WHY USING ACTIVATED PLATELETS AND PATIENT’S SERUM IN AESTHETIC MEDICINE.

ABSTRACT: Over the past decade, there has been a steady trend for patients to seek less invasive procedures for facial rejuvenation. The introduction of new and more predictable soft tissue fillers has led to an increased acceptance of those treatments over surgical standard procedures. However, the ideal soft tissue filler has not yet been found...

Recently, the Health Secretary (Andrew Lansley) acting after concerns raised in The Times has promised that dermal fillers and Anti-ageing injections will be subjected to tighter regulation as part of a review of the lax rules governing cosmetic surgery. He has asked Sir Bruce Keogh, medical director of the NHS, to come up with plans to improve regulation for cosmetic surgery, specifically highlighting the problem of dermal fillers.

Doctors have seen a tremendous increase in the understanding of platelet physiology. Platelet preparations are convenient in that they are autologous and non-immunogenic, non-infectious, non-carcinogenic, can be easy to prepare and are made from an abundant and readily available source.

PLATELET ORIGIN, MORPHOLOGY, AND DISTRIBUTION
Platelets are formed when cytoplasmic fragments of megakaryocytes (which are a very large type of white blood cell), in the bone marrow, are released into the circulation as they age. They are round or oval in shape, approximately 2 μm in diameter. The platelet is metabolically more active than the red blood cell and has a variety of functions. Platelets play an important and not fully understood role in the formation of the blood clot. Platelets also store and transport several chemicals, including serotonin, epinephrine, and histamine and they phagocytize foreign bodies. They reside in the spleen and the plasma which contains other constituents: salts, glucose, amino acids, vitamins, hormones...

Platelets lack nuclei but contain organelles and structures such as mitochondria, microtubules, and granules (alpha, delta and lambda). There are approximately 50 to 80 alpha-granules per platelet, each bound by a unit membrane.

PLATELET ROLE IN WOUND HEALING
In the wound healing environment, platelets will degranulate within 10 minutes of the formation of the clot, releasing growth factors into the local environment; 95% of the pre-synthesized and existing platelet growth factors are present in the wound within the first hour. However, normal platelets will continue to synthesize and release growth factors over the next 7-10 days, thus supporting angiogenesis, collagen production and other aspects of wound healing.

Numerous proteins are contained within the alpha-granules of platelets including:
3 isomers of platelet-derived growth factor (PDGF)
   - Chemo attractive to Mesenchymal Stem Cells and endothelial cells.
   - Differentiation for fibroblasts and osteoblasts.
   - Up regulate effects of other growth factors on cells such as macrophages.
- Mitogenes of mesenchymal stem cells promote the synthesis of the extra cellular matrix

**transforming growth factor (TGF)**-[1 and 2],

- Promotes cell mitosis
- Significantly increases type I Collagen production in tendon -Favours the synthesis of collagen.
- Sheath fibroblast
- Stimulation of DNA synthesis, proliferation of various types of cells.

**vascular endothelial growth factor (VEGF),** Stimulates angiogenesis, chemo attractive for osteoblasts

**epidermal growth factor (EGF),**

- Important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR
- Induce epithelial development and promote angiogenesis
- Stimulates proliferation and differentiation of epidermis cells, co-stimulating angionegesis.

**platelet factor 4 (PF4),**
**interleukin (IL)-1,**
**platelet-derived angiogenesis factor (PDAF),**
**platelet-derived endothelial growth factor (PDEGF),**
**epithelial cell growth factor (ECGF),**
**insulin-like growth factor (IGF),**
**osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin (TSP).**

In addition the activated thrombocytes have onto their surface a multitude of signalisation molecules: CD9, CD-W17, CD41, CD42a-d, CD51, CD-W60, CD61, CD62P, CD63

As the direct platelet influence begins to subside, macrophages, which arrive by means of vascular ingrowth stimulated by the platelets, assume responsibility for wound-healing regulation by secreting their own factors. Thus, the platelets at the repair site ultimately set the pace for wound repair.

