Latest Applications of Amniotic Fluid Stem Cells

A New Breakthrough in Aesthetic Medicine & Orthopedics
Objectives

To give you an understanding of why and how amniotic fluid can help patients in various indications.
• In the early days of gestation, amniotic fluid is composed of water and electrolytes.

• At 12-14 weeks, it transforms into a much more complex fluid.

• Now it has water, electrolytes, proteins, carbohydrates, lipids, phospholipids, hyaluronic acid, a plethora of growth factors and stem cells.

• It also possesses antibacterial, anti-inflammatory properties. Its anti-adhesion qualities are also valuable in organ regeneration for skin & bone regeneration.
The Problem

Low Knowledge
Patients know very little about latest stem cell applications

Treatment
Current treatment limitations hinder best possible clinical outcomes

Decision Making
Factors like poor awareness, oscillating costs and physician scarcity make it difficult to decide.

Cost
Treatment costs are high but it not communicated to the patients that actually they are saving money in maintenance & aftercare.

Variety
It is usually good to multiple options for any problem, but it may cause a dilemma if you don’t know what you need and what you want.
Prioritize

Establish the superiority of AF derived stem cell treatment over the conventional one.

Communicate

Let the patient know the benefits it offers and disadvantages (if any)

Implement

Perform the procedure e.g. transplantation and topical applications.

Solution

Organ regeneration is the ideal solution for most of the skin and orthopaedic conditions requiring medical interventions.
What Is Regenerative Medicine?

As our world continues to evolve, the field of regenerative medicine follows suit. Although many modern day therapies focus on synthetic and natural medicinal treatments for skin, bones, brain and all other vital organs’ repair, many of these treatments and prescriptions lack adequate results or only have the ability to slow the progression of diseases or injuries.

Regenerative medicine seeks to replace tissue or organs that have been damaged by disease, trauma, or congenital issues, vs. the current clinical strategy that focuses primarily on only treating the symptoms.
Concentrations in the Field of Regenerative Medicine

1. Tissue Engineering and Biomaterials

2. Cellular Therapies

3. Medical Devices and Artificial Organs
Concentrations in the Field of Regenerative Medicine

1. Tissue Engineering and Biomaterials

Tissue engineering is a strategy where biologically compatible scaffolds are implanted in the body at the site where new tissue is to be formed.
Concentrations in the Field of Regenerative Medicine

2. Cellular Therapies

Many millions of adult stem cells are found in every human. Our body uses stem cells as one way of repairing itself. Studies have illustrated that if adult stem cells are harvested and then injected at the site of diseased or damaged tissue, reconstruction of the tissue is feasible under the right circumstances.
3. Medical Devices and Artificial Organs
In cases where an organ fails, the predominant clinical strategy is to transplant a replacement organ from a donor. Using circulatory support as an example, there are technologies in various stages of maturity, initially using ventricular assist devices (VADs) as a bridge to a heart transplant, and now there are VADs that are used for long-term circulatory support (destination therapy).
Stem cell biology, construed in its broadest sense, has forced medicine to view development and disease, and subsequent potential therapies, from an entirely different perspective.

We have learned that there is an inborn plasticity and flexibility “programmed” into the organism and its organ systems. The repository of this plasticity is thought to be the stem cell—the most primordial cell in the body and in any given structure.
Tackles Core Problem

focusses on replacement, repair and regeneration of cells, tissues and organs.

Safe Differentiation

Amniotic fluid stem cells are broadly multipotent, that is, they can differentiate into all embryonic germ lineages, but they do not form tumors.

Tested

a suitable cell source for tissue engineering and their ability for the repair of muscle, cartilage and bone defects have been tested in established animal models.

Authentic

the ability to generate progenitors of a wide range of lineages render AFSC attractive candidates for regenerative medicine-based treatments against both congenital and acquired disorders.

