PaiNweek

Stem Cells & Regenerative Medicine

Jay Joshi, MD, DABA, DABA-PM, FABA-PM

Disclosures

- None

PaiNweek

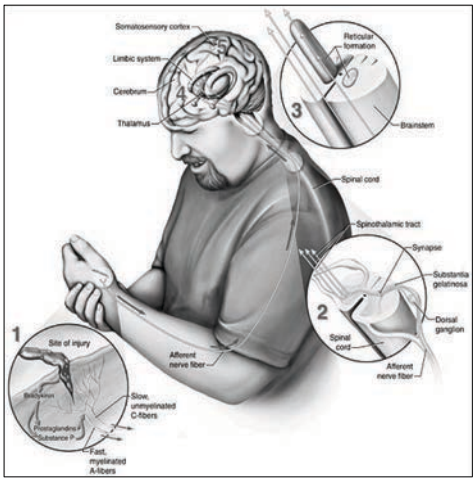
Outline

- Review the overview of inflammation
- Define regenerative medicine
- Review the history of stem cells
- Differentiate different types of stem cells
- Discuss autologous stem cells
- Discuss non autologous stem cells
- Compare non stem cell regenerative products

PainWeek

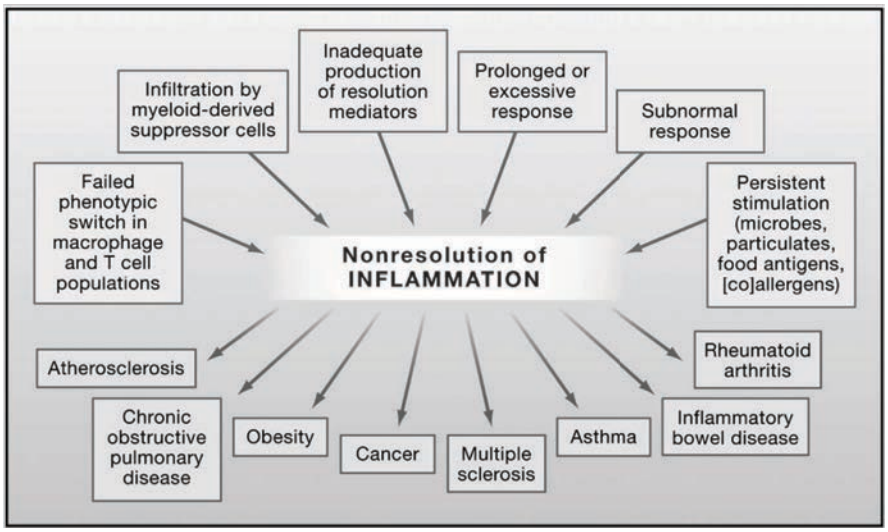
Pain is the First Sign of Inflammation

- Inflammation can be provoked by injuries caused by physical, chemical, and biologic agents
- Classic signs of inflammation are pain, heat, redness, swelling, and loss of function
- Impact of Pain:
  - Delay healing
  - Decrease appetite
  - Increase stress
  - Disrupt sleep and concentration



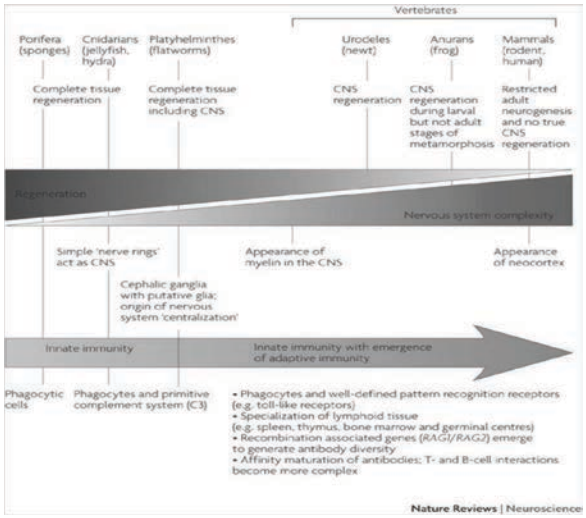
PainWeek

Non-resolving "Inflammation"



Nathan and Ding Cell, 140:871-882, 2010

Evolution of CNS and Immune System



Popovich et al. 2008. Nature Reviews Neuroscience 9, 481-493

What is Regenerative Medicine?

