J. Appl. Cosmetol. **35,** 153-161 (July/December 2017)

Correction of defects in the flaws of Bone Tissue Facial, through the use of Tricalcium Phosphate or Calcium Hydroxyapatite with Plasma Gel. A Clinical Assessment Study

F. Svolacchia, M.D., Ph.D.[1] Student, L. Svolacchia, M.D.[2] Student

[1] Department of Anatomical, Histological, Medical and Legal Sciences and the Locomotive Equipment, Section of Human Anatomy, Laboratory of Experimental Morphology at the University of Rome "La Sapienza", Italy

[2] Student, Medicine and Surgery, University of Rome "La Sapienza", Italy

Received: *October, 2017*

Key words: *Allografts; Antiaging agents; Chronoaging; Zygomatic; Malar and maxillary bone reconstruction; Autologous growth factors; Bone substitutes; Ceramics; Demineralized bone matrix (dbm); Hydroxyapatite; β tricalcium phosphate (β-tcp);*

Summary

The aging process as it is observed by all patients begins after the first third of life and involves all the tissues, even for the depletion of adult stem cells responsible for the reintegration of lost tissues. The epidermis and dermis tend to thin, fatty tissue loses consistency, and bone tissue changes. The impoverishment of the tissue layer leads to typical wrinkles and folds that can be observed on the face, typically as early as 40 years. The purpose of this study was to clinically evaluate the volume increase near zygomatic and maxillary bone by using tricalcium phosphate powder or calcium hydroxyapatite combined with a gel derived from autologous plasma in order to contribute to the attenuation of the anesthetics of bone hypovolumetry.

The hypothesis of scientific work was that by depositing exclusively injecting a plasma compound with calcium hydroxyapatite or tricalcium phosphate at the zygomatic, malar and maxillary levels, taking care to scratch the periosteum with the tip of the needle, bone to supplement what deposited through the injection, it could have a longer duration and three-dimensional effect of aesthetic correction together with an integration with the bone itself, unlike what happens with absorbable fillers now in use.

All volunteer patients have been studied after obtaining regular informed consent. Patients were photographed at 0, immediately after treatment and after four months demonstrating the perfect integration of injected material in the absence of pathological inflammatory responses.
This study, launched in November 2011, showed that tricalcium phosphate and calcium hydroxyapatite combined with an autologous gel derived from platelets is perfectly tolerated as biomaterial in the volumetric integration of bone tissue.

Riassunto

Il processo di invecchiamento cosi come viene percepito dai pazienti inizia dopo il primo terzo della vita e coinvolge tutti i tessuti, anche per il depauperarsi delle cellule staminali adulte deputate al reintegro dei tessuti persi. L'epidermide ed il derma si assottigliano, il tessuto adiposo perde di consistenza ed i tessuti ossei si modificano. L'impoverimento dello strato dei tessuti porta alle tipiche rughe e pieghe osservabili sul viso, in genere già verso i 40 anni. Lo scopo di questo studio è stato quello di valutare clinicamente l'aumento volumetrico in prossimità dell'osso zigomatico e malare effettuato mediante l'ausilio della polvere di fosfato tricalcico o di idrossiapatite di calcio uniti ad un gel derivato dal plasma autologo al fine di contribuire all'attenuazione dell'inestetismo delle ipovolumetrie ossee.
L'ipotesi di lavoro scientifico era quella che depositando con metodo esclusivamente iniettivo un composto di plasma unito ad idrossiapatite di calcio o fosfato tricalcico a livello zigomatico, malare e mascellare, avendo cura di scalfire il periostio con la punta dell'ago e permettendo all'osso di integrare quanto depositato attraverso l'iniezione, si potesse avere una durata maggiore nel tempo e tridimensionalmente della correzione estetica unitamente ad una integrazione con l'osso stesso, diversamente da quanto accade con i filler riassorbibili oggi in uso.
Le pazienti sono state fotografate a tempo 0, immediatamente dopo il trattamento e dopo quattro mesi dimostrando la perfetta integrazione del materiale iniettato in assenza di risposte infiammatorie patologiche.
Lo studio ha permesso di dimostrare la perfetta integrazione delle ceramiche con i tessuti

