Bone Regeneration of the Maxillofacial Region Through the Use of Mesenchymal Cells Obtained by a Filtration Process of the Adipose Tissue

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Abstract: In this work, the authors will discuss about a new protocol and rapid alternative to isolate the mesenchymal stromal cells from the stromal vascular fraction, without the use of collagenase, to regenerate the bone tissue in the maxillo-facial region. This method employs a device that allows the isolation and concentration of stromal vascular fraction by means of lipoparticles, which separate the lipid component and fragments of the extracellular matrix. The innovative element consists in using a filtration device instead of a centrifuging device to separate the different components. The purpose of this work was to illustrate the results obtained with the above-mentioned method in a series of 8 patients suffering from cystic neoformations maxillary or mandibular.

Key Words: Bone regeneration, mesenchymal cell, stem cell

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The stromal vascular fraction (SVF) of adipose tissue appears to be a rich source of somatic stem cells (mesenchymal stromal cells [MSC]) and angiogenic factors.1,2 Mesenchymal stromal cells are multipotent adult stem cells that can be isolated from various human tissues: the most analyzed are isolated from bone marrow and adipose tissue.3,4 Over the past 10 years, several studies have been conducted on platelet-rich plasma (PRP) and stem cells derived from adipose tissue, because of their potential and wide application in the medical field, from cardiovascular research to applications for the treatment of corneal detachment.

Adipose tissue contains 100,000 mesenchymal cells per gram of fat, which can be taken through direct withdrawal by liposuction. Compared with what provides the bone marrow, adipose tissue appears to be more accessible, abundant, and reliable for the isolation of stem cells. The speed of preparation is the key to publicize and maximize the surgical application of the MSC. Various methods to isolate stem cells from adipose tissue are present in the literature. The most used technique regards the method of centrifugation.

In this work, we will discuss about a new protocol and rapid alternative to isolate the MSC from the SVF, without the use of collagenase, to regenerate the bone tissue in the maxillo-facial region. This method employs a device that allows the isolation and concentration of SVF by means of lipoparticles, which separate the lipid component and fragments of the extracellular matrix. The innovative element consists in using a filtration device instead of a centrifuging device to separate the different components. The purpose of this work was to illustrate the results obtained with the above-mentioned method in a series of 8 patients suffering from cystic neoformations maxillary or mandibular.

METHODS

From September 2014 to February 2015 we have treated 8 patients with maxillary cysts and/or mandibular odontogenic. In some patients, the pathology has been an occasional finding during x-ray inspection. We included these patients in our experimental protocol, once we got their formal approval.

The patients' statistics were: 4 women, and 4 men, ages ranging from 32 to 57. In 5 patients the cystic lesion was located in hemimandible (3 symphysial and 2 body mandibular). In the 3 remaining patients the cystic lesion was localized in the upper jaw (in 2 patients in the premaxilla and in 1 patient at the level of the draft dog). Before the execution of Mysen (Mysen LLC, Wilmington, DE), the surgical procedure carried out the removal of the tumor and the consequential histological test; in 3 patients extraction of dental elements was considered necessary.

In the same operative session, liposuction in the abdominal area has been performed to collect adipose tissue. By means of liposuction technique, 10 cc of adipose tissue has been extracted and placed into the dedicated disposable device. The filtration procedure we adopted takes only a few minutes and it is executed in contained sterile environment free from external contamination risks. Once the cellular preparation, we placed it on an antigen-free bone scaffold used to fill the bone defect (mandible or jaw defects). After

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that, a careful suturing of the mucosa has been carried out. Patients have been then managed through a wide spectrum antibiotic therapy. Most of the patients experienced a postoperative period of about 2 days (Figs. 1–4).

**RESULTS**

All the treated patients had periodic check-up for medication and surgical sutures removal. The regional site conditions were more than satisfactory from both a clinical and functional point of view.

We did not find any patient of active infection even after suspension of antibiotic therapy. The radiological follow-up has been performed with Rx orthopantomography after 1, 3, and 6 months from surgery; newly formed bone was visible just after 1 month.

As a further step, 3 months from surgery we have performed a small biopsy to obtain a microscopic evaluation for the newly formed bone. Macroscopically, the sample showed a compact bone, as the histological test later confirmed (Figs. 5 and 6).

**DISCUSSION AND CONCLUSION**

Over the past 10 years, several studies have been conducted on PRP and stem cells derived from adipose tissue, because of their potential and wide application in the medical field, from cardiovascular research to applications for the treatment of corneal detachment.