- Regenerative medicine is a branch of medicine that deals with the process of replacing, repairing, and restoring normal tissue and function.
- Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and implanting them when the body cannot heal itself.
- Regenerative medicine involves stem cells and growth factor products.

PAINWEEK

Regenerative Medicine Therapies

Healing Environment

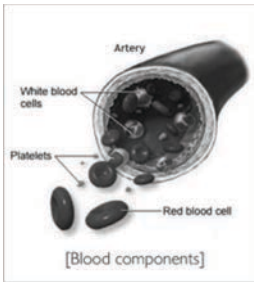
- NSAIDS
- Steroids
- Synthetic Hyaluronic Acid
- Platelet Rich Plasma (PRP)
- Amniotic Fluid Liquid Suspension
- Amniotic Fluid + ECM Liquid Suspension
- Wharton's Jelly Liquid Suspension

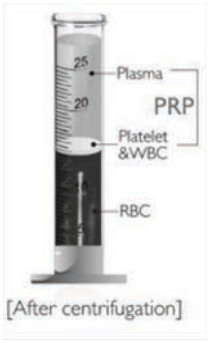
Cellular Products

- Lipoaspirate Concentrate
- Bone Marrow Aspirate Concentrate
- Umbilical Cord Blood
- Umbilical Cord Mesenchymal Stem Cells (MSC)

PRP

- Platelet-rich plasma (PRP) is defined as "autologous blood with concentrations of platelets above baseline levels, which contains at least seven growth factors"
- PRP is taken directly from a patient's own blood and then injected into the affected area
- PRP contains growth factors that trigger localized inflammation, collagen production, and other regenerative processes
- Used since 1987





PAINWEEK

PRP Advantages and Disadvantages

- Advantages
  - Autologous
  - Relatively cheap
  - Can be reproducible geographically
- Disadvantages
  - Side effects may occur, such as swelling at the injection site, increased pain and stiffness, and infection
  - Unwanted products, such as white blood cells, certain cytokines, inflammatory cells, and infections can exist in the PRP

PAINWEEK

PRP Advantages and Disadvantages

- Disadvantages (continue)
  - Many questions still exist, such as:
    - Indications, i.e. when should this treatment be used?
    - If it is an effective treatment for osteoarthritis, should it be used in the early stages of osteoarthritis or only when all other options are exhausted?
    - What are the optimal concentrations of platelets and white blood cells?
    - How much platelet-rich plasma should be injected?
    - Do certain additives, such as thrombin, make the PRP more effective?
    - When and with what frequency should injections be given? Is one injection enough?
    - What is the best rehabilitation protocol to use after PRP injection?

PAINWEEK

Brief History of Stem Cells

- Adult stem cell research began about 40 years ago
- Stem cell discoveries in 1960s:
  - Bone marrow contains 2 populations of stem cells
    - Hematopoietic stem cells – forms all blood cell types
    - Bone marrow stromal cells – mixed cell population that generates bone, cartilage, fat and fibrous connective tissue
  - Rat brain contains two regions of dividing cells, which become nerve cells
- Stem Cell Discoveries in the 1990s
  - Neural stem cells are able to generate the brain's three major cell types:
    - Astrocytes, Oligodendroglial cells, Neurons

PAINWEEK

Brief History of Stem Cells

- 1998 - Researchers first extract stem cells from human embryos
- 1999 - First Successful human transplant of insulin-making cells from cadavers
- 2001 - President Bush restricts federal funding for embryonic stem-cell research
- 2002 - California ok stem cell research
- 2004 - Harvard researchers grow stem cells from embryos using private funding
- 2004 - Ballot measure for $3 Billion bond for stem cells
- 2009 - Rabbit umbilical cord stem cells completely abolish rat mammary carcinomas with no evidence of metastasis or recurrence hundred days post- tumor cell inoculation
- 2013-2017 - National Pain Centers has Multiple Firsts with Autologous and Non-Autologous Stem Cell products

