

Review Article

Soft and hard-tissue augmentation with platelet-rich plasma: Tissue culture dynamics, regeneration and molecular biology perspective

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ABSTRACT

Platelet-rich plasma (PRP) has been extensively used in maxillofacial and oral surgery with predictable clinical outcomes. PRP has been used for hard and soft tissue regeneration. Anecdotal data indicate that PRP enhances the early wound-healing cascade by the interactions of activated platelet-released growth factors with the extra cellular matrix with potential potent anabolic affects. The processing of autologous PRP is highly variable and the types of propriety kits, centrifuges and vials available are numerous. Regarding facial rejuvenation and PRP, initial results are short-lived, inconsistent and further maintenance treatment is needed regarding facial wrinkle amelioration, as is the case with other fillers. It is not clear if "neocollagenesis" occurs after PRP rejuvenation therapy. Drawbacks of activated PRP, if used in the facial area, include the potential to micro-thrombosis in the region of the anterior facial vein, closed compartment syndrome and release of pro-inflammatory proteolytic activators from leucocytes. Compared to conventional culture mediums, unrefined and undiluted PRP may not be biologically suitable as a cell transport medium (i.e., for fibroblasts, keratinocytes and neural cells). Our *ex vivo* studies confirm potent TC mitogenic stimulation of human fibroblasts, keratinocytes, chondrocytes, neural tissue and myoblasts. There are no clinical reports of the application of PRP for repair of complex shoulder rotator cuff lesions. The authors describe the biology of platelet-rich plasma, potential application in dermal regeneration and rotator cuff surgery as an adjunct to conventional surgery for large or previous failed surgery, tissue physiological response to PRP and the molecular biology of PRP relevant to the shoulder surgeon.

Key words: Platelet derived factors, growth factors, platelet regeneration, rejuvenation, platelet rich plasma, rotator cuff repair, shoulder surgery.

PLATELET-RICH PLASMA (PRP) AND TISSUE REGENERATION BY GROWTH FACTORS

Scientists and clinicians have a vested interest in the field of soft tissue regeneration, rejuvenation by fibroblasts, keratinocytes and neural tissue, through the application of activated platelet-derived cytokines or growth factors in selected patients in

the clinic. Potential benefits to patients by the intraoperative application of PRP include reduced capillary bleeding and oozing in surgical flaps, reduced need for drains, reduced postoperative pain and swelling, accelerated postoperative recovery time and improved wound healing.^[1-5] These local effects are related to and orchestrated by the trophic and anabolic nature of platelet releasate following activation in the wound.^[1-5] Seven anabolic and trophic factors identified

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in platelet-rich gel have now been described, the best known include platelet-derived factor (PDGF) and transforming-growth factor-beta 1.^[1-5] Other important secretory proteins that influence wound healing and derived from alpha-granules of activated platelets include, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), vitronectin (Vn), fibronectin (Fn), fibrinogen (Fg) and thrombospondin-1 (TSP-1).^[1,5] These factors can supposedly increase the rate of collagen deposition, angiogenesis, fibroblast proliferation, extra cellular matrix synthesis relevant to wound healing and soft tissue regeneration.^[1-5]

PRP can be defined as “an autologous concentration of human platelets in a small volume of plasma”.^[1] This concentrate contains the trophic growth factors that are released once the platelets therein are activated either by calcium chloride, thrombin or fibrinogen.^[1] All seem equally effective in activating the platelets *ex vivo*. In the process, a gel is created that can be used as a suitable carrier for bone chips that are destined to be used in cranio-facial interventions such as sinus augmentation or lifting.^[1] For these uses, a kit is not necessary and the blood vials can be bought separately, that makes the procedure cost-effective. The plasma poor plasma fractionate (PPP) has potent sealant properties.^[1] For this application, a two nozzle syringe system, containing blood vials is available (Regen[®]). These platelet factors are not present in bovine calf serum or conventional mediums such as Eagle's or DMEM. The side-room preparation of PRP has been documented and has been simplified by the availability of tabletop high speed centrifuges; however, not all centrifuges and blood vials render equivalent concentrate of PRP.^[1] Paying attention to fine detail and avoiding contamination, is important during the generation of PRP.^[1,5] The authors prefer to do the centrifuging and plasma/cell separation as aseptically as possible in a laminar flow hood. A mobile laboratory is also highly effective and practical and can be taken to the specialist's rooms. The apparatus can be conveniently installed in an office side-room or adjacent to an operating theatre. In order to ensure quality assurance of the PRP, designated FDA or CE approved automated platelet concentrate systems and qualified medical personnel must be utilized.^[1] The surgeon needs to determine how much PRP he or she wants to generate for the particular procedure.^[1] This is possible in a 30 minute period and can easily be performed by a suitably qualified and board certified clinical technologist who assists the surgeon. Upholding aseptic principles during cell separation is mandatory to avoid contamination.^[1] Briefly, venous blood is obtained from the patient by venesection of the median cubital forearm vein, shortly before the surgical procedure.^[1] Surgery activates the platelets, so timing of the venesection is important.^[1,5] The blood is collected and inserted into special designated vials containing an anticoagulant (Regen[®]). Storage of the blood should be avoided due to loss of platelet activity. Medium speed centrifugation concentrates the platelets into a small volume without fragmentation. The layer containing the PRP, which can be differentiated from the

PPP, is aspirated into a sterile tube. A second spin is preferable to obtain PRP. For the platelet growth factors to be released, platelets have to be activated.^[1] This is intentionally induced shortly before use of the gel and affected by addition of calcium chloride and / or thrombin to the platelet concentrate.^[1] Use of these activators needs specialized medical supervision and control to avoid cardiac arrhythmias and thrombosis. Ongoing CME training courses at specialist level are needed to highlight the hematological components of PRP and the mechanism of action. The gel thus created, is available for soft tissue augmentation during surgery (i.e. tendon repair or facelift) or admixture with a bone graft exclusively for specialists trained in the art.^[6-9] Because of the potential dangers of facial augmentation with PRP, compared to the use of Botox[®], legislation excludes untrained practitioners from giving such injections. The addition of PRP is intended to improve and enhance bone grafting as well as take, by facilitation of greater and denser bone regeneration.^[6-9] The potential advantages of the biological approach of PRP are safety, quick release of platelet-derived growth factors, autologous nature of the preparation and avoidance of animal and human disease transmission.^[1-5]

