Importance of Stem Cell Transplantation in Cleft Lip and Palate Surgical Treatment Protocol

Marcelo Paulo Vaccari Mazzetti, MD, MSc, Nivaldo Alonso, MD, PhD,† Ryane Schmidt Brock, MD, MSc,‡ Alexandre Ayoub, MD,§ Sally Mizukami Massumoto, MSc,|| and Lilian Piñero Eça, PhD¶*

Abstract: Cleft lip and palate is a congenital malformation that requires a multidisciplinary treatment that evolves pediatrician, obstetrics, fetal medicine, genetics, plastic surgery, orthodontics, speech therapist, nursery, and psychology. Actually, the authors believe that it could be possible to ad protocols to use stem cells.

The intrauterine diagnosis leads to preborn parental orientation and better parental collaboration to accept a precocious multidisciplinary treatment. After birth the authors' protocol is: orthodontic devices, phonoaudiology, and surgical procedures.

The authors' cleft lip and palate reconstructive surgery protocol demands several steps and begins at 4 to 6-month old with rhinocheiloplasty and soft palate closure at the same moment. The treatment sequence involves the hard palate surgery (8–18 months after the first surgical step), alveoloplasty (after 10 years old), and secondary rhinoplasty (after 14 years old).

New ideas to use stem cells and blood from the umbilical cord and also blood from placenta are discussed to improve final surgical results. Maternal stem cells are easy to collect, there are no damage to the patient and mother, it is autologous and it could be very useful in the authors' protocol.

Nine patients with clef lip and palate were operated and had stem cells from umbilical cord blood and placenta blood injected into the bone and soft tissue during the primary procedure (rhinocheiloplasty).

The stem cells activity into soft tissue and bone were evaluated. Preliminary results have shown no adverse results and improvement at the inflammatory response. A treatment protocol with stem cells was developed. It had a long time follow-up of 10 years.

Key Words: Cleft lip, cleft palate, reconstructive surgery, stem cell

(*J Craniofac Surg* 2018;29: 1445–1451)

From the *Brazilian Plastic Surgery Society, Brazilian Cleft Lip and Palate Society, Plastic Surgery Division at Darcy Vargas Children Hospital; †Brazilian Plastic Surgery Society, Brazilian Craniofacial Surgery Society, Plastic Surgery at São Paulo University; ‡Brazilian Plastic Surgery Society, Plastic Surgery Division at Darcy Vargas Children Hospital; §Brazilian Cryogenic Center (BCC); ||Umbilical Cord Blood Laboratory of Brazilian Cryogenic Center (BCC); and ¶Facial Defects Studies and Research Center, São Paulo, Brazil.

Received January 10, 2018.
Accepted for publication May 10, 2018.

Address correspondence and reprint requests to Marcelo Paulo Vaccari Mazzetti, MD MSc, 475, Ceci Avenue, São Paulo, SP 04065-000, Brazil; E-mail: consultasvaccari@ig.com.br

The authors report no conflicts of interest.

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000004766

Lip and palate clefts are congenital craniofacial malformations that compromise the lip individually, hard or soft palate or even the 3 structures together.[1]

The treatment of the cleft lip and palate is multidisciplinary and must be coordinated by a unique philosophy. The research for ideal surgical techniques is a constant goal. To find the best way to correct anatomical tissue repositioning is a big challenge. The use of stem cells and regenerative medicine open new possibilities of improving the final results in different pathways.[2]

The use of regenerative medicine by tissue engineering with mesenchymal stem cells was studied at least for 10 years and many researches demonstrated the capacity of bone marrow, dental pulp, umbilical cord blood and adipose tissue of being source of osteoblastic, adipogenic and chondrogenic cell lines.[3–5]

The mesenchymal stem cells produce hematopoietic and non-hematopoietic growth factors, chemokines, and cytokines and have also immunomodulatory functions.[3]

Prenatal ultrasonography during pregnancy is a fundamental fetal analysis and is commonly done in many countries. This examination can identify the gestational age, placenta location and viability, and congenital deformities.[6]

The gestational diagnosis of cleft lip and palate allows a precocious treatment with parent orientation, involvement of fetal medicine, obstetricians and plastic surgeons. The family is guided to be prepared to birth and to the extensive treatment of this craniofacial malformation that demands several steps and a group of specialists.

There are a large number of surgical protocols and techniques[7] and this fact suggests to us that the ideal result was not reached. Looking for these ideal results it was imagined a possibility to use stem cells in order to improve surgical results in soft, osseous, and cartilaginous tissues.

There are some studies that demonstrated osseous and soft tissue improvement results when it was used stem cells at the surgical procedure.[8,9]

The main objective of this study was to evaluate the effect of injection of stem cells from the umbilical cord, umbilical cord blood and placenta blood in surgical wound healing after primary lip and palate surgery in cleft lip and palate patients.

