

Tissue Regeneration

Dr Alain Gondinet of ReGen Lab reviews cell therapies using platelet rich plasma autologous cellular regeneration

This article describes the potential for doctors to use platelet rich plasma in their daily practice in the aesthetic fields of mesotherapy, nappage and fillers.

A MULTIDISCIPLINARY PROGRAMME FOR SKIN REJUVENATION

From the centrifuged blood of the patient, we can obtain, in a specific tube, a plasma, with platelets and white cells separated from the red cells by a gel. Once we have this plasma, we can inject it into the patient's skin in different ways: nappage, mesotherapy and/or fillers. But before starting the protocol for aesthetics injections, we have to understand what autologous cell regeneration (ACR) means, and what the components of the PRP are.

WHAT IS AUTOLOGOUS CELL REGENERATION?

Autologous cell regeneration is the bio-stimulation of the cells of the dermis using an injection technique which utilises the patient's own bio-active platelet rich plasma (PRP), including leucocytes. The aim of these injections is to stimulate and regenerate aged and damaged skin and hypodermal tissues, and to produce a new dermis.

WHAT IS PLATELET RICH PLASMA?

Platelet rich plasma is an autologous concentration of human platelets in a small volume of plasma measured as 1,000,000 platelets per mm^3 or two to three times the native concentration of whole blood at a pH of 6.5-6.7 (whole blood pH is 7.0-7.2).

It is also referred to as autologous platelet gel, plasma rich growth factors (PRGFs) or autologous platelet concentrate. PRP is a concentration of the fundamental protein growth factors which have been proved to

Figure 1: Growth factors acting on 'healing cascade'

Factor	Name	Principal source	Effects
PDGF aa PDGF bb PDGF ab	Platelet derived growth factors	Activated thrombocytes Activated thrombocytes	Mitogenes of mesenchymal stem cells promote the synthesis of the extracellular matrix
TGF-alpha TGF-beta	Transforming growth factors	Activated thrombocytes	Stimulation of DNA synthesis, proliferation of various types of cells. Favours the synthesis of collagen
IGF-I IGF-II	Insulin-like growth factors	Activated thrombocytes	Stimulates proliferation and differentiation of osteoblasts
EGF	Epidermal growth factor	Activated thrombocytes	Stimulates proliferation and differentiation of epidermis cells, co-stimulating angiogenesis
VEGF	Vascular endothelial growth factor	Leucocytes and endothelial cells	Stimulates angiogenesis and chemo-attraction of osteoblasts

In addition, the activated thrombocytes have on their surface a multitude of signalisation molecules, for example: CD9, CD-W17, CD31, CD41, CD42a-d, CD51, CD-W60, CD61, CD62P, CD63

Figure 2: Benefits of PRP reported in the 'healing cascade'