PRP: In support of its use in dermal regeneration

Autologous PRP, under optimal clinical conditions, can accelerate healing of both hard and soft tissue, because the biological preparation is a vector for cell growth factors.^[10,11,13-16] PRP is widely used in cranio-maxillo-facial and oral surgery and will not be discussed further.^[1,11,12]

From an electron microscopic point of view, PRP gel consists of two components: A fibrillar element and a cellular component that contains human platelet cells.^[1-5] This unique morphological structure is theoretically capable of acting as a transport vehicle for cells (of various lines) that may be complementary to soft/hard tissue regeneration.^[1,5] Single case studies and anecdotal reports indicate that PRP can favourably influence outcome regarding wound healing and repair of chronic wounds, nonresponsive to conventional treatment.^[1] Soft tissue regeneration and enhancement by PRP has been reported in the following clinical scenarios:

- Surgical repair of torn Achilles tendons in man and other ligaments.^[17-19]
- Enhanced and accelerated tissue repair in nonhealing wounds of the lower equine limb.^[19]
- Treatment of diabetic foot ulcers.^[1]
- Face-lift surgery (rhytidectomy), because of the hemostatic properties of PRP.^[1,20-24]
- Cosmetic facial surgery induced alopecia (alopecia due to hair follicle loss in the side burn areas).^[1]
- Enhancement and facilitation of skin sensory nerve recovery postsurgery. This is due to increased capillary in-growth, collagen synthesis that supports nerve regeneration.^[1]
- Reduction in skin wound healing complications after upper and lower eyelid blepharoplasty.^[1]

- Increased “take” of free dermal fat grafts destined for facial augmentation and lipoatrophy.^[1] This is supposedly due to increased lipoblast and lipocyte survival.^[1] It has also been proposed that capillary in-growth into the graft is enhanced.^[1]
- Periodontal disease (increased wound healing in periodontal tissue).^[11,12]
- Stand-alone facial rejuvenation therapy for wrinkles, solar aged skin, by direct injection infiltration of the PRP into the dermis (in malar and nasolabial regions). Because the dermis is thin, this goal is not achieved consistently and the PRP dissipates into the adjacent hypodermis in the hands of the senior author. This will contribute to a nonresponse. The anatomical venous drainage and variations in the region of the forehead, glabella, nasolabial and mandibular regions are especially relevant to aesthetic caregivers and plastic surgeons. Reason is that facial veins have communication with the cavernous and pterygoid venous sinuses.^[25] Also and of great importance is that the superficial veins of the face are valveless and potentially have retrograde communication with intracranial venous sinuses and intervertebral venous plexus.^[25] Of relevance is the fact that the facial vein that is sited at the medial angle of the eye, communicates via the superior ophthalmic vein with the cavernous sinus.^[25] If secondary or iatrogenic infection, arises in the “danger triangle of the face” after PRP injection or filler, there exists a very definite, but albeit small, possibility of thrombophlebitis of the facial vein, side branches and cavernous venous sinus.^[25] Because PRP is generated by the use of thrombin, care must be taken to avoid and anticipate precipitating, accelerated and harmful thrombosis of the superficial veins in susceptible recipients (i.e. angular vein) with potential retrograde extension into the orbit and cavernous sinus. Cosmetic surgeons and physicians should be aware of these important applied and clinical anatomical details.^[25] Any patient who develops the following clinical scenario after the facial injection of a filler or biological such as PRP should be deemed to have a cavernous sinus thrombosis: orbital pain and periorbital edema.^[26] Associated cranial nerve palsy is almost always present in the experience of the senior author and involves the third, fourth, sixth and upper two divisions of the fifth cranial nerves.^[26] Bilateral ocular signs may follow due to thrombus propagation into the contralateral cavernous sinus.^[26] Because activated platelets are present in PRP, thrombus formation could well occur in small facial veins of the hypodermis and the angular veins, especially in susceptible high-risk persons (i.e., previous thrombosis and pulmonary embolism). Special care should be exercised in higher risk individuals, based on these anatomical facts, when injecting PRP in the face especially in the regions of the zygomatic arches and nasolabial regions in close proximity to large dural venous sinuses. In the event of cavernous sinus thrombosis, general principles of stroke therapy will apply including anticoagulation.^[26] Informed consent, regarding these matters and patient aspirations

is important, when considering the use of fillers and PRP. Because PRP contains small quantities of red blood cells, sequestration in one area of the skin could, in a small percentage of cases, result in hemosiderin deposition and hyperpigmentation not related to melanin deposition. Compared to aged spots, hemosiderin-related dermal staining, due to capillary laceration, is not responsive to phototherapy and takes time to fade. Iatrogenic-induced thrombophlebitis of the facial vein by biologicals and other fillers, needs to be carefully avoided. The needle tract in the nasolabial fold lies in close proximity to the facial vein, trigeminal nerve and facial artery.^[25] Knowledge of the anatomical drainage of the facial vein in relation to the nasolabial fold is important [Figure 1]. Following PRP use, bruising and discoloration can occur, because of capillary disruption and thrombosis in the dermis and hypodermis during injection and patients should be warned of this possibility that can occur and that could last for a few days. Hardening and induration of the facial skin overlying the injectate is explainable by traumatic fat necrosis and or capillary thrombosis. These complications are not exclusive to PRP and may occur after the use of fillers.

- Amelioration of stretch marks.^[20]
- Enhanced healing of anterior cruciate ligaments (ACL) in experimental porcine models.^[17]

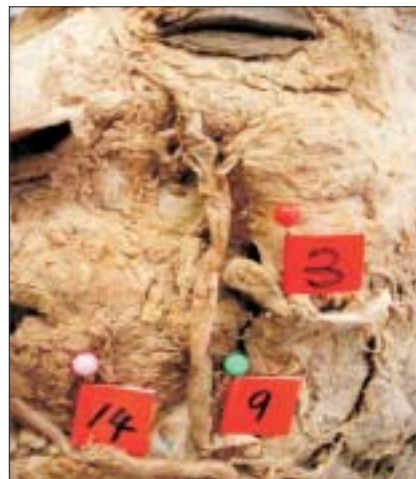


Figure 1: Human cadaveric dissection showing the facial vein (marker 9) in relation to the excised nasolabial loose connective tissue of the facial prominence, parotid duct (marker 3) and facial artery (marker 14). There is free communication with this vein and the intracranial circulation. Thrombophlebitis of the facial vein (that is valveless) may extend into the angular vein by retrograde tracking orbit and cranial dural venous sinuses, thereby reaching the intervertebral plexus (IVVP). Inadvertent injection of a biological (PRP) or filler may track to the orbit, dural venous sinuses or systemic circulation. The anatomical position of the facial vein must be born in mind when dermal or hypodermic injections are made on the face (because the epidermis is thin). Danger area for iatrogenic puncture is between the upper border of m. zygomaticus major and the orbital ridge. The IVVP is associated with temperature regulation and brain function by nature of central connections