**PLATELET-RICH PLASMA (P.R.P)**
Platelet-rich plasma is defined as a small volume of plasma of autologous blood having a platelet concentration above baseline. It is therefore inherently safe and free of transmissible diseases such as HIV and hepatitis. Because it is a concentration of platelets, it is also a concentration of the 7 fundamental protein growth factors proved to be actively secreted by platelets to initiate all wound healing.

PRP development via specific centrifugation process has been greatly simplified so that now it can be easily used in the medical office setting. However, the centrifugation process must be sterile and precisely suited to platelet separation from red blood cells and their sequestration in high concentrations without lysing the platelets or damaging them so that they no longer can actively secrete their growth factors.
How Does PRP Work?
PRP works via the degranulation of the alpha granules in platelets, which contain the synthesized and pre-packaged growth factors. The active secretion of these growth factors is initiated by the clotting process of blood and begins within 10 minutes after clotting. More than 95% of the pre-synthesized growth factors are secreted within 1 hour. That generates the formation of 3D fibrin mesh and platelet cohesion (via calcium and thrombin) which in turn will lead to:
- Local stem cell proliferation and
- Local tissue stem cell differentiation and therefore enhanced tissue regeneration and tissue remodelling.
- Enhanced proliferation and differentiation of keratinocytes that are essential for a smooth complexion.
- Enhanced release of collagen from activated fibroblasts, thus restoring the extra cellular matrix (ECM) and enhancing thickening of the dermis.

The growth factors within the alpha granules of platelets are incomplete because they must be soluble. When the clotting process activates the platelets, the growth factors are secreted from the cell through the cell membrane. Then the protein growth factors are completed to a bioactive state by the addition of histones and carbohydrate side chains to these proteins.

Therefore, PRP must be developed in an anti-coagulated state and should be used on the graft, flap, wound, or within 10 minutes of clot initiation.
PRP has been shown to remain sterile and the concentrated platelets viable for up to 8 hours once developed in the anti-coagulated state and placed on a sterile surgical table.

What is the Safety of PRP?
Because it is an autogenous preparation, PRP is inherently safe and therefore free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever, and Cruetzfeld-Jacob disease (CJD) (“mad cow disease”).
The importance of this knowledge is that the PRP growth factors never enter the cell or its nucleus, they are not mutagenic, and they act through the stimulation of normal healing, just much faster. Therefore, PRP has no ability to induce tumour formation and has never done.

Plastic surgery
Autologous platelets are especially useful for the soft tissue and bony reconstruction encountered in facial plastic and reconstructive surgery. Valbonesi et al. who used autologous fibrin-platelet glue in 14 patients with skin and soft tissue losses caused by recent trauma or chronic pathology has concluded: Their use results in a decrease in operative time, necessity for drains and pressure dressings, and incidence of complications as well as reduced infections and length of hospital stay.

Anti-inflammatory properties with reduced oedema and ecchymosis was associated with the use of autologous platelet gel in 8 women after deep plane rhytidectomy. Autologous platelet-rich gel was also shown to be effective in stopping capillary bleeding in the surgical flaps of a series of 20 patients undergoing cosmetic surgery (face lifts, breast size changes or neck lifts).

Wound healing (ulcers)
As early as 1990, autologous human platelet-derived wound healing factors (HPDWHF) were proposed to regulate wound healing of recalcitrant skin ulcers by promoting the formation of granulation tissue in the early healing phase. This conclusion was based on studies on 23 patients with 27 skin ulcers who had shown no signs of healing after an average period of 25 weeks conventional wound care. Strikingly, 100% healing was seen an average of 10 weeks after the application of HPDWHF.

Foot ulceration is a common complication of diabetes. The wounds are often multifactorial but arise in the setting of peripheral neuropathy and/or vascular complications. Platelet releasate has been used on thousands of patients over a ten year period in the USA, and an analysis of results for these patients in an American Health Service database allowed Margolis et al. to conclude that use of platelet releasate is of proven efficacy, especially for patients with more severe wounds.

