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Research

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A combined use of Chondrocytes Micro Grafts (CMG) Mixed with Platelet Rich Plasma (PRP) in Patients Affected by Pinch Nose Deformity

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Abstract

Background: The combined use of autologous chondrocytes micro-grafts (CMG) mixed with Platelet Rich Plasma (PRP) is an alternative that opens a new era in this field.

Materials and Methods: At the Department of Plastic and Reconstructive Surgery, University of Rome "Tor Vergata", Italy, 15 patients, affected by pinch nose deformity underwent nasal alar reconstruction with chondrocytes micro-grafts gentle poured onto PRP in solid form. A CT scan control was performed after 12 months. Pearson's Chi-square test was used to investigate difference in cartilage density (CD) between native and newly formed cartilage.

Results: The constructs of CMG-PRP subcutaneously injected resulted in cartilage tissue with adequate central nutritional perfusion. Postoperative follow-up evaluation has shown optimal aesthetic results and improvement of nasal obstruction. These composite grafts provide functional support to the alar cartilages, usually collapsed in pinch nose deformity because of excessive resection during previous surgery.

Conclusion: This report demonstrated that chondrocytes micro-grafts derived from nasal septum poured onto PRP in solid form are a useful method for cartilage regeneration in patients affected by external nasal valve collapse.

Keywords

Chondrocytes auto-graft; Cartilage regeneration; Cartilage tissue engineering

Introduction

For therapeutic cartilage regeneration, the use of chondrocytes micrografts (CMG) mixed with platelet-rich plasma (PRP) has not been studied for recapitulating chondrogenesis. PRP has been demonstrated to be effective in the treatment of soft tissue defects and pattern hair loss [1-4]. It has been demonstrated that three-dimensional culture system in type I collagen scaffold and the addition of multiple growth factors contained in PRP, in the culture medium induced proliferation and a robust chondrogenesis of adult stem cells *in vitro* [5,6]. Direct mixing of chondrocytes with PRP leads to shrinking and deformed cartilage formation in vivo [7] owing to poor mechanical stability and rapid degradability. In particular the use of chondrocytes micrografts represents a micro-invasive procedure and grafts are more flexible to fill the lesions with various shapes. Today the main problem in transferring the experimental protocols of tissue engineering in the routine clinical practice is the identification of accessible sites where an adequate amount of stem cells are collected [8,9]. In addition, the need to specifically define technical procedure and its safety is an essential factor, as occurring for stem cell application in breast reconstruction and soft tissue defects [10-13].

Although within the human body there are several "niches" inhabited by a significant number of stem cells [14-16], often these are not easy to access. The nasal septum represents a niche housing chondrocytes easily accessible without limited morbidity of the anatomical site after collection of the micrografts. Ruikai Ba et al. [17] developed the cell bricks technique, which cultured a chondrocyte sheet and cut such a cell–Extra Cellular Matrix complex into multiple small fragments (cell bricks). They found that chondrocyte bricks significantly inhibited vascular infiltration into PRP gels and slowed their degradation, thus maintaining the framework and shape of the PRP grafts [18]. They hypothesized that the cell brick-enriched PRP gel could be an ideal injectable niche for adults stem cells, which is expected to regenerate biological cartilage tissues with persistent cartilaginous phenotype, less deformation and uniform histological structure [18].

Currently, in this study, we investigated the in vivo performance of chondrocytes in cell brick-enriched PRP gels, and evaluated the persistence of a stable chondrogenic phenotype.

Chondrocytes can be obtained by enzymatic digestion and/or mechanical disaggregation: in the first method, the cartilage tissue is harvested under sterile conditions, digested with appropriate enzymes, and then the resulting cell suspensions are seeded in culture dishes containing a special medium supplemented with necessary additives and then incubated. Finally, the resulting colonies are sub-cultured before confluence and the cells are stimulated to differentiate. In the second procedure, chondrocytes are isolated by the mechanical centrifugation of cartilage (Rigenera Method) in which the cartilage is treated as any other connective tissue subjected to grafts, with a phase of collection and a phase of mechanical disaggregation of the tissue without manipulating the matrix. Rigenera protocol produces millions of viable micro-grafts and filters them with a cut-off of 50 microns, in order to promote the discharging of old differentiated cells and the enrichment of young progenitors cells contained within the cartilage.

Materials and Methods

Patients

A total of 15 patients aged 23-67 years, affected by external nasal valve collapse, the so-called pinched nose deformity, were treated from January 2014 to September 2015 at the Plastic and Reconstructive Surgery Department of "Tor Vergata" University, Rome.

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The preoperative study was carried out through a complete clinical examination of the nasal pyramid and of the nasal cavities through anterior rhinoscopy, a photographic examination in three projections and CT scans.