PAINWEEK

Types of Stem Cells

Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells

PAINWEEK

Potential Uses of Stem Cells

- Basic research – clarification of complex events that occur during human development & understanding molecular basis of cancer
- Biotechnology (drug discovery & development) – stem cells can provide specific cell types to test new drugs
- Cell based therapies:
  - Regenerative therapy
  - Stem cells in gene therapy
  - Stem cells in therapeutic cloning
  - Stem cells in cancer

PAINWEEK

Homologous Cell and Tissue Products-HCT/Ps

- In 1980's the FDA asserted authority of human tissue
- In 1993 the FDA created two pathways for regulating homologous tissue and cell products by statue with Part 1270 of Title 21, CFR (Codes of Federal Regulations)
- Section 361: Minimally manipulated tissues and cells, intended for homologous use only, and not combined with another article, with some exceptions
  - HCT/Ps labs are accredited by American Association of Tissue Banks (AATB)
- Section 351: Biological products derived from living material– human, animal or microorganism – applicable to the prevention, treatment or cure of a disease
  - These products meet the definition of a Therapeutic Biologic Application (BLA)
  - Regulated by the FDA Center for Drug Evaluation and Research (CDER)

PAINWEEK

Autologous Pros and Cons

Pros

- Known source of MSCs
- Reduced risk of rejection or inflammation
- Reduced potential for bacteria or virus transr

Cons

- **Requires a surgical procedure**
- **Additional capital, disposable, surgical and time costs**
- **Potential morbidity complications**
- **Low concentrations**
  - **Lipoaspirate 4,500 – 450,000 MSCs per CC**
  - **BMAC – 30 – 300 MSCs per CC**

PaiNweek

Current Autologous Therapies

Adipose Derived Adult MSCs

- Adult stromal cells intended for regenerative therapy can be isolated from the patient's adipose tissue
- Mesenchymal stem cells may differentiate into the cells that make up bone, cartilage, tendons, and ligaments, as well as muscle, neural and other progenitor tissues, they have been the main type of stem cells studied in the treatment of diseases affecting these tissues
- The number of stem cells transplanted into damaged tissue may alter efficacy of treatment
- It takes approximately 60cc's of adipose to obtain 1cc with over a million cells.

PaiNweek

Current Autologous Therapies

Bone Marrow Aspirate Concentrate (BMAc)

- Marrow stromal cells have been used for a while in orthopedics (i.e. knee microfracture surgery)
- BMAc produces a very dilute MSC population but still show promise for joint repair
- BMAc are usually transferred with growth factors for success
- Unknown which component is more efficacious

PaiNweek

Non-Autologous Pros and Cons

Pros

- High concentrations of MSCs
  - Umbilical Cord 2 million per CC
- Epigenetically young cells
- Quick, easy and reproducible
- No capital, surgical or time costs
- No known complications

Cons

- Potential for bacteria or virus transmission
  - Less than blood transfusion
- Logistics and handling considerations
  - Products must be shipped and stored -200º C

PaiNweek

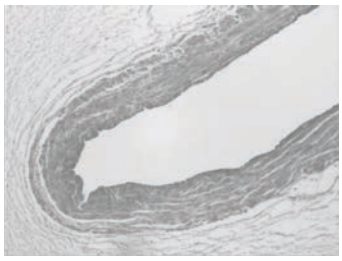
Allogenic Available Products (HCT/Ps)

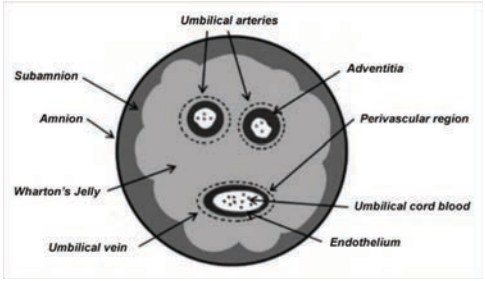
- Umbilical Cord Derived MSCs
  - HCT/P product with the potential to replace Lipoaspirate and Bone Marrow Aspirate Concentrate products
- Umbilical Cord Tissue Matrix
  - Injectable matrix rich in HA, Cytokines, Growth Factors and Proteins
- Amniotic Liquid Suspension
  - Injectable matrix rich in HA, Cytokines, Growth Factors and Proteins
- Amniotic Membrane
  - Tissue product used extensively for wound management, burns and soft tissue repair

PAINWEEK

Wharton's Jelly

- Wharton's Jelly is a mucous connective tissue within the umbilical cord, which originates in the extraembryonic mesoderm and is composed of myofibroblast-like stromal cells, collagen fibers, proteoglycans, and has very high concentrations of hyaluronic acid and chondroitin sulfate.