INTRODUCTION

The face aging that is observed after the first third of life is characterized by a progressive intensification of folds and relaxations caused by the loss of tissue (1), especially at the upper corners of a hypothetical triangle, called beauty, which have the apices the zigomatic area.
This process also includes the remodeling of zygomatic and maxillary bone that contribute to changes in the skull structure. In fact, age depletes not only the dermis and the subcutaneous tissue but also the bone (2).
Bone resorption significantly increases the typical appearance of time on the face, especially at zygomatic, maxillar and mandibular levels, affecting negatively the hypothetical but characteristic geometric formation (3). Patients in whom these signs of time may be evident may be aesthetic benefit from treatment with tricalcium phosphate or calcium hydroxyapatite injected directly in contact with this bone formulation as a starting point for subsequent epidermal, dermal and hypodermic integration.

MATERIALS AND METHODS

This study started on November 7, 2011 with the first session and I used a microporous hydroxyapatite with granules of between 70/ 100 microns or a microporous tricalcium phosphate with 30/40 micron granulation.
The choice of the implant to be used on patients was quite random. It pushed me to use these materials on the skull bone structures, to certify that the inorganic component of all mineralized tissue tissues, such as bone, is predominantly composed of tricalcium phosphate salts or hydroxyapatite, together with other inorganics such as carbonates calcium and sulphates present in inferior amounts and perfectly biocompatible (5) (12) and it is known that bone weight is represented by 60-70% of calcium hydroxyapatite.
Differences between the two materials can be identified in their chemical composition: tricalcium phosphate has a calcium/phosphate ratio of 1.5 and has a crystalline structure while hydroxyapatite corresponds to tribasic calcium phosphate with a calcium/phosphate ratio of 1.67 equal to bone mineral. In maxillofacial surgery, hydroxyapatite has been and is used in the form of shaped blocks in bone defects, in the post traumatic reconstruction of the face, especially of the orbital, zygomatic and maxillary bone portions. It is also used as a block or as a powder.
The ability of hydroxyapatite to bind to bone has been demonstrated in several studies, as well as its biocompatibility and peculiar properties to promote cell differentiation to form new bone (3,5,6,7). No toxicity or flogistic phenomena have been detected at clinically relevant positioning or remotely, and perfect biocompatibility has also been demonstrated for tricalcium phosphate in soft tissues without epidermal or dermal inflammatory inflammation (18), exactly as reported for calcium hydroxyapatite (9,10,11).
Scientific opinions on calcium hydroxyapatite resorption are contrasting, while some authors in their studies have confirmed that, unlike tricalcium phosphate, it does not respond to absorption (11,12,13), other authors have argued that it also goes meeting the same resorption typical of tricalcium phosphate (5,14,15,16,17). But anyway, we can certainly assert that both materials are absorbable.
In this study, I could personally observe a slower clinical development of hydroxyapatite reabsorption than tricalcium phosphate, but better solubilization of the latter in the plasma, though both can be perfectly utilized by the procedure described.

Procedure

For the solubilization of tricalcium phosphate powders and calcium hydroxyapatite contained in ampoules and sterile, 2 cc of plasma derived from 10 cc of blood from a vein extraction supplemented with 0.5 cc of sodium citrate was used to prevent clotting and centrifuged.
Once red blood cell separation is obtained, the plasma in the amount of 2 cc was mixed with the materials studied directly in ampoules containing 500 mg of product, strictly observing sterility and aseptic charges. After solubilization inside the ampoules, the materials were taken with a syringe and placed in a dry sterilizer for the time and temperature required for coagulation. Subsequently, the compound thus obtained was passed several times within a two-way tap to achieve its homogenization and distribution as much as possible.
The plasma gel coupled with the ceramics thus obtained was injected to patients via a 21G or 22G needle on the malarial, zygomatic and maxillary anatomic areas very slowly and taking care to scratch the periosteum during injection. Particular attention was paid to the execution, to the infrared, zygomatic and facial holes, and to the mental hole, whose shuttering with the material of the study would have had repercussions at the level of nervous, sensory and motor endings on the facial musculature (Fig. 7). Detailed informed consent was obtained and patients photographed before and after treatment. The average age was 51 years.
Checkups and evaluations were carried out after 15, 30, 60, 90 and with a 120 day follow-up visit. The degree of correction obtained for the treatment was objectively evaluated using an analog scale 0-10 (0= no correction; 5= satisfactory correction; 10= total correction). The degree of satisfaction and effectiveness was obtained subjectively, asking patients if there was an itching, pain, or burning sensation during and after the procedure.
All patients reported a total correction of 15, 30 and 45 days and a very good 120-day correction. The best parameters were observed in patients treated with calcium hydroxyapatite.
All patients stated only a burning sensation during the injection of a few seconds. No one has stated a foreign body sensation or pruritus reported clinical details worthy of being interpreted as referring to the procedure under study (Fig. 1-2-3-4-5-6)