Amniotic Fluid Derived Stem Cells

AF stem cells can be expanded, differentiated and used together with scaffolds for engineering of tissues and organs.
AF is safe, effective and has no significant risk of GVHD (graft vs host disease)

Other benefits include
- Richest sources of growth factors and interleukins.
- AF can improve many areas of an aging face, damaged skin and hair follicles.
- The orthopedic applications are diverse and clinically proven.
Aesthetic dermatology is becoming a vital and important branch of medicine. To meet and anticipate patient needs, it is necessary to ensure that the technologies being invested in are tried and true and “work the way they say they will.”

Dermatological Applications of AF Stem Cells & AF Mesenchymal stem cells
Major Benefits in Dermatology

AF is a fountain of youth, full of growth factors healthy rebuilding proteins & peptides, anti-inflammatory & anti-aging molecules.

**Research**
More than 200 growth factors, cytokines and peptides have been isolated from AF

**Ease of Application**
In severe cases of skin trauma, amniotic membrane can be topically applied to accelerate healing and it reduces the need for skin grafting.

**No Ethical Conflict**
It offers an ethical use of stem cells & GFs to be used in medicine unlike embryonic stem cells which have ethical issues.
What are Growth Factors & How they Work

Growth factors are an extremely heterogeneous group of proteins that regulate the growth and differentiation of various cell types including skin, bone and nerve cells. They also act as chemical messengers or signaling factors (1).

Problem

• Skin aging is primarily due to a decrease in collagen I content, fragmentation of collagen fibrils, and elastosis (accumulation of Elastin in skin).
• Skin cells are damaged due to exposure & environmental factors.

Role of GFs

• Growth factors and cytokines are included in several cosmetic products intended for skin rejuvenation because of their ability to promote collagen synthesis. (2)
• Gf’s repair damaged cells & recruit other cells to assist in repair.
• They regulate many aspects of cellular function, including survival, proliferation, migration and differentiation.
fibroblast cells are the "construction workers" of skin, the source of GF. A decline in the number of fibroblast cells results in:

- less production of collagen, ECM, self repairing
- decreased concentration of growth factors (3)

Skin loses around 1% of the number of fibroblast cells and 1% of its thickness per year. Fibroblasts number and GF production drops with age and sun exposure (4).

Sun damaged skin has 35% fewer capillaries (3) This is why GF are needed to augment the results of skin treatments in older, smokers or sun damaged patients (5).
The role of growth factors in differentiation of stem cells provides an avenue for creating an unlimited supply of embryonic-like stem cells, bypassing the current ethical issues.
Researchers are enhancing their already significant understanding of how growth factors affect stem cell expansion and differentiation. Once they can completely control the fate of pluripotent stem cells, which is influenced by both physical (attachment factors) and biochemical cues (growth factors), they will be able to direct these cells to become the specialized cells that make up all the tissue in the body. This enables subsequent use of stem cells in cell-based therapies, drug development, and disease modeling.

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Sub-types</th>
<th>Functions</th>
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</table>
| Activins/Inhibin | Activin A, Activin B, Inhibin | • Mesodermal induction  
• Neural cell differentiation |
| BMPs | BMP-2, BMP-2a, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8b, BMP-10 | • Bone formation  
• Induction of ventral mesoderm |
| FGF | FGF acidic, FGF basic, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-12, FGF-16, FGF-17, FGF-18, FGF-19, FGF-20, FGF-21, FGF-22, FGF-23 | • Cell proliferation, differentiation and migration  
• Embryonic development and angiogenesis |
| IGF | IGF-I, IGF-II | • Maintenance of pluripotency, differentiation and proliferation of myeloid cells  
• Promotion of neural stem cell self renewal, neurogenesis and cognition |
| TGF-beta | TGF-β1, TGF-β2, TGF-β3 | • Maintenance and differentiation of embryonic stem cells and somatic stem cells |
| Wnt | Wnt-1, Wnt-2, Wnt-7a | • Cell survival, proliferation and polarity  
• Tissue homeostasis, tissue patterning and cell fate |
The results of in vitro and clinical studies suggest that cosmetic products containing growth factors, cytokines, matrikines, or matrikine-like peptides can enhance the production of collagen and other ECM molecules and promote skin rejuvenation. (7)