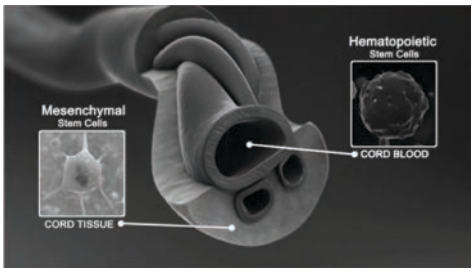




PAINWEEK

Mesenchymal Stem Cells Derived from Wharton's Jelly

- Wharton's Jelly-derived mesenchymal stem cells (WJ-MSC) have the following advantages
  - About 131 million births worldwide annually provide a large supply
  - There is no need for invasive harvesting methods
  - Their rate of proliferation is more than other sources
  - They can be collected easily at the time of child birth
  - They are not associated with ethical concerns
  - They are immune privileged
  - They have non-tumorigenic properties



PAINWEEK

Benefits of Mesenchymal Stem Cells

- Their potential to migrate to sites of inflammation caused by tissue injury
- Their potential to differentiate into different cell types
- Their potential to release different bioactive molecules that can stimulate the recovery of injured cells
- Their ability to prevent inflammation and accomplish immunomodulatory functions

PAINWEEK

Cryopreservation of Human MSC for Clinical Use

- For cells to remain viable, they must be stored at -200° C.

P. Mazur, American Journal of Physiology - Cell Physiology Sep 1984, 247 (3) C125-C142

Hunt CJ, Cryopreservation of Human Stem Cells for Clinical Application: A Review. Transfusion Medicine and Hemotherapy. 2011;38(2):107-123. doi:10.1159/000326623

Yong KW, Wan Safwani WK, Xu F, Biopreserv Biobank. 2015 Aug;13(4):231-9. doi: 10.1089/bio.2014.0104

PAINWEEK

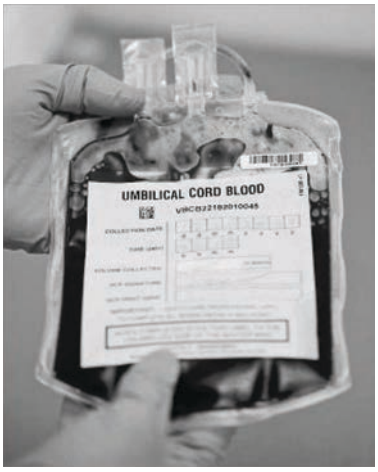
Umbilical Cord Blood

- Umbilical cord blood derived allografts have been recently introduced with claims of millions of cells per milliliter of product
- Cord blood will not yield therapeutic numbers of MSCs unless the blood samples from many donors are pooled or the MSCs from a single donor are expanded in culture (Divya, et al., 2012)
- The FDA strictly forbids both procedures for CFR 1271 section 361 HCT/Ps as this makes the product “more than minimally manipulated.”
- The only other explanation for the claim of millions of cells in these products is that these companies are using Peripheral Blood Mononuclear Cells (PBMC, hematopoietic cells, CD34+)
- Processing is accomplished by ficoll or hetastarch and will yield hundreds of millions of cells, however, only a small fraction of those cells would be considered stem cells (either hematopoietic or MSCs)

PAINWEEK

Umbilical Cord Blood vs. Umbilical Cord Matrix

- Hematopoietic Stem Cells are the most commonly cryopreserved stem cells, usually for autologous applications
  - Umbilical cord blood differentiate into blood cells
- Lining Stem Cells are isolated from the umbilical cord lining
  - Committed to an epithelial lineage.
- Mesenchymal Stem Cells are isolated from the umbilical cord tissue stroma (Wharton's Jelly)
  - Multipotent in nature and can differentiate into specialized cells from the three germ layers
- White/Blood Cells are not defined as stem cells
  - Commonly found in Umbilical Cord Blood products and could cause potential issues with the donor recipient



