

Scientific Rationale for Use of Cytokine-Based Topical Adjuvants in Aesthetic Medical Practice

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Cytokines - Biosignals of Life

Accumulating evidence suggests that growth and development, wound healing and regeneration, and aging and senescence share a common set of molecular signaling mediators, collectively called “cytokines”. Included in the term are hundreds of proteins, peptides, and glycoproteins divided into families of molecules: cytokines, growth factors, interleukins, interferons, and others. “Cocktails” of cytokines derived from cells in culture (e.g. fibroblasts or stem cells) and from plasma concentrates (e.g. PRP) are being employed as therapeutic agents for aging skin.

However, not all cytokines are regenerative; many function as immune system stimulants that promote tissue destruction, fibrosis, and scarring. A deeper understanding of cytokines and their role in anti-aging therapeutics will help the reader discern when such treatments are likely to be beneficial, or potentially counterproductive, in aesthetic practice.

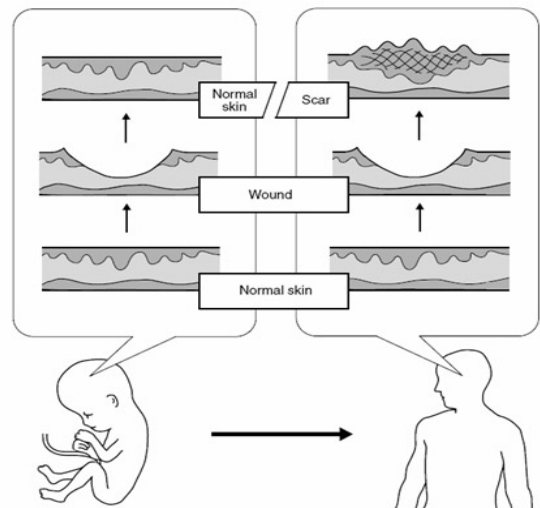
Cytokines are effective at very low concentration. In contrast to protein hormones that circulate in nanomolar (10^{-9} M) concentrations, cytokines are found in picomolar (10^{-12} M) amounts. Each individual cytokine exerts its effect through activation of a specific corresponding cell membrane receptor. Activation initiates a cascade of intracellular events that alters cell function, up-regulating or down-regulating specific gene activity. Cellular behavior is not controlled by individual cytokines but rather the net pattern of numerous molecules having complementary and/or competitive influence.

Cytokines can act within a cell, on the surface of the same cell that produces them, or on other cells in the vicinity. Nearby cell activation is termed *paracrine* signaling. Topical use of cytokines mimics paracrine signaling.

The Healing Process and Cytokine Control

All life processes are ultimately dependent on biosignaling. This is especially true of the process of healing which consists of three major phases. 1) The *inflammatory* phase is the body’s natural response to injury. At injury, blood vessels in the wound bed contract, platelets release pro-inflammatory cytokines, and a clot is formed. After hemostasis, blood vessels dilate to allow antibodies, white blood cells, growth factors, enzymes and nutrients to reach the wounded area. The predominant cells of inflammation are phagocytic neutrophils and macrophages which autolyse devitalized tissue and pathogens. 2) During *proliferation*, granulation tissue comprised of collagen and extracellular matrix develops including a new network of blood vessels to provide nutrients and oxygen. Epithelial cells finally resurface the wound. 3) *Maturation* occurs after the wound has closed and involves remodeling of collagen from type III to type I. This phase lasts months.

Fetal skin heals scar free with replacement of skin appendages (hair follicles). Adult skin heals with scar tissue and has no appendages. An extremely brief inflammatory phase is the hallmark of fetal healing. Of all the cytokines involved in skin healing, TGF β -3 (transforming growth factor beta three) is the one that most distinguishes fetal from adult healing. The fetus has abundant TGF β -3 which is strongly anti-inflammatory and promotes increased collagen turnover resulting in abundant pliable collagen III, Adult healing has a prolonged inflammatory phase with abundant stiff collagen type I.