RESULT

All patients were satisfied with the treatment received. Specifically, the three-dimensional increase in volume and soft tissue support has been highlighted. I can say that induction treatment of neocollagenogenesis for the formation of a fibrous capsule in injection sites with this method has yielded clinically relevant results between the very good and the excellent, as the photos show (Fig. 1-2-3-4-5-6).
Through the traumatic scratching of the periosteum by means of the needle tip, it is possible to integrate the ceramics contained in the gel and the bone tissue; gel obtained through plasma thermal coagulation combined with hydroxyapatite or calcium phosphate. Soft tissues in contact with tricalcium phosphate supplemented a prolonged stimulatory effect on fibroblasts with induction of a fibrosis so as to activate a three-dimensional correction of deep wrinkles or slides (17) close to the zygomatic-malarial or maxillary bone.

Fig. 1 *Correction with deposition of tricalcium phosphate on the lower edge of the outer face of the mandible (mandibular angle).*

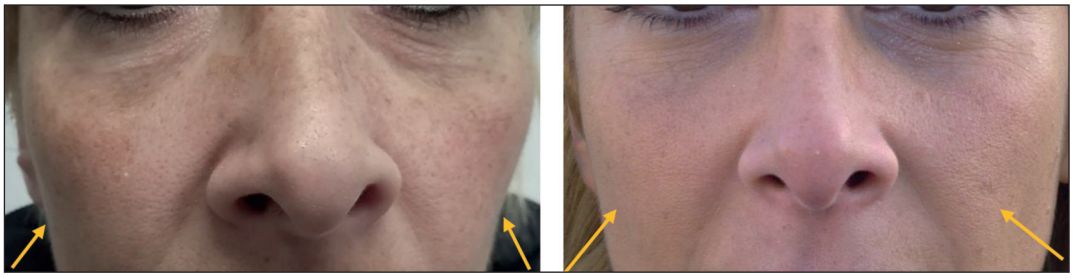

Fig. 2 *Correction of the zygomatic arch, the lateral face zygomatic bone and the zygomatic bone process maxillary level with deposition of calcium hydroxyapatite, correction to 60 days.*

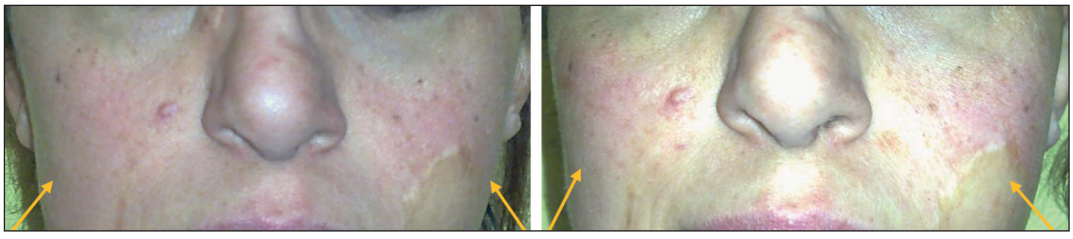

Fig. 3 *Zigomatic area correction with deposition on the side of the zygomatic bone and at the level of the zygomatic process of the maxillary bone with calcium phosphate, 60-day correction.*