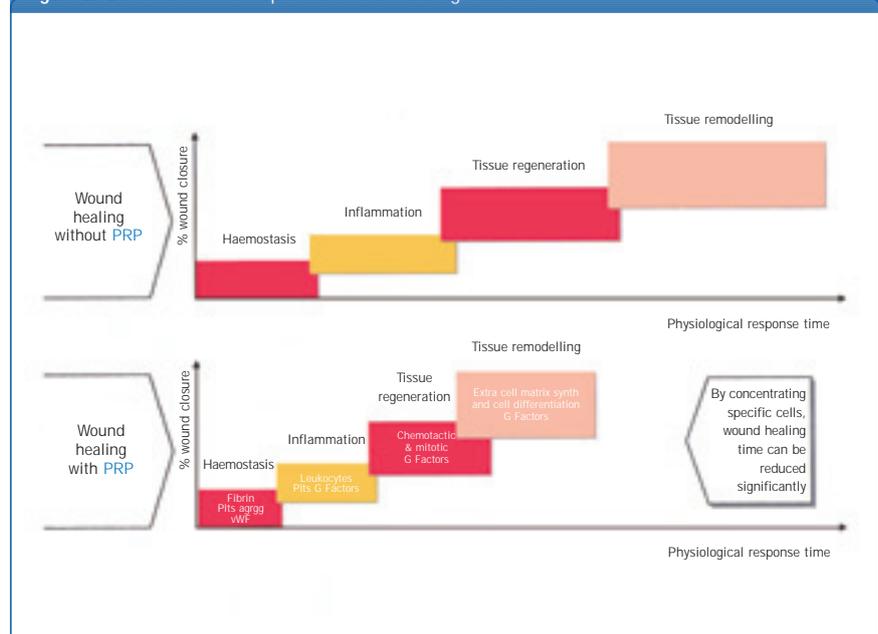
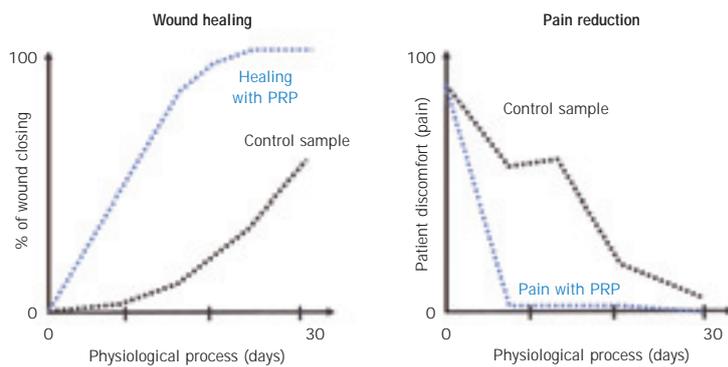
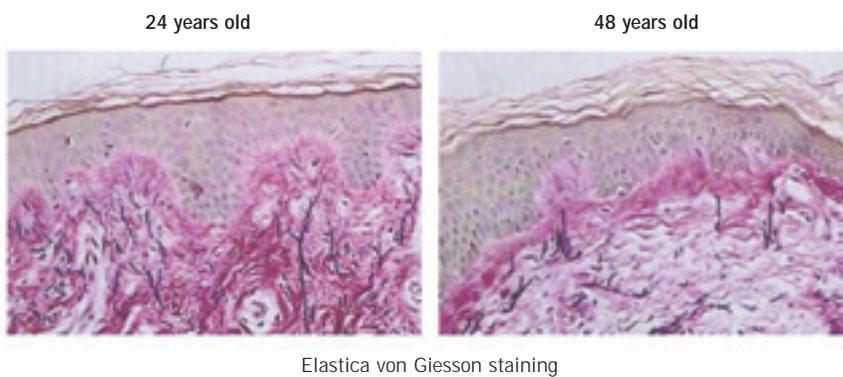


Figure 3: Visible effect in time on healing and discomfort (randomised study US)



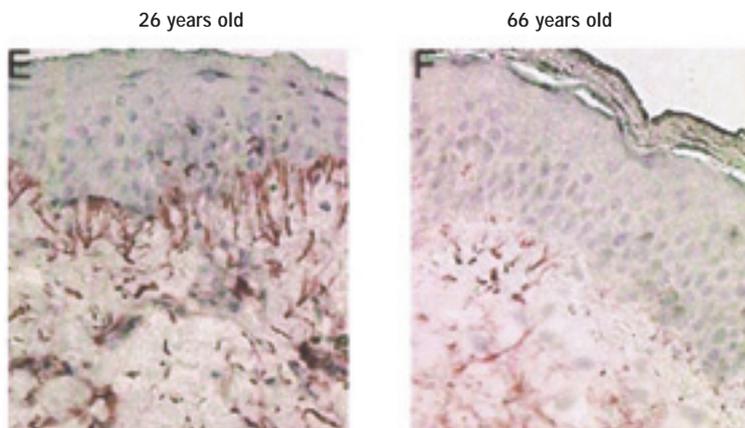
Marx, Monteleone, Ghurani and Marx, University of Miami, Journal of Oral & Maxillofacial Surgery, 58: p54, 2000

Figure 4: What happens when we get older?