Cells Cultured for Use in Anti-aging Products

Dating back more than a decade, human fibroblast culture has the longest history of use in topical skincare products. More recently, human stem cells have been utilized, specifically mesenchymal stem cells (MSC) of both adipose and bone marrow origin, and parthenogenetic stem cells derived from chemical stimulation of human ova to induce repetitive cellular division. One product uses cytokines harvested from co-culture of fibroblasts and adipose derived MSCs. Aside from possible antioxidant value, and because of the complete lack of proved scientific rationale for their use as biosignals for human tissues, botanical “stem cells” are not considered in this discussion.

Conditioned media is the name given to the “spent” nutrient broth in which cells have been cultured. It contains the biosignals produced by the cells during culture and is used as an active ingredient after ultrafiltration to remove cells and debris. One exception is parthenogenetic stem cells, which are lysed

through multiple freeze-thaw cycles, and used as an active ingredient that contains all constituent cell parts, cytoplasm, enzymes and proteins of all sorts, some of which degrade cytokines. Conditioned media methods permit production and isolation of cytokines for specific therapeutic purposes i.e. “designer cytokine cocktails”. Lysed cells compare to lytic whole tissue (“meat grinder”) techniques.

Of the several cell types mentioned, strong evidence supports the selection of bone marrow derived mesenchymal stem cells (BM-MSCs) as the preferred cell type to obtain cytokines for use in topical anti-aging products for the skin.

The Role of BM-MSCs in Healing

Over the past several years, researchers determined that BM-MSCs play a critically important role in tissue healing throughout the body. Rather than residing passively within the bone marrow, BM-MSCs routinely “patrol” through injured and non-injured tissue. Chemokines released from injured cells further stimulate the process. As the figure below illustrates, BM-MSCs are well known to be capable of differentiating into several cell types including myocytes, chondrocytes, osteocytes, fibroblasts, etc. In fact, BM-MSCs are the predominant cells research focuses on to grow body parts in the laboratory, treat

When repair is complete, BM-MSCs that differentiate remain as part of the new tissue. Reminiscent of 911 emergency responders, some return to the bone marrow, ready to be called into action again. Similar command and control ability is not observed in adipose MSCs, fibroblasts, or parthenogenic embryonic stem cells.

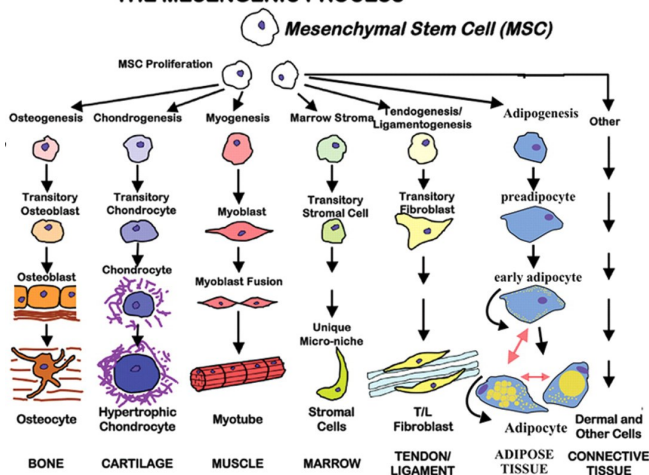
Because aging is the accumulated damage of many small injuries over time, the unique role of bone marrow derived MSCs in directing and orchestrating healing makes them particularly suitable for use in advanced anti-aging skincare formulations. As explained below, their anti-inflammatory cytokine pattern is another compelling reason.

The Inflammation – Aging Connection

Inflammation is considered a major contributing factor to the development of many diseases and degenerative conditions in the elderly, including cancer. So well accepted it has been given a name - “inflammaging.” Chronic inflammation is pro-aging for the whole organism, and especially skin.

The importance of this concept with regards to biosignals used in products intended for chronic application to the skin is that all growth factors and cytokines are either inherently pro-inflammatory, anti-inflammatory, or neutral. Because cultured cells produce many different cytokines, the collective pattern from each type of cell can also be net pro-inflammatory, net anti-inflammatory or neutral. The chart below details the inflammatory character of the major cytokines and growth factors involved in healing.