PAINWEEK

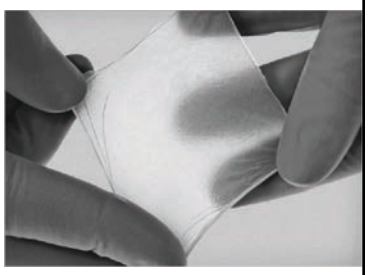
Umbilical Cord Blood Product Concerns

- Graft-versus-host disease (GVHD)
  - Can affect many different parts of the body including the skin, eyes, mouth, stomach, and intestines
- Occurs because of differences between the immune system of the host's body and the donated cells
- Types of GVHD
  - ACUTE GVHD: Usually develops in the first 3 months and affects the skin, stomach, intestines, and liver
  - CHRONIC GVHD: Usually develops in 3-6 months

PAINWEEK

Amniotic Membrane

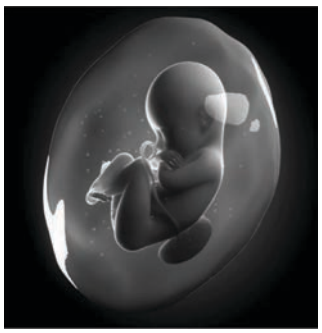
- Amniotic Membrane (AM) is the inner layer of the placenta that surrounds the baby during pregnancy
- AM is a universal transplant
- Unique structural and compositional properties that facilitate natural wound healing
- AM Allograft is composed of a complex extracellular matrix that is dehydrated and terminally sterilized
  - Decellurized dehydrated human amniotic membrane (ddHAM)
- It is derived from the placentas of normal, full term pregnancies



PainWeek

Amniotic Fluid

- Amniotic fluid can be used to increase the volume of lubricating and shock absorbing fluid in joints
- Prevents the formation of new adhesions after closed manipulation of joints
- Induce immune tolerance and aids wound healing
- Amniotic fluid has cosmetic applications, specifically as an anti-wrinkle agent
- Amniotic fluid contains cytokines, hyaluronic acid, various proteins, and growth factors

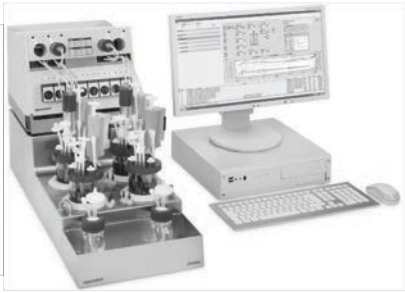


PainWeek

Cell Differentiation Equipment

- The most common methods to characterize cells include:
  - Cell Counter
  - Flow Cytometry
  - Cell Expansion





PAINWEEK

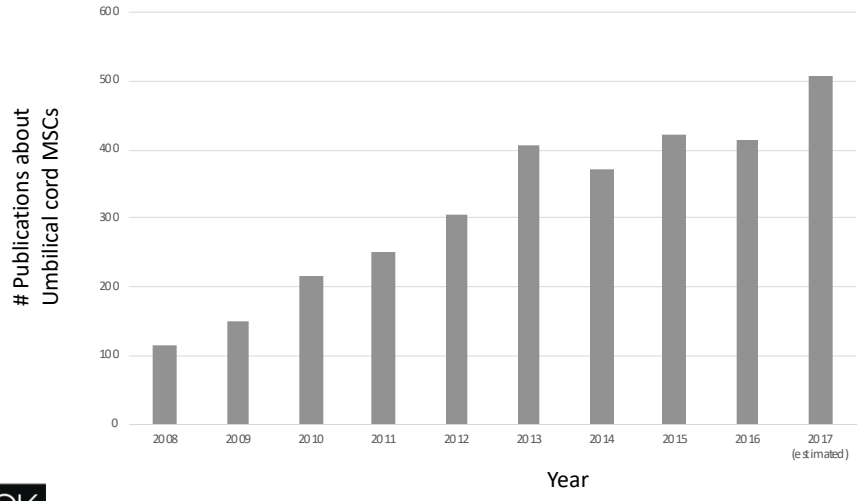
Differentiation Between Cell Types

- Flow Cytometry
  - The International Society of Cellular Therapy (ISCT) recommends use of Flow Cytometry to identify cell types by using specific markers
- The markers that need to be present to help identify MSCs include
  - CD73
  - CD90
  - CD105
- The markers that identify Hematopoietic Stem Cells, White/Blood and Lining Cells include
  - CD14
  - CD34
  - CD45