Elastica von Giesson staining

Figure 5: Elastolysis: degeneration of elastic fibres



Specific immuno-elastin staining

be actively secreted by platelets to initiate all wound healing. PRP includes three blood proteins known for acting as cell adhesion molecules: fibrin, fibronectin and vitronectin. Because all constituents retrieved in PRP are autologous in origin, the risk of a local immunological reaction is ruled out.

The preparation of the PRP required for treatments is simple, rapid and

advantageous. When we inject the PRP into the skin, we increase the rate of healing and tissue restructuring. Let's have a look at the physiology of the healing process:

- During the ageing process, the body reacts with a series of repairing steps. The healing cascade that we can see during any injury of the skin shows that there are specific

components of blood plasma, such as thrombocytes and leukocytes, which play an important role in every phase of tissue regeneration

- The leukocytes and the activated platelets begin to release signalisation proteins (chemiokines), which promote the migration of the macrophages from the conjunctive tissue
- The macrophages have to clean up to start tissue regeneration

The alpha granules of the activated thrombocytes release more and more growth factors, inducing the migration and the proliferation of non-differentiated mesenchymal cells. These stem cells become cells with specific functions. The leukocytes also release a growth factor (VEGF), which induces the creation of new blood vessels (angiogenesis).

Adhesion molecules, polypeptides, enzymes, vitamins and oligo-elements retrieved in the plasma, show a crucial role in the recruitment and the differentiation of stem cells. These molecules are necessary to support the high level of metabolism associated with these activated cells.

In Figure 3 we can see how the wound healing process has been shortened thanks to the PRP, and the pain reduction with PRP treatment.

In Figures 4 and 5, we can see the differences of structure in the papillary dermis, and specifically the degeneration and the lack of the elastic fibres responsible for elastolysis. At the same time, we have degradation via metalloproteinase-1, which is induced by UV light and smoking.

FIVE MAJOR STEPS IN THE ACR REGENERATION PROCESS

Intra dermal and hypodermal injections of autologous cellular rich plasma acts as a bio-scaffold which comprises a 'structure and signals' process including:

- Formation of a tri-dimensional fibrin network

- Release of growth factors by thrombocytes and leucocytes in their biologically determined ratios
- Chemo-attraction of macrophages and stem cells
- Stem cell proliferation (mitosis)
- Stem cell differentiation

THE ADVANTAGES OF THE AUTOLOGOUS CELL REGENERATION THERAPIES

There are distinct advantages of ACR therapies today for patients and doctors:

- Autology: no allogenic (donor) treatment
- Tissue regeneration and rejuvenation
- Safety: non-allergenic and free from concerns over transmissible diseases such as HIV, Hepatitis B & C, CJD and so on