The authors cut the septum in strips during rhynoplasty. The strips were gently collected and dissociated using Rigenera System (HBW srl, Turin, Italy) in 1.2 ml of physiologic solution. After a specific centrifugation the suspension was harvested from the system and infiltrated onto PRP gel.

The product obtained (PRP gel mixed with chondrocytes) was applied on the defect in external nasal valve collapse (Figure 1F). After 12 months follow-up radiographic examinations were done with the use of CT scans.

PRP preparation

PRP was prepared with approval of the transfusional service from a small volume of blood (18 ml) according to the method of the Cascade-Selphyl-Esforax system (Figure 2A-C) (Aesthetic Factors, LLC, Wayne, NJ, www.selphyl.com), with modifications, and from 60 ml of blood according to the P.R.L. Platelet Rich Lipotransfert system (CORIOS Soc. Coop, San Giuliano Milanese, Italy, www.corios.it), with modifications, using PRP alone (C-punt; Biomed Device, Modena, Italy, www.biomeddevice.it) without the combined use of stromal vascular fraction cells. In the Cascade-Selphyl-Esforax procedure, we used centrifuge 1,100 g for 10 minutes (Figure 2A); in the P.R.L. Platelet Rich Lipotransfert system and C-Punt System, we used centrifuge 1,200 RPM for 10 minutes). Then, autologous PRP, not activated (9 ml) obtained by Cascade-Selphyl procedure, was switched with 10-ml tubes containing Ca^{2+}. The authors obtained PRP in solid form (Figure 1A) after the second spin of PRP activated with Ca^{2+}.

Autologous PRP, not activated, obtained by the C-Punt Biomed Device procedure after centrifugation (20 ml), was inserted in a light selector device. At the end of the procedure, 9 ml of PRP was harvested.

Micro-grafts chondrocytes preparation

Micro-grafts of chondrocytes were prepared from a Rigeneracons® (CE certified Class I). After the extraction of the nasal septum (Figure 3A and B) during rhynoplasty, the authors cut the septum (Figure 3C and D) in strips (2 mm × 2 mm) (Figure 3E and F). The strips was dissociated using Rigeneracons® System (HBW srl, Turin, Italy) (Figure 2D and E) in 1.2 ml of physiologic solution (Figure 2F). After one minute of centrifugation at 80RPM the cellular suspension was harvested from the system and infiltrated onto PRP gel (Figure 1A).

The aim is to disaggregate a small piece of cartilage (septum cartilage strips) and select a cell population with a size of 50 micron. These cell populations were analyzed by the authors in hystological evaluation section.

Cytospin and alcian-PAS staining

Chondrocytes, isolated by Rigenera System (HBW srl, Turin, Italy), were made to adhere on a glass slide by cytospin, then Alcian-PAS staining was performed (Ventana-Roche Diagnostics, Milan, Italy). Positive cells were counted in the total area under a light microscope (Eclipse E600, Nikon, Japan) and microphotographs captured by DXM1200F Digital camera (Nikon) using ACT-1 software (Nikon).

Histological evaluation

Excisional tissue was obtained from four randomly selected patients after 6 months from surgery. Microscopic evaluation of routinary Haematoxylin-Eosin stained paraffin embedded sections [19] was performed to morphologically analyze cartilage tissue.

Results

Clinical and Instrumental observation

In this study, we reported the results with the use of composite autologous chondrocytes micro-grafts with PRP, as exemplified by the following case. Nasal obstruction was very marked in these patient; external nose analysis showed external nasal valve collapse

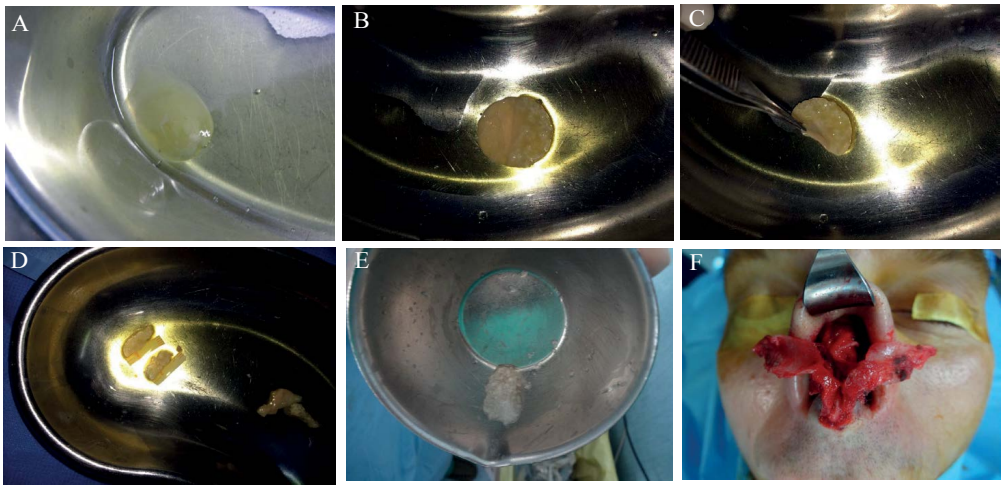

Figure 1: (A) Membrane of PRP in solid form; **(B)** Membrane of PRP in solid form mixed with chondrocytes (left part) and with strips (right parts); **(C)** Mixing of the part injected with chondrocytes with the part containing strips; **(D)** Re-modelling of the membrane obtained; **(E)** Micrografts; **(F)** Open Tip access with exposition of the alar cartilage.

