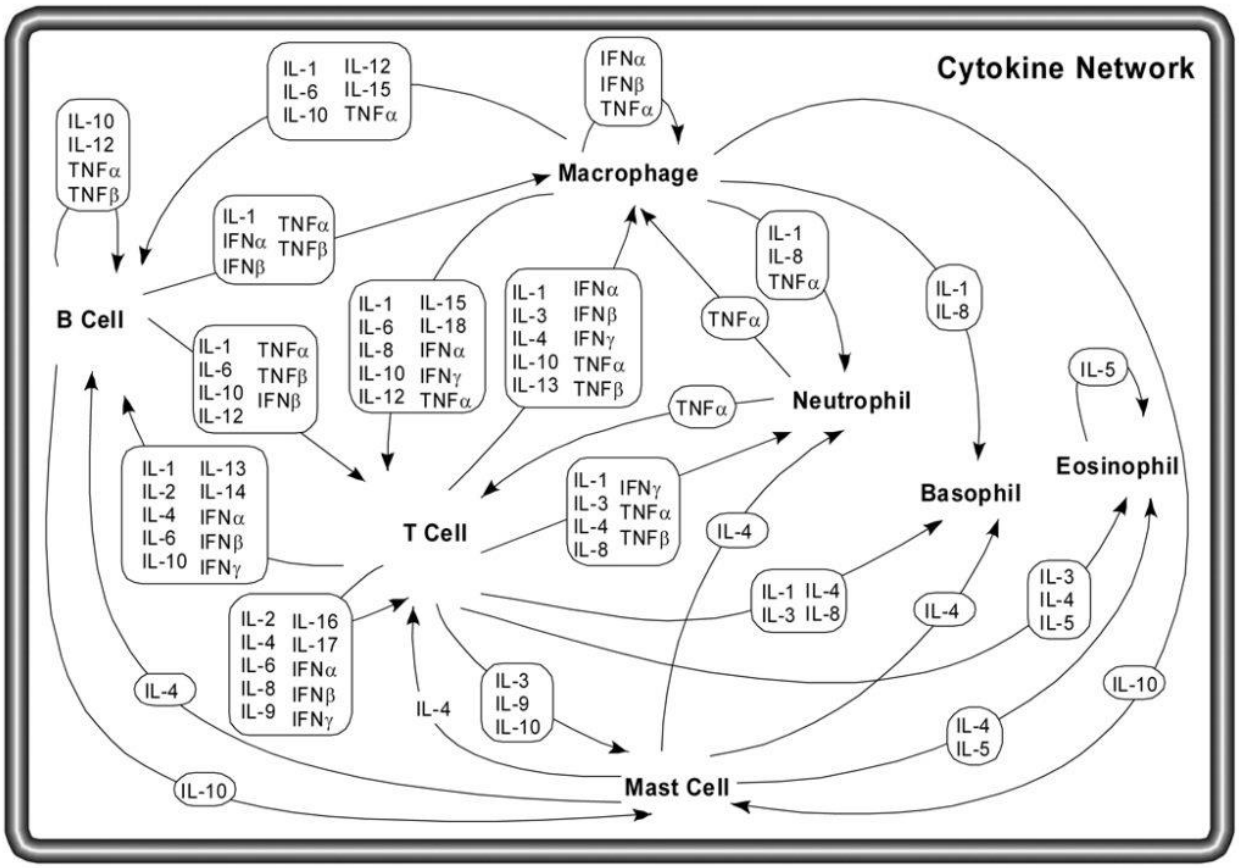
Safety Of Topical Biosignals in Skincare

Topical skincare products containing cell culture-derived and/or synthesized human growth factors and cytokines have been available to consumers for two decades. The first such product contained conditioned media derived from cultured fibroblasts isolated from infant foreskins. Since then, products containing conditioned media from cultures of other human cell types has been marketed including fibroblasts from fetal skin and mesenchymal stem cells isolated from bone marrow, fat, umbilical cord, placenta and embryos.