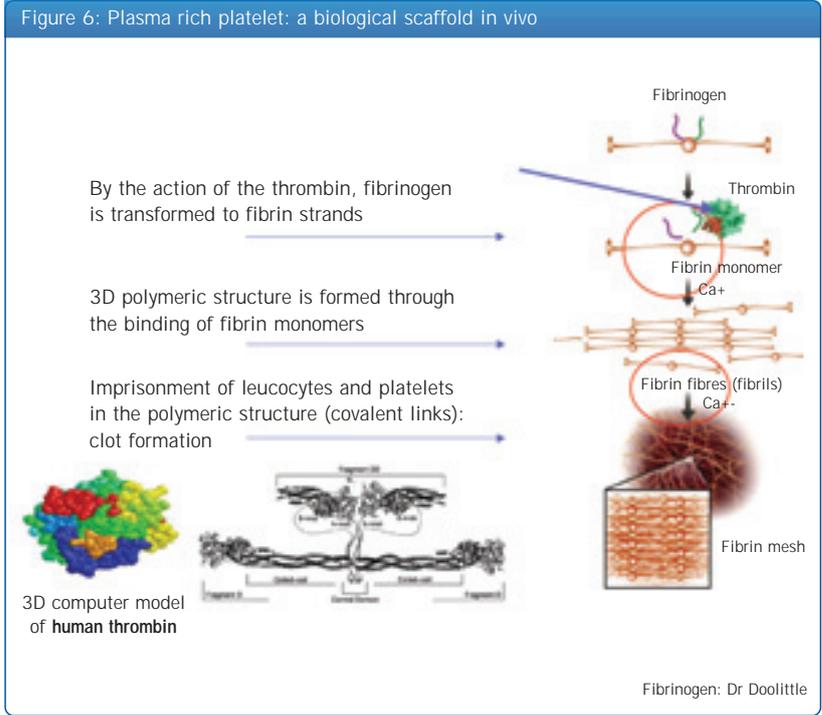


Figure 7: Ultra micro photo of a mesh Dr Susan Lim

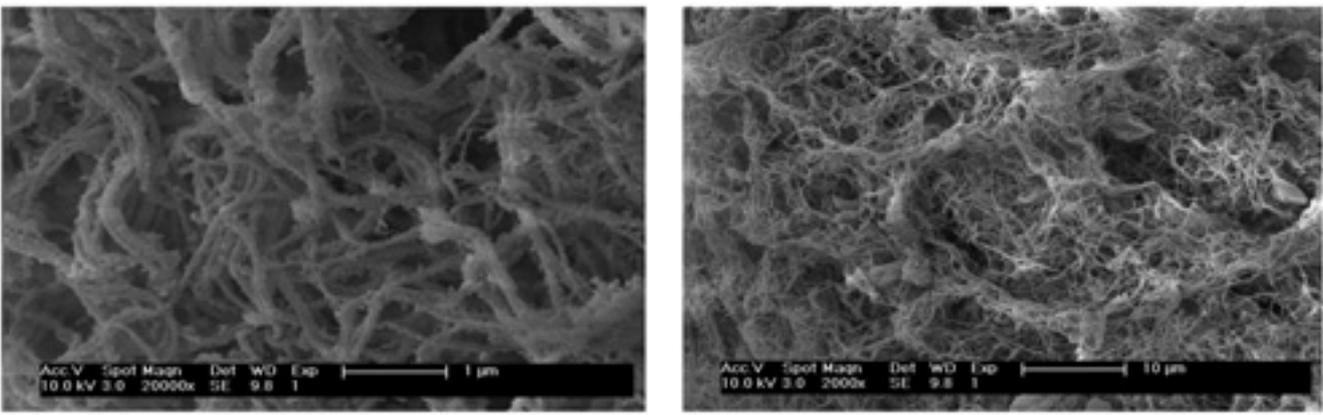


Figure 8: *In vivo* biologic scaffold – ‘structure and signals’ (1)