**Wrinkles and skin rejuvenation**

Following the results and published studies with PRP in surgery, dentistry, ophthalmology.. Cosmetic practitioners started mid-2000 to use PRP for skin rejuvenation, dark circles under eyes, hair growth, etc.

But apart from a meta-analysis published in 2007 by Borzini and Mazucco, only the subjective aspect has been traditionally evaluated either based on the patient or physician’s subjective level of satisfaction. The G. Amgar study (2011) uses biometric parameters for scientifically measurable means of assessing the results from the PRP injections.

- Skin hydration was made after compensating for the ambient humidity level. The hydration was expressed by a standard index.
- Anisotropy measures the distribution of micro-lines in 360°, and is expressed as a percentage. Following marked swelling of the skin, a number of micro-lines on the surface of the skin can be observed. The projection and distribution of these micro-lines two-dimensionally in all directions (360°) is indicative of young skin. The older the skin, the more these lines will change orientation and become parallel.
- TEWL is a non-invasive measurement of the amount of epidermal water lost or evaporated. It is expressed in grams per hour per square metre of cutaneous surface. TEWL reflects the integrity of the stratum corneum.

“The study tracked 37 patients for a period of 3 weeks following PRP treatment, and 27 for an additional 10 weeks post-PRP treatment. A good anti-ageing effect was assessed by measured anisotropy values of -24.1% and -16.9%, respectively. Additionally, a cross-analysis involving the initial anisotropy readings demonstrated further improvement. The anisotropy correlates were -33% and -39.7%, respectively, if the treatment is provided to patients who would mostly benefit from it (anisotropy > 30 %). The study also demonstrated that the effects of one PRP treatment could last for up to 10 months.”

Latest update:

The study published by the Department of Dermatology, College of Medicine, Chungnam National University, Daejeon, Korea.(November 2011) was concentrating not only on the effects of activated platelet-rich plasma (aPRP) but also on the activated platelet-poor plasma (aPPP).

They investigated on the remodelling of the extracellular matrix, a process that requires activation of dermal fibroblasts, which is essential for rejuvenation of aged skin.
Their results proved that aPRP and aPPP both stimulated cell proliferation, with peak proliferation occurring in cells grown in 5% aPRP. Additionally, aPRP and aPPP increased the expression of type I collagen, MMP-1 protein, and mRNA in human dermal fibroblasts. And they concluded: “aPRP and aPPP promote tissue remodelling in aged skin and may be used as adjuvant treatment to lasers for skin rejuvenation in cosmetic dermatology”.

**Conclusion:** After more than 300 treatments in 2011, my personal experience is in total agreement with the Korean study. When we centrifuge the whole blood, the result is 2 folds: a PRP and a PPP fraction. Why discarding the PPP? It is already there, does not cost anything in time or material and obviously can be of use as even if not that rich in platelets still contains interesting elements for the skin.

The review of my last 100 treated patients with serum and platelets (PRP AND PPP) over the last 5 months shows a return rate (satisfaction) of 63%. But over the last two years those 100 patients accumulated 240 treatments. Interesting enough none of them were prompted to come back for more treatments.

Results were shown very fast on: Skin tone/skin radiance, pores sizes, little lines, naso-labial folds, Décolleté, Scars (even keloids), Hair loss. It is a very versatile, easy to use treatment. It can be used by topical application only, mesotherapy, deeper injections, mixed with almost every type of dermal filler, as a stand-alone treatment or post ablative laser. It is proven, safe, potent and without any risk of major side effect, No risk of infection (correctly used), no risk of granuloma, no risk of cancerous cell spreading. Also it is cheap compare to lasers (ablative or not) or other hi-tech equipment designed to rejuvenate the skin and stimulate collagen production.

Do we have now the “perfect” dermal filler...?

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