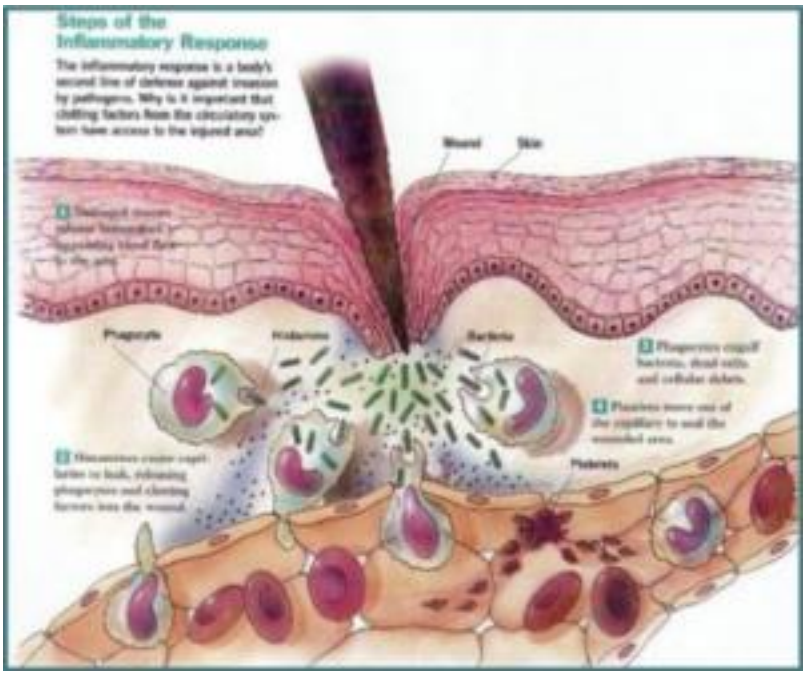


Abundant peer-reviewed literature documents the aesthetic improvements in skin appearance when topical products containing human growth factors and cytokines are added to daily skin care regimens. Differences, however, in the secretome (the “pattern” of biosignals secreted by cells in culture) exist among the various cell types. Analysis of conditioned media makes it possible to determine the relative amounts of growth factors and cytokines produced, and whether the pattern from a specific cell type results in a net pro-inflammatory, anti-inflammatory or neutral effect.

The Human Cytokine “Language” is Hugely Complex and Primarily Focused on the Inflammatory Process

Human bio-signals are necessary for life itself and play their most important role in the initiation and modulation of the inflammatory process. However, whereas inflammation was lifesaving throughout most of our evolutionary history, when life was under constant assault through microbial attack, often from the most minor of traumas. Modern hygiene, vaccinations, antibiotics and sterile equipment and procedures have eliminated this need much of the time. Yet our genetic heritage continues to initiate

a robust inflammatory response, regardless of the cause of the trauma. Despite use of scrupulous sterile techniques that eliminates microbial exposure and risks, our bodies undergo several days of painful inflammation following even the most minor surgery.



Use of Human Growth Factors & Cytokines in Minimally Invasive Procedures

With modern science, it is now possible to harness the power of the biosignal network to exploit their healing benefits yet prevent some of the downsides associated with inflammation. Topically applied bio-signals are increasingly used in dermal microneedling, RF microneedling and recovery following light therapy such as fractional CO_2 laser resurfacing. Sources are autologous PRP (platelet rich plasma) and commercially available products containing biosignals derived from laboratory cell cultures and laboratory synthesis as described above. Claimed benefits are enhanced healing, reduced healing time and improved aesthetic outcomes. In the case of bone marrow stem cell derived conditioned media products, the net anti-inflammatory pattern is cited as potentially helpful in preventing or reducing post-inflammatory sequelae such as fibrosis and excessive pigmentation (PIH.)