BIOSTIMULATORY EFFECTS OF PRP

PRP has many theoretical, unique and biological mechanisms of actions, albeit not fully understood, albeit relevant to the practice of orthopedic surgery. These are enumerated as follows:

- Stimulation of cell proliferation from tendon explants in culture.^[18,27-28] Tendons has been cultured in explant fashion in PRP.^[18] In these studies, the cultured tendons showed enhanced expression of the matrix collagen molecules COL1, COL3A1 and COMP, with no increase in catabolic molecules MMP-3 and MMP-13.^[18] This reflects an anabolic effect of PRP on tendon metabolism, tendon matrix gene expression and matrix synthesis.^[18] Some studies recommend that tissue culture should be affected in PRP-enriched mediums of less than 40%.^[18] This is the experience of the authors. The monolayer of leucocytes may have to be extracted and discarded because of the presence of proinflammatory proteolytic mediators, such as *neutral proteases* and *acid hydrolase's* contained in white blood cells.^[18] These are potentially catabolic or proinflammatory mediators released by white cells and possibly explains the increased post-application pain, observed by the authors, in wounds treated with PRP. This observation has to be carefully considered proactively and weighed up if PRP is injected into a closed space, as the mononuclear white blood cells have the potential to incite an undesirable cutaneous inflammatory reaction.^[18] Both are proteolytic enzymes, promote tissue dissociation and are capable of modifying proteins and proteoglycans in ECM, in preparation for calcification. The end result may be increased deposition of *fibrous scar tissue (scarification)* that is incorrectly attributed to physiological skin tightening.
- Stromal cell proliferation in culture.^[28]
- Increased epithelial regeneration, enhancement of dermal collagen deposition.^[1,29,30-31]
- Stimulation of fibroblasts in cell culture.^[31]
- Prevention of ecchymosis and hematomas.^[1]
- Enhanced proliferation of nucleus pulposis cartilaginous cells in tissue culture.^[10]
- Prevention of surgically induced alopecia by increase in capillary in-growth and hair follicle survival.^[1]
- Improved hemostasis by the interaction of fibrin-fibronectin-vibronectin cell adhesion molecules contained in PRP.^[1]
- Increase of capillaries, collagen and nerve in-growth from deep fascia.^[1]
- Improved angiogenesis.^[1]

Molecular biology of PRP, related to living cells, tissue culture and photo-light biomodulation

Details of normal cell morphology and cytocavitary network integration have been previously documented and will not be reviewed here.^[32] For facial rejuvenation, apart from the use of

cosmeceuticals and living cells, PRP and photo-light therapy are currently under study to treat fine-line wrinkles.^[20,33] The mechanism of action differs for the three treatment modalities. But, the common goals are to augment the face appearance, reduce laxity and improve facial complexion due to a loss of collagen, extra cellular matrix and elastin.

PRP, autologous fibroblast and other cell transplantation

After a period of *ex vivo* tissue culture, concentrated, multiplied and expanded fibroblasts from the same person is injected into a designated dermal recipient region (i.e., face) to enhance "neocollagenesis" and skin tightening.^[33,34] This is still a hotly debated topic (because uncontrolled skin histology is difficult to interpret and ultrasonic collagen scanning questionable) and there are many sceptics. The response rate in the hands of the authors is about 60% despite excellent cell yields and viability with poor results being recorded in persons over 60-years and those with irreversible photo-aging. Repeated fibroblast cell injections are needed as well as maintenance therapy at varying intervals. In the modern era of noninvasive, walk-in and walk-out technology, taking a skin biopsy may be too cumbersome for the patient. The Achilles heel of this cell therapy approach is the long waiting time to proliferate cells and the definitive loss of cells after deposition into the recipient dermal site. It is postulated that many of the engrafted cells are rapidly phagocytosed by resident dermal macrophages and the rest are washed away into the veins and lymphatics. The outcome is a failed graft. What percentage of cell retention is achieved remains unknown. This loss of cell retention explains the poor results in some patients, which cannot be predicted before cell therapy. Also, from a biological point of view or tissue cultures perspective, it is not certain if the new engrafted donor fibroblasts or "resident" fibroblasts of the recipient are responsible for enhancing the facial rejuvenation. Another unanswered question is; can the newly cultured cells consistently stimulate quiescent dermal fibroblast metabolism due to the aging process and solar damage? Possibly, fibroblast cell retention can be enhanced by suspending the fibroblasts in PRP prior to injection, thus utilizing a PRP-growth factor transport vehicle. But undiluted and enriched PRP could be cytotoxic to the newly released cells by trypsin digestion and therefore viability studies are critical to predict outcome cell morphology and phenotype quantification by SEM, ICC and flow cytometry. PRP may subtly change the differentiation and phenotype in TC. Cell cultures are not always 100% pure and are occasionally contaminated by keratinocytes derived from deep hair follicles. At this time, it is not known if PRP and cells, will be able to reduce apoptosis of engraftment by the dermal proteolytic enzymes of the *caspase cascade*.^[32] It is uncertain now if PRP can reverse the activity of *caspase* regulators such as Bcl-2 and IAP protein families.^[32] Also, it is unclear if the growth factors in PRP will provide sufficient extracellular signals to stimulate fibroblast cell proliferation *in vivo* after engraftment. It is well-established that human cells

