Jaw bones regeneration using mesenchymal stem cells.

A single-center experience

Ann Ital Chir, 2018 89, 1: 20-23
pii: S0003469X18027896
Epub Ahead of Print - December 21
free reading: www.annitalchir.com

Walter Colangeli, Umberto Riccelli, Amerigo Giudice, Ida Barca, Davide Caruso, Daniela Novembre, Claudio Tortosa, Raffaella Cordaro, Maria Giulia Cristofaro

Department of Clinical and Experimental Medicine, Maxillofacial Surgery Unit (Head: Prof. M.o Giudice), University of Magna Graecia, Catanzaro, Italy

Jaw bones regeneration using mesenchymal stem cells. A single-center experience.

PURPOSE: *Mesenchymal stem cells (MSC), which are multipotent stromal cells, are considered to be a promising resource in tissue engineering and tissue regeneration. MSCs have been used to generate new maxillary bone with clinically successful results. The aim of this study was to determine the role of MSC in bone regeneration procedures in patients with benign maxillary lesions.*
METHODS: *A study was conducted on five patients treated for maxillary bone defects resulting from biopsy of benign lesions at the University Hospital of Magna Græcia, Catanzaro, Italy from January 2015 to October 2016. MSC from autologous bone marrow were used for bone regeneration. The bone mineral density was compared, using the Hounsfield scale, before and after treatment. Follow-up was monthly for six months, and the patients underwent a computed tomography scan of the maxilla at 6 months.*
RESULTS: *Five patients, who underwent biopsy of osteolytic odontogenic benign tumors, were included in the study. There were no intraoperative or postoperative complications. The mean volume of the newly formed bone was 2.44cm3 (range 2,0-3,1) and the mean bone density was 1137 Hounsfield Units (range 898-1355).*
CONCLUSIONS: *Bone regeneration with MSC from autologous bone marrow appears to be a valid treatment option for maxillary bone defects.*

KEY WORDS: Bone regeneration, Mesenchymal stem cells, BM-MSC, Upper jaw, Mandible

Inroduction

Embryonic stem cells are derived from blastocysts and are considered to be pluripotent cells as they are able to form all the body s cell lineages. Mesenchymal stem cells (MSC) are one type of adult stem cells, that are able to give rise to tissues of mesodermal origin such as dentin, bone, or periodontal ligament [1]. Bone regenerative potential of MSC was first evaluated in bone defects in animals [2]. Recent studies have evaluated the bone mineral density, in patients who underwent bone regeneration with particulate cancellous bone and marrow (PCBM) and platelet-rich plasma (PRP), with a micro-computed tomography (CT) scan [3]. To evaluate the maxillary defects repaired with regenerated bone we used a software algorithm measuring and comparing the three-dimensional (3D) volume defect and bone density.

Materials and Methods

The present study was conducted on patients who underwent biopsy of maxillary and mandibular lesions sec-

Pervenuto in Redazione Ottobre 2017.Accettato per la pubblicazione Novembre 2017.
Correspondence to: Maria Giulia Cristofaro, University "Magna Grecia", Department of Clinical and Experimental Medicine, Maxillo-Facial Surgery Unit, 88100 Catanzaro, Italy (e-mail: cristofaro@unicz.it)

ondary to benign dentigerous cyst, followed by bone regeneration at the University Hospital of Magna Graecia, Catanzaro, Italy from January 2015 to October 2016. The inclusion criteria were as follows: upper maxillary or mandibular bone defect from osteolytic odontogenic benign tumors, volume defect > 2cm^3 (calculated from CT scan using Osirix software), no comorbidities, no smoker (Fig. 1) Informed consent was obtained from all patients. The resection methods consisted of marginal resection performed by the same maxillofacial team (Fig. 2). MSC from autologous bone were used to repair the maxillary defects. In all patients 40 ml of bone marrow (BM) aspirate was obtained from the superior posterior iliac spine (Fig. 3). RegenKit Extracell (RegenLab SA Lausanne, Switzerland) was used to obtain a concentration of autologous stem cells. Preparation of autologous BM extracted from the iliac crest:
– aspiration of BM from the iliac crest;
– four sampling points (trocar insertion location moved);
– filling volume in Regen THT tubes, 5 ml per tube, final volume before centrifugation approximately 20 ml;
– only centrifugation 3400 rpm x 8 minutes (greater recovery of stem cells);
– Centrifuge Regen Centrigel H-19 F.
The cells were suspended in separating gel and concentrated for engineering. Based on the final volume of autologous stem cell concentrate (about 14/15 ml) 5ml of gluconate calcium was added if the cells needed to be gelled (Fig. 4). We put the mixture in the maxillary