Overall Safety Record

The safety record of this class of products is remarkable. Searches of Google Scholar, PubMed and the FDA Adverse Event Reporting System (FAERS) do not yield published case reports, peer-reviewed studies or reported adverse events. This lack of evidence suggests that topical skin application of human growth factors and cytokine containing products have a favorable risk-benefit ratio.

Nonetheless, some medical and esthetic practitioners continue to be wary, not using or recommending such products and cautioning others against their use. One recurring concern is their “potential” to accelerate or induce cellular dysplasia or malignant transformation. Evidence that such a phenomenon occurs is lacking.

Published Examples of Clinical Safety

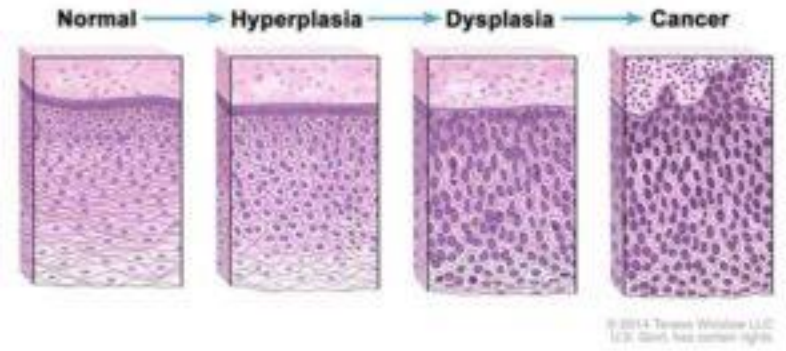
Safety and efficacy of a growth factor and cytokine-containing topical product in wound healing and incision scar management after upper eyelid blepharoplasty: a prospective split-face study. Clin Ophthalmol. 2016; 10: 1223–1228.
A growth factor and cytokine-containing product was used in a prospective split-face study on patients who underwent bilateral upper eyelid blepharoplasty. Product was applied to one eyelid incision for 12 weeks and no product to the other eyelid. At all-time points, all subjects thought eyelids treated with product had a better scar and overall appearance than fellow eyelids (P<0.5.) Investigator assessment of erythema and pigmentation revealed less erythema and pigmentation in treated eyes at the weeks 6 and 10.

Safety and Efficacy of Growth Factor Concentrate in the Treatment of Nasolabial Fold Correction: Split Face Pilot Study. Indian J Dermatol. 2015 Sep-Oct; 60(5): 520.
We developed growth factor concentrate (GFC) from platelets and evaluated their clinical outcome in nasolabial folds. 80 patients were divided into two groups. Group I (20) received bilateral single injection of GFC and group II (60) received single injection of GFC on the right side of the face and platelet-rich plasma (PRP) on the left side of the face. Objective clinical assessment and subjective satisfaction scale was determined for overall improvement at the end of the study. Overall improvement score analysis showed that GFC was significantly superior to PRP (P < 0.001). The results showed that the single application of GFC is highly effective and safe.

Intradermal injections of a hair growth factor formulation for enhancement of human hair regrowth – safety and efficacy evaluation in a first-in-man pilot clinical study. Journal of Cosmetic and Laser Therapy. Volume 20, 2018 – Issue 6
Research has shown the efficacy of hair growth factors in hair regrowth. We describe the intradermal injections of a recombinant, bioengineered hair formulation, containing growth factors, into the scalp skin, for enhancement of hair regrowth and evaluate its efficacy. The formulation contains vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor, keratinocyte growth factor, thymosin β4, and copper tripeptide-1 suspended in a sterile injectable vehicle. Significant reduction in hair fall was seen in 83% of the patients. Microscopic image evaluation showed that most patients had a decrease in the number of vellus hairs, increase in number of terminal hairs, and increase in shaft diameter.