(Figure 4A), deficit of supratip projection. Anterior rhinoscopy showed a deviated nasal septum and bilateral stenosis of the internal valves. The composite graft, was applied on the external nasal valve collapse, in the alar cartilage side by fixing with absorbable stitches. Postoperative follow-up evaluation has shown optimal aesthetic results (Figure 4B) and improvement of nasal obstruction. These composite grafts provide functional support to the alar cartilages, usually collapsed because of excessive resection during previous surgery. Transcolumellar open-tip access was necessary to allow for better visualization of the valve collaspe, alar cartilage and for fixation of the cartilaginous structures, to allow for placement of unexposed absorbable stitches.

In the CT scans it shows the pre-operative situation in comparison with the regenerated site in post-operative image. Figure 1B-E shows the preparation.

Histological observation

Chondrocytes isolated by Rigenera System, were stained with Alcian-PAS and counted under a light microscope. The mean of cell yield was 9,088.30 ± 788.86 cells/ml of digestion.

In addition, microscopic analysis of excisional fragments, in two patients, showed the persistance of healthy cartilage tissue surrounded by a fibrous connective tissue layer in which newly formed capillaries spread and penetrate into cartilage.

Discussion

The regenerative surgery opens new challenges for correction of nasal deformity. An injectable approach for cartilage regeneration could meet today's demand for micro-invasive surgery [20]. Zhu et al. [18] showed that cell bricks (CB) formed by fragmented chondrocyte macro-aggregates could stabilize the PRP gel efficiently in vivo and support chondrogenesis with stable morphology. They found that such an injectable complex could support chondrogenesis of bone marrow mesenchymal stem cells (BMSCs) in vivo, and demonstrated that such a completely biological graft could meet the requirement of nasal augmentation. The most important finding in this study is that BMSCs embedded in chondrocyte brick-enriched PRP gel underwent persistent chondrogenesis, and hypertrophic translation was prevented [18]. In addition, BMSCs are capable to promote angiogenesis through the secretion of growth factors, in particular VEGF [21]. Angiogenesis is a crucial event for tissue regeneration [22], as well as for tumor growth [23,24].

This finding provides a micro-invasive approach for cartilage regeneration, indicating the promising future of cell bricks for clinical application. A surprising phenomenon presented in study of Ba, et al. [17] is that BMSCs mixed into CB-PRP gel guided fast angiogenesis in BMSC regions and prevented central necrosis of the whole graft. Their previous experiment encountered the problem that chondrocytes mixed into CB-PRP gels resulted in obvious necrosis in the interior of constructs, which could be attributed to the robust anti-angiogenic potential of chondrocytes. Their results confirmed that BMSCs in CB-PRP gels presented higher VEGF expression than chondrocytes in CB-PRP gels, which was regarded to contribute the angiogenesis in BMSCs regions in PRP grafts.

Here we demonstrated that Rigenera System is a useful method to isolate human chondrocytes and when cells were injected with PRP *in vivo* in patients affected by nasal valve collapse.

The constructs of chondrocytes micro-grafts-PRP that were subcutaneously injected resulted in a persistent cartilage tissue with appropriate morphology, adequate central nutritional perfusion without central necrosis or ossification, and further augmented nasal dorsum without obvious contraction and deformation.

In addition, microscopic analysis of excisional fragments showed the persistence of healthy cartilage tissue with the formation of new capillaries penetrating into cartilage.

Appropriate scaffolds combined with growth factors can significantly improve the survival and differentiation of the transplanted stem/progenitor cells [6,19,25-31].