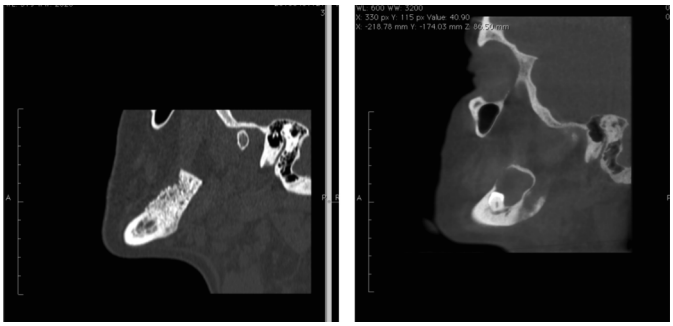

Fig. 2: Preopetive and postoperative tc scan.

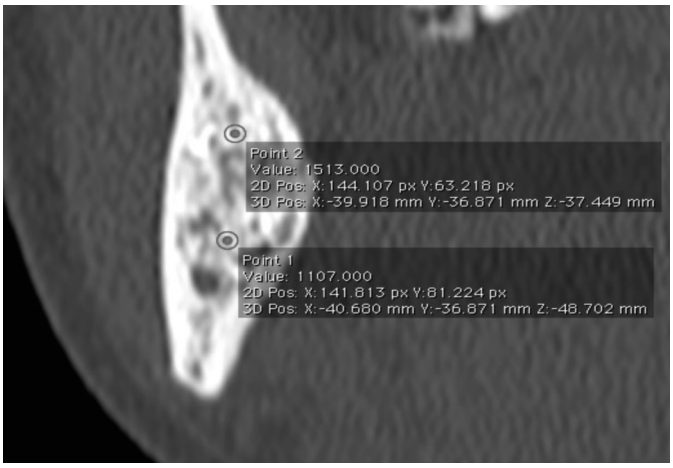

Fig. 3: tc scan at 6 months with hu evaluation.

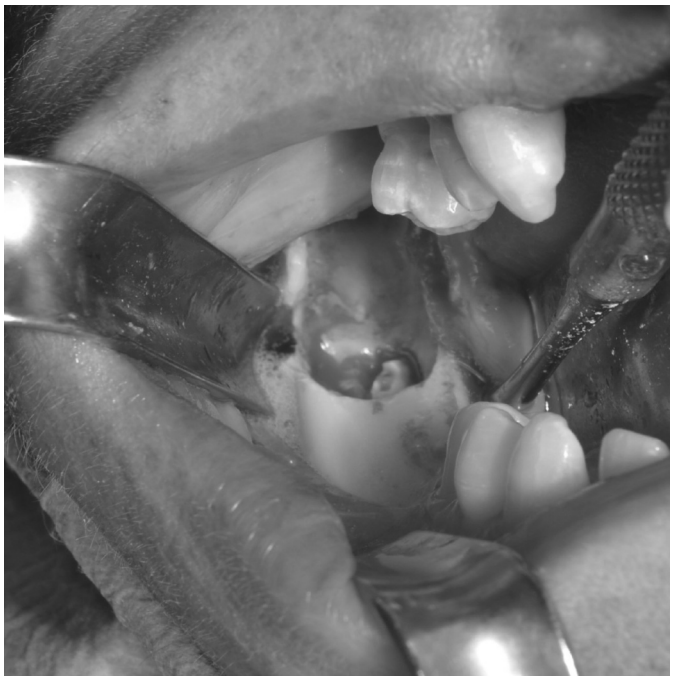

Fig. 1: Intraoperative view.

Fig. 4: Mesenchymal stem cells with autologous bone.

lesion and closed the defect with absorbable sutures. We performed a computed tomography (CT) scan at 6 months (Fig. 5) to evaluate:
– volume of newly formed bone;
– bone density using the Hounsfield scale (Osirix software)

TABLE I: *Patient data*

Patients	Location	Age years	Sex	Volume of lesion from CT scan cm^3	Bone density surgery pre-surgery HU	Bone density surgery post-surgery – CT scan HU
1	Mandible	49 Y	F	2,4	161	1167
2	Mandible	41 Y	F	3,1	195	1355
3	Mandible	55 Y	M	2,2	55	1284
4	Upper jaw	59 Y	M	2,0	190	985
5	Upper jaw	43 Y	F	2,5	254	898

CT= Computed tomography; HU= Hounsfield units

Results

There were 5 patients, 3 women and 2 men with a mean age of 49,4 years (range 41-59 years). Patient data is shown in Table 1. The procedure was well tolerated by all patients. There were no intraoperative or postoperative complications. No dehiscence of the surgical wound or infection of the surgical site was observed. Pain was well controlled. The patients underwent a clinical follow-up for six months that showed progressive improvement of the mucosa layer. A CT scan was performed at 6 months and showed excellent bone regeneration in all patients. We evaluated the volume of newly formed bone (mean volume 2.44 cm^3, range 2.0–3.1) and bone density quality using the Hounsfield scale. All patients had very high density new trabecular bone with a mean density of 1137 Hounsfield units (HU), (range 898-1355), (Table I).

