

Amniotic Fluid

A New [Breakthrough](#) in Aesthetic Medicine



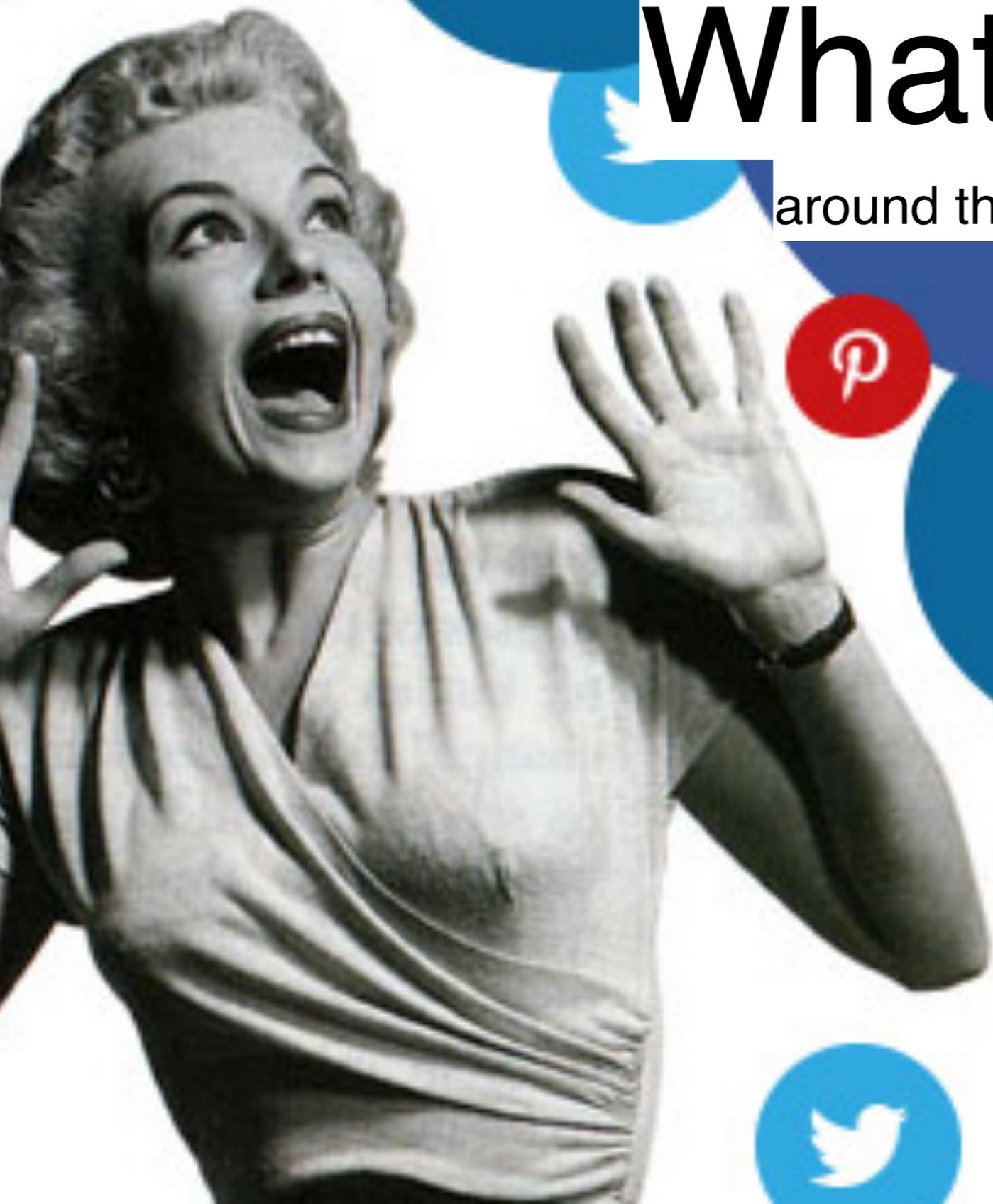
- to give you an understanding of why and how to start using amniotic fluid in your aesthetic practice.



What is Amniotic Fluid?

