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Soft Tissue Augmentation of the Face With Autologous Platelet-Derived Growth Factors and Tricalcium Phosphate. Microtomography Evaluation of Mice

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Background: The platelets used in oral, maxillofacial, and plastic surgery are generally grouped as concentrated platelet-rich plasma. The general principle of production consists of a centrifugation, making it possible to eliminate red blood cells, then acellular plasma, to preserve only the concentrated platelets.

Objective: The aim of the present study was that micro porous tricalcium phosphate (β -TCP) mixed with autologous platelet-derived growth factors could be an alternative to fat and hyaluronic acid which are widely used for oral and maxillofacial soft tissue augmentation.

Methods: Ten female, 6 to 8-week-old black-haired mice were selected. On 1 cheek was injected the gel of tricalcium phosphate/autologous platelet-derived growth factors, while on the other cheek, was left empty and was used as control. The animals were killed after 8 weeks. Investigator evaluation was based on microtomography observation and comparison of control and test.

Results: The microtomography technique demonstrated amorphous radiopaque images projected in the soft tissue parts of each paramedian region of the right cheek, in those sites corresponding to the injection of the gel of tricalcium phosphate/autologous platelet-derived growth factor. Eight weeks after surgery, β -TCP granules were clearly visible with most remaining within the cheek. The margins of the β -TCP granules were clear and not diffused within the vicinity of the tissues.

Conclusion: The results indicate that micro β -tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ mixed with autologous platelet-derived growth factors material was able to create a lasting three-dimensional soft tissue augmentation and is a promising biomaterial for soft tissue augmentation as a scaffold for cells.

Key Words: Autologous platelet-derived growth factors, full-face rejuvenation, skin rejuvenation, soft tissues augmentation, tricalcium phosphate

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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. Soft tissue augmentation is frequently required for the reconstruction of perioral skin tissues in facial deformities, trauma, or for intraoral ridge defects,^{1,2} causing functional troubles and/or esthetic complaints. Several techniques for managing such deformities have been proposed, either by soft or by hard tissue augmentation.^{1–4} Soft tissue augmentation with absorbable injectable filling agents in the facial region is rapidly increasing in popularity.² In addition, the proven safety of these products substantially contributed to its widespread use. Although rare, complications do occur.³ A novel use for filling agents is to exploit their volume-occupying properties to replace lost subdermal fat and remodel maxillary and mandibular bone. Dermal fillers are commonly subdivided on the basis of their turnover in the skin temporarily, semipermanent (duration is often longer than 18 months but the exact time frame is unknown), and permanent with a wide range of choices as well as specific indications. An autologous source for promotion of soft tissue deposition in areas of depletion is desirable. The main clinical protocols, which make it possible to use autologous growth-inducing agents, are generally part of the platelets' concentrated family. The platelets used in oral, maxillofacial, and plastic surgery are generally grouped as PRP (concentrated platelet-rich plasma).⁴ 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 centrifugation, making it possible to eliminate red blood cells, then acellular plasma, to preserve only the concentrated platelets. Tricalcium phosphate (β -TCP) is a rather new material in aesthetic medicine as it is dispersed in hyaluronic acid with a stable preparation for a certain period of time. It is a biostructuring medicine used to obtain a reaction from organisms through mechanisms of insult and lesion. This insult is an extraneous body and occurs in polylactic and tricalcium phosphate: a mechanism of reaction and reparation that provides the results. It is composed of microparticles of synthetic ceramic, biocompatible, biodegradable, immunologically inert, with a diameter of 45 μm .

The hypothesis of the present study was that micro porous tricalcium phosphate (β -TCP) mixed with autologous platelet-derived growth factors (APDGF) could be an alternative to fat and hyaluronic acid which are widely used for oral and maxillofacial soft tissue augmentation.

METHODS

Subject Animal

The black-haired mice used in this study were obtained from the laboratory of University of Chieti-Pescara, Italy. Ten female, 6 to

8-week-old black-haired mice were selected. The procedures were approved by Inter-Institutional Ethics Committee for Animal Experimentation of University of Chieti-Pescara, Italy (Prot.06/2012/CEISA/PROG31), and this study was performed according to the European community guidelines (E.D. 2010/63/UE).

