Topical Cytokines & Growth Factors in Anti-Aging and Aesthetic Medical Practice

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Cytokines - Biosignals of Life

Embryogenesis, growth and development, wound healing and regeneration, and aging and senescence share a common set of bio-molecular intercellular signaling mediators, collectively called cytokines. Included in the term are hundreds of proteins, peptides, and glycoproteins which are divided into "families" of molecules - cytokines, growth factors, interleukins, interferons, and others.

In 2001, the first skincare products containing bio-signals derived from laboratory culture of human cells (fibroblasts) became available. Today, products containing bio-signals derived from culture of human adipose, bone marrow and umbilical stem cells are also marketed, as are kits to produce autologous PRP (platelet rich plasma), another source of topical bio-signals used in skin aesthetics.

More than 500 published articles confirm the value of topical bio-signals in improving the health and appearance of aging skin. Newer work by the authors and others demonstrates value when used in conjunction with aesthetic procedures such as dermabrasion, microneedling, RF microneedling, and fractional laser resurfacing.

The pattern, i.e. the relative composition and concentration of cytokines, produced by a cell culture is dependent upon the genetic destiny of the cell type being cultured, which is also directly related to its physiologic role in life. The patterns produced by fibroblasts, and stem cells of adipose, bone marrow or umbilical cord origin, are substantially and significantly different from one another. Most practitioners are unaware of these differences; despite the fact they have direct impact upon the dermatologic response that results from topically applied products.

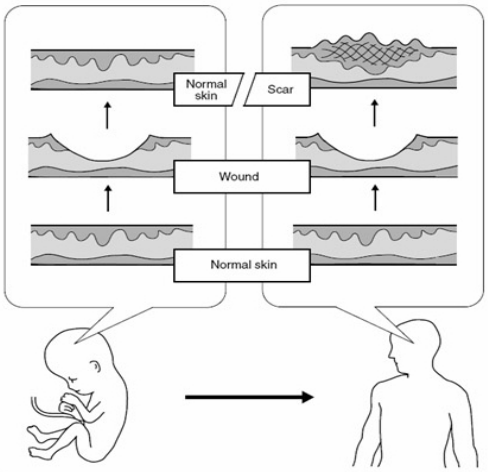
Some, but not all, cytokines are regenerative; many function as immune system stimulants that promote tissue destruction, fibrosis, and scarring. A deeper understanding of cytokines and their disparate physiologic effects will assist the reader in discerning when such treatments are likely to be beneficial in aesthetic practice, or potentially counter-productive,

Cytokines are effective at extremely low concentrations. In contrast to protein hormones that circulate in nanomolar (10^{-9} M) concentrations, cytokines are found in picommolar (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. Moreover, cellular behavior is not controlled by individual cytokines but rather the net pattern of numerous molecules having complementary and/or competitive influence. Cytokines can activate receptors on the surface of the same cell that produces them, known as *autocrine* signaling, or on cells in their immediate vicinity. Nearby cell activation is termed *paracrine* signaling. Topical use of cytokines in skincare and procedure recovery mimics paracrine signaling.

Cytokine Control of the Healing Process

All life processes are ultimately dependent on bio- signaling. This is particularly 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 resurface the wound. 3) *Maturation* occurs after the wound has closed and involves remodeling of type III collagen into type I collagen. This phase lasts months.

Fetal skin heals scar-free, complete with regeneration 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, TGFβ-3 (transforming growth factor beta three) is the one that most distinguishes fetal from adult healing. The fetus has abundant TGFβ-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 Type I collagen.

Cell Types Cultured for Use in Anti-Aging Products

Human fibroblast bio-signals have the longest history of use in topical skincare products. More recently, human stem cells have been utilized, specifically adipose derived mesenchymal stem cells (AD-MSC), bone marrow derived mesenchymal stem cells (BM-MSC), umbilical cord mesenchymal stem cells (UC-MSC), and parthenogenic stem cells, which are human ova chemically induced to repetitive cellular division. Aside from possible antioxidant value, and because of the lack of scientific proof for their use as bio-signals for human tissues, botanical "stem cells" are not included in this discussion.

Conditioned media is the name given to the nutrient broth in which cells have been cultured, after ultrafiltration removal of all cells and cell remnants. That is the form in which cell culture derived bio-signals are most commonly used as a skincare product ingredient. An exception is parthenogenic 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. At least one product contains lysed fibroblasts.