ISCT
International Society for Cellular Therapy

PAINWEEK

Research Growth



PAINWEEK

Challenges to Regenerative Medicine

- FDA restrictions
- Insurance coverage
- Physician variability
- Product variability
- False claims
- Counterfeit services and products

PAINWEEK

References

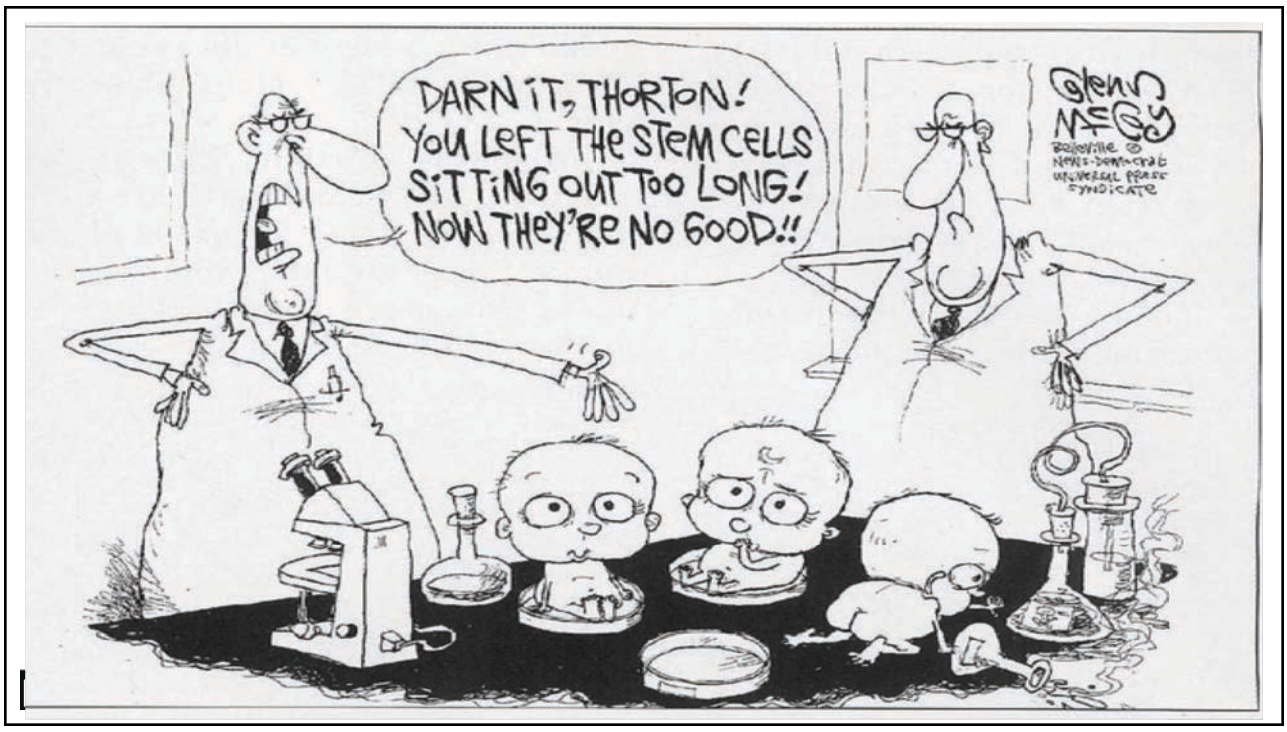
- Cornwell KG, Landsman A, James KS. Extracellular matrix biomaterials for soft tissue repair. Clin Podiatr Med Surg. 2009;26(4):507–523
- Lyons AB, Chipps LK1, Moy RL, Herrmann JL, Dehydrated human amnion/chorion membrane allograft as an aid for wound healing in patients with full-thickness scalp defects after Mohs micrographic surgery. JAAD Case Rep. 2018 Aug 15;4(7):688-691. doi: 10.1016/j.jdcr.2018.03.015
- Ganatra MA. Amniotic Membrane in Surgery. J Pak Med Assoc. 2003;53(1):29–32
- Litwiniuk M, Grzela T, Amniotic membrane: new concepts for an old dressing. Wound Repair Regen. 2014 Jul-Aug;22(4):451-6. doi: 10.1111/wrr.12188.
- Akle C, Adinolfi M, Welsh KI, Leibowitz S, McColl I. Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. Lancet. 1981;2(8254):1003–1005
- Hori J, Wang M, Kamiya K, Takahashi H, Sakuragawa N, Immunological characteristics of amniotic epithelium. Cornea. 2006 Dec;25(10 Suppl 1):S53-8
- Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res. 2000;70(3):329–337
- Sharma N, Kaur M, Agarwal T, Sangwan VS, Vajpayee RB, Treatment of acute ocular chemical burns. Surv Ophthalmol. 2018 Mar - Apr;63(2):214-235. doi: 10.1016/j.survophthal.2017.09.005
- Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea. 2000;19(3):348–352
- Thomasen H, Schroeter J, Reinhard T, Seitz B, Steuhl KP, Meller D, Good practice procedures for acquisition and preparation of cryopreserved human amniotic membranes from donor placentas. Ophthalmologe. 2017 Dec 12. doi: 10.1007/s00347-017-0626-4
- Heckmann N, Auran R, Mirzayan R, Am J Orthop Application of Amniotic Tissue in Orthopedic Surgery. (Belle Mead NJ). 2016 Nov/Dec;45(7):E421-E425
- Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. J Cell Physiol. 1999;179(3):325–335