All animals were anesthetized by an intramuscular injection (average dose, 8 mL/kg) of a mixture of ketaminium hydrochloridum and xylazine hydrochloridum diluted with saline (2.1 mL of 50 mg/mL Ketalar [Parke-Davis, Zaventem, Belgium], 0.3 mL of 2% Rompun [Bayer, Leverkusen, Germany], and 3.4 mL of 0.9% NaCl). This protocol provided good anesthesia during treatment.

Test Procedure

This study now evaluates in vivo, a micro β -tricalcium phosphate $Ca_3(PO_4)_2$ (Skin-hyxa; Italfarmacia srl, Rome, Italy) architecture with 45 μ m mixed with the autologous platelet-derived growth factors. In the present study, we used high-resolution microtomography (micro-CT) for the evaluation soft tissues augmentation in mice. Explants harvested after 8 weeks were examined with micro-CT for macroscopical appearance.

Approximately 0.1 cc of blood was collected into collection tubes via standard venipuncture using a 27-gauge butterfly needle. The tubes containing sodium citrate 3.8% were then centrifuged at 1100 PRM for 4 minutes with centrifuge machine (GFOne; UBGEN, Padova, Italy). The total time of preparation from venipuncture to injection was approximately 8 minutes. Approximately 0.02 cc of APDGF was produced per tube. This activated suspension was mixed together tricalcium phosphate. We consider the addition of 20% APDGF to β -TCP offers a better tricalcium phosphate grafting survival, a less bruising and inflammatory reaction. The liquefaction of platelet-derived with tricalcium phosphate makes the application easier (Fig. 1A). The mice then injected intradermally or subdermally below the cheek regions through 22 gauge needles, respectively, as needed to achieve the most soft tissues augmentation and optimal correction, using mainly a linear threading technique (Fig. 1B). On 1 cheek was injected the gel of tricalcium phosphate/autologous platelet-derived growth factors, while the other cheek, was left empty and was used as control. Total treatment time (from venipuncture to completion of treatment) was less than 12 minutes in most patients. The animals were killed, with an overdose of intravenous pentobarbital after 8 weeks. Investigator evaluation was based on micro-CT observation and comparison of control and test.

The micro-CT system, (Skyscan 1172 100kV 50 mm FOV) involves rotation of the vertically positioned animal and employs a stationary tube and an X-ray detector, which permits the use of a high flux rotatinganode X-ray source (Philips SRO 09 50) with a dual 0.3/1.0 mm focal spot. The flux from the system is sufficient to support exposures as short as 10 msec.

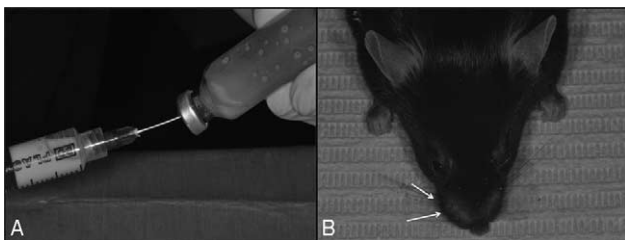


FIGURE 1. (A) Preparing the gel of tricalcium phosphate/autologous platelet-derived growth factors. (B) Injection of the autologous platelet-derived growth factors mixed with micro porous tricalcium phosphate produces a soft tissues augmentation (arrows).



FIGURE 2. (A) In test site (T), the micro-CT 3D view, are shown amorphous radiopaque images (arrows) projected in the soft tissue parts of the paramedian region of the right cheek, in sites corresponding to the gel of tricalcium phosphate/autologous platelet-derived growth factors injection points. (B) In control site (c), the micro-CT 3D view, there are no biomaterials detected in the soft tissues. (C) Micro-CT 2D slice showed a good volume after 8 weeks in the cheek right. (D) The margins of the β -TCP granules were clear, with no diffusion to the tissues in the vicinity (arrows). micro-CT, microtomography.