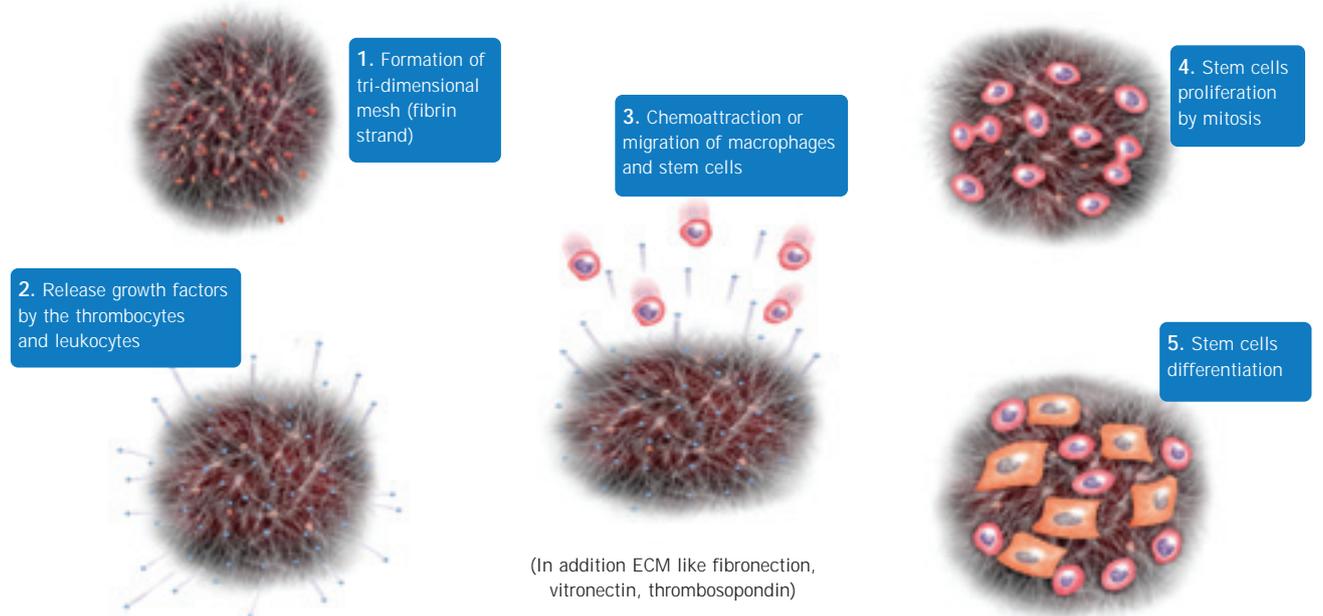


Figure 9: ACR harvesting protocol

The protocol for the preparation of the plasma is very simple:
Withdraw (vacuum allows 8ml max per tube)

⇒ Centrifuge: 3,000rpm x 8 minutes

ACR tube plasma yield = 4ml

- Faster healing: no fibrosis formation compared to the fillers, for example
- Easy to use: dermal and hypodermal injections require only one syringe and one needle
- Convenience: ACR harvesting can be performed in doctor's offices
- A sterile and registered specific kit for the blood puncture, the preparation of the plasma and the materials

- Cost effectiveness: one ACR-REGEN kit delivers between 4 and 12ml of PRP (one to three tubes)

THE ACR CONTRA INDICATIONS

Platelet dysfunction syndrome can lead to:

- Critical thrombocytopenia
- Hypofibrinogenaemia
- Haemodynamic instability
- Sepsis
- Acute and chronic infections
- Chronic liver pathology
- Anti-coagulation therapy (for fillers applications)

ACR HARVESTING PROTOCOL

With the 5ml syringe, withdraw the 4ml of plasma in the tube separated from the red cells by the gel, then adapt the meso needle (4mm/27 G) to your syringe. The plasma is then ready to be injected with the nappage mesotherapy technique.

Materials and Methods

The mesotherapy practiced with the nappage technique is injected into the papillary dermis (1.5 to 2.5mm)

For fillers therapy, we need to increase the volume of the wrinkles; this is known as volumetric therapy. To have volume, the activity of the thrombin released by the thrombocytes needs to increase to start the healing process seen before. Thrombin will be activated when we add a small quantity of calcium chloride (CaCl₂).

