispcd.JISPCD\_359\_16. Epub 2017 Feb 21.

### Human Amnion Membrane: Potential Applications in Oral and Periodontal Field.

Mohan R1, Bajaj A1, Gundappa M2.

**Author information** 

#### **Abstract**

Human amniotic membrane (HAM) is derived from the fetal membranes which consist of the inner amniotic membrane made of single layer of amnion cells fixed to collagen-rich mesenchyme attached to chorion. HAM has low immunogenicity, anti-inflammatory properties and their cells can be isolated without the sacrifice of human embryos. Amniotic membrane has biological properties which are important for the experimental and clinical applications in managing patients of various medical specialties. Abundant, natural and wonderful biomembrane not only protects the foetus but also has various clinical applications in the field of dermatology, ophthalmology, ENT surgery, orthopedics and dental surgery. As it is discarded post-partum it may be useful for regenerative medicine and cell therapy to treat damaged or diseased tissues. **KEYWORDS:** 

Biomembrane; human amnion membrane; placental allografts; scaffold; tissue engineering

Acta Cir Bras. 2017 Feb;32(2):125-139. doi: 10.1590/s0102-865020170205.

### Amniotic membrane as an option for treatment of acute Achilles tendon injury in rats.

<u>Nicodemo MC</u>1, <u>Neves LR</u>2, <u>Aguiar JC</u>3, <u>Brito FS</u>4, <u>Ferreira I</u>5, <u>Sant'Anna LB</u>6, <u>Raniero LJ5</u>, Martins RÁ7, Barja PR8, Arisawa EA9.

#### **Author information**

#### **Abstract**

#### **PURPOSE:**

To evaluate the effect of human amniotic membrane (hAM) fragment on inflammatory response, proliferation of fibroblast and organization of collagen fibers in injured tendon.

#### **METHODS:**

Sixty rats were divided into 3 groups: C - surgical procedures without tendon lesion and with simulation of hAM application; I - surgical procedures, tendon injury and simulation of hAM application; T - surgical procedures, tendon injury and hAM application. These groups were subdivided into four experimental times (3, 7, 14 and 28 days). The samples underwent histological analysis and ATR-FTIR spectroscopy.

#### **RESULTS:**

Histological analysis at 14 days, the T group showed collagen fibers with better alignment. At 28 days, the I group presented the characteristics described for the T group at 14 days, while this group presented aspects of a mature connective tissue. FT-IR analysis showed a clear distinction among the three groups at all experimental times and groups T and I presented more similarities to each other than to group C.

#### **CONCLUSION:**

Acute injury of tendon treated with human amniotic membrane fragment showed a faster healing process, reduction in inflammatory response, intense proliferation of fibroblasts and organization of collagen fibers.

<u>Plast Reconstr Surg Glob Open.</u> 2016 Dec 7;4(12):e1132. doi: 10.1097/GOX.00000000001132. eCollection 2016.

#### Management of a Complex Excoriation Disorderinduced Wound with a Viable Cryopreserved Placental Membrane.

Bain MA1, Vincent J1. **Author information** 

#### **Abstract**

Excoriation disorder (ED), also known as dermatotillomania, is a condition characterized by repeated "skin picking" that leads to the formation of skin lesions. Because of the similarity of its symptoms to obsessive compulsive disorder, ED is classified as a subcategory of obsessive compulsive disorder by Diagnostic and Statistical Manual of Mental Disorders Fifth Edition. Although the majority of the self-inflicted wounds are not clinically significant, many wounds lead to social and occupational dysfunction by becoming infected, chronic, and life threatening. This report describes the successful use of a viable intact cryopreserved human amniotic membrane in conjunction with selective serotonin re-uptake inhibitors in treating an ED patient who presented with a large calvarial wound of 3-year duration that had failed previous extensive medical and surgical interventions.

World J Plast Surg. 2017 Jan;6(1):33-39.

## Comparison of Characteristics of Human Amniotic Membrane and Human Adipose Tissue Derived Mesenchymal Stem Cells.

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#### Abstract

#### **BACKGROUND:**

Mesenchymal stem cells (MSCs) are ideal candidates for treatment of diseases. Amniotic membranes are an inexpensive source of MSCs (AM-MSC) without any donor site morbidity in cell therapy. Adipose tissue derived stem cells (ASCs) are also suitable cells for cell therapy. There is discrepancy in CD271 expression among MSCs from different sources. In this study, the characteristics of AM-MSC and ASCs and CD271 expression were compared.

#### **METHODS:**

Adult adipose tissue samples were obtained from patients undergoing elective surgical procedure, and samples of amniotic membrane were collected immediately after caesarean operation. After isolation and expansion of MSCs, the proliferation rate and viability of cells were evaluated through calculating DT and MTT assay. Expression of routine mesenchymal specific surface antigens of MSCs and CD271 was evaluated by flow cytometry for both types of cells.

#### **RESULTS:**

The growth rate and viability of the MSCs from the amniotic membrane was significantly higher compared with the ASCs. The low expression of CD14 and CD45 indicated that AM-MSC and ASCs are non hematopoietic cells, and both cell types expressed high percentages of CD44, CD105. The results revealed that AM-MSC and ASCs expressed no CD271 on their surfaces.

#### CONCLUSION:

This study showed that amniotic membrane is a suitable cell source for cell therapy, and CD271 is a negative marker for MSCs identification from amniotic membrane and adipose tissue.