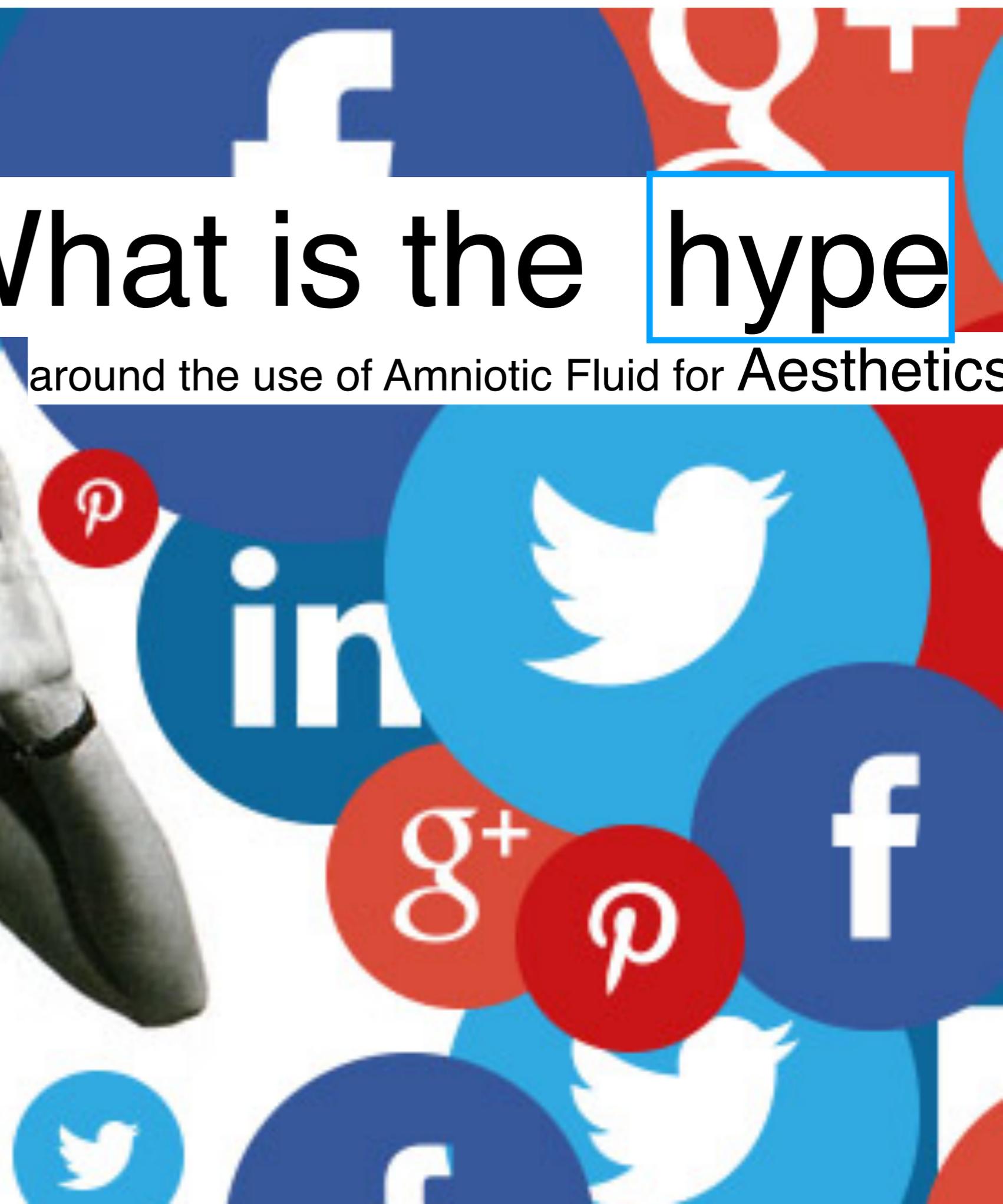
Amniotic fluid (AF) in the first days of gestation comes primarily from the mother. Initially it is composed of water and electrolytes. It transforms at age 12 -14 weeks into a much more complex fluid. At this point it has water, electrolytes, proteins, carbohydrates, lipids, phospholipids, hyaluronic acid, a plethora of growth factors and stem cells.

AF also contains antibacterial, anti-inflammatory properties. Its anti-adhesion qualities are also valuable in organ regeneration For skin regeneration the best age for amniotic fluid collection is after age 25 weeks when the fetal skin keratinization is complete.



What is the **hype**

around the use of Amniotic Fluid for Aesthetics



Amnio can improve many areas of an aging face, damaged skin and hair follicles. If properly obtained it is safe, effective and has no significant risk of GVHD.

AF is one of the **richest** sources of growth factors and interleukins found.



The main reasons amnio has become so popular is the ease of collection and the growing body of scientific evidence showing its consistent effectiveness in regenerating skin and other organs.