THE MESENGENIC PROCESS



cardiac failure, cardiac infarction, strokes and other medical conditions. Indeed, as part of their role in healing injury some BM-MSCs may differentiate into specific tissue cell types. That, however appears to be a secondary function.

Evidence suggests the primary role BM-MSCs play in tissue healing is to act as command and control of the entire process, something they accomplish through production of cytokines that affect local cells at the site of injury and transient cells, such as leukocytes, that migrate to the injury. BM-MSCs participate in the healing process by: 1) controlling and modulating inflammation; 2) stimulating white blood cells to remove debris; 3) triggering division of resident cells to produce more cells; 4) promoting resident cells to produce substances to create intercellular matrix – e.g. collagen, elastin, etc.; 5) differentiating into specific kinds of tissue cells needed for repair.

Inflammatory Cytokines – Acute	Inflammatory Cytokines – Chronic	Anti-Inflammatory Cytokines	Cytokine Growth Factors	Proteases & Inhibitors
IL-1 α	IL-2	IL-4	PDGF	MMP's
IL-1 β	IL-3	IL-10	VEGF	TIMP's
IL-6	IL-5	IL-13	TGF- α	
IL-8	IL-7	IL-16	TGF- β 1	
IL-11	IL-9	Wisp-2	TGF- β 2	
Ep	IL-12	IL1-RA	IFN γ	
G-CSF	IL-14	IL18-RA	FGF-b	
EGF	IL-15	TNF α -RA	FGF-a	
TNF- α		TGF- β 3	IGF-1	
		HGF	KGF	
			EGF	

Cytokines & Related Factors in Healing & Regeneration

Cytokine Patterns also Affect Facial Fat

Volume loss of the face contributes to signs of aging. Transfer of autologous fat, with or without stem cells, is therapeutic and done alone or in conjunction with lifting procedures. Experimental evidence demonstrates that pro-inflammatory cytokines promote adipose tissue destruction, while anti-inflammatory cytokines support adipose tissue expansion. Genetic predisposition, solar radiation, and exposure to

chemicals leading to cellular free radical generation, all exert their effects through the production of inflammatory cytokines.

Platelet rich plasma or fibrin matrix has been used as an adjunct to facial fat transfer. However, PRP contains pro-inflammatory and anti-inflammatory cytokines and growth factors with a net pro-inflammatory pattern (see top illustration next column.) Although high levels of VEGF assist with vascularization of transplanted fat, high levels of FGF β 1 and β 2 are associated with fibrotic healing. When applied only at the time of fat transfer, this is a transient exposure and likely insignificant.

More important is the local tissue cytokine balance at the graft site during the weeks and months post-procedure, particularly if topical cytokine products are regularly applied. Use of sunscreens and avoidance of chemical stressors improves the net anti-inflammatory cytokine balance within facial skin. Additional improvement can be achieved with topical application of cytokines that are predominantly anti-inflammatory.