#### **KEYWORDS:**

Adipose tissue; Amniotic membrane; CD 271; Mesenchymal stem cells

Cytotherapy. 2017 May;19(5):603-616. doi: 10.1016/j.jcyt.2017.02.003. Epub 2017 Mar 9.

## Human amniotic mesenchymal stromal cell transplantation improves endometrial regeneration in rodent models of intrauterine adhesions.

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#### Abstract

#### **BACKGROUND AIMS:**

Intrauterine adhesion (IUA) is a common uterine cavity disease characterized by the unsatisfactory regeneration of damaged endometria. Recently, stem cell transplantation has been proposed to promote the recovery process. Here we investigated whether human amniotic mesenchymal stromal cells (hAMSCs), a valuable resource for transplantation therapy, could improve endometrial regeneration in rodent IUA models.

#### **METHODS:**

Forty female Sprague-Dawley rats were randomly assigned to five groups: normal, sham-operated, mechanical injury, hAMSC transplantation, and negative control group. One week after intervention and transplantation, histological analyses were performed, and immunofluorescent and immunohistochemical expression of cell-specific markers and messenger RNA expression of cytokines were measured.

#### **RESULTS:**

Thicker endometria, increased gland numbers and fewer fibrotic areas were found in the hAMSC transplantation group compared with the mechanical injury group. Engraftment of hAMSCs was detected by the presence of anti-human nuclear antigen-positive cells in the endometrial glands of the transplantation uteri. Transplantation of hAMSCs significantly decreased messenger RNA levels of pro-inflammatory cytokines (tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$ ), and increased those of anti-inflammatory cytokines (basic fibroblast growth factor, and interleukin-6) compared with the injured uterine horns.

Immunohistochemical expression of endometrial epithelial cells was revealed in specimens after hAMSC transplantation, whereas it was absent in the mechanically injured uteri.

#### **CONCLUSIONS:**

hAMSC transplantation promotes endometrial regeneration after injury in IUA rat models, possibly due to immunomodulatory properties. These cells provide a more easily accessible source of stem cells for future research into the impact of cell transplantation on damaged endometria.

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#### **KEYWORDS:**

amnion; endometrium; intrauterine synechiae; mesenchymal stromal cell transplantation; regeneration

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## Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review.

<u>Jirsova K</u>1, <u>Jones GL</u>2. **Author information** 

#### **Abstract**

The use of amniotic membrane in ophthalmic surgery and other surgical procedures in the fields of dermatology, plastic surgery, genitourinary medicine and otolaryngology is on the increase. Furthermore, amniotic membrane and its epithelial and mesenchymal cells have broad use in regenerative medicine and hold great promise in anticancer treatment. Amniotic membrane is a rich source of biologically active factors and as such, promotes healing and acts as an effective material for wound dressing. Amniotic membrane supports epithelialization and exhibits anti-fibrotic, anti-inflammatory, anti-angiogenic and anti-microbial features. Placentas utilised in the preparation of amniotic membrane are retrieved from donors undergoing elective caesarean section. Maternal blood must undergo serological screening at the time of donation and, in the absence of advanced diagnostic testing techniques, 6 months postpartum in order to cover the time window for the potential transmission of communicable diseases. Amniotic membrane is prepared by blunt dissection under strict aseptic conditions, then is typically transferred onto a nitrocellulose paper carrier, usually with the epithelial side up, and cut into multiple pieces of different dimensions. Amniotic membrane can be stored under various conditions, most often cryopreserved in glycerol or dimethyl sulfoxide or their mixture with culture medium or buffers. Other preservation methods include lyophilisation and airdrying. In ophthalmology, amniotic membrane is increasingly used for ocular surface reconstruction, including the treatment of persistent epithelial defects and non-healing corneal ulcers, corneal perforations and descemetoceles, bullous keratopathy, as well as corneal disorders with associated limbal stem cell deficiency, pterygium, conjunctival reconstruction, corneoscleral melts and perforations, and glaucoma surgeries.

#### **KEYWORDS:**

Amniotic membrane; Graft indications; Preparation; Procurement; Properties; Storage

J Cardiothorac Surg. 2017 Jan 26;12(1):6. doi: 10.1186/s13019-017-0567-7.

## Anti-inflammatory properties of amniotic membrane patch following pericardiectomy for constrictive pericarditis.

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**Author information** 

#### **Abstract**

#### **BACKGROUND:**

Since constrictive pericarditis is most often idiopathic and the pathophysiology remains largely unknown, both the diagnosis and the treatment can be challenging. However, by definition, inflammatory processes are central to this disease process. Amniotic membrane patches have been shown to possess anti-inflammatory properties and are believed to be immune privileged. Due to these properties, amniotic membrane patches were applied intraoperatively in a complicated patient presenting with constrictive pericarditis.

#### **CASE PRESENTATION:**

A patient with a history of multiple cardiac surgeries presented with marked fatigue, worsening dyspnea and sinus tachycardia. He was found to have constrictive physiology during cardiac catheterization, with cardiac MRI demonstrating hepatic vein dilatation, atrial enlargement and ventricular narrowing. After amniotic membrane patch treatment and pericardiectomy, post-operative cardiac MRI failed to demonstrate any appreciable pericardial effusion or inflammation, with no increased T2 signal that would suggest edema.

#### **CONCLUSIONS:**

Given the positive results seen in this complex patient, we suggest continued research into the beneficial properties of amniotic membrane patches in cardiac surgery.

#### **KEYWORDS:**

Amniotic membrane patch; Constrictive pericarditis; Orthotopic heart transplant