Discussion

Achieving a successful and well-functioning reconstruction of craniofacial deformities still remains a challenge. With the recent advances in stem cell research, cell-based tissue engineering strategies moved from the bench to the patients' bedside. MSC, multipotent stromal cells, are considered to be a promising resource in tissue engineering. Minimal criteria for identifying these cells have been established by the International Society for Cellular Therapy 3. They must be plastic-adherent during culture in standard conditions, express cell surface markers such as CD105, CD73 and CD90 and not express CD45, CD34, CD14, CD11b, CD79a, CD19 or HLA-DR.

They must also be proven to differentiate into osteoblast, adipocyte and chondroblast lineages. MSC are supposed to act not only through direct bone formation, but also due to paracrine effects: releasing cytokines, producing extracellular matrix and promoting angiogenesis. MSC in combination with biomaterials have great potential that has already been proven in animal studies and in the first studies involving human subjects [4] BM was the first source reported to contain MSC and the most thoroughly investigated. Differentiation of BM-MSC into osteogenic, chondrogenic, adipogenic, hepatogenic, cardiogenic and neurogenic lineages was documented [5,6]. A hybrid-type bone substitute was created with cryopreserved MSC and autologous serum, and placed subcutaneously in nude mice [7] .The follow-up showed good osteogenic potential of cryopreserved cells and thus the possibility of banking them for bone grafting. However, invasive donation procedures (although with reduced donor site pain in comparison to traditional autologous bone grafting), as well as reports concerning age-related deterioration in proliferation and differentiation capabilities, may hinder the clinical use of BM-MSC [8,9]. The earliest studies on BM-MSC did not show any decline in BM-MSC potential with aging [10-12], but more recent papers on both human and animal BM-MSC showed that the stem cells do undergo the process of senescence and thus their differentiation and proliferation capabilities decrease with advancing donor age [13]. Bellows et al. [14] demonstrated reduced self-renewal capability of rat osteoprogenitors, while Tokalov et al. [15] showed age-related decrease of the MSC population in a rat model. Mareschi et al.[16] expanded BM-MSC from both adults and children, and observed differences in cell growth with favorable population doubling time in the pediatric donors and different cell morphology in adults, probably resulting in decreased proliferation capacity. In another experiment, made by Stenderup et al.[17], MSC were differentiated to the maximal life span and a decrease in viability and population doubling rate of human adult-derived MSC was detected with no differences in the mean telomere length in early passages. MSC derived from maxillofacial sources are thought to have outstanding greater proliferative and osteogenic capacity than other from iliac spine [18]. They can be easily obtained and thus are attractive as an autologous stem cell source. One of the major advantages of maxilla-derived stem cells is that their proliferation potential is probably unaffected by donor age, and only population doubling time seems to increase with donor age [19]. BM-MSC has already been used in alveolar cleft regeneration in ani-

mal models [20-22] and in the first clinical cases [23]. The increasing demand for implant-based prosthetic rehabilitation in patients with maxillary atrophy or osteolytic maxillary lesions, as in our study, has led to a search for an alternative to autologous bone for grafting purposes. In this context, the use of BM-derived MSC in combination with autologous bone, for benign osteolytic maxillary lesions, was investigated as a method to induce endogenous bone regeneration in a way that can adequately reproduce the osseoinductive effects of autologous bone. In the present study we evaluated the quality of maxillary bone regeneration from autologous BM-MSC. Satisfactory bone formation has been observed even in previous studies with the use of PRP and PCBM. Histological examination is the most common method for evaluating trabecular bone. Micro-CT is used to focus on bone microstructures without destruction of the specimens. We introduced a method that includes a software algorithm to evaluate the HU of the new bone based on CT scan data.

Conclusions

Our results suggest that bone regeneration using MSC from autologous BM is a valid treatment option for maxillary bone defects. Further studies with larger patient cohorts are needed to confirm our findings.

References

1. Huang GT, Gronthos S, Shi S: *Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine*. J Dent Res, 2009; 88:79806.

2. Gładysz D, Hozyasz KK: *Stem cell regenerative therapy in alveolar cleft reconstruction.* Archives of Oral Biology, 2015; 60(10) 1517-532.