The PRP is a concentration of human autologous platelets in a small volume of plasma. The PRP works through the degranulation of alpha-granules in platelets; it must be developed in a state anticoagulant and applied within 10 minutes from the process beginning. After the initial 10 minutes, the platelets synthesize and secrete additional proteins to balance their life cycle. The positive effects of PRP have been experimentally demonstrated. Successful applications have been obtained in the field of reconstructive surgery aimed to fill bone defects. As an autologous preparation, PRP is inherently safe in relation to the transmission of diseases such as HIV, hepatitis, West Nile fever, and Creutzfeldt–Jacob syndrome, even if its security is still topic of debate for scientific community.

The adipose-derived stem cells (ASCs) are derived from human adipose tissue that can be sampled by direct sampling or, more commonly, through liposuction. Compared with what provides the bone marrow, adipose tissue appears to be more accessible, abundant, and reliable for the isolation of stem cells. Adipose tissue contains 100,000 mesenchymal cells per gram of fat. After the absorption of collagen and the subsequent centrifugation of collected tissue, the SVF is obtained. Within the stromal vascular fraction we find undifferentiated multipotent cells characterized by a mesenchymal nature and negative hematopoietic markers. Reconstructive plastic surgery represents the first direct expression of cell-based therapies, as such a kind of surgery presents low risks of systemic complications. Nevertheless, the speed of preparation is the key to endorse and maximize the surgical applications of ASCs. The SVF is a raw preparation, quite rapid to be obtained. SVF can provide ASCs for specific points of application, even if it contains other types of cells (endothelial cells, muscle cells, fibroblasts, etc.). Thus, in some patients of application, the purity of ASCs could be sacrificed in favor of surgical convenience.
MSCs can be isolated on the basis of their adherence to the crop area without executing lysis tissue with collagenase. The described cells can play a key role in the medical-regenerative field, also because of the low level of complexity of the procedures related to the isolation and culture.

In the literature we find several techniques for the isolation of stem cells derived from adipose tissue. The technique of rapid isolation presented in 2009 proposes a method of advanced isolation using a saline solution in less than 30 minutes. This process produces an abundant population of adipose stem cells with:

- differentiation potential
- proliferative cycle
- cell surface markers

almost indistinguishable from mesenchymal stem cells obtained following the traditional method.

The common isolation procedures of mesenchymal cells derived from adipose tissue are based on separation of the stromal vascular fraction, from which, by a process of collagenase digestion and cell filtration, is obtained a cell suspension that is extensively cultivated. The time needed for incubation and washing is not compatible with the operating timing. Therefore, new methods for cell separation are needed to optimize time and respect the physical-chemical properties of the cells.

The presented method employs a device that includes suitable tools capable of guaranteeing isolation and concentration of SVF by liposuptions, as it can separate the liquid component and the not desired fragments of the extracellular matrix.

The innovative element consists in the using of a filtration device instead of a centrifuging device to separate the different components.

Filtration is actually regarded as a safe and effective method to separate cells from a liquid suspension.\(^5\) Filtration systems for processing stem cells are available since years, for example for human placental/umbilical cord blood.\(^6\) Anyhow, no filtration system for processing stem cells from abdominal fat tissue has been reported in the literature.

Some of the numerous advantages are the following:

- Permits the sample management in a closed and safe environment, thus reducing the risk of contamination and the processing time.

- Permits the careful selection of the cellular component on the basis of the size.

- Permits to wash, separate, and concentrate the sample within the system, allowing to obtain a final ready-to-use product enriched of somatic multi powerful stem cells.

Therefore, the described device allows to get in a few minutes a concentration of regenerative cells, also applicable in the intraoperative, saving lengthy and costly processes; besides, it allows obtaining growth factors very useful to the tissue regeneration process and mesenchymal cells multipowerful.

Bone deficits may be linked to problems related to:

- malformations
- traumatic factors
- oncology factors
- infections

The aim of our work was to verify the effectiveness of transplantation of MSC cells through the use of a scaffold, derived by animal bones. The scaffold has been inserted in the place of bone deficit. MSCs cells have been applied on the scaffold.

We have monitored every single patient from both clinical and functional points of view. All the patients were inspected by means of x-ray OPT at intervals of 1, 3 and 6 months after surgery, to gradually evaluate the formation of the newly formed bone. After 3 months from the operative sessions, we performed a bone biopsy to histological analyze the bone formation progress: all the samples presented the formation of lamellar newly formed bone.

REFERENCES

9. Russell LB, Hunsicker PR, Russell WL. Comparison of the genetic effects of equimolar doses of ENU and MNU, while the chemicals differ dramatically in their mutagenicity in stem cell spermatogenesis both exhibit very high mutation rates in differentiating spermatogonia. Mutat Res 2007;616:181–195