A novel skin cream containing a mixture of human growth factors and cytokines was recently marketed for skin rejuvenation. The cream significantly reduced periorbital and perioral wrinkles, as well as improved skin texture of the chin after one month of treatment, which confirms the beneficial use of growth factors and cytokines for skin rejuvenation reported in 2 earlier studies. (8)

This study demonstrates that addition of a topical formulation of growth factors and cytokines to a basic skin care regimen reduces the signs of photoaging. (9)

A soluble human extracellular matrix material with growth factors and proteins was safe and clinically effective in reducing anti-aging effects in this group of female subjects aged 35-65 years as measured by both investigator assessments and subjects' self-assessments. (10)
List of GFs with each GF working on a specific function

TGF-Beta
B1, B2 & B3
- Stimulates collagen, glucosamine & glycogen production.
- Increases fibronectin synthesis.
- Inhibits matrix degradation.
- Facilitates cell chemotaxis.

PDGF
Platelet Derived GF
- Stimulates angiogenesis.
- Promotes wound healing.
- Removes scarred tissue.

GM-CSF
Granulocyte-Monocyte Colony Stimulating Factor
- Increases proliferation & differentiation of hematopoietic cell lines.
- Improves monocyte & macrophage functioning.
- Increases leukocyte activity.
List of GFs with each GF working on a specific function (continued)

<table>
<thead>
<tr>
<th>Interleukins</th>
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<tr>
<td>IL-3, IL-6, IL-7 &amp; IL-8</td>
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- Enhances cell defence.
- Regulate cell homeostasis.
- Act as anti-inflammatory agents to improve wound healing.
# Key Terms in Cosmetic Dermatology

<table>
<thead>
<tr>
<th>Terms</th>
<th>Features &amp; Characteristics</th>
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<tbody>
<tr>
<td>Collagen</td>
<td>Collagen is the protein that gives our skin structure and strength. Our skin is made up of almost 80% collagen. With age we lose collagen and the firmness of our skin deteriorates.</td>
</tr>
<tr>
<td>Elastin</td>
<td>Elastin is what gives our skin the elasticity. With age the elasticity is reduced and lost especially on face. Face receives more sun exposure than the rest of the body. Skin becomes thinner and fragile.</td>
</tr>
<tr>
<td>New Cell Turnover</td>
<td>The skin is the largest organ in the body. Needs to renew itself as the dead cells are being shed. With age this process gets slower. Causing the skin to become drier and thinner.</td>
</tr>
<tr>
<td>Loss of Subcutaneous (Hypodermis Layer) Fat</td>
<td>The protective fat of the hypodermis layer that helps give the body structure. It is lost over time as we age. This loss also makes the skin thin and wrinkled.</td>
</tr>
</tbody>
</table>
Cell cultures of AFSC have been differentiated into fat, bone, muscle, heart, skin, lung and neural tissue. Commercially available AF is usually acellular.

- **Platelet Derived Growth Factor (PDGF)**
  Released by the activated platelets. Powerful chemoattractant

- **Transforming Growth Factor - Beta (TGF-B)**
  Plays a major role in matrix formulation and healing

- **Vascular Endothelial Growth Factor (VEGF)**
  Stimulates endothelial growth and angiogenesis

- **Fibroblast Growth Factor (FGF)**
  Family of growth factors involved in angiogenesis, wound healing

- **Epidermal Growth Factor (EGF)**
  Linked to angiogenesis and collagen deposition at wound sites. Shown to stimulate wound repair in fibroblast and epithelial cells

- **Insulin-like Growth Factor - 1 (IGF-1)**
  Cellular recruitment. Orchestrator of cellular proliferation
Amniotic fluid exists as a promising donor source of stem cells for the treatment of multiple clinically relevant conditions.

**Comprehensive**
AF contain all the components to fully alter the health of multiple tissues and organs.

**Versatile**
Based on morphology and growth characteristics, AFSC can differentiate to many cells but most easily develop into epithelial cells and fibroblasts.