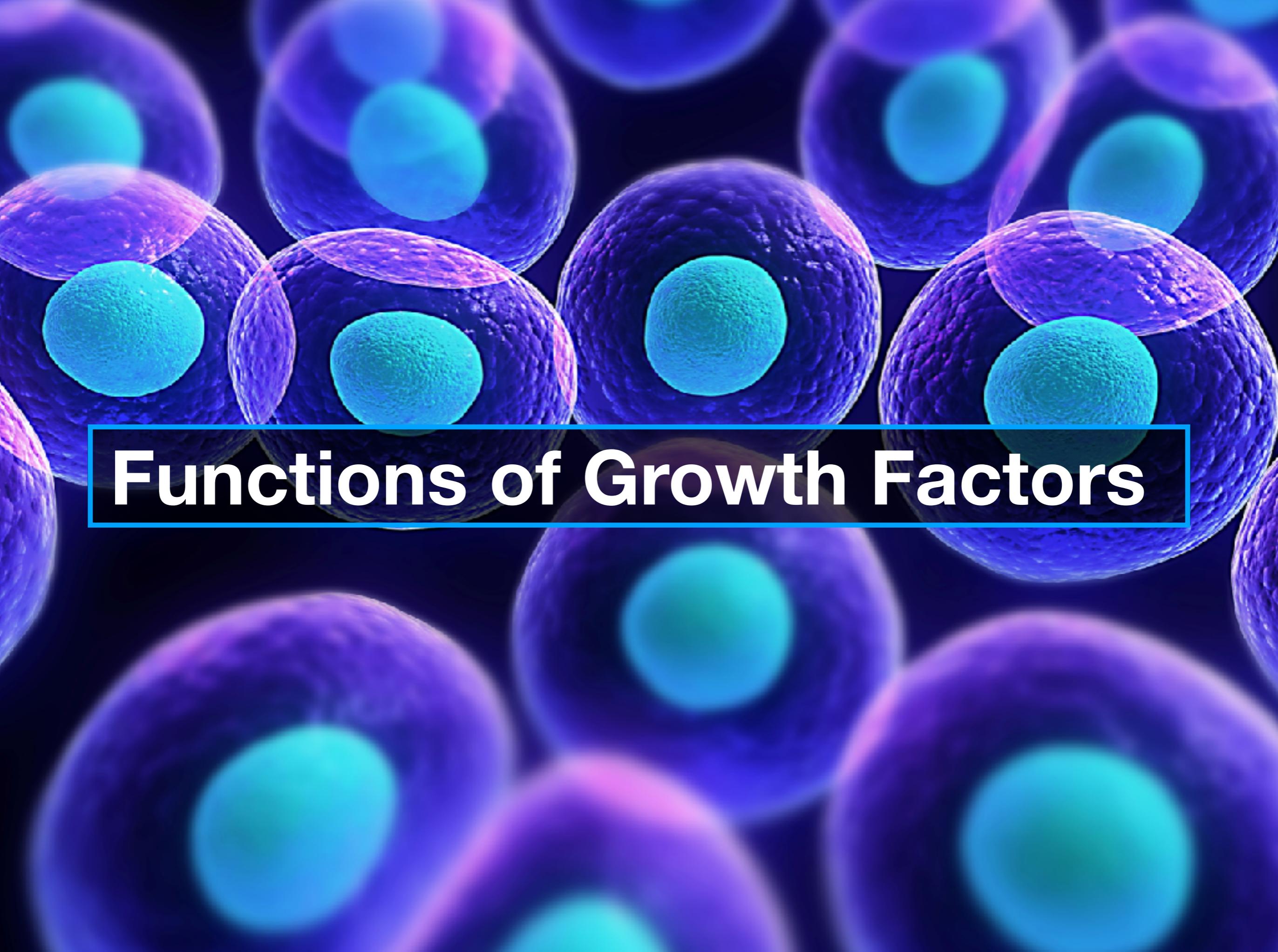
The scientific interest and active study of Amniotic fluid began decades ago; Today consumers are more aware and better educated on the alternative therapies that can regenerate without needing to operate.

The mayor benefit of amnio is the larger percentage and representation of growth factor types. In severe cases of skin trauma, amniotic membrane can be topically applied to accelerate healing and provide a scaffolding without need for skin grafting.

Amnio presents a way to apply biologics to medicine without ethical conflict . We know this fluid is a fountain of youth , full of growth factors healthy rebuilding proteins anti-inflammatory and anti-aging molecules.

Based on Sophisticated analysis at advanced research centers worldwide, over 200 growth factors, cytokines and peptides have been isolated from this fluid.

Put in prospective, adult PRP only has a fraction!!!

A microscopic view of numerous cells, likely fibroblasts or epithelial cells, arranged in a dense, overlapping pattern. Each cell has a prominent, bright blue nucleus and a textured, purple cytoplasm. The cells are set against a dark background, making the glowing nuclei stand out. The overall appearance is that of a cell culture or a tissue section stained with fluorescent dyes.

Functions of Growth Factors

Growth Factors (GF) are usually proteins that bind to receptors on the cell surface. GF primarily activate proliferation and differentiation of stem cells. GF are chemical messengers or signaling factors.

