

Platelet-rich fibrin during facial lipostructure

To evaluate fibrin PRF clots, **Frédéric Braccini**, **Jérôme Paris** and **David M Dohan** discuss their research in developing a plate and immunising concentrate for the optimisation of adipocyte grafts in accordance with Dr Sydney Coleman's technique of facial lipostructure

The filling of tissue for facial rejuvenation can be challenging. The aim is to restore harmonious volumetric facial ratios by regaining the contours of cheekbones, drooping jaw lines and nasolabial folds. Adipocyte grafts can return the skin to a state of brightness and to a volume that deteriorates gradually during ageing.

To ensure that a tissue graft remains stable, it must be integrated into the receiver site. This integration has to—as for all living tissue—take place in the form of a matrix and a tissue fusion.

Fat grafts have always had difficulties in inducing the necessary neoangiogenesis, which subsequently had significant resorption. Non-reabsorbable, exogenous material such as silicone was introduced as early as the 1950s but was suspended years later owing to harmful effects (migration, granulomas).

Hyaluronic acid fillers and other formulations are popular but absorb quickly and large volumes present cost issues.

The adipocyte graft technique, described by the American plastic surgeon Sydney Coleman, deals with the problem of resorption. The fat tissue that is grafted is centrifuged before re-injection, meaning it can be sufficiently broken apart without damaging the adipocytes to stimulate the neosynthesis of an extracellular matrix and to facilitate the colonisation of the graft by endothelial cells. This centrifugation stimulates the preadipocytes, cells allowing the reconstruction of the grafted tissue. Like the Latin expression: *destruam et aedificabo*—“I destroy and I build up”—Dr Coleman's technique requires decomposition of preexistent tissue to allow its *in situ* reconstruction.

Growth-inducing agents to improve the sustainability of adipocyte grafts have never really been developed. The graft-



60-year-old patient before bilateral facial lipostructure with PRF (left), two months (middle) and eight months post-operative



HIV positive patient, aged 38, before bilateral lipostructure with PRF (left), immediate postoperative (middle) and six months' post-operative

ed adipocytes, being very differentiated, are not sensitive to the causes of proliferation likely to be induced by these molecules. The main clinical protocols, which make it possible to use autogenous growth-inducing agents, are generally part of the platelets' concentrated family.

The platelets used in oral, maxillofacial and plastic surgery are generally grouped as cPRP (concentrated platelet-rich plasma). According to the literature, many protocols exist, but they are all supported by specific methods originating from fibrin glues, which are not uncommon in plastic surgery. The general principle of production consists of a double centrifugation, making it possible to eliminate red blood cells, then acellular plasma, to preserve only the concentrated platelets. The blood sample must be made under anticoagulation, and bovine thrombin (with calcium chloride) is generally used to make polymers *in situ* PRP, in the same way as making a simple fibrin adhesive.

The clinical effects of PRP are not that different from those usually produced by fibrin adhesives, suggesting perhaps a fibrin matrix is more effective than the platelets' cytokines and are greatly loosened in this biological adhesive. Besides this adhesive effect, it has a proficient haemostatic effect on the diffused bleeding of the parenchymas and reduces the pain and post-operative oedemas, resulting in a better angiogenesis induced by the fibrin matrix.

For medico-legal reasons of blood handling, PRP protocols have not been developed in France. The platelet-rich fibrin (PRF) technique developed by Choukroun and Al in 2001 in the line of the first studies is used. The PRF technique is simple: blood is taken without anticoagulant from a 10ml tube and then centrifuged directly using moderate forces (400g). In the centre of the tube, a fibrin PRF clot is formed, containing most of the tube's platelets and leucocytes. The clot can be used as a filling material—it can be cut into small pieces and mixed with a graft (usually with osseous grafts), or it can be pressed between two swabs, removing the serum to preserve the membranes (used in oral surgery and in tympanoplasties).

When mixed with an osseous graft, the PRF is used as biological bond of the grafted particles, and the fibrin matrix leads to the vascularisation of the graft. We took this further and mixed the PRF with another type of graft to maintain it *in situ* without reabsorption. The first experiment resulted in a positive effect of the PRF on the proliferation of cultivated

preadipocytes. The mature adipocytes are not that sensitive to the cytokine platelets, but the incorporation of fibrin clots to the fat mass allowed a better graft vascularisation.