A phase III study to evaluate the safety and efficacy of recombinant human epidermal growth factor (REGEN-D™ 150) in healing diabetic foot ulcers. WOUNDS. 2006;18(7):186-196.
A phase III clinical trial was carried out to determine the safety and efficacy of recombinant human epidermal growth factor in healing diabetic foot ulcers. Skin biopsy was done at baseline and after treatment to evaluate the degree of healing. Parameters, such as increase in collagen tissue, granulation tissue formation, skin epithelization, and microbial growth, were analyzed. In the gel-treated group, at the end of 10 weeks, 69% of the ulcers healed, while in the placebo group, only 21% healed in 10 weeks. The study demonstrated the clinical safety and efficacy of rhEGF in accelerating healing of diabetic foot ulcers.

Differences between Normal and Malignant Cells

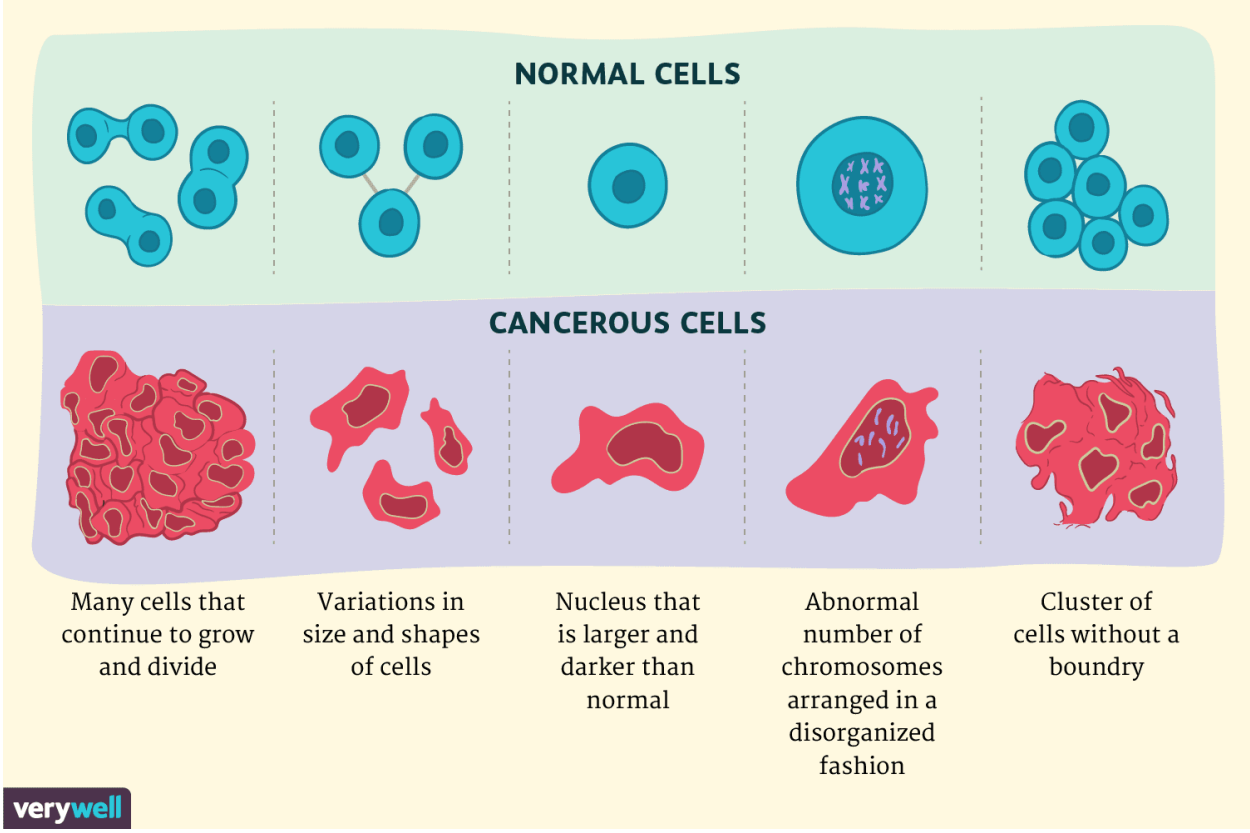


Cancer is a genetic disease caused by changes to genes that control the way cells function, especially how they grow and divide. Such genetic aberrations may be inherited or acquired. Because cancer is a complex disease, it is usually a combination of abnormalities that lead to a cancerous cell, rather than a single mutation or protein abnormality. Cancers of infancy and early childhood aside, it is felt that development of cancer is a process that takes many years, even decades.