In a study of Brunelli et al. [25], the micro-grafts obtained by dental pulp poured onto collagen sponge are useful method for bone regeneration in atrophic maxilla. Dental stem/progenitor cells

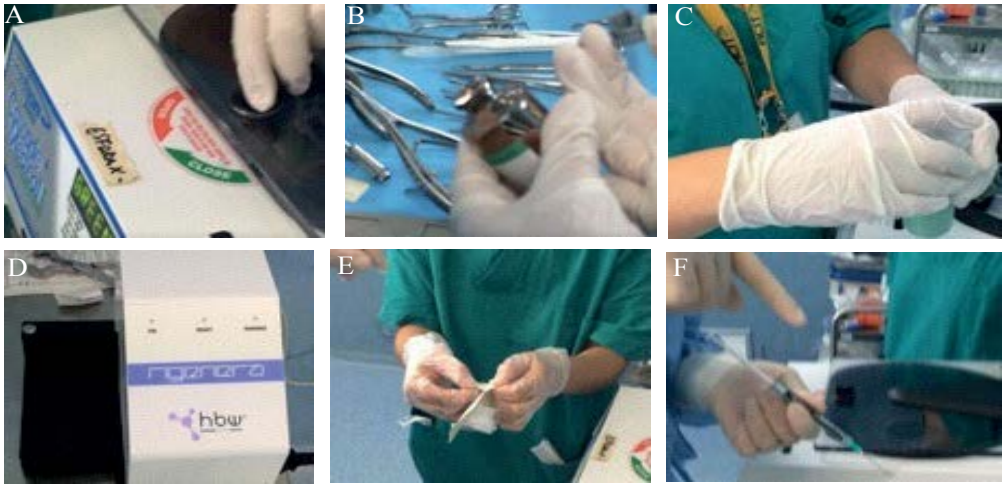

Figure 2: (A) Cascade Centrifuge; **(B)** Tube extracted by Cascade kit for membrane preparation containing CaCl; **(C)** Phase of preparation; **(D)** Rigenera Centrifuge; **(E)** Rigenera Kit; **(F)** Syringe with 1ml of solution containing chondrocytes.

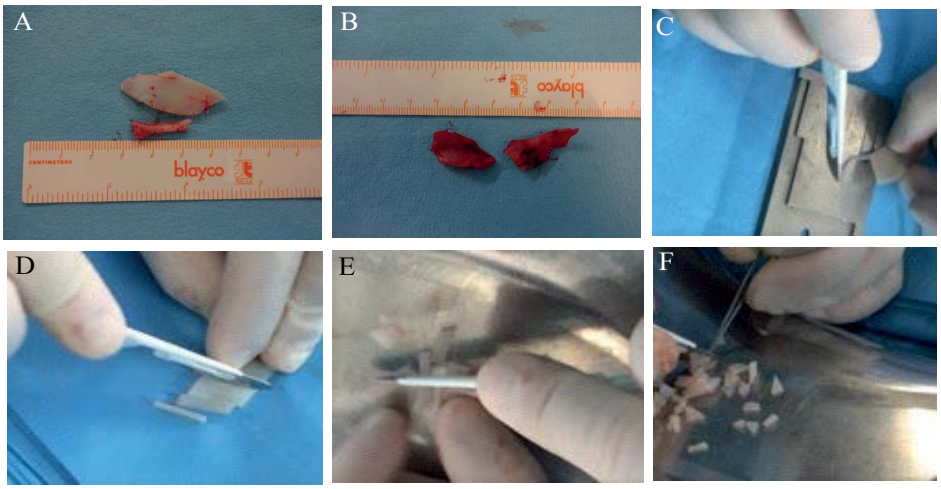

Figure 3: (A) Original fragment of the cartilage extracted from the septum during rhinoplasty; **(B)** Fragment of the septum cartilage remodeled; **(C)** Phase of the septum cutting; **(D)** Strips of the cartilage septum; **(E)** Selection of the strips; **(F)** Selected 2 mm × 2 mm strips.

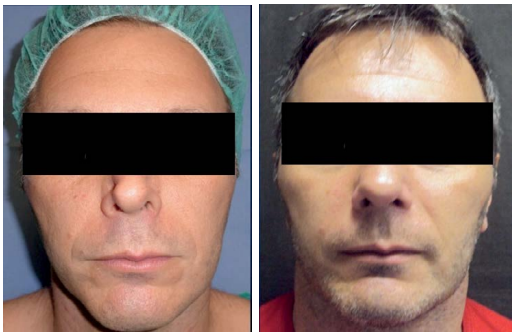

Figure 4: (A) Pre-operative in frontal projection with right nasal valve collapse; **(B)** Post-operative situation after 1 year in frontal projection.

(DPSCs) collected from dental pulp can be differentiated in vitro and then transplanted with biomaterial scaffolds into the host without immunologic rejection [32,33]. Graziano et al. [34] observed DPSCs performances on different scaffolds, such as PLGA or poly (lactic-co-glycolic acid) 85:15, hydroxyapatite chips (HA), and titanium. Results showed that stem cells exerted a different response, depending on the different type of textured surface [35]. Thus, cells cultured on the concave textured surface had better cell-scaffold interactions and were induced to secrete factors that, due to their autocrine effects, quickly lead to osteogenic differentiation, bone tissue formation, and vascularization.

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