**Effective**
Robust reduction of infarct volumes by 92% and reduced local inflammation. (11)
Improved reference memory. (12)
Summary of the most important markers identified in AFCs and AMCs by the use of transcriptomic, proteomics, secretome, and immunophenotypic analyses. Proteins identified in more than one study are marked in bold. (13)
There are a lot of orthopedic conditions and injuries that presently have limited treatment options available. Here regenerative technologies come up as a ray of hope among surgeons for such conditions by functionally repairing the tissues and organs using growth factors, stem cells and products developed by genetic engineering with the advancement in the stem cells research field.

Orthopedic Applications of AF Stem Cells & AF Mesenchymal stem cells
What orthopedic condition is?

• Orthopedic condition basically refers to the injuries and diseases of the body's musculoskeletal system. This complex system includes your bones, joints, ligaments, tendons, muscles, and nerves. It allows you to move, work, and be active.
Aging, exercise, sports and injuries cause excessive wear and tear of the body. As we age, the process of repair is slowed down due to reduced production of Mesenchymal Stem Cells (repair cells). This causes the joints’ elastic tissue to become stiff and lose its elasticity, thereby increasing its susceptibility to damage. This problem can be treated with stem cell therapy, where your own body’s cells can be used to repair and promote healing of degenerated or injured joints.
## ORTHOPAEDIC CONDITIONS

<table>
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<tr>
<th>Injury and Mechanical Derangement</th>
<th>Metabolic and Endocrine Disorders</th>
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<tbody>
<tr>
<td>Congenital and Developmental Abnormalities</td>
<td>Tumors and Lesions that mimic them</td>
</tr>
<tr>
<td>Infection and Inflammation</td>
<td>Neurological Disorders</td>
</tr>
<tr>
<td>Arthritis and Rheumatic Disorders</td>
<td>Muscle Weakness</td>
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</table>
Role of AFSC Therapy in Orthopedics

- Osteoarthritis
- Spinal cord injuries
- Degenerative vertebral discs
- Bone fractures
- Chronic tendonitis
- AFSC Therapy
Researchers have found that Mesenchymal Stem Cells (MSC’s) obtained from the AF have the capacity to detect changes in the body which are causing pain or dysfunction, and to respond and communicate with the local environmental needs to provide the correct therapy needed for healing. These stem cells are commonly referred to as “repair cells” for their ability to heal injured organs or body parts. Their primary functions are:

- Acceleration of tissue repair process.
- Stimulation of tissue regeneration.
- Decrease inflammation in the affected area.
- Prevention of further cell death.
- Creation of new blood vessels.
General Procedure of AFSCs & AFMSCs
Amniotic Fluid Stem Cells in Bone Healing

AFMSCs are able to differentiate into osteoblasts under the influence of growth factors:

- **BMPs**: Bone morphogenetic proteins
- **PDGF**: Platelet-derived growth factor
- **Transforming growth factor beta**
- **IGF**: Insulin-like growth factor
- **Fibroblast growth factor PTH**: parathyroid hormone
Orthopedic Applications of Stem Cells

**Non-union FRACTURES**

Non-union is a serious complication of a fracture and may occur when the fracture moves too much, has a poor blood supply or gets infected.

**Bone Fillers & Substitutes**

Bone grafting represented 500,000 procedures per year in the United States, and more than 2 millions in the world.

**Avascular Necrosis**

Avascular necrosis (AVN), also called osteonecrosis, aseptic necrosis or ischemic bone necrosis, is a condition that occurs when there is loss of blood to the bone.

**Cartilage, Tendon, Ligament & Arthritis**

A recent study says as many as 91 million Americans may really have arthritis – when you add together those who are officially diagnosed plus those who report obvious symptoms but haven’t been diagnosed.
Other Less Common Orthopedic Conditions Warranting AFSCs & AFMSCs Potential Use