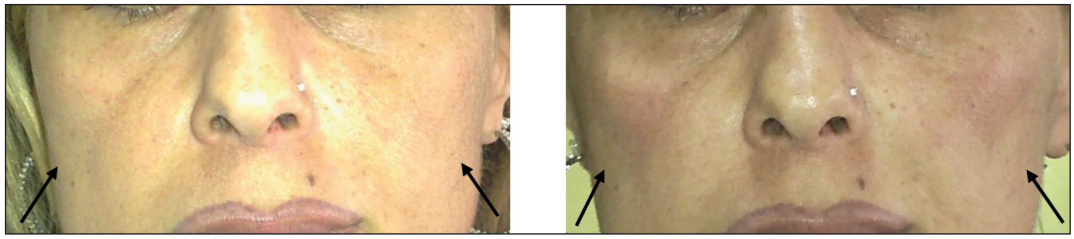

Fig. 4 *Zigomatic area correction with deposition on the side of the zygomatic bone and zygomatic process of the maxillary bone with calcium phosphate, 45-day correction.*

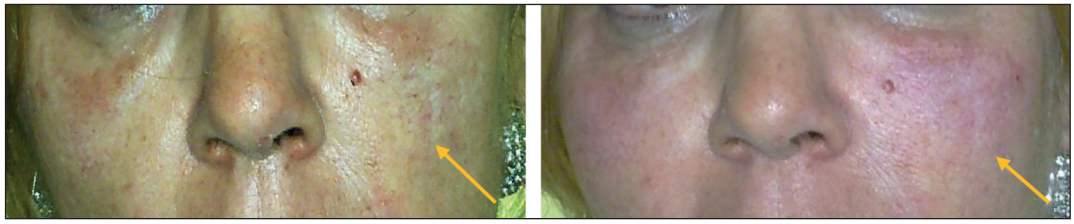

Fig. 5 *Correction of zygomatic area with deposition of tricalcium phosphate on the lateral side of the zygomatic bone and at the level of the zygomatic process of the maxillary bone. Time 0.*

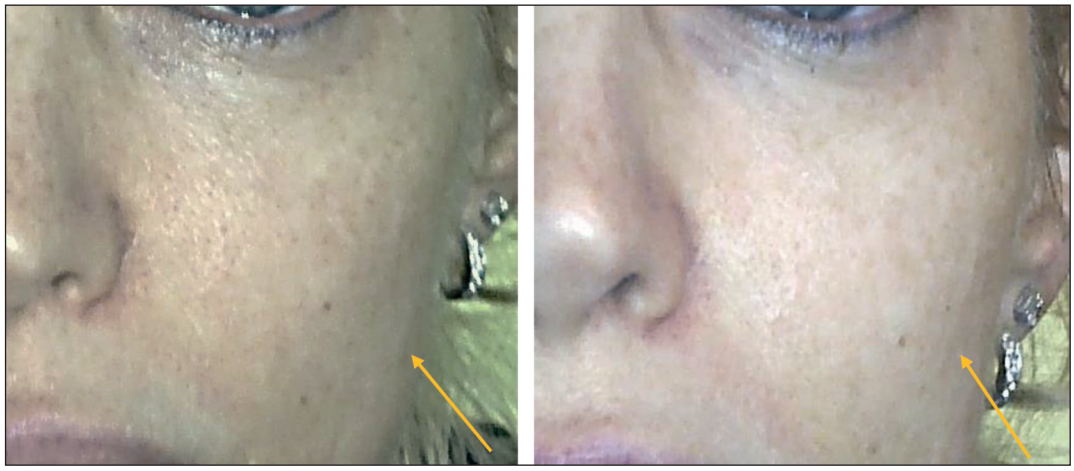

Fig. 6 *Correction of the zygomatic arch, the lateral face zygomatic bone and the zygomatic bone process maxillary level with deposition of calcium hydroxyapatite, correction to 120 days.*

DISCUSSION

Since the late 90's, dermo-epidermal and adipose treatment has also been treated with growth factors derived from plasma derived platelets for centrifugation of venous removal. For these indications there are many extraction protocols that involve different procedures such as that related to the radius of the centrifuge, the tube used or the possessed medical devices, but the general

principle is to separate the red part from the plasma and obtain a centrifuge containing three sections: PRP, Plasma Platelet and Poor Plasma Platelet.