PAINWEEK

References

- Walraven M, Gouverneur M, Middelkoop E, Beelen RH, Ulrich MM, Altered TGF-β signaling in fetal fibroblasts: what is known about the underlying mechanisms. Wound Repair Regen. 2014 Jan-Feb;22(1):3-13. doi: 10.1111/wrr.12098. Epub 2013 Oct 17.
- King AE, Paltoo A, Kelly RW, et al. Expression of natural antimicrobials by human placenta and fetal membranes. Placenta. 2007;28(2–3):161–169
- Talmi, Y, Sigler, L, Inge, E, Finkelstein Y, Zohar Y. Antibacterial Properties of Human Amniotic Membranes. Placenta. 1991;12(3):285–288
- Mermet I, Pottier N, Sainthillier JM, et al. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Repair Regen. 2007;15(4):459–464
- Adly OA, Moghazy AM, Abbas AH, et al. Assessment of amniotic and polyurethane membrane dressings in the treatment of burns. Burns. 2010;36(5):703–710
- Salehi SH, As'adi K, Mousavi SJ, Shoar S, Evaluation of Amniotic Membrane Effectiveness in Skin Graft Donor Site Dressing in Burn Patients. Indian J Surg. 2015 Dec;77(Suppl 2):427-31. doi: 10.1007/s12262-013-0864-x
- Lorusso R, Geraci, L, Masellis, M. The treatment of superficial burns with biological and synthetic material: frozen amnion and biobrane. Annals of the MBC. 1989;2(2):79–84
- Li Z, Maitz P, Cell therapy for severe burn wound healing. Burns Trauma. 2018; 6: 13. doi: 10.1186/s41038-018-0117-0
- Niknejad H, Peirovi H, Jorjani M, et al. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater. 2008;15:88–99
- Woodall BM, Elena N, Gamboa JT, Anterior Cruciate Ligament Reconstruction With Amnion Biological Augmentation. Arthrosc Tech. 2018 Mar 19;7(4):e355-e360. doi: 10.1016/j.eats.2017.10.002
- Johnson HL. Observation on the prevention of post operative peritonitis and abdominal adhesions. Surg Gynac Obstet. 1927;XLV:612
- Kerry Rennie, Andrée Gruslin, Markus Hengstschläger, et al., “Applications of Amniotic Membrane and Fluid in Stem Cell Biology and Regenerative Medicine,” Stem Cells International, vol. 2012, Article ID 721538, 13 pages, 2012

PAINWEEK


DARN IT, THORTON!
YOU LEFT THE STEM CELLS
SITTING OUT TOO LONG!
NOW THEY'RE NO GOOD!!