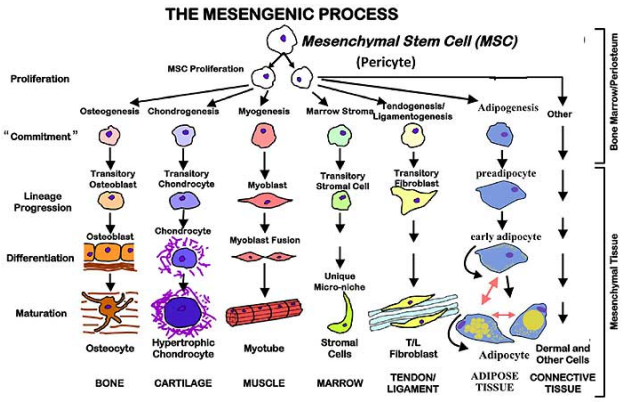
Use of conditioned media permits laboratory production and isolation of cytokines for specific therapeutic purposes i.e. "designer cytokine cocktails". Of the several cell types mentioned above, abundant evidence supports bone marrow derived mesenchymal stem cells (BM-MSC) as the preferred cell type to obtain cytokines for use in topical anti-aging products, and post-procedure adjuvants for the skin. Some of the most compelling evidence pertains to the preeminent role these stem cells play in tissue repair and regeneration throughout life.

Command and Control of Healing by BM-MSCs

Over the past decade, researchers determined that BM-MSCs play a critically important role in all tissue healing. Like other cellular elements in the blood, BM-MSCs migrate into the vasculature where they gain access to the entire body. Chemokines released from injured tissues further stimulate the process, attracting BM-MSCs from distant sites.

BM-MSCs are capable of differentiating into multiple cells types including myocytes, chondrocytes, osteocytes, fibroblasts, etc. (see below) In fact, BM-MSCs are the predominant cells research focuses on to grow body parts in the laboratory, treat 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.



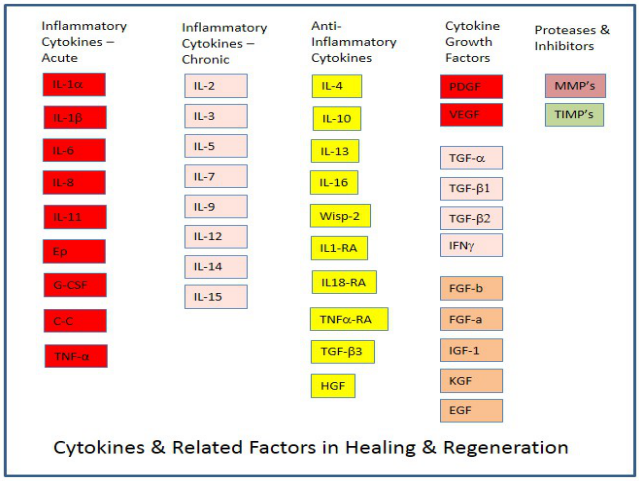
When repair is complete, BM-MSCs that differentiate remain as part of the new tissue. Others, reminiscent of 911 emergency responders, return to the bone marrow, ready to be called into action again. Similar command and control function is not observed in adipose or umbilical cord MSCs, fibroblasts,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

Because skin aging results from the accumulated damage of innumerable small injuries over time, the unique role of bone marrow derived MSCs in directing and orchestrating healing makes them particularly well-suited for use in advanced anti-aging skincare and procedure recovery formulations. Their anti-inflammatory cytokine pattern is a particularly compelling reason.

The Inflammation – Aging Connection

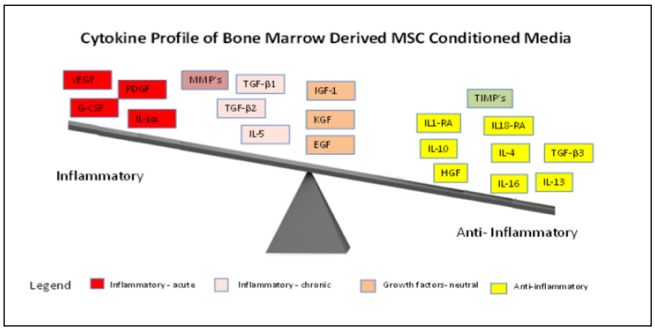
Inflammation is a major contributing factor in the diseases and degenerative conditions of the elderly, including cancer. So well accepted is the connection, it has been given a name - "inflammaging." Chronic inflammation is especially pro-aging in the skin.