(i.e., fibroblasts) have a built-in limitation on the times these cells can divide.^[32] This has been confirmed in our laboratory and supports existing literature. Intracellular mechanisms can limit cell proliferation, that is relevant to living cell fibroblast transplantation.^[32] CKI proteins are thought to play a crucial role here.^[32] Therefore, it is important to remember the phenomenon of replicative cell senescence after fibroblast cell therapy.^[32] It has been suggested that telomeres (repetitive DNA sequences and associated proteins at the terminal ends of chromosomes) are the cause of replicative cell senescence.^[32] The role of PRP and telomeres and *telomerase* remains to be clarified. Possibly enforcing expression of *telomerase* in cultured human fibroblasts, by using genetic engineering techniques, including adhesion molecule-based culture wells and PRP may be able to combat fibroblast cell senescence. Possibly the p53-dependent cell-cycle arrest can be overcome, that is typical of senescence. More research and development is needed. Promising short-term outcome has been experienced after autologous chondrocyte, keratinocyte, fibroblast and myoblast transplantation and has been observed by us. At two years, durable resurfacing of deep femoral cartilage damage has been demonstrated by MRI in four selected patients. In two patients, aged 68 and 86 very satisfactory cardiac function has been recorded after two years by autologous myoblast transplantation. In one, the ejection fraction increased from below 10% to more than 35%, at two years and he still enjoys a good quality of life. Other units have also documented favorable medium term outcome after autologous chondrocyte, myoblast and fibroblast transplantation and cell therapy. With new advances in TC and tissue engineering guided by accurate quantification technology, better clinical outcomes can be expected. The future of autologous fibroblast transplantation for facial rejuvenation and correction of wrinkles, is currently in the balance, since the recent introduction of plasma skin regeneration technology as antiaging modality.

PRP as stand alone treatment for amelioration of facial rhytids

The physiological and trophic effect of PRP is based on the release of numerous anabolic growth factors from platelet α -granules.^[18] Tissue culture studies show that PRP can enhance gene expression of matrix molecules such as collagen.^[18] PRP is also capable of stimulating fibroblast proliferation *ex vivo* in experimental models and thereby increasing total protein synthesis.^[18] For the moment it is uncertain if PRP can enhance fibroblast-generated elastin production and myofibroblast stimulation as seen after nonthermal photolight therapy. Compared to phototherapy for facial rejuvenation, PRP differs in the biological mechanism of action on the dermis. PDGF is a mitogen present in activated PRP. Extracts of platelets can serve instead of serum, to stimulate fibroblast proliferation *ex vivo*.^[32] The authors have the same experience in our laboratory. PDGF plays a major role in stimulating cell division during wound healing, including fibroblasts.^[32] Also, TGF- β 1 can promote cell proliferation, survival, differentiation and migration.^[32]

Albert's *et al.*, have shown that mitogens (such as released by activated platelets), control the rate of cell division by acting in the G1 phase of the cell cycle.^[32] Possibly, mitogens can reverse the inhibitory effects of Cdk activity, thereby allowing the S-phase to begin. This is affected by binding to cell-surface receptors to initiate a complex array of intracellular signals that penetrate deep into the cytoplasm and nucleus.^[32] However, it still remains to be shown if the mitogen signalling in PRP can activate the small GTPase RAS, that leads to activation of the *MAP KINASE cascade*.^[32] If PRP mitogens can stimulate *Myc*, also needs to be quantified.^[32] *Myc*, is important, as it plays a major role in stimulating the transcription of genes that increase cell growth.^[32] When considering PRP as stand alone therapy for facial rejuvenation of wrinkles, these biochemical pathways need to be taken into account. The dermis is very thin on the sides of the face and tricky for the doctor to keep the injectate accurately in this layer. It is predicted that a substantial amount of the PRP injectate migrates during and after injection into the hypodermis that is rich in adipose tissue. This will inevitably result in PRP stimulated adipose-derived fibroblasts and not fibroblasts of dermal origin.

Tissue culture, cell dynamics and PRP

In our laboratory we are unable to enhance early cell proliferation potential and cell division (within the first four days) of keratinocytes, fibroblasts, corneal limbal cells, neural tissue and myoblasts in primary cell explant culture enriched with PRP. We utilize both static and dynamic TC technology. We concur with Albert's *et al.*, (2002) that cell anchorage operates in G1 of the cell cycle. Also, we confirm that confluent fibroblast monolayers, no longer proliferate.^[32] However, increased addition of diluted PRP (40%) strongly stimulates cell proliferation in the presence of monolayers (keratinocytes, fibroblasts and skeletal myoblasts) [Figure 2]. For the moment it is not established if PRP will affect the differentiation and phenotype of the cultured cell lines. Possibly, the enhanced growth is related to replenishment of mitogens by the PRP, for which the cells compete.^[32] Of the five cell lines studied in our laboratory, human fibroblasts appear to be the most responsive to culture enrichment with PRP [Figure 2]. Strict adherence to aseptic technique is needed within a laminar flow hood when processing cultures with PRP [Figure 3]. We are in agreement with Albert's *et al.*, (2002) that extracellular growth factors that stimulate cell growth bind to receptors on the cell surface and activate intracellular signalling pathways.^[32] Also, one of the most important intracellular signalling pathways activated by growth factor receptors involves the enzyme PI3-kinase. The activation of PI3-kinase leads to the activation of several protein kinases, including S6 kinase.^[32] Protein synthesis therefore is increased following activation of phosphorylated S6 kinase, increase in mRNA translation which stimulates cell growth.^[32] Stimulation of e4e also results in increased mRNA translation.^[32] We reserve opinion regarding the viability of transplantation of noncultured keratinocytes and fibroblasts without a definitive period of tissue culture. Establishment of

active intracellular signalling pathways is not established within a few days of cell dissociation and culture and may facilitate rapid phagocytosis or apoptosis of the newly engrafted cells and a failure to imbed and engraft. We are uncertain if PRP can enhance cell engraftment or “take” under these circumstances. During the early phase of cell proliferation and growth, the cell cytoskeleton is established. As observed in our cell lines, the cytoskeleton consists of microtubules, actin filaments and intermediate filaments and has been documented before.^[32] We have not established if the enhanced cell growth by PRP interferes with the protein filaments of the cytoskeleton and cytocavitary network.