- Turn a variety of cellular activities “on” and “off”
- Repair damaged cells
 - Recruit other cells to assist in repair
- Signal angiogenesis
 - Build ECM: Collagen, elastin, fibronectin

- 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
- 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

Sun damaged skin has 35% fewer capillaries³

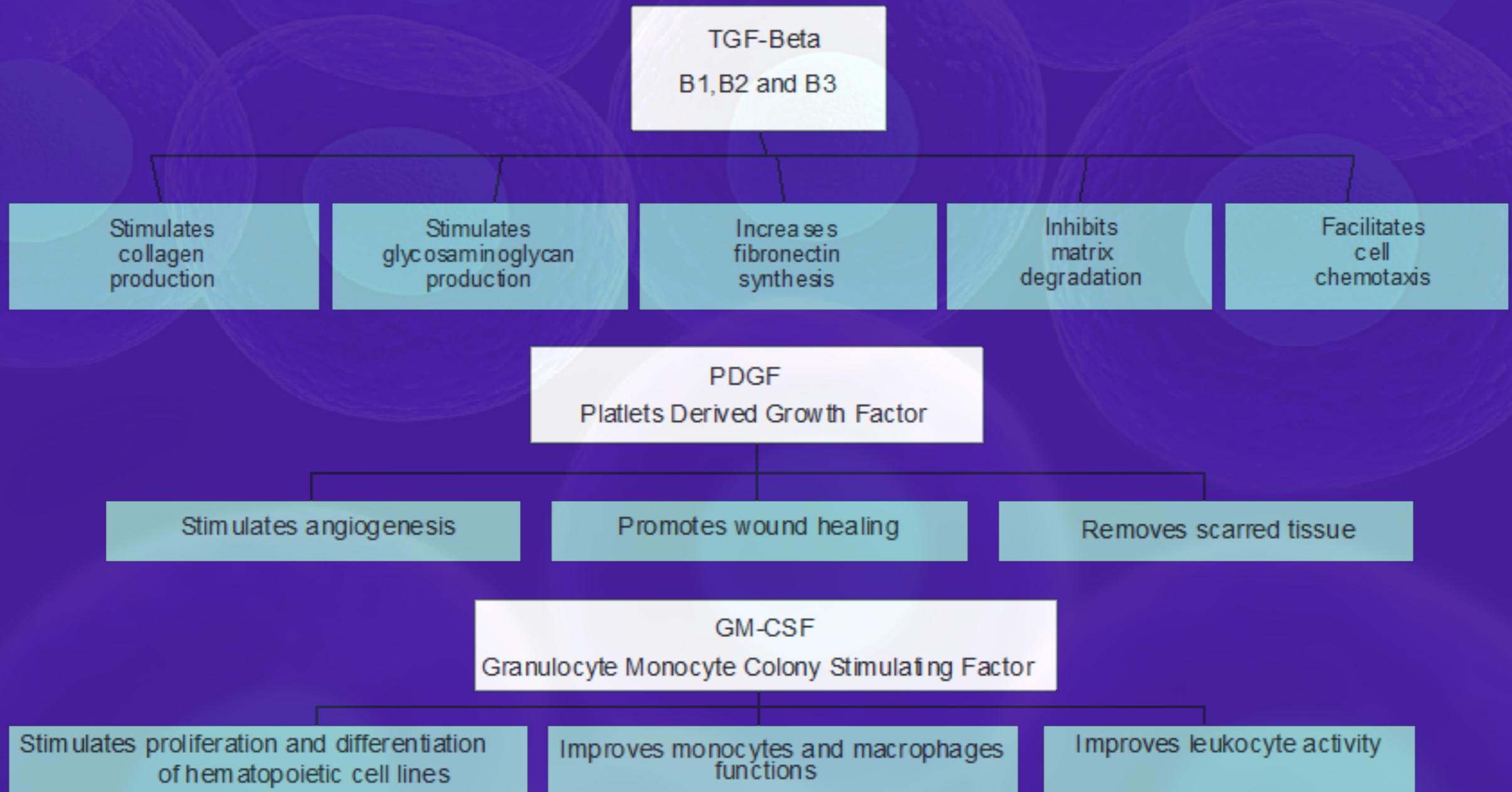
This is why GF are needed to augment the results of skin treatments in older, smokers or sun damaged patients

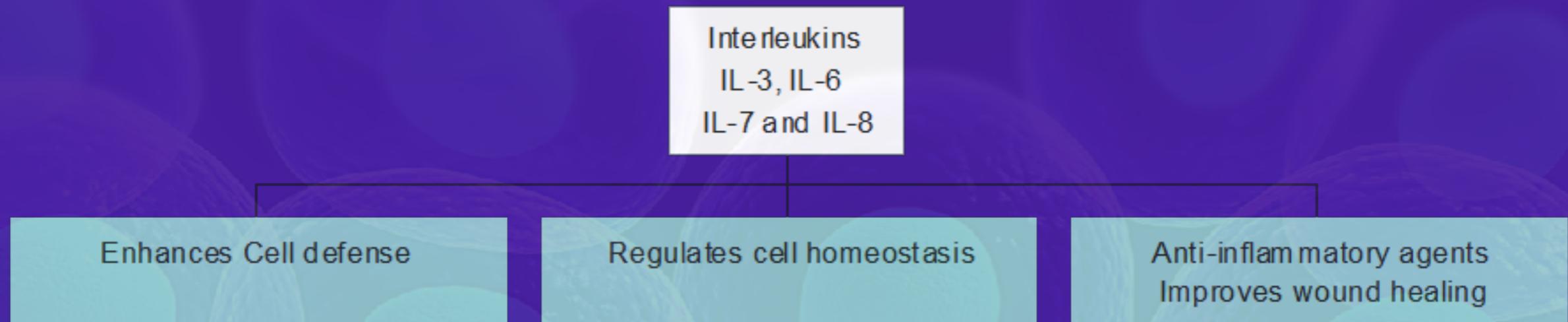
1. Molecular Aspects of Dermatology, GC Priestley, ed. John Wiley & Sons, NY 1993