Normal and cancer cells are very different, including:

- **Growth**—Normal cells stop reproducing when enough are present. Cancer cells reproduce rapidly and continuously.
- **Cellular Communication**—Normal cells respond to signals sent from other nearby cells and stop growing. Cancer cells do not respond to these signals.
- **Invasiveness**—Normal cells stop growing when they encroach on nearby tissues. Cancer cells ignore these cells and invade nearby tissues.
- **Cell repair and cell death**—Normal cells are either repaired or die (undergo apoptosis) when they are damaged or get old. Cancer cells are either not repaired or do not undergo apoptosis
- **Stickiness**—Normal cells secrete substances that make them adhere together in a group. Cancer cells fail to make these substances, and can "float away" to locations nearby, or through the bloodstream or system of lymph channels to distant regions in the body.
- **Appearance**—Under a microscope, normal cells and cancer cells look very different. Cancer cells exhibit much more variability in cell size and shape. Because of excess DNA, their nuclear genetic material looks darker and larger.
- **Maturation**—Normal cells mature. Cancer cells, because they grow rapidly and divide before cells are fully mature, remain immature.
- **Evading the immune system**—When normal cells become damaged, the immune system identifies and removes them. Cancer cells are able to evade (trick) the immune system long enough to grow into a tumor by escaping detection or secreting chemicals that inactivate immune cells that come to the scene.
- **Functioning**—Normal cells perform the function they are meant to perform, whereas cancer cells may not be functional at all.
- **Blood supply**—Normal cells undergo angiogenesis as part of normal growth and development and when new tissue is needed to repair damaged tissue. Cancer cells undergo angiogenesis even when growth is not necessary.
- **Evading growth suppressors**—Normal cells are controlled by growth (tumor) suppressors. Mutations that result in tumor suppressor genes being inactivated allow cancer cells to grow unchecked.

- **Energy Source**—Normal cells get most of their energy through the production of ATP in the Krebs cycle which requires oxygen. Cancer cells produce most of their energy in the absence of oxygen using anaerobic metabolism.
- **Mortality/Immortality**—Normal cells have a lifespan. When their telomeres become too short, the cell can no longer divide and dies. In cancer cells, telomerase lengthens the telomeres so that the cell can divide indefinitely—essentially becoming immortal.
- **Genomic instability**—Cancer cells often have an abnormal number of chromosomes and the DNA becomes increasingly abnormal as it develops a multitude of mutations. Some of these are driver mutations, promoting the transformation of the cell to be cancerous.



Multiple Changes are Needed for any Cell to Become Cancerous
The genetic changes that cause cancer may be inherited from one's parents or arise during a person's lifetime. Cancer-causing environ-mental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun.

Some studies suggest that being exposed to ultraviolet (UV) radiation and the sensitivity of a person's skin to UV radiation are risk factors for skin cancer. UV radiation is the name for the invisible rays that are part of the energy that comes from the sun. Sunlamps and tanning beds also give off UV radiation.

Risk factors for nonmelanoma skin cancer:

- Being exposed to natural sunlight or artificial sunlight (such as from tanning beds) over long periods of time.
- Having a fair complexion – skin that burns easily, light-colored eyes, red or blonde hair
- Having actinic keratosis.
- Past treatment with radiation.
- Having a weakened immune system.
- Being exposed to arsenic.