Cytokine Patterns of PRP and Cell Cultures

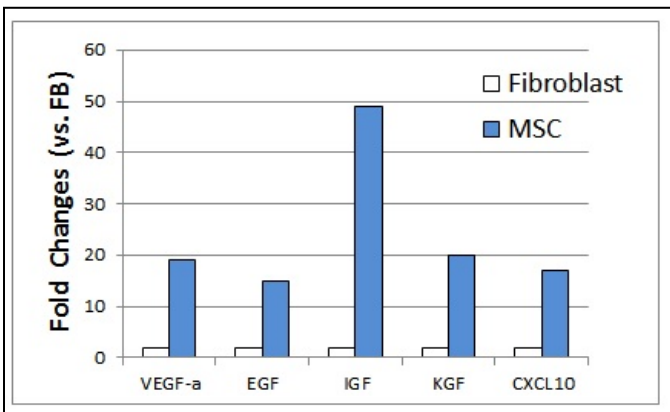
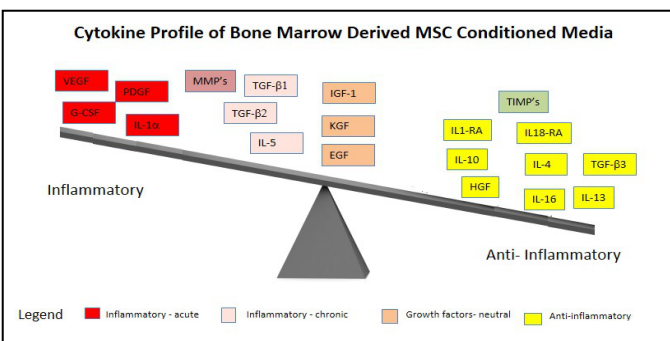
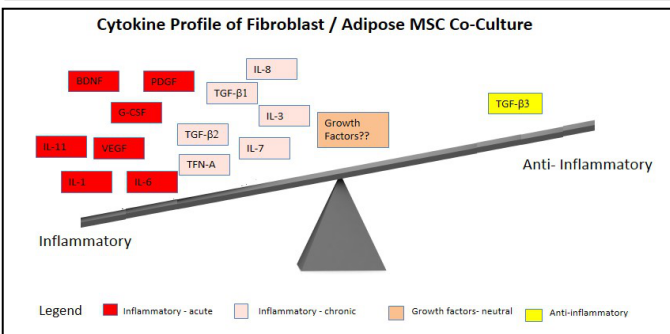
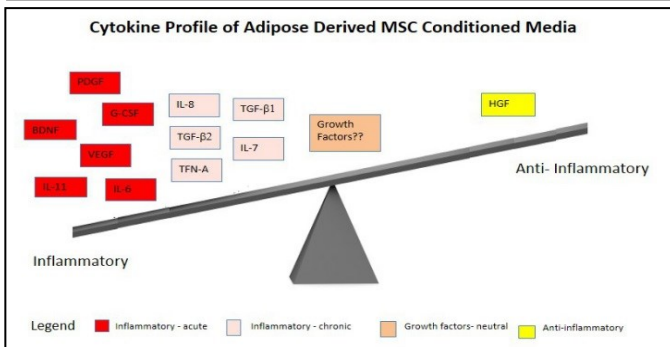
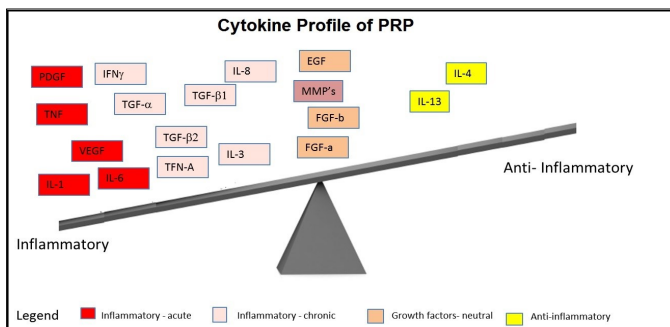
The “balance beam” illustrations in the next column depict the net effect of the cytokine patterns produced by: 1) PRP; 2) cultures of adipose derived mesenchymal stem cells; 3) co-culture of adipose MSCs with fibroblasts, and; 4) culture of BM-MSCs. These are selected because conditioned media from these three types of cell cultures are used as active ingredients in anti-aging skincare products currently being marketed. PRP is used in the “Vampire Facial.”

The PRP cytokine pattern, and those from culture of adipose MSCs, alone or co-cultured with fibroblasts, are pro-inflammatory. For adipose MSCs, this is not surprising since fat is now considered an endocrine “organ” that secretes pro-inflammatory cytokines. Excess visceral fat is a recognized risk factor for many of the medical conditions associated with aging. Studies confirm obese women have higher rates of breast cancer and suffer more recurrences of their disease.

Because inflammation is proved to be pro-aging, it is reasonable to infer that conditioned media with more anti-inflammatory effect will provide greater anti-aging benefit. Chronic application of a pro-inflammatory cytokine pattern should be seen as counterproductive to that goal.