Each growth factor or cytokine is inherently pro-inflammatory, anti-inflammatory, or neutral. Because cultured cells produce many 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.

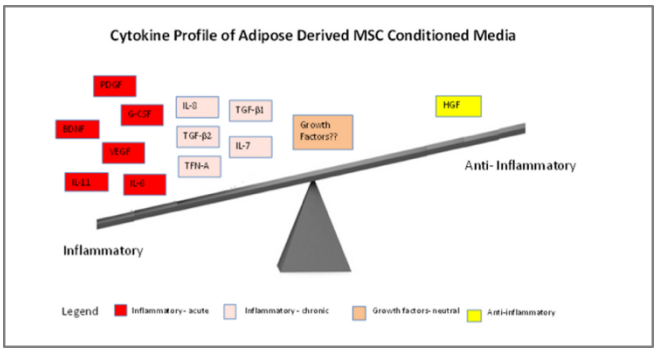


Cell Culture & PRP Cytokine Patterns

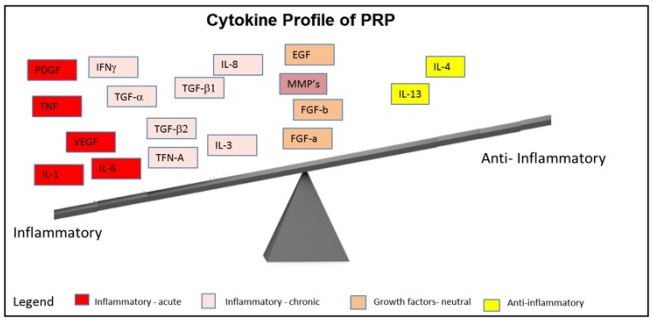
The "balance beam" diagrams below illustrate the net effect of the bio-signals contained in conditioned media produced by laboratory culture of AD-MSC and BM-MSC, and contained in PRP.



Anti-inflammatory pattern of BM-MSC conditioned media



Pro-inflammatory pattern of AD-MSC conditioned media

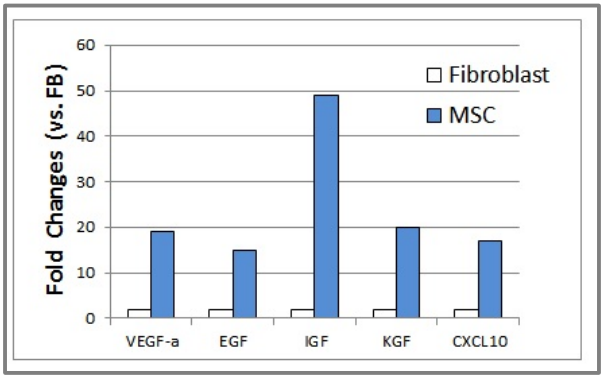


Pro-inflammatory pattern of PRP (platelet rich plasma)

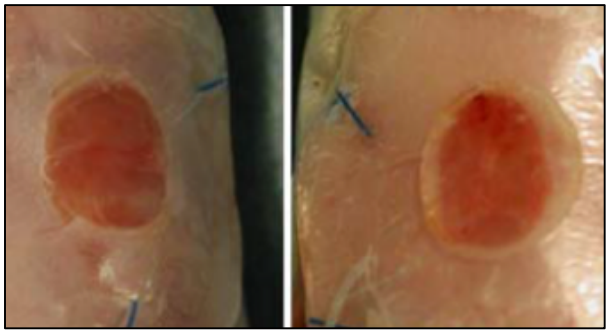
If anti-inflammatory effect is a desired goal of topically applied bio-signals, BM-MSC appears to be the preferred cell type to culture.

Fibroblasts are Very Poor Producers of Bio-signals

Fibroblasts produce collagen and intercellular matrix but are poor secretors of important pro-healing anti-inflammatory growth factors and cytokines. Depending on the bio-signal measured, fibroblasts produce 1/15 to 1/50 the amount produced by BM-MSCs. Fibroblasts, under keratinocyte stimulation, however, are prolific producers of pro-inflammatory IL-6 and IL-8. Moreover, fibroblast conditioned media had essentially no effect in promoting healing of full thickness rodent wounds, whereas BM-MSC conditioned media resulted in significant fibroblast proliferation and migration into the wound during a 14-day trial.