PRP in combination with photo-light therapy (PLT) for skin augmentation

PRP outcomes rely on the mitogen stimulation of resident cells after dermal injection. In tissue culture, PRP can stimulate fibroblast proliferation and collagen release.^[18] PRP has been

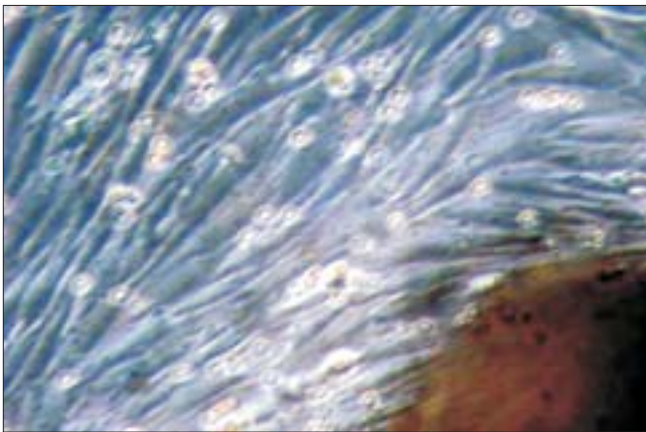


Figure 2: Phase-contrast micrograph of fibroblasts in TC (Olympus inverted microscope). Monolayer of human dermal-derived fibroblasts, plated on adhesion molecule-enriched wells and following stimulation with 40% autologous PRP (day 21, cell culture). Immunomarkers were positive for Type 1 collagen



Figure 3: Aseptic technique during tissue culture in a laminar flow hood is imperative to avoid contamination of cultures, especially when working with blood components

used in the clinic to ameliorate nasolabial lines and fine wrinkles.^[20] Compared to cell therapy with living fibroblasts, the effect may not be paracrine-induced. Data is still needed to explain the effect of PRP on *in vivo* collagen synthesis, collagen contraction in the dermis and stimulation of myofibroblasts. Objective histological data is lacking to support the concept of “neocollagenesis”. Currently, PRP is given as a single treatment, but maintenance treatment (needing further injections) may be imperative to sustain the initial transient results. However, few recipients are enthusiastic to undergo repeated facial dermal injections with modified blood. Reinjection has been needed in our study. It may take months before facial regeneration becomes visible, definitive and measurable. For these reasons, aesthetic clinicians have been compelled to opt for complimentary, adjunctive, multimodal therapy, plus PRP, to ensure lasting results. This is a very costly exercise especially if noninvasive skin stimulation by IPL and low level lasers is needed. Currently, PRP injections for facial wrinkles show no scientifically proven advantage in regenerative capacity over nonablative lasers or radiofrequency devices. The molecular basis for the use of nonablative photolight therapy differs from the application of PRP. The latter relies on cell stimulation by anabolic growth factor or mitogen stimulation and the former by unique activation of photoacceptors, mitochondrial and ATP stimulation and oxidative respiration in the dermis. The regenerative efficacy of low energy photon therapy has been documented (*in vivo* and *ex vivo*).

- Enhanced wound healing.^[35]
- Activation of fibroblast proliferation in-culture.^[35]
- Upregulation of collagen production. This is mediated by up-regulating TGF-beta 1.^[36]
- Increase in myofibroblast stimulation.^[37,38]
- Increased expression of collagen and elastic fibres in certain experimental models.^[39]

Because the clinical results, of anecdotal studies, suggest that a single dose of PRP for facial regeneration and solar damage is only transient, has compelled cosmetic physicians to combine PRP treatment with adjunct radiofrequency, IPL or low-level laser therapy. Advanced solar induced elastosis cannot be reversed by a single application and maintenance therapy is needed. The two treatments are theoretically complimentary and possibly synergistic. However, low energy photon therapy alone has distinct advantages over PRP, in that the biomodulation can be repeated more often, increases elastic fibre expression and myofibroblast stimulation. The latter two elements play key roles in facial rejuvenation of the solar-aged face. Photo-biomodulation and photo-biostimulation differs from the biological action of PRP in that at cellular level, activation of mitochondrial respiratory chain components occurs together with cellular proliferation and cytoprotection. Quantification of the mechanism of action of PRP in facial rejuvenation is problematical and use of the Dermascan, Skin Visio™ and Dermatoscope in our laboratory are difficult to interpret from an objective scientific point of view. There is no proof-

of-science and therefore more research in this area is needed before clinical trials are commenced. It has been reported that photostimulation induces a cascade of signalling events initiated by the initial absorption by cytochrome oxidase. This results in an increase in oxidative metabolism, cell proliferation and enhanced healing.^[35-38] Other important molecular changes include improvement of vascularity, stimulation of collagen production, release of ATP and increased RNA and DNA synthesis. Therefore, compared to the use of PRP, the primary tissue response after laser light therapy is more fully understood by scientists. The primary response on the cytochavitory mitochondria, photon absorption by cytochromes, increase in ATP synthesis and energy are well-documented.

PRP: Addressing dermal elastic tissue loss in solar elastosis and cutaneous aging

It is proposed that PRP and other biologicals can potentially shorten the "healing cascade" of the inflammatory process i.e., reduction in the stages of hemostasis, inflammation, tissue regeneration and tissue remodelling. This process may well be facilitated by the secretory proteins released by activated platelets in PRP. Five major steps in the biological regeneration process in which PRP is used are explained as follows. Intradermal and hypodermal injection of autologous cellular rich plasma acts theoretically as a bio-scaffold that comprises a "structure and signals" process including:

- Formation of a tri-dimensional fibrin network.
- Release of growth factors by thrombocytes and leucocytes.
- Chemo attraction of macrophages and resident stem cells.
- Stem cell proliferation (mitosis).
- Stem cell differentiation.

But the photo aged facial skin has many and complex facets needing multimodal treatment to affect at best, a partial reversal that a sole modality cannot effect. For the moment there is no proof-of-science to confirm positive outcomes and complete reversal of the extra cellular matrix (ECM) dysfunction induced by aging. Currently, there are no comparative studies showing a more efficacious effect of PRP over IPL / laser in improvement of facial fine-lines, solar elastosis and wrinkles. PRP, other biologicals or fillers are currently unable to convincingly reverse the following components of the photo-aged skin.

- Restoration or improvement of photo-oxidative dermal damage, resulting in epidermal thinning.
- Photo-oxidative ECM matrix damage, resulting in elastotic and dermal changes.
- At molecular level, influence the ability of aged or "sluggish" fibroblasts and keratinocytes to respond to changes in their environment.
- Papillary dermal microfibrillar network remodelling observed in solar elastosis.
- Thinning of the stratum spinosum, flattening of the dermal-

epidermal junction and senescence of keratinocytes.