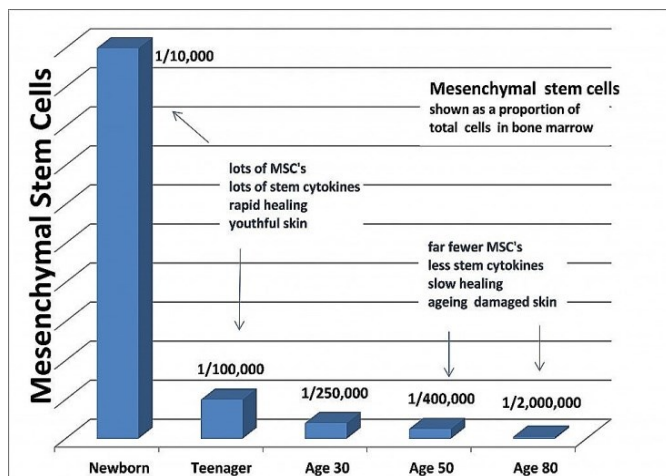
Further confirmation that BM-MSCs are the preferred cell type for topical skincare is found in a study that compared the effect on wound healing of conditioned media from fibroblasts to BM-MSCs. Bone marrow cells proved superior.

The accompanying graph to the right was taken from that study and shows the relative production of important cytokines from the two cells types during culture. (FB refers to fibroblast conditioned media; MSC to bone marrow mesenchymal stem cell conditioned media.)



Aging Effects on the BM-MSC Population

As the illustration below demonstrates, a dramatic decline in the population of BM-MSCs occurs with advancing age. A logical inference is a concomitant reduction in pro-healing and anti-inflammatory cytokines also occurs which may contribute to the slower and less efficient healing commonly seen in older individuals. This decline supports the notion that supplemental topical application of pro-healing anti-inflammatory cytokines derived from laboratory culture of BM-MSCs can result in healthier, younger-looking skin.



Producing Stem Cytokines in the Laboratory

Culturing of stem cells is a mature science with reliable and reproducible methods that yield consistent results. BM-MSCs are obtained from healthy young donors (average age 22 years), and separated from other bone marrow cells using sophisticated automated techniques at highly FDA regulated facilities. They are then cultured in the laboratory under strict sterile conditions, generation after generation (termed "passages" in the jargon), until morphologic evidence of differentiation into terminal cell types (typically fibroblasts). The process is technologically demanding yet straight forward and reproducible.

Cytokines are continuously produced as the cells grow and divide during culture. The cytokine pattern can be modulated using methods that result in preferential production of the cytokines most beneficial in the healing of skin. Lipid encapsulation of conditioned media into nanoliposomes enhances penetration of the stratum corneum.

BM-MSC Derived Cytokine Skin Rejuvenation

Topical application of a net anti-inflammatory cytokine admixture produced by BM-MSCs in culture rejuvenates skin in a way that mimics what would occur if the native population of BM-MSCs was replenished. In essence, what is lacking in vivo is fabricated ex vivo using a bioreactor in which BM-MSCs from healthy young human donors are cultured.

In the autumn of 2011, forty-five women and four men participated in a multi-month trial of an anti-aging formulation containing BM-MSC derived cytokines (AnteAGE). Twelve parameters of skin health and appearance were measured including:

Tone	Dryness	Brightness
Softness	Pore size	Blotchiness
Redness	Age spots	Fine lines
Texture	Color blend	Deep lines

The results of the trial were highly positive, subjectively and objectively. 60% of subjects considered AnteAGE the best anti-aging product they had ever used. Another 32% considered it better than most products they had tried. Four trial subjects with chronic facial redness and episodic "flare-ups" reported the anti-inflammatory effect of AnteAGE was sufficient to allow them to discontinue all other management measures.

Anecdotal "Pro-healing" User Experience

Confirming the pro-healing anti-inflammatory effect of AnteAGE, many users report noticeable benefit on the speed and quality of healing with application of AnteAGE to a variety of injuries. The first such report was when a clinical trial participant, who regularly incurred minor lacerations and abrasions from her craft hobbies, noted accelerated healing when AnteAGE was applied after injury.

Subsequent anecdotes reported benefit following thermal burns, sunburns, "road rash" from bicycle falls and following clinical procedures such as dermabrasion, microneedling, laser resurfacing, and IPL. A facial chemical peel treatment failed to induce desquamation for one woman who applied AnteAGE serum immediately after treatment.

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