At the base of PRP we find the buffy coat consisting of a more nebulous honey, the richest of platelets and leukocytes. Low density lipoproteins (LDLs) that interact with fibroblasts receptors and activate other stimulatory pathways float at the top of PPP, and should also be used in regenerative treatments.

Growth factors for pathology have been used for years to promote and accelerate the healing of both acute open wounds and chronic lesions, such as in the case of the decubitus, and to accelerate functional recovery related to locomotor functions such as tendinopathies and injuries of professional athletes. They are located within the alpha granules contained in the platelets. They include, IGF-1, PDGF, bFGF, TGFbeta 1, TGF alpha, VEGF.

The cell target of these GFs in aesthetic and regenerative medicine can be recapitulated as follows: VEGF directed towards dermal papilla, EGF towards outer follicular sheath, FGF towards fibroblasts and keratinocytes, TGF beta1 and TGF alpha towards dermal papilla, fibroblasts and keratinocytes, PDGF towards fibroblasts. Not only, in dense granules of platelet there are serotonin, histamine, ADP, and ATP, which of particular importance in regenerative medicine .

The use of PRP dentistry was proposed by Dr. Anitua while as a biostimulant for dermis and epidermis by Dr. Victor Garcia. Dr. Scarano has proposed a mix with tricalcium phosphate for soft tissue treatment.

Here the plasma gel was considered as a vehicle for positioning both calcium hydroxyapatite and tricalcium phosphate powder on the bone tissue by means of a deep injection, as a three-dimensional support without any conditioning on the tissues of GFs (20).

In fact, it must be emphasized that both the time it takes to prepare the plasma with tricalcium phosphate powder or hydroxyapatite powder up to its injection, and to heat it up within the sterilizer for the time required to coagulate the plasma to have the gel produces a total ineffectiveness of the GFs on the tissues surrounding the injection site, as the protein denaturation that already occurs at 41 degrees is abundantly exceeded by the 80 degrees required to form the gel, making them no longer active on the tissues. It is therefore presumed that no effect can be induced by the growth factors present in platelets on the surrounding tissues, since they have been rendered ineffective during plasma gelling.

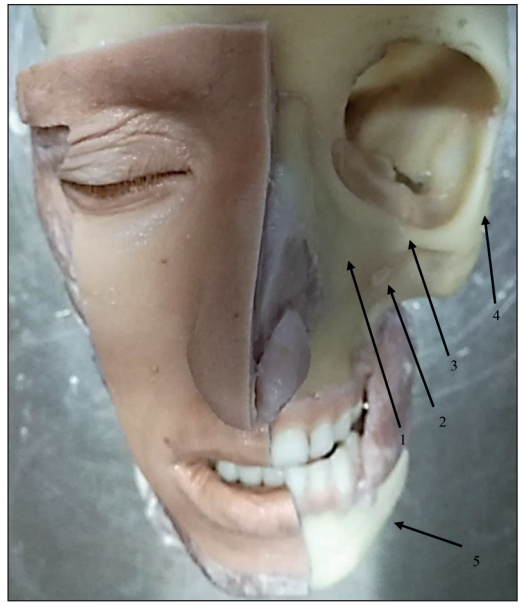

Fig. 7 *Ceramic Positioning Areas: 1-3-4-5. Forum Infraorbital: 2.*

Declaration

For this publication I declare that there is no conflict of interests.