MATERIALS AND METHODS

ACR regeneration kits have potential complications, including:

- Intra-vascular injections (thrombus) – watching out for the peri-orbital area
- Nerve trauma
- Haematoma/bruising
- Secondary infection

Figure 10: Depth of injections

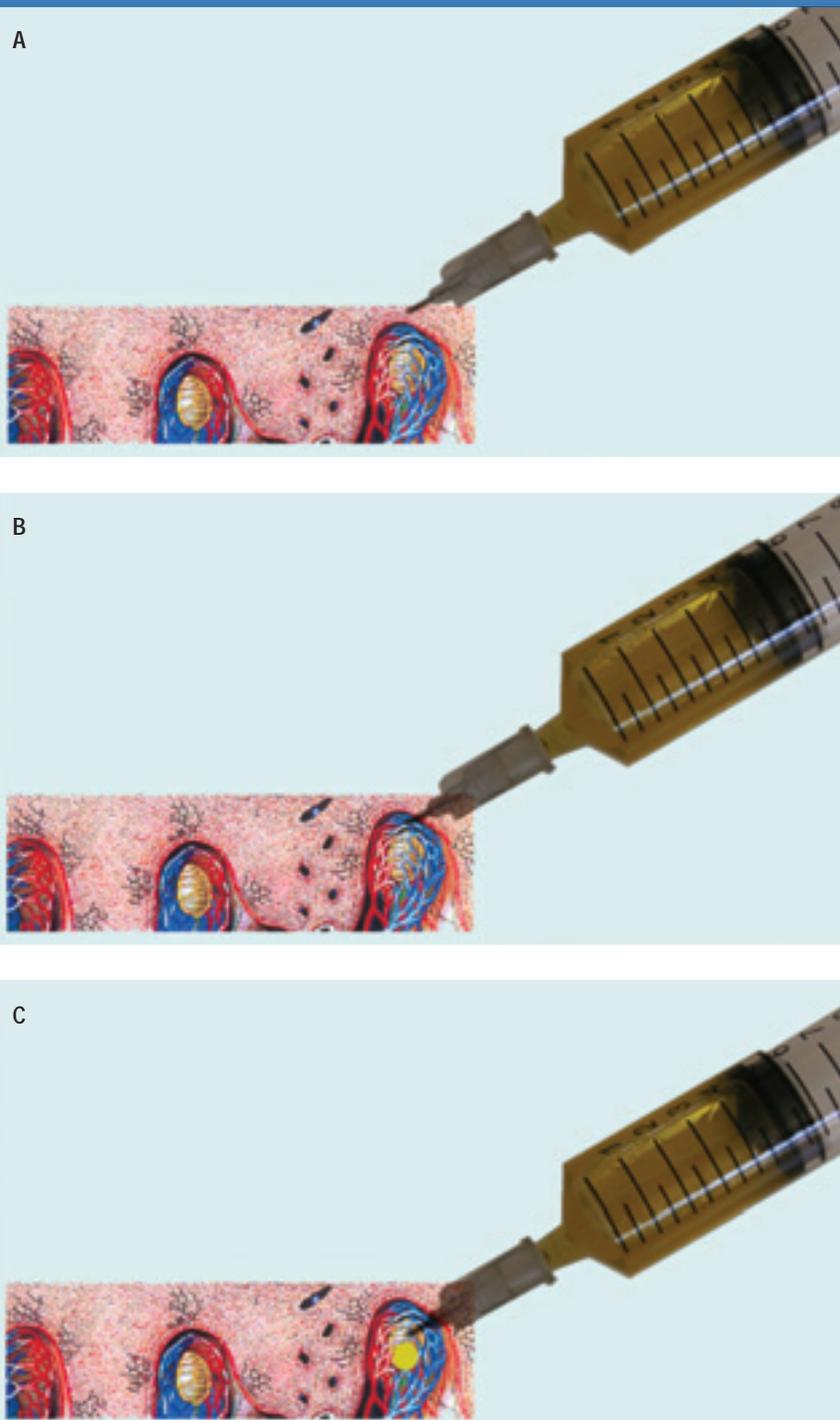
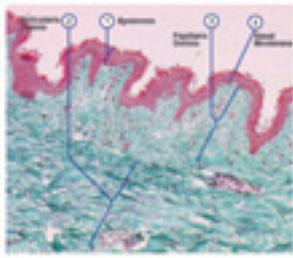


Figure 11: Dermis



Papillaris and reticularis dermis

- Micro-circulation with terminal capillaries ansa
- Plenty of nerve endings
- Immune cells: plamocytes, mastocytes
- Mitochondria

- Collagen and elastic fibres
- Fibroblasts at the origin of the production of collagen, elastin, glycoproteins

Figure 12: Injections – PRP nappage



How?

- Nappage technique: no pain, no bleeding
- Needle (4mm/27G)

When?

- First session: Do
- Next sessions: D15/M1
- One session per year

Figure 13: PRP autologous cell mesotherapy actions

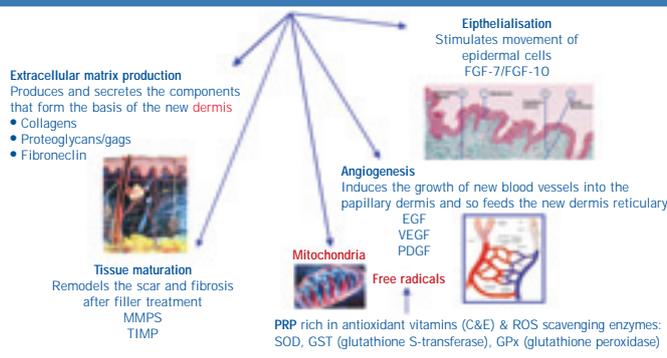


Figure 14: Autologous thrombin preparation

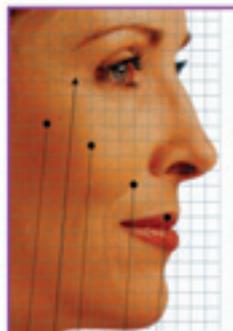
Centrifugation of the three tubes (5mn/3,000) from the kit

Then the specific ATS tube is added

1ml ethanol
+
0.4ml CaCl2

The injections of PRP and thrombin in the fillers indications are different from the well-known pharmaceutical fillers made of cross-linked hyaluronic acid (bacterial biofermentation origin). The PRP injected in the wrinkles is different, since the idea is not to fill the wrinkles but to stimulate the fibroblasts which will produce a new

Figure 15: Fillers – facial applications



- Cheeks
- Eyelids
- Chin
- Naso-Labial
- Lips
- Forehead

Technique guides and advice

1. Layer specific transplant
2. 'Tenting' of skin
3. 'Cul-de-sac' and needle bevel up
4. Over-correction 10 to 20 per cent
5. Avoid under-dosing
6. Minimal-trauma technique (long needle: 13mm/30G)
7. Intra-dermal injection = blanching

- First session: D0
- Second session: M3
- One session per year

About the author



Dr Alain Gondinet graduated as a medical practitioner from Necker-Enfants Malades in 1975. In 1986 he founded the cosmetics

company Bionuclea and invented Mesogel. He went on to co-found ARPHA, offering marketing and advertising services to the pharma industry. In 2002 he developed the concept of Antioxidative MesoTherapy with Dr André and became founding vice-president of the Association for the Development of Anti-oxidant Therapies. He works as a medical consultant for a number of companies worldwide and has contributed several articles to leading journals.

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collagen (see Figures 3 and 4, page 62) biostimulatin, and regenerating a new dermis and a moisturising and renewing the skin. The patient must be told that the volume will decrease in three weeks, but during this period fibroblasts have started the process of regeneration and rejuvenation.

CONCLUSION

Aesthetic treatment is one of the biggest global markets and increases dramatically

every year. Cell therapy with PRP allows patients to be treated with sterile materials in safe, secure and efficient ways. Tissue regeneration (autologous), thanks to the growth factors and the stem cells present in the plasma, is the future of medicine in the 21st century.

Reference

1. Gibbins J and Mahaut-Smith P, *Platelets and Megakaryocytes*, vol 2, 2004