2. Arch Dermatology 138 (11): 1462-70, 2002

3. Growth Factors and Wound Healing, Ziegler, Pierce, Herndon, ed. Springer-Verlag. NY 1997

Specialized Growth Factor Chart





This is a specific list of Growth Factors with each Growth Factor working on a specific function in harmony with each other.

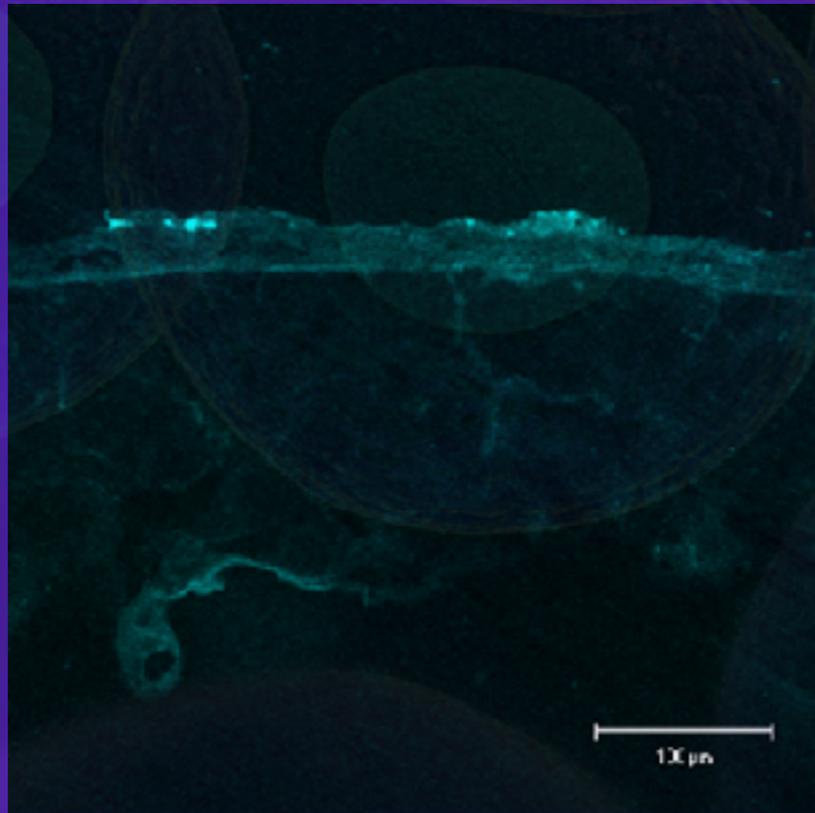
- **Collagen** – Collagen is the protein that gives our skin structure and strength. As our skin is made up of almost 80% collagen. As we age we lose collagen and the firmness of our skin deteriorates with that loss.
- **Elastin** – Elastin is what gives our skin the elasticity. As we age the elasticity is reduced and lost especially in the area of our faces since this area generally receives more sun exposure than the rest of our bodies. With both a loss of elastin and collagen, the skin will naturally become thinner and more fragile.
- **New Cell Turnover** – The skin, being the largest organ in the body, is constantly in a state of renewing new skin cells as the dead ones are being shed. As we start to age this process also slows, causing the skin to become drier and thinner.
- **Loss of Subcutaneous (Hypodermis Layer) Fat** – The protective fat of the hypodermis layer that helps give the body structure is lost over time as we age. This loss is also a cause of the appearance of thinning skin.

Today there is a great deal of disparity in blogs and other medical forums about the presence of stem cells in amnio.(AFSC)