METHOD

This study was approved by the Facial Defects Study and Research Center Ethics Committee (Protocol 007/2007) and all parents received a Consenting Form and an explanatory class of the conventional surgical treatment protocol. Two weeks later they returned with the authorization of their children accession in this study. It was clear all the time about the possibility to decline the participation at this study.

From 2007 to 2016, 9 newborn patients with cleft lip only or associated with palate cleft were diagnosed by prenatal ultrasonography and had their umbilical cord and placenta blood collected at

TABLE 1. Classification of the Patients Cleft of Stem Cell Group and Control Group, Age at the First Surgical Procedure and Gender

Patients Number	Stem Cell Group (SCG)—Cleft Classification	Stem Cell Group (SCG)—Gender	Stem Cell Group (SCG)— Age of First Procedure	Control Group (CG)— Cleft Classification	Control Group (CG)—Gender	Control Group (CG)— Age of First Procedure
1	Incomplete unilateral lip cleft	Female	5 mo	Complete unilateral lip and palate cleft	Female	5 mo
2	Incomplete unilateral lip cleft	Female	4 mo	Complete unilateral lip and palate cleft	Male	6 mo
3	Complete unilateral lip and palate cleft	Male	5 mo	Complete unilateral lip cleft	Female	5 mo
4	Complete unilateral lip cleft	Female	5 mo	Incomplete unilateral lip cleft	Female	5 mo
5	Complete unilateral lip cleft	Male	6 mo	Complete unilateral lip and palate cleft	Female	4 mo
6	Complete bilateral lip and palate cleft	Male	5 mo	Complete bilateral lip and palate cleft	Male	5 mo
7	Complete unilateral lip and palate cleft	Female	6 mo	Complete unilateral lip and palate cleft	Male	6 mo
8	Complete unilateral lip cleft	Male	5 mo	Complete unilateral lip cleft	Female	5 mo
9	Complete unilateral lip and palate cleft	Female	5 mo	Incomplete unilateral lip cleft	Male	5 mo

birth, the least 3 of them had also the umbilical cord collected. This group of patients was designed Stem Cell Group (SCG).

Other group of 9 patients with similar clinical presentation of cleft lip and palate was chosen to be compared with the Stem Cell Group and was submitted to the same surgical procedures without the use of stem cells. This other group was designed Control Group (CG). Each patient of the Control Group was evaluated after birth, for this reason it was impossible to collect stem cell at birth.

The surgical procedures were done by the same plastic surgeon and the techniques used were the same to both groups. The clinical presentation of the patients is demonstrated in Table 1.

Both groups of patients were submitted to orthopedic treatment with palatal device since their birth, as it is part of the treatment protocol of the Facial Defects Study and Research Center.

In all cases of Stem Cell Group, the mother's blood was collected to virologic analysis.

The first case had the umbilical and placenta blood collected after cord clamping, with a needle at the vein, connected to 1 bag storage (Fig. 1). It was maintained at a 4°C temperature transport pack and sent to the laboratory where the material was prepared.

At the laboratory, the bag storage was processed in a closed system (Sepax – Biosafe), spared and cryoprepared into 1 bag. After the second patient, we started to storage into 2 to 10 small bags.

These bags are submitted to a gradative freezing in a Freezaal machine and storage in a liquid nitrogen tank with the controlled temperature of negative 188°C.

The difference of the first patient to the other cases was that the cells were frozen in multiple (2–10) bottles.

When the surgery was scheduled, at least 1 bottle was thawed in ambient air, washed and centrifugated in 1500 rpm for 10 minutes. The cell pallet formed was aspirated and prepared with saline solution in a total volume of 1 mL.

These total nucleated hematopoietic cells were marked with CD34 (antigen expressed on stem and progenitor cells) to determine the stem cell number.

The patients were submitted to the first surgical procedure at 4 to 6 months old. It was done the cheiloplasty, posterior palatoplasty, and primary rhinoplasty as described previously.[10] At the end of the surgical sutures, the first 4 patients had the stem cells in Dubelco Modified Eagle Medium solution combined to hyaluronic acid and injected with a 1 mL syringe and a needle 0.45 × 13 at the osseous alveolar gap, into the lip muscle and subcutaneous and into posterior palate depending on the cleft defect (Figs. 2 and 3).

The patients number 5 to 9 had the 1 mL stem cell in saline solution injected into the lip, muscle, and subcutaneous, when the patient had only the cheiloplasty done, 0.5 mL stem cells in saline solution was injected into the lip muscle and subcutaneous and 0.5 mL was mixed with collagen powder, washed with saline solution, and inserted into the palatal lateral incisions and in midline incision between the closed posterior palate and open anterior palate.

At the second surgical procedure, anterior palatoplasty, the stem cells are injected under the mucoperiosteal flap associated with washed collagen powder.

The analysis was done with clinical examination and photographs at the immediate postoperative, 1 week, 1 month, 1 year, 5 years, and 10 years of postoperative. Tomography was done at 2 years follow-up only in the first patient.

The comparison with the control patients was clinical and observed 5 different aspects: lip soft tissue inflammatory process