References

1) **F. Svolacchia F. (2013)** Uso delle Cellule Staminali Mesenchimali Adulte in Medicina Rigenerativa da Sole od in Associazione ai Fattori di Crescita: le Certezze e le Prospettive, Medicina Rigenerativa, Prevenzione degli inestetismi, pag. 1-21 (docvadis.it).
2) **Vashi NA, De Castro Maymone MB, Kundu RV. (2016)** Aging differences in ethnic skin, *J. Clin. Aesth. Dermatol.,* **9(1):**31-38.
3) **Levine RA, Garza JR, Wang PTH, Hurst CL, Dev VR. (2003)** Adult facial growth: applications to aesthetic surgery, *Aesth. Plast. Surg.,* **27(4):**265-268.
4) **Vamze J, Pilmane M, Skagers AJ. (2015)** Biocompatibility of pure and mixed hydroxyapatite and α-tricalcium phosphate implanted in rabbit bone, *J. Mater. Sci. Mater. Med.,* **26(2):**73.
5) **Evian CI, Al-Maseeh J, Symeonides E. (2003)** Soft tissue augmentation for implant dentistry. *The Compendium of Continuing Education in Dentistry,* **24(3):**195–208.
6) **Rutkowski JL, Thomas JM, Bering CL, et al.** Analysis of a rapid, simple, and inexpensive technique used to obtain platelet-rich plasma for use in clinical practice. *J. Oral Implantol.,* **34(1):**25-33
7) **Hamilton B, Knez W, Eirale C, Chalabi H. (2010)** Platelet enriched plasma for acute muscle injury. *Acta Orthopaedica Belgica,* **76(4):**443-448.
8) **Orly I, Kerebel B, Abjean J, Heughebaert M, Barbieux I. (1989)** Hydroxyapatite biomaterial implanted in human periodontal defects: an histological and ultrastructural study. *Bull Group Int. Rech. Sci. Stomatol. Odontol.,* **32(2):**79-86.
9) **Kent JN. (1986)** Reconstruction of the alveolar ridge with hydroxyapatite. *Dent. Clin. North Am.,* **30(2):**231-57.
10) **Weinlaender M. (1991)** Bone growth around dental implants. *Dent. Clin. North Am.,* **35(3):**585-601.
11) **Hoogendoorn HA, Renooij W, Akkermans LM, Visser W, Wittebol P. (1984)** Long-term study of large ceramic implants (porous hydroxyapatite) in dog femora. *Clin. Orthop. Relat. Res,* **187:**281-8.
12) **Verburg AD, Klopper PJ, van den Hooff A, Marti RK, Ochsner PE. (1988)** The healing of biologic and synthetic bone implants. An experimental study. *Arch. Orthop. Trauma Surg.,* **107(5):**293-300.
13) **Klein CP, Patka P, den Hollander W. (1989)** Macroporous calcium phosphate bioceramics in dog femora: a histological study of interface and biodegradation. *Biomaterials,* **10(1):**59-62.
14) **Kwong CH, Burns WB, Cheung HS. (1989)** Solubilization of hydroxyapatite crystals by murine bone cells, macrophages and fibroblasts. *Biomaterials,* **10(9):**579-84.
15) **Grégoire M, Orly I, Menanteau J. (1990)** The influence of calcium phosphate biomaterials on human bone cell activities. An *in vitro* approach. *J. Biomed. Mater. Res,* **24(2):**165-77.
16) **Piattelli A, Cordioli GP, Trisi P, Passi P, Favero GA, Meffert RM. (1993)** Light and confocal laser scanning microscopic evaluation of hydroxyapatite resorption patterns in medullary and cortical bone. *Int. J. Oral Maxillofac. Implants,* **8(3):**309-15.

17) Närhi TO, Jansen JA, Jaakkola T, de Ruijter A, Rich J, Seppälä J, Yli-Urpo A. (2003) Bone response to degradable thermoplastic composite in rabbits. *Biomaterials,* **24(10):**1697-704.

18) Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. (2016) Soft Tissue Augmentation of the Face With Autologous Platelet-Derived Growth Factors and Tricalcium Phosphate. Microtomography Evaluation of Mice. *J. Craniofac. Surg.,* **27(5):**1212-4.

19) Scarano A, Ceccarelli M, Marchetti M, Piattelli A, Mortellaro C. (2016) Soft Tissue Augmentation with Autologous Platelet Gel and β-TCP: A Histologic and Histometric Study in Mice. *Biomed. Res Int.,* :2078104. doi.org/10.1155/2016/2078104

20) Svolacchia F. (2012) Trattamento con fattori di crescita derivati dal plasma ricco in piastrine o biostimolazione con fattori di crescita autogeni, Medicina Estetica magazine, **1(2):** 6-9.

Author Address:

Fabiano Svolacchia
Via Gran Sasso, 14
00048 Nettuno (RM) - Italy
E-mail: fabiano.svolacchia@alice.it