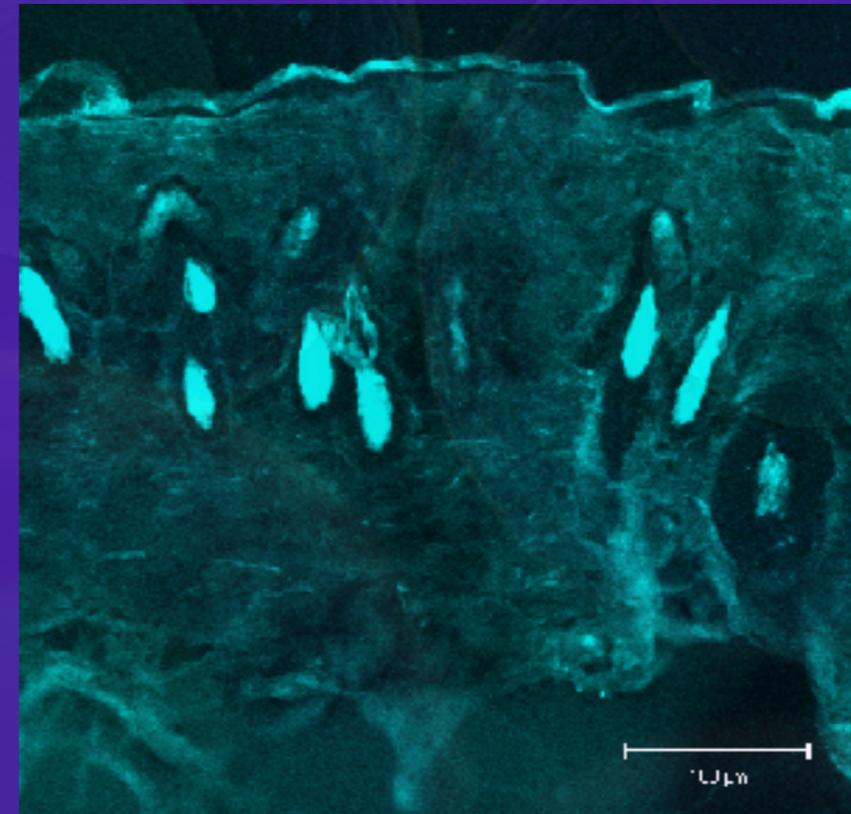
AFSC only make up 0.9 to 1.5% of all the cells in amnio. In addition, conventional tools available to clinicians and used in ordinary labs do not have the Sophisticated technology needed to confirm their presence .

In properly equipped research labs it has been shown that AFSC replicate in as little as 36 hours maintaining normal karyotype without alteration in telomeres when taken to 250 generations.!!!

Needle Based Trans-Dermal Delivery Study



Solution applied without Derma Stamp



Solution applied with Derma Stamp

Solution penetration study with Hoechst 33342 (Glowing Substance)

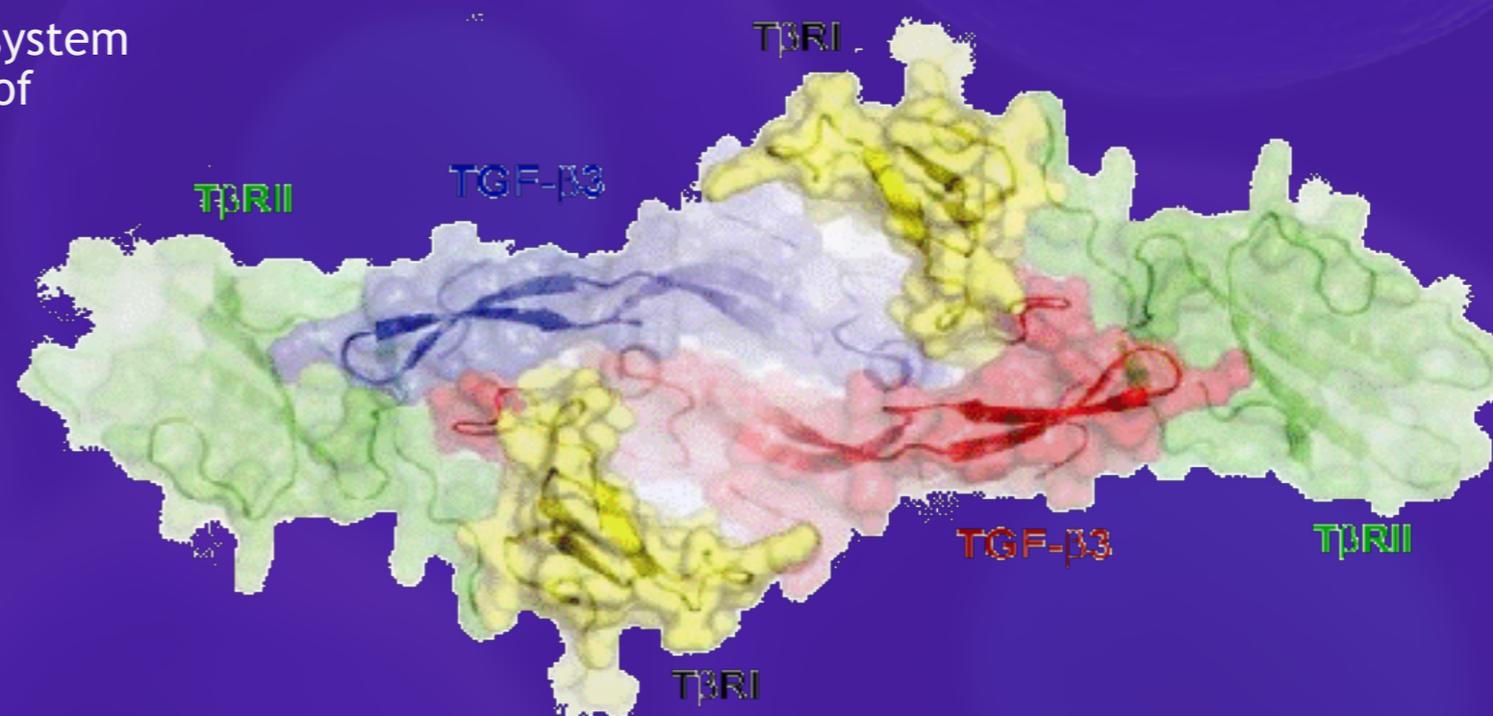
How does Science define Growth Factors & Cytokines?