- Restoration of reduced oxytalan fibers by Fibulin-2.
- Reversal of the age-induced lyses of elaunic fibers, solar elastosis, elastolytic degeneration and hypodermal atrophy resulting in less stretchability, resilience, more laxity of the skin and prone to wrinkles.
- Restoration of the loss of dermal fibroblasts or activation of quiescent or senescent fibroblasts.
- Restimulation of wrinkle markers: filaggrin, keratohyalin granules and transglutaminase-1.
- Restoration of depleted collagen (type I and III) in organized bundles at the level of the dermal-epidermal layer or the papillary dermis.
- Metalloproteinase activities of senescent dermal fibroblasts that contribute to the age-related atrophy of ECM architecture.
- Restimulation of reduced melanocyte density and Langerhan's numbers as seen in aging skin^[40-51].
- Reversal of advanced skin aging histopathology^[51-64].

PRP: Potential impact of dermal and hypodermal injection of PRP on anatomical facial structures

Tracking of PRP injectate in the nasiolabial region (because of the mild enzymatic action), intended for a volumetric and aesthetic effect, from the dermis into the hypodermis may result in a closed compartment syndrome. This may compromise flow in the anterior facial vein, that runs over the facial prominence en-route to the retromandibular vein. Embolization of active factors into this valveless venous conduit (initially via the deep dermal-reticular capillary network), needs further study. Doppler studies show potential reversal of flow in this vein depending on the position of the patient. Inadvertent injection superior to the line of the parotid duct may embolize GF into the orbit, frontal diploic vein or cranial circulation. Tracking of active GF, inferior and medial to the *m. zygomaticus major* may enter the pterygoid plexus via the deep facial vein and side branches (i.e., palpebral and nasal). In the nasolabial line the anterior facial is fairly superficial and can come into play with the tip of the needle unknowingly, during hypodermic injection of PRP.

APPLICATION OF PRP IN ORTHOPAEDIC SURGERY

PRP has been successfully applied in maxilla-facial and oral surgery regarding sinus augmentation, autologous bone transfer, socket surgery and face-lifts.^[1,7,9,20] The supplementation of suture repair with collagen-PRP hydrogel in an ACL-animal model has been reported by orthopaedic surgeons from Harvard Medical School.^[17] In this model the following was shown four weeks after surgery:

- Improvement in load at yield
- Improvement of maximum load
- Improvement in linear stiffness

This group were therefore able to show improvement of biomechanical properties, regarding wound healing and the use of PRP after tendon repair.^[17] Other orthopedic research groups have reported their experience with PRP:

- Human tendon cell proliferation is enhanced in tissue culture with PRP.^[40] Similar reports were documented in a rat Achilles tendon transection model and highlights the importance of platelet growth-factors in the early phase of regeneration.^[41]
- PRP induced regeneration of bone in experimental models.^[42]
- PRP has the potential to reduce the inflammatory process thereby promoting tissue regeneration.^[42] PRP has been shown to modify monocyte-mediated proinflammatory cytokine/chemokine release.^[42]
- PRP upregulates proteoglycan and collagen synthesis in ECM of cultured cartilage cells.^[10] This may well be important for the regeneration of intervertebral discs in man.^[10]
- PRP can enhance chemotaxis and cell proliferation of human osteoblastic cell line SaOS-2 *in vitro*.^[12]
- PRP can enhance the expression of cartilage oligomeric matrix protein (COMP).^[18]
- PRP potential to enhance integration of bone allografts in experimental models.^[28]
- PRP upregulation of osteoblastic MG63 cell line.^[14]
- Experimental evidence that PRP can mediate fracture healing in diabetes.^[43]

PRP: Potential for enhanced rotator cuff healing relevant to the shoulder surgeon

Burkhart and co-workers from the University of Texas Health Science Centre, Texas and De Beer of the Shoulder Institute, Cape Town, are pioneers in arthroscopic rotator cuff repair by humeral-head footprint reconstruction.^[44-48] The success of the arthroscopic approach has been based on a solid base regarding the local anatomy of the humeral head and tendon footplates [see illustrations 4-6] The durability of the arthroscopic approach and drawbacks of rotator cuff repair, are not disputed.^[46-50] Burkhart *et al.*, have demonstrated the challenges of arthroscopic revision rotator cuff repair, for which a previous rotator cuff repair had failed.^[48] Of interest in their series, was the reported occurrence of massive recurrent rotator cuff tears.^[48] In these patients, extensive arthroscopic dissection and mobilization of the rotator cuff was required and needed to delineate the tear margins to effect repair.^[48] It is postulated, based on our laboratory research, that the supplementation of arthroscopic or open suture repair with PRP in such cases may further facilitate healing of the degenerative cuff. Obviously, more experimental work is indicated, but the PRP-hypothesis for enhancement of rotator cuff healing is based on the following scientific and biological observations:

- Beneficial results observed in maxilla-facial surgery regarding soft and hard tissue augmentation with PRP.^[1-5]
- Beneficial effects of PRP on tendon healing and proliferation

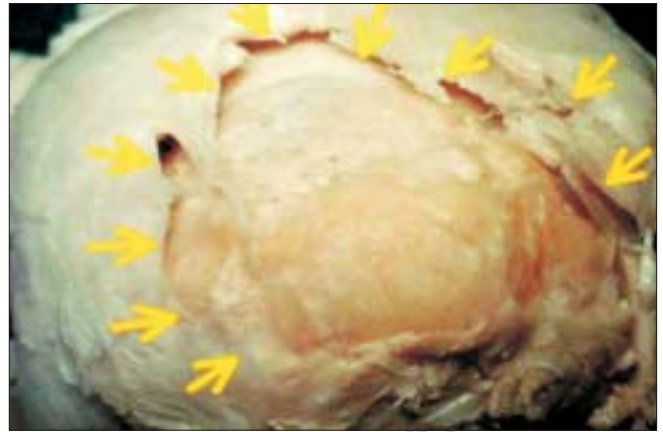


Figure 4: Human cadaveric dissection of the shoulder and viewed laterally, showing a large rotator cuff tear in a young female. The cause of the pathology is unknown. Yellow arrows delineate the edges of the tear



Figure 5: Osteology specimen, viewed from laterally, showing shoulder rotator cuff tendon foot plates of the m.supraspinatus (2), m.infraspinatus (3), m.teres minor (4, but not clearly visible) and m.subscapularis muscles(1)