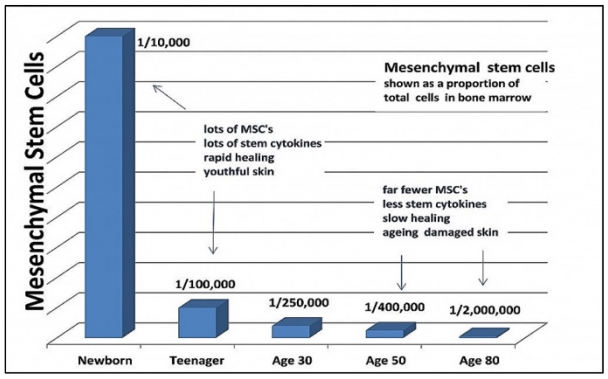


Fibroblast vs. BM-MSC bio-signal concentrations



Healing effect Fibroblast vs. BM-MSC conditioned media

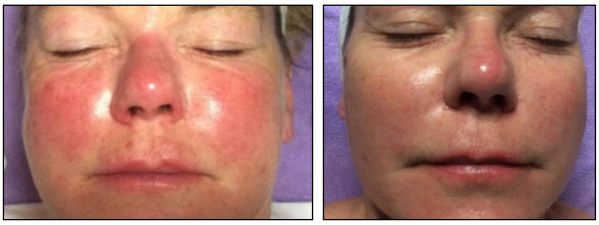
Effects of our Declining BM-MSC Population



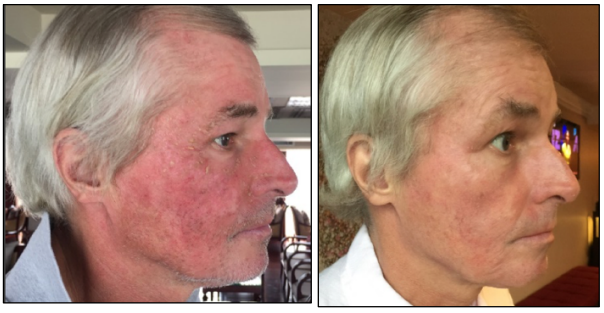
The natural dramatic decline in BM-MSCs that occurs with age causes corresponding major reductions in pro-healing anti-inflammatory bio-signals; slower, less robust healing with increased inflammation results.

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. As one might predict, topical augmentation with BM-MSC conditioned media products has proved beneficial in both general anti-aging and in enhancing recovery from aesthetic procedures. Skin convincingly *acts* younger and healthier. The evidence base is growing.

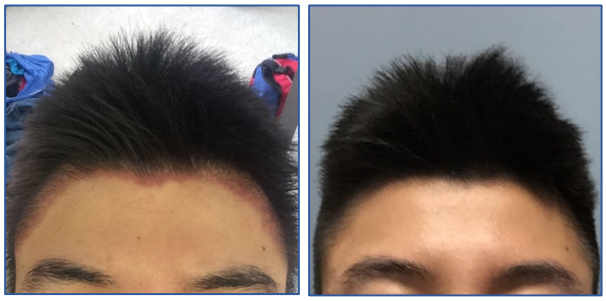
Examples of Anti-inflammatory Responses



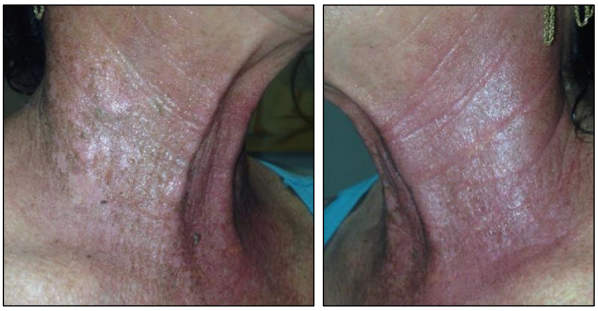
Improved redness: 30 days bid topical BM-MSC product



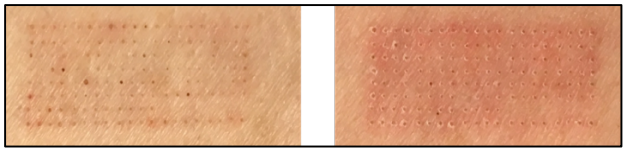
Flare-up improved @ 48 hours - BM-MSC product qid



Improved steroid-induced dermatitis @ 14 days bid



Accelerated healing of R neck over L with BM-MSC product - 3 days post-fractional CO2 laser resurfacing



100% power Venus Viva RF microneedling @ 4 days with BM-MSC product BID on L, none on R photo courtesy of Venus Concept

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