3. Muneharu Iwamoto, Akira Matsuo, Noriko Kato, Sawako Takeuchi, Hidetoshi Takahashi, Satoru Hojo, Hiroshige Chiba: *Computed tomographic evaluation of bone quality of the mandible reconstructed by particular cellular bone and marrow combined with platelet rich plasma*. Oral Science International, 2009; 6(2):63-72.

4. Simmons PJ, Torok-Storb B: *CD34 expression by stromal precursors in normal human adult bone marrow*. Blood, 1991; 78:2848–853.

5. Dominici ML, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E: *Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement*. Cytotherapy, 2006; 8 (4): 315-17.

6. Branco de Sousa E, Ladeira Casado P, Moura Neto V, Leite Duarte ME, Pinheiro Aguiar D: *Synovial fluid and synovial membrane mesenchymal stem cells: Latest discoveries and therapeutic perspectives*. Stem Cell Research & Therapy, 2014; 5:112.

7. Sugimoto T, Yamazaki Y, Kumazawa K, Sone Y,Tak A: *The significance of performing osteogenic differentiation in human bone tissue-derived mesenchymal stromal cells.* J Oral Tissue Engin, 2013; 11(2):103-12.

8. Matsuo A, Yamazaki Y, Takase C, Aoyagi K, Uchinuma E: *Osteogenic potential of cryopreserved human bone marrow-derived mesenchymal stem cells cultured with autologous serum.* J Craniofac Surg, 2008; 19(3):693-700.

9. Gimbel M, R Ashley,. Kawamoto HK, Bradley JP: *Repair of alveolar cleft defects: Reduced morbidity with bone marrow stem cells in a resorbable matrix*. J Craniofac Surg, 2007; 18:895-901.

10. Wu Y, Chen L, Scott PG, Tredget EE: *Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis*. Stem Cells, 2007; 25:2648-659.

11. Stenderup K1, Justesen J, Eriksen EF, Rattan SI, Kassem M.: *Number and proliferative capacity of osteogenic stem cells are maintained during aging and in patients with osteoporosis.* J Bone Miner Res, 2001; 16(6):1120-129.

12. Leskelä HV, Risteli J, Niskanen S, Koivunen J, Ivaska KK, Lehenkari P: *Biochemical and biophysical research communications.* 2003; 311(4):10013.

13. Bianchi G, Banfi A, Mastrogiacomo M, et al.: *Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2*. Experimental Cell Research, 2003; 287(1):98-105.

14. Kretlow JD1, Jin YQ, Liu W, Zhang WJ, Hong TH, Zhou G, Baggett LS, Mikos AG, Cao Y: *Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells*. BMC Cell Biol, 2008; 9:60.

15. Bellows CG1, Pei W, Jia Y, Heersche JN*: Proliferation, differentiation and self-renewal of osteoprogenitors in vertebral cell populations from aged and young female rats*. Mech Ageing Dev, 2003; 124(6):747-57.

16. Tokalov SV, Gruner S, Schindler S, Wolf G, Baumann M, Abolmaali N: *Age-related changes in the frequency of mesenchymal stem cells in the bone marrow of rats.* Stem Cells Dev, 2007; 16:439-46.

17. Mareschi L, Ferrero I, Rustichelli D, Aschero S, Gammaitoni L, Aglietta M, Madon E, Fagioli F: *Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow*. J Cell Biochem, 2006; 97:744-54.

18. Stenderup K1, Justesen J, Clausen C, Kassem M: *Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells*. Bone, 2003; 33(6):919-26.

19. Liu Y, Hu J, Wang S: *Mesenchymal stem cell-mediated treatment of oral diseases*. Histol Histopathol, 2014; 29(8):1007-15.

20. Steindorff MM, Strempel J. F, Winkel A, Kühnel M, Stiesch M: *Differences of isolated dental stem cells dependent on donor age and consequences for autologous tooth replacement.* Archives of Oral Biology, 2014; 59(6): 559-67.

21. Motoko Yoshioka, Kotaro Tanimoto, Yuki Tanne, Keisuke Sumi, Tetsuya Awada, Nanae Oki, Masaru Sugiyama, Yukio Kato, and Kazuo Tanne: *Bone regeneration in artificial jaw cleft by use of carbonated hydroxyapatite particles and mesenchymal stem cells derived from iliac bone*. International Journal of Dentistry, 2012.

22. Ou XR, Jian XC, Lin G: *An investigation of restoration of alveolar cleft with engineered bone.* Zhonghua Zheng Xing Wai Ke Za Zhi, 2007; 23(1):29-31.

23. Tavakolinejad S, Ebrahimzadeh Bidskan A, Ashraf H Hamidi Alamdari D: *A glance at methods for cleft palate repair.* Iran Red Crescent Med J, 2014; 16(9): e15393.