Figure 6: Cadaveric dissection specimen viewed from posteriorly, showing a small rotator cuff tear (arrow) in relation to fibres of m.subscapularis (19), m.supraspinatus (7) and m.infraspinatus (3)

ex vivo.^[40,41]

- Stimulation of fibroblast growth *ex vivo* by PRP and therefore collagen release as well as the biological effects on the ECM.^[31]
- Stimulatory effects on osteoblast cultures *ex vivo*.^[8,12]
- Stimulatory effects on angiogenesis.^[1]
- Stimulatory effect on chondrocyte physiology.^[10]

In conclusion, there is scientific evidence, albeit anecdotal, that PRP can modulate and influence the early healing cascade relevant to the regenerative capacity of soft and hard tissue. These observations may well be relevant to the shoulder surgeon when considering repair of large rotator cuff tears. In these cases, the application of PRP in addition to conventional surgery may enhance suture repair and healing of the rotator cuff.

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REFERENCES

1. Marx RE. Platelet-rich plasma: Evidence to support its use. *J Oral Maxillofac Surg* 2004;62:489-96.
2. Pietrzak WS, Eppley BL. Platelet rich plasma: Biology and new technology. *J Craniofac Surg* 2005;16:1043-54.
3. Della Valle A, Sammartino G, Marenzi G, Tia Mariano M, Di lauro AE, Ferrari F, Lo Muzio L. Prevention of post-operative bleeding in anti-coagulated patients undergoing oral surgery: Use of Platelet-Rich Plasma gel. *J Oral Maxillofac Surg* 2003;61:1275-8.
4. Freymiller EG, Aghaloo TL. Platelet-rich plasma: Ready or not? *J Oral Maxillofac Surg* 2004;62:484-8.
5. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from Platelet-Rich-Plasma: Implications for Wound healing. *Plast Reconstr Surg* 2004;114:1502-8.
6. Robiony M, Polini F, Costa F, Politit M. Osteogenesis distraction and Platelet-Rich Plasma for bone restoration of the severely atrophic mandible: Preliminary results. *J Oral Maxillofac Surg* 2002;60:630-5.
7. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet Rich Plasma: Growth factor enhancement for bone grafts. *Oral Surg* 1998;85:638-46.
8. Kanno T, Takahashi T, Tsujisawa T, Ariyoshi W. Platelet-Rich Plasma enhances human osteoblast-like cell proliferation and differentiation. *J Maxillofac Surg* 2005;63:362-9.
9. Marx RE, Carg AK. Dental and craniofacial applications of platelet-rich plasma. Quintessence Books: Chicago; 2005.
10. Aakeda K, An HS, Pichika R, Attavia M, Thonar EJ, Lenz ME, *et al.* Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and annulus fibrosus cells cultured in alignate beads. *Spine* 2006;31:959-66.
11. Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, *et al.* Platelet-rich plasma contains high levels of platelet-derived growth factors and transforming growth factor-beta and modulates the proliferation of periodontally related cells *in vitro*. *J Periodontol* 2003;74:849-57.
12. Graziani F, Cei S, Ducci F, Giuci MR, Donos N, Gabriele M. *In vitro* effects of different concentration of PRP on primary bone and gingival cell lines. Preliminary results. *Minerva Stomatol* 2005;54:15-22.
13. Grotendorst GR, Rahmanie H, Duncan MR. Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation. *FASEB J* 2004;18:469-79.
14. Kawase T, Okuda K, Wolff L, Yoshie H. Plasma-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells *in vitro*. *J Periodontol* 2003;74:856-64.
15. Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM, Ince C. Influence of the application of platelet-enriched plasma in oral mucosal wound healing. *Clin Oral Implants Res* 2007;18:133-9.
16. Roukis TS, Zgonis T, Tiernan B. Autologous platelet-rich plasma for wound and osseous healing: A review of the literature and commercially available products. *Adv Ther* 2006;23:218-37.
17. Murray MM, Spindler KP, Abreu E, Mullwer JA, Nedder A, Kelly M, *et al.* Collagen-platelet-rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. *J Orthop Res* 2007;25:81-91.
18. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, *et al.* Platelet-rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res* 2007;25:230-40.
19. Carter CA, Jolly DG, Worde CE, Hendren DG, Kane CJ. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. *Exp Mol Pathol* 2003;74:244-55.
20. Chajchir A, Fabrizio D, Chajchir G, Celi E. Growth factors in plastic surgery. *Aesth Plast Surg* 2005;29:295-9.
21. Marlovits S, Mousavi M, Gäbler C, Erdős J, Vécsei V. A new simplified technique for producing platelet-rich plasma: A short technical note. *Eur Spine* 2004;13:S102-6.
22. Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. *Facial Plast Surg* 2002;18:27-33.
23. Eppley BL, Pietrzak WS, Blantou M. Platelet-rich plasma. A review of biology and applications in plastic surgery. *Plast Reconstruct Surg* 2006;118:147e-59e.
24. Man D, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg* 2001;107:229-39.
25. Moore KL, Dalley AF. Clinically orientated anatomy. Lippincott Williams and Wilkins: Philadelphia; 2006.
26. Walton J. Brain's diseases of the nervous system. 10th ed. Oxford University Press: Oxford; 1983.
27. Everts PA, Hoffmann J, Weibrich G, Mahoney CB, Schonberger JP, van Zundert A, *et al.* Differences in platelet growth factors release and leucocyte kinetics during autologous platelet gel formation. *Transfus Med* 2006;16:363-8.
28. Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, *et al.* Platelet-derived growth factors enhance proliferation of human stromal stem cells. *Biomaterials* 2003;24:3095-100.
29. Kocaomer A, Kern S, Klueter H, Bieback K. Human AB-serum as well as thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for the expansion of mesenchymal stem cells from adipose tissue. *Stem Cells* 2007;14:535-40.
30. Holten-Andersen MN, Brunner N, Christensen IJ, Jensen V, Nielsen HJ. Levels of tissue inhibitor of metalloproteinases-1 in blood transfusion components. *Scand J Clin Lab Invest* 2002;62:223-30.
31. Liu Y, Kalen A, Risto O, Wahlstrom O. Fibroblast proliferation due to exposure to a platelet concentrate *in vitro* is pH dependent. *Wound Repair Regen* 2002;10:336-40.
32. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *Molecular Biology of the Cell*. 4th ed. Garland Science: New York; 2002. p. 1019-20.
33. Boss WK, Usal A, Chernof G, Keller GS, Lask GP, Fodor PB. Autologous cultured fibroblasts as cellular therapy in plastic surgery. *Clin Plast Surg* 2000;27:613-26.
34. Du Toit DF, Geldenhuys KM. Biotechnological anti-aging cell-therapy treatment of facial wrinkles with cultured human fibroblasts. *The Specialist Forum* 2005;5:38-46.
35. Pourzarandian A, Watanabe H, Ruwanpura SM, Aoki I, Ishikawa