<table>
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<tr>
<th>MENISCUS</th>
<th>INTERVERTEBRAL DISC DEGENERATION</th>
<th>Sacro-iliac Joint Dysfunction</th>
<th>Total Knee or Hip Arthroplasty</th>
<th>Periprosthetic Fractures</th>
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<tr>
<td>Recommendations</td>
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<tr>
<td>Stem cell therapy is an attractive option for the treatment of intractable diseases.</td>
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<td>Its use is based on sound biological principles.</td>
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<td>Many of these studies have shown good results.</td>
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FDA Approval of Various SC Treatments
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<tr>
<th>Treatment (Sourced from)</th>
<th>Indications</th>
</tr>
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<tbody>
<tr>
<td>ALLOCORD/ CLEVECORD/ HEMACORD/ DUCORD (HPC Cord Blood)</td>
<td>For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.</td>
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<tr>
<td>LAVIV (Azficel-T)</td>
<td>Indicated for improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults.</td>
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<tr>
<td>GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)</td>
<td>Indicated for topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults.</td>
</tr>
<tr>
<td>IMLYGIC (talimogene laherparepvec)</td>
<td>Indicated for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery.</td>
</tr>
<tr>
<td>KYMRIAH (tisagenlecleucel)</td>
<td>Indicated for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse.</td>
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<tr>
<td>LUXTURNINA (adeno-associated virus vector-based gene therapy)</td>
<td>Indicated for the treatment of patients with confirmed Biallelic RPE65 mutation-associated retinal dystrophy. Patients must have viable retinal cells as determined by the treating physician(s).</td>
</tr>
<tr>
<td>PROVENGE (sipuleucel-T)</td>
<td>For the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer.</td>
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<tr>
<td>YESCARTA (axicabtagene)</td>
<td>Indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma</td>
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</table>
Stem cell products have the potential to treat many medical conditions and diseases. But for almost all of these products, it is not yet known whether the product has any benefit—or if the product is safe to use.

If you're considering treatment in the United States:

Ask if the FDA has reviewed the treatment. Ask your health care provider to confirm this information. You also can ask the clinical investigator to give you the FDA-issued Investigational New Drug Application number and the chance to review the FDA communication acknowledging the IND. Ask for this information before getting treatment—even if the stem cells are your own.

Request the facts and ask questions if you don’t understand. To participate in a clinical trial that requires an IND application, you must sign a consent form that explains the experimental procedure. The consent form also identifies the Institutional Review Board (IRB) that assures the protection of the rights and welfare of human subjects. Make sure you understand the entire process and known risks before you sign. You also can ask the study sponsor for the clinical investigator’s brochure, which includes a short description of the product and information about its safety and effectiveness.

If you're considering treatment in another country:

Learn about regulations that cover products in that country.

Know that the FDA does not have oversight of treatments done in other countries. The FDA typically has little information about foreign establishments or their stem cell products.

Be cautious. If you’re considering a stem cell-based product in a country that may not require regulatory review of clinical studies, it may be hard to know if the experimental treatment is reasonably safe.
FDA has Approved Numerous Stem Cell–Based Treatments for Clinical Trials

• A 2013 report from the Pharmaceutical Research and Manufacturers of America lists 69 cell therapies as having clinical trials under review with the FDA, including 15 in phase 3 trials.

• The therapeutic categories represented in these trials include cardiovascular disease, skin diseases, cancer and related conditions, digestive disorders, transplantation, genetic disorders, musculoskeletal disorders, and eye conditions, among others. (17)
## Overview of Risk Factors & Risks Associated with AFSC Therapy

<table>
<thead>
<tr>
<th>Risk factors or hazards</th>
<th>Identified risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic factors (Origin of cells e.g. autologous vs. allogenic, diseased vs. healthy donor/tissue)</td>
<td>Rejection of cells</td>
</tr>
<tr>
<td>Cell characteristics - Differentiation status</td>
<td>Disease susceptibility</td>
</tr>
<tr>
<td>Extrinsic factors - Lack of donor history</td>
<td>Disease transmission</td>
</tr>
<tr>
<td>Manufacturing and handling</td>
<td>Reactivation of latent viruses &amp; Cell line contamination</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td>Undesired immune response (e.g. GVHD)</td>
</tr>
<tr>
<td>Wrong Indication</td>
<td>Unintended physiological and anatomical consequences (e.g. arrhythmia)</td>
</tr>
<tr>
<td>Use of immune suppressive Drugs</td>
<td>Lack of efficacy</td>
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</tbody>
</table>
References

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Epidemiology of Fracture Nonunion in 18 Human Bones.


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