- Growth factors are very specific peptides (proteins) that emit biological signals involved in the regulation of cell growth and function.^{1,3}
- For Example: TGF- β is a potent stimulator of collagen production, promotes the synthesis of extracellular matrix proteins called glycosaminoglycan's (GAG's), and inhibits extra cellular matrix degradation (thinning of your skin).^{1,2,3}
- Cytokines are associated with immune system cells and play a key role in modulation of inflammation
- For example: PDGF (Platelet-derived growth factors) and GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), can stimulate extracellular matrix production, reduce inflammation, and promote the formation of new blood vessels.^{1,3}

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Function of Cytokines and GF's

Cytokine	Function
TGF-beta	<ul style="list-style-type: none"> Stimulates collagen, elastin and fibronectin production
(b1, b2, b3)	<ul style="list-style-type: none"> Stimulates fibroblast collagen and glycosaminoglycan production Increases fibronectin synthesis Inhibits matrix degradation Facilitates cell chemotaxis (healthy communication environment)
PDGF	<ul style="list-style-type: none"> Stimulates fibroblast collagen and glycosaminoglycan production Stimulates angiogenesis (new blood vessel growth) Stimulates wound contraction Recruits endogenous regenerative cells to region
GM-CSF	<ul style="list-style-type: none"> Improves leukocyte function Activates neutrophils, eosinophils, and monocytes/macrophages Recruits endogenous regenerative cells to region
Interleukins	<ul style="list-style-type: none"> Involved with Immunological Functions
(IL-3,IL-6, IL-7, IL-8, and IL-16)	<ul style="list-style-type: none"> Regulate hematopoietic and endothelial cell differentiation and proliferation Involved with microenvironment homeostasis Regulate inflammation Immune cell response to pathogens

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.

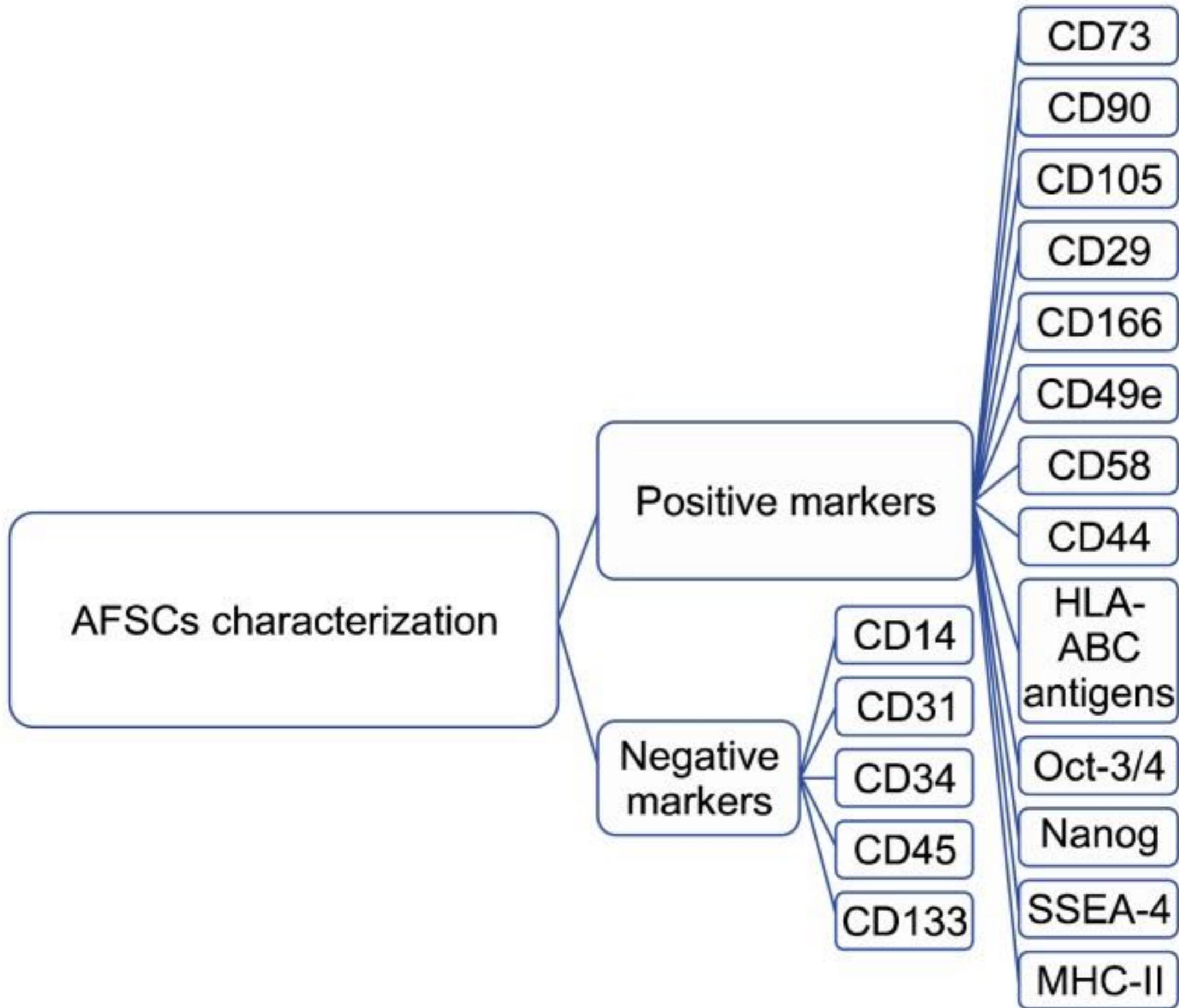
Cell cultures of AFSC have been differentiated into adipogenic, osteogenic, myogenic, cardiomyocytic, skin, lung and neural tissue. Commercially available amino however is considered acellular.