Risk factors for melanoma skin cancer:

- Being exposed to natural sunlight or artificial sunlight (such as from tanning beds) over long periods of time.
- Having a fair complexion – skin that burns easily, light-colored eyes, red or blonde hair
- Having a history of many blistering sunburns, especially as a child or teenager.
- Having several large or many small moles.
- Having a family history of unusual moles (atypical nevus syndrome).
- Having a family or personal history of melanoma.
- Being white.

While fair complexion is a risk factor, people of color can also contract skin cancer.

Role of Inflammation in Carcinogenesis Well-Established

Regardless of the etiologic contributor, resultant inflammation is considered a major influencer of carcinogenesis.

The Role of Inflammation in Skin Cancer. Inflammation and Cancer (book) 2014, pp 437-469
Cancer is an environmental disease and skin cancer (melanoma and non-melanoma) is the most common of all cancers. Epidemiological and experimental evidence suggest “chronic inflammation” to be one of the hallmarks in solar ultraviolet radiation and several other environmental agent-mediated skin cancers. The identification of transcription factors, mainly nuclear factor-kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1 alpha (HIF-1α) and their gene products i.e. prostaglandins, cyclooxygenase-2 (COX-2), cytokines [tumor necrosis factor- alpha (TNF-α)], chemokines [CXC-chemokine ligand (CXCL)] and chemokine receptors suggest a critical role of inflammation in skin carcinogenesis.

Inflammation: Gearing the journey to cancer. Mutation Research/Reviews in Mutation Research. Volume 659, Issues 1–2, July–August 2008, Pages 15-30
Chronic inflammation plays a multifaceted role in carcinogenesis. Mounting evidence suggests that persistent inflammation functions as a driving force in the journey to cancer. The possible mechanisms include induction of genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumor neovascularization, invasion through tumor-associated basement membrane and metastasis, etc.

*Chemopreventive Strategies for Inflammation-Related Carcinogenesis: Current Status and Future Direction***.** Int J Mol Sci. 2017 Apr; 18(4): 867.
A sustained and chronically inflamed environment is characterized by the presence of heterogeneous inflammatory cellular components, including neutrophils, macrophages, lymphocytes and fibroblasts. These infiltrated cells produce growth stimulating mediators (inflammatory cytokines and growth factors), chemotactic factors (chemokines) and genotoxic substances (reactive oxygen species and nitrogen oxide) and induce DNA damage and methylation. Therefore, chronic long-term inflammation serves as an intrinsic niche for carcinogenesis and tumor progression.

Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. In Vivo. 2014 Nov-Dec;28(6):1005-11.
Data on melanoma from the majority of countries show a rapid increase of the incidence of this cancer. The incidence rate of melanoma is greater in women than men until they reach the age of 40 years. By 75 years of age, the incidence is almost 3-times as high in men versus women. The most important and potentially modifiable environmental risk factor for developing malignant melanoma is the exposure to ultraviolet (UV) rays because of their genotoxic effect. Artificial UV exposure may play a role in the development of melanoma

Basal cell carcinoma: pathogenesis, epidemiology, clinical features, diagnosis, histopathology, and management. Yale J Biol Med. 2015 Jun 1;88(2):167-79.
Basal cell carcinoma (BCC) is the most common skin malignancy with exposure to sunlight the most important risk factor. A variety of treatment modalities exist and are selected based on recurrence risk, importance of tissue preservation, patient preference, and extent of disease.

*Pathways connecting inflammation and cancer***.** Curr Opin Genet Dev. 2008 Feb;18(1):3-10.
Chronic and persistent inflammation contributes to cancer development and can predispose to carcinogenesis. Hallmarks of cancer-associated inflammation include the presence of infiltrating leukocytes, cytokines, chemokines, growth factors, lipid messengers, and matrix-degrading enzymes. Schematically, two interrelated pathways link inflammation and cancer: (1) genetic events leading to neoplastic transformation promote the construction of an inflammatory milieu; (2) tumor-infiltrating leukocytes, in particular macrophages, are prime regulators of cancer inflammation. Thus, an intrinsic pathway of inflammation (driven in tumor cells), as well as an extrinsic pathway (in tumor-infiltrating leukocytes) have been described and both contribute to tumor progression.

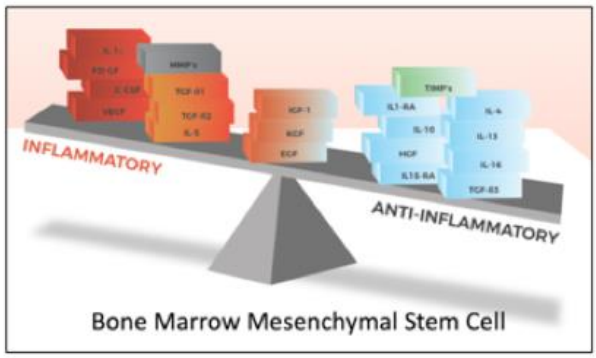
Molecular pathways linking inflammation and cancer. Curr Mol Med. 2010 Jun;10(4):369-73.
Inflammatory conditions in organs increase the risk of cancer. An inflammatory component is present also in the microenvironment of tumors that are not epidemiologically related to inflammation. Recent studies have begun to unravel molecular pathways linking inflammation and cancer. An intrinsic (driven by genetic events that cause neoplasia) and an extrinsic (driven by inflammatory conditions which predispose to cancer) pathway link inflammation and cancer.

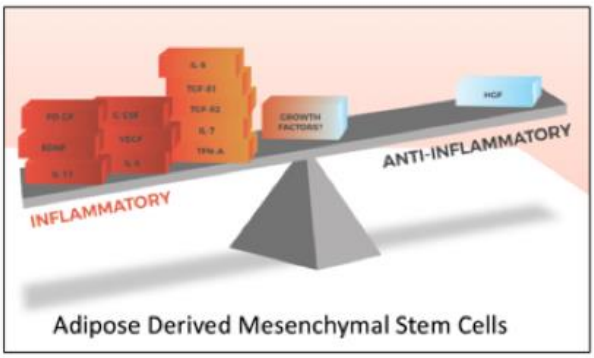
Molecular pathways in cancer-related inflammation. Biochem Med. 2011;21(3):264-75.

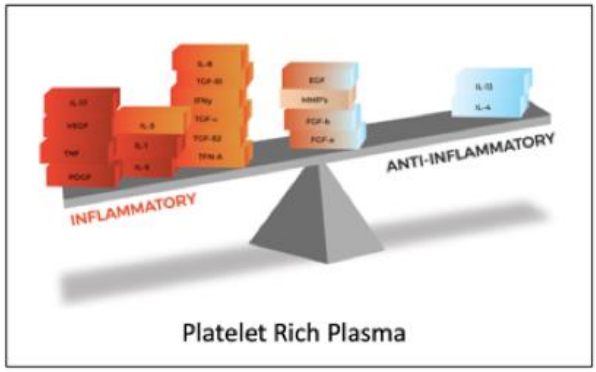
Accumulating evidence shows chronic inflammation is associated to increased risk of cancer. Extensive investigations over the past decade have uncovered many of the important mechanistic pathways underlying cancer-related inflammation. Smoldering inflammation is a component of the tumor microenvironment and is a recognized hallmark of cancer. Thus, cancer-related inflammation represents a target for innovative diagnostic and therapeutic strategies.

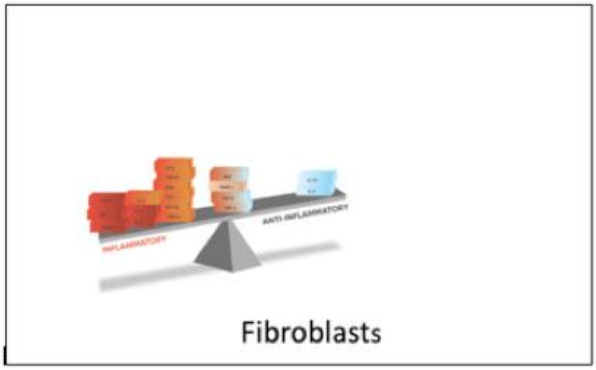
Inflammation, a key event in cancer development. Mol Cancer Res. 2006 Apr;4(4):221-33. Several recent studies have identified a key modulator in driving inflammation to cancers. Besides this transcription factor, essential in regulating inflammation and cancer development, an inflammatory microenvironment inhabiting various inflammatory cells and a network of signaling molecules are also indispensable for the malignant progression of transformed cells, which is attributed to the mutagenic predisposition of persistent infection-fighting agents at sites of chronic inflammation.

Differences in Secretomes of Cells Cultured for Conditioned Media & Platelet Rich Plasma
The "balance beam" diagrams below depict the net inflammatory effects of cytokine patterns from different cell culture sources and platelet rich plasma.









Of the secretomes depicted above, only that of bone marrow mesenchymal stem cells has a net anti-inflammatory pattern. The others – fibroblasts, adipose derived stem cells, and PRP- have net pro-inflammatory patterns, with the one for fibroblasts indicating very poor secretory capacity. Umbilical, placental and embryo stem cells also produce net pro-inflammatory secretome patterns.

Based upon the knowledge that chronic inflammation is a recognized "contributor" or "initiator" of the malignant transformation of skin cells, it seems prudent to consider products containing biosignals produced from or replicating the pattern seen in cultures of bone marrow mesenchymal stem cells

for daily skincare. This is not the same as saying other cell type secretomes *are* carcinogenic; only that their secretome has a net pro-inflammatory effect.

Published literature supports the anti-inflammatory effect of bone marrow mesenchymal stem cells:
Secretome from bone marrow mesenchymal stem cells: A promising, cell-free therapy for allergic rhinitis. Med Hypotheses. 2018 Dec; 121:124-126.
Bone marrow mesenchymal stem cells (BMSCs) are a population of adult stem cells with multipotential differentiation capability, low immunogenicity, and immunoregulatory effects. The unique immunoregulatory properties of BMSCs make them hold great promise in the treatment of chronic inflammation and immune disorders through a paracrine mechanism of anti-inflammatory and anti-allergic effects. The stem cell secretome is defined as the set of molecules secreted to the extracellular space. The secretome such as conditioned media (CM) obtained from BMSCs contains various bioactive molecules and vesicular elements, which may act as therapeutic mediators to support their immunoregulatory effects. Therefore, we hypothesize that the BMSCs secretome may represent a promising treatment for AR by anti-allergic effects via the paracrine mechanism.

Immunomodulatory effects of bone marrow-derived mesenchymal stem cells on pro-inflammatory cytokine-stimulated human corneal epithelial cells. PLoS One. 2014 Jul 8;9(7)
Investigated was the modulatory effect of rat bone marrow mesenchymal stem cells (MSC) on human corneal epithelial cells (HCE-T) stimulated with pro-inflammatory cytokines interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) in an in vitro co-cultured model. MSC attenuated expression of IDO through both NF-κB transcription and TGF-β1 signaling pathways. Co-culture of HCEC with MSC therefore provides a useful in vitro model to study the anti-inflammatory properties of MSC on corneal epithelium.

Mesenchymal stem cell-conditioned medium prevents radiation-induced liver injury by inhibiting inflammation and protecting sinusoidal endothelial cells. J Radiat Res. 2015 Jul;56(4):700-8.
MSC-conditioned medium (MSC-CM) was generated from rat bone marrow-derived MSCs. MSC-CM also reduced the secretion and expression of inflammatory cytokines and increased the expression of anti-inflammatory cytokines. MSC-derived bioactive components could be a novel therapeutic approach for treating radiation-induced liver injury.