RESULTS

The micro-CT technique demonstrated amorphous radiopaque images projected in the soft tissue parts of each paramedian region of the right cheek, in sites corresponding to the gel of tricalcium phosphate/autologous platelet-derived growth factors injection points (Fig. 2A). This finding was present in all 10 slice 2D (Fig. 2C), the filler was seen more clearly in 3D reconstruction (Fig. 2A and D). A good visual correlation was found between the appearance and dimensions of the filler visible in the virtual CT slices and in the macroscopic pictures of the cheek.

Micro-CT scanning demonstrated a good volume after 8 weeks. Volumetric analysis shows an average of $4 \times 4 \times 2$ mm (Fig. 2D).

Eight weeks after surgery, β -TCP granules were clearly visible with most remaining within the cheek. The margins of the β -TCP granules were clear and not diffused within the vicinity of the tissues.

DISCUSSION

Autologous platelet-derived growth factors have been used over the last several years as an effective treatment in various surgical and medical fields.^{5,6} Platelets are an excellent source of growth factors (GFs) in their naturally occurring and biologically determined ratio, and are successful in acute wound healing. The application of autologous platelet-derived growth factors has been proven to enhance early wound healing and healing in diabetic ulcers.⁷ Concentrated platelet preparations have been used clinically since the 1990s to simulate the native wound healing environment compared with that after isolated growth factor application. There is also substantial clinical proof of autologous platelet-derived growth factors in other areas of medicine. For example, platelet gel is widely used in orthopedics and oromaxillofacial surgery.^{8,9} Platelets are products of megakaryocytes and respond to vascular injury by aggregating. In vivo, platelets attach to nodes of a fibrin scaffold that form at the site of injury. Platelet activation leads to the development of pseudopods, aggregation, and ultimately platelet degranulation. Alpha granules within the platelets release, via exostosis, a multitude of GFs that act as chemoattractants and mitogenic agents. These growth factors include vascular endothelial growth factor, platelet-derived growth factor, epidermal growth factor, insulin-like growth factor-1, basic fibroblast growth factor, transforming growth factor-b1, transforming growth factor a, platelet-activating

factor, thrombospondin, platelet thromboplastin, coagulation factors, serotonin, histamine, hydrolytic enzymes, and endostatin.¹⁰ They are released in specific ratios and work in concert and in a specific order to attract inflammatory cells, fibroblasts, as well as to stimulate collagen deposition and endothelial budding. These features will lead to appropriate wound healing.

In 2010, Ceccarelli has proposed the use of platelet growth factors in dermal biostimulation.¹¹ Obviously what was originally designed for topical application will be reviewed in the light of the introduction by injection into the dermis. After injection into the dermis and subcutaneous layers, the platelets are activated endogenously by the body's own coagulation factors such as thrombin and collagen. This leads to platelet degranulation, releasing platelet GFs such as platelet-derived growth factor, insulin-like growth factor, epidermal growth factor, and TGF- β . Activated platelets also release proteins such as the adhesive glycoproteins fibrin, fibronectin, and vitronectin. These proteins and GFs interact with cells in the subcutaneous tissues, such as fibroblasts, endothelial cells, and stem cells. After binding to their cellular receptors, they activate intracellular signaling events mediating cell proliferation, migration, survival, and production of extracellular matrix proteins. This results in tissue rejuvenation.¹² Its application for superficial or deep dermal stimulation leads to skin rejuvenation. While $\text{Ca}_3(\text{PO}_4)_2$ determines an increase of volume for a mild tissue reaction with a formation of fibrotic tissue. This reaction is normally cold. This biostimulation is safe, creates an immediate and long-lasting volumetric effect and a natural result. It is easy to perform and the procedure has virtually no side-effects and high levels of patient satisfaction. Face rejuvenation with autologous platelet-derived growth factors is a promising, easy technique, performing favorably in all small skin wrinkles, as well as in skin texture and elasticity.

The results indicate that micro β -tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ mixed with autologous platelet-derived growth factors material was able to create a lasting three-dimensional soft tissue

augmentation and is a promising biomaterial for soft tissue augmentation as a scaffold for cells.

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