Clearly the AFSC contain all the components to fully alter the health of multiple tissues and organs.

Progenitor cells in amnio were found back in 1993. Amnio cells have mesenchymal stem cell surface marker profile. The first suggestion that AFSC were present was indirectly , based on finding dystrophin, a skeletal muscle protein. MSC appear at about 16-20 weeks by 15-17 weeks neurogenic lines are detectable.

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





The FDA monitoring and approval biologics is in process and changing quickly as more patients and scientist share the convincing data of effectiveness and safety of their use over traditional invasive procedures.

Recently Blue Cross published their support of biologics in ortho applications. The viability and finding of AFSC in amnio is real but very small which works on our favor as the FDA considers the amnio to be stem cell free. It is categorized as a biologic allograft. It requires us to follow the FDA good tissue practice and regulations.

If you choose to use Aminio in your aesthetic practice it is imperative to use a product that is minimally manipulated eg comes directly from a certified tissue transplant center and it has been fully tested pre and post collection for viral and bacterial pathogens. The amnio must come as a transplant product and kept frozen till ready to use.

Applications as per the FDA are limited to homologous sites. That translates into organs that are in direct contact with the amniotic fluid such as skin, cornea, respiratory system, renal such as kidney bladder and the urogenital area.

Risks

Unlike PRP, amnio is not autologous therefore it is most crucial that it be obtained from a certified USA tissue bank. In addition be aware that it is approved for limited applications under the FDA criteria, it is temperature labile and can be damaged by incorrect injection techniques . I hope this lecture gives you the boost needed to start your amnio practice. I have found amnio to be irreplaceable in correcting scars, acne pits and aged lower lids. I applaud you for sharing your time here to expand the horizon of non-invasive regenerative medicine.

Applications

Now I hope you can appreciate even more the magnitude of the regenerative potential of the variety of GF and other molecules in amino. The issue now is to establish protocol for its use. We know the micro needling is a good way to introduce GF into the skin. The challenge however with amnio is its heat instability. I encourage you to use it as you would an injectable filler.

It has great promise in mid face improvement and rejuvenation of thin tissue such as periorbital.

Compared to PRP it has the advantage of having a more complete and complex molecular profile. In addition it is a louder message to the treated tissue since it comes from a healthy young human. It eliminates a common issue in PRP ,the health and age of its donors. Amnio is a perfect product.

The Fibroblast

- Dermal fibroblast cells are the "construction workers" of skin. A decline in the number of fibroblast cells results in:
 - less collagen production
 - decreased concentration levels of growth factors
- Skin loses around 1% of the number of fibroblast cells and 1% of its thickness per year.
- The rate of decline in fibroblast cell number and function is increased by sun exposure, recent studies have also shown that sun damaged skin has 35% fewer blood capillaries than normal skin³; thus receiving less nutrients and oxygen than normal healthy skin.

1. Molecular Aspects of Dermatology, GC Priestley, ed. John Wiley & Sons, NY 1993

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[Stavros P. Loukogeorgakis](#) [Paolo De Coppi](#)

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Applications of Amniotic Membrane and Fluid in Stem Cell Biology and Regenerative Medicine

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