Method

Between May 2005 and June 2006, 32 patients (seven men and 25 women underwent a lipostructure (Coleman method) using PRF. The patients' age ranged from 39–72 years; the average age was 59 years. For 22 patients, lipostructure was carried out in an isolated way and joined with a cervico-facial face-lift and/or a blepharoplasty for 10 patients. No patient had to undergo a second lipostructure.

For an isolated lipostructure, the intervention is done under neuroanalgesia in ambulatory surgery. Surgical procedures are performed under general anaesthesia. For one patient the surgery was more difficult, owing to HIV treatment.

Lipostructures are classically carried out according to the protocols described by Dr Coleman. Fat tissue is extracted from the inner side of the knees and is supplemented with a paraumbilical extraction if necessary. The extraction is done using a specific aspiration nozzle with a diameter of 3mm and 15cm length, with foam ends and openings at both sides. A 10ml syringe is screwed onto the nozzle. The vacuum in the syringe is created manually and gradually to avoid a build-up of pressure on the adipocytes. There are multiple tubes leading out to limit traumatism and bleeding. On average, 60cc of fat is extracted: this varies between patients and depends on the indication and the quantity needed for the re-injection.

The purification process then follows by means of centrifugation of extracted tissue. The 10ml syringes are sealed using stoppers and are placed in the centrifugal machine. Centrifugation is carried out for three minutes at the speed of 3000 RPM. After centrifugation, the contents of the syringe are divided into three layers:

- the upper oily sample of adipocytes—this consists of triglycerides from the damaged adipocytes and has the lowest density. This part, usually eliminated, is kept to humidify the membranes with PRF;
- the bottom sample, containing primarily the blood debris, which ceases when the syringe is withdrawn; and
- the middle sample, which contains the adipocytes to be grafted. On average, the graft makes up 6cc of the syringe. After purifying the fat it is transferred into 1ml syringes without coming into contact with air.

To produce the PRF, 40–60ml of venous blood is taken in four to six tubes without anticoagulant (Becton Dickinson's Vacutainer 10ml). These are immediately centrifuged in 10 minutes at 3000 RPM in centrifugal machines according to the protocol described by Choukroun et al. The activation of coagulation and centrifugal forces create three layers in the tube: a red blood corpuscle base at the bottom, a plasmatic acellular in surface and a clot of fibrin PRF charged in platelets in the middle.

Clots of PRF are collected using forceps. These are separated from the red blood corpuscle base and are placed into a cup. The fibrin PRF clot is cut out in fragments of 1–2mm, which are mixed with the oil sample produced by fat centrifugation. This mixture is put into a 1ml syringe and is placed using a nozzle (in line with Dr Coleman's method of tunnelisation) on the principal sites to be grafted before the installation of purified fat.

Once cut out and mixed, each clot represents a volume of injection of approximately 1cc, half made up of fibrin PRF matrix, and the remainder in a liquid state (plasmatic serum and purified sample of adipocytes).

On average, four to six clots of PRF are sufficient to treat a complete face: six clots for complete lipostructure; four clots for lipostructure with cervico-facial face-lift. The deposit of

PRF before the injection of the fat grafts is used for preparation and activation of the site for the graft.

In accordance with Dr Coleman's technique, the grafted tissue is placed in small amounts with each passage of the nozzle. Cannulas of various forms and lengths are used—all with a smooth stump to limit the risk of haematoma, and with lateral injecting apertures to avoid an intravascular injection. It is important to perform much tunnelling. All layers are grafted, starting at the deepest point. The closing of the incisions is done using 6-0 sutures.

In isolated lipostructures there are three main sites of re-injection: the cheekbones, cheeks and the chin. Each zone receives, on average, 8cc per cheekbone, 7cc per cheek and 5cc for the chin.

With multiple lipostructures, on average an additional 10cc of the solution is used per temple, 4cc for the upper lip, 2cc for the lower lip and 3cc for the nasolabial fold. When the adipocytes graft is associated with a cervico-facial facelift procedure or a blepharoplasty, the adipocytes graft is always performed last to allow for adjustment and redistribution of volumes. Moreover, this avoids traumatising the transplanted fat tissue during surgery.