- I. Effect of low level Er:YAG laser irradiation on cultured human gingival fibroblasts. *J Periodontol* 2005;76:187-93.
36. Yu HY, Chen DF, Wang Q, Cheng H. Effects of lower fluence pulsed dye laser irradiation on production of collagen and the mRNA expression of collagen genes in cultured fibroblasts in vitro. *Chin Med J Engl* 2006;119:1543-7.
 37. Pinheiro AL, Pozza DH, Oliveira MG, Weismann R, Ramalho LM. Polarized light (400-2000 nm) and non-ablative laser (685 nm): A description of the wound healing process using immunohistochemical analyses. *Photomed Laser Surg* 2005;23:485-92.
 38. Medrado AR, Pugliese LS, Reis SR, Andrade ZA. Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. *Lasers Surg Med* 2003;32:239-44.
 39. Pugliese LS, Medrado AP, Reis SR, Andrade Zde A. The influence of low-level laser therapy on biomodulation of collagen and elastic fibers. *Pesqui Odontol Bras* 2005;17:307-13.
 40. Anitua E, Andia I, Sanchez M, Azofra J, del Mar Zalduendo M, de la Fuente M, *et al*. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF by human tendon cells in culture. *J Orthop Res* 2005;23:281-6.
 41. Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. *Acta Orthop* 2006;77:806-12.
 42. El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, Van Dyke TE. Platelet rich plasma: Growth factors and pro- and anti-inflammatory properties. *J Periodol* 2006;78:661-9.
 43. Ghandi A, Doumas C, O'Connor JP, Parsons JR, Lin SS. The effects of local platelet rich plasma delivery on diabetic fracture healing. *Bone* 2006;38:540-6.
 44. Burkhart SS, Klein JR. Arthroscopic repair of rotator cuff tears associated with large bone cysts of the proximal humerus: Compaction bone grafting technique. *Arthroscopy* 2005;21:1149.
 45. Burkhart SS, Lo IK. Arthroscopic rotator cuff repair. *J Am Acad Orthop Surg* 2006;14:333-46.
 46. Brady PC, Arrigoni P, Burkhart SS. Evaluation of residual rotator cuff defects after *in vivo* single-versus double-row rotator cuff repairs. *Arthroscopy* 2006;22:1070-5.
 47. Smith CD, Alexander S, Hill AM, Huijsmans PE, Bull AM, Amis AA, *et al*. A biomechanical comparison of single and double-row fixation in arthroscopic rotator cuff repair. *J Bone Joint Surg Am* 2006;88:2425-31.
 48. Lo IK, Burkhart SS. Arthroscopic revision of failed rotator cuff repairs: Technique and results. *Arthroscopy* 2004;20:250-67.
 49. Wolf EM, Pennington WT, Agrawal V. Arthroscopic rotator cuff repair: 4-10-year results. *Arthroscopy* 2004;20:5-12.
 50. Brislin KJ, Field LD, Savoie FH 3rd. Complications after arthroscopic rotator cuff repair. *Arthroscopy* 2007;23:124-8.
 51. West MD. The cellular and molecular biology of skin aging. *Arch Dermatol* 1994;130:87-95.
 52. Suwabe H, Serizawa A, Kajiwa A, Ohkido M, Tsutsumi Y. Degenerative processes of elastic fibers in sun-protected and sun-exposed skin: Immunoelectron microscopic observation of elastin, fibrillin-1, amyloid P component, lysozyme and alpha1-antitrypsin. *Pathol Int* 1999;49:391-402.
 53. Kurban RS, Bhawar J. Histologic changes in skin associated with aging. *J Dermatol Surg Oncol* 1990;16:908-14.
 54. El-Domyati M, Attia S, Saleh F, Brown D, Birk DE, Gasparro F, *et al*. Intrinsic aging vs photoaging: A comparative histopathological, immunohistochemical and ultrastructural study of skin. *Exp Dermatol* 2002;11:398-405.
 55. Bosset S, Barre P, Chalon A, Kurfust R, Bonte F, Andre P, *et al*. Skin aging: Clinical and histopathologic study of permanent and reducible wrinkles. *Eur J Dermatol* 2002;12:247-52.
 56. Bouissou H, Pieraggi MT, Julian M, Savit T. The elastic tissue of the skin. A comparison of spontaneous and actinic (solar) aging. *Int J Dermatol* 1988;27:327-35.
 57. Iurassich S, Pedana MA. Photoaging of the skin and occupation: Correlation between clinical ultrasound and histological findings. *Med Lav* 2005;96:419-25.
 58. Watson RE, Griffiths CE, Craven NM, Shuttleworth CA, KIELTY CM. Fibrillin-rich microfibrils are reduced in photoaged skin. Distribution at the dermal-epidermal junction. *J Invest Dermatol* 1999;112:782-7.
 59. Contet-Audonneau JL, Jeanmaire C, Pauly G. A histological study of human wrinkle structures: A comparison between sun-exposed areas of the face, with or without wrinkles and sun protected areas. *Br J Dermatol* 1999;140:1038-47.
 60. Wulf HC, Sandby-Moller J, Kobayashi T, Gniadecki R. Skin aging and natural photoprotection. *Micron* 2004;35:185-91.
 61. Smith L. Histopathologic characteristics and ultrastructure of aging skin. *Cutis* 1989;43:414-24.
 62. Yaur M. Molecular mechanisms of skin aging. *Adv Dermatol* 1995;10:63-76.
 63. Bogle MA, Arndt KA, Dover JS. Evaluation of plasma skin regeneration technology in low-energy full-facial rejuvenation. *Arch Dermatol* 2007;143:168-74.
 64. Bogle MA. Plasma skin regeneration technology. *Skin Therap Lett* 2006;11:7-9.

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