Lastly, for two patients treated by bilateral simple lipostructure, the pretreatment of the site of the graft with PRF was performed unilaterally only—even if volumes of injected fat on each side were thereafter identical.

The post-operative evolution of patients was followed up during at least one year, with clinical examination associated with photographic analysis. Results of patients having undergone an associated surgery (face-lift, blepharoplasty) are most delicate to analyse. Evaluation of results is done in each area, by examining, in particular, the three facial zones that give the best results: the cheekbone, the cheek and the chin.

Preliminary results

The results of procedures such as Dr Coleman's lipostructure are difficult to evaluate in the absolute, because their perception remains subjective. However, in this series of 32 patients, all were satisfied with the result and no additional grafting was necessary. If it is difficult to evaluate the amount of fat reabsorption without performing pre- and post-operative MRI. However, it was noted that the reabsorption was insignificant; there was no massive reabsorption requiring a secondary lipostructure.

In the two patients treated with a



40-year-old patient before bilateral facial lipostructure (top). PRF is injected in the unilateral left side (middle). At nine months' post-operative (bottom), the aesthetic outcome appears slightly asymmetrical

“ A biomaterial of cicatrisation such as the PRF will enter a wider field of application ”

unilateral use of PRF, a light aesthetic asymmetry was noticeable. Four months' post-operative one-half of a face treated with PRF appeared more inflated than the other side treated without.

The results of Dr Coleman's procedure are subjective and operator-dependent. Results are rather unforeseeable and the graft's stability rate after three months varies in the literature. Using PRF with the grafted tissue enabled us to stabilise our results. It is difficult to demonstrate results objectively, unless systematically performing MRI on a much larger study of patients with or without PRF.

However, in our research the contribution of PRF did not necessitate remedial work as the patients were satisfied with their results. Moreover, our procedure did not result in significant oedemas or bruises.

In general, a significant percentage (about 10%) of patients present with oedemas—approximately 6% had bruises at the end of four weeks. In our study, there was no case of an oedema or prolonged bruising. It is possible that the grafting treatment using PRF is the way forward. Indeed, the deposit of a fibrin matrix in the grafted areas makes it possible to induce a better angiogenesis and thus a better vascular and lymphatic drainage. The risks of bruising and oedemas may also be reduced.

The under-correction is the most frequent complication, which may be due to an undervaluation by the surgeon at the time of the intervention, or an excess of reabsorption of the adipocytes.

The quality and stability of the graft through time are the most difficult factors to control, as important reabsorption reaching 50–70% can occur. It is particularly the case on bad surfaces having an atrophied vascularisation or weakened cicatricial capacities as the result of smoking or HIV. Patients with HIV may benefit from lipoatrophy, but optimal results may represent an additional challenge.

The first in vitro studies highlighted an acceleration in the proliferation of pre-adipocytes put in contact with a PRF

membrane. If the cytokines platelets help accelerate the cicatrisation, the deposit of a cicatricial fibrin screen will have effects on the incorporation of grafted tissue. The presence of a fibrin membrane charged with platelets and leucocytes cytokines is thus the best chemotactic factor and is supported by the proliferation of the endothelial cells.

Looking forward

A fast neovascularisation of grafted tissue is a major parameter for cellular survival and limits tissue resorption. Dr Coleman's technique, which is done predominantly by small fat-tissue deposits, is an important factor in the results: if the graft is too big it cannot be vascularised and decreases. Lastly, it is important to consider that fibrin is an excellent trap for the original circulating cells, as well as the immune cells, ending in a better immunisation of the purified graft.

This first clinical study brings the opportunity to use concentrated platelets for adipocyte grafts. The PRF is a platelet concentrate and can quickly and easily be produced at a minimal cost using only a natural blood clot without biochemically modifying the blood. Knowing the extent of the uses of the surgical additives containing fibrin in plastic surgery, it shows that in the years to come a biomaterial of cicatrisation such as the PRF will enter a wider field of application, even if it is still necessary to carry out further studies to validate its indications.

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PRF is produced using a centrifugal machine of table PC-02 (Process, Nice, France). The result is a fibrin clot rich in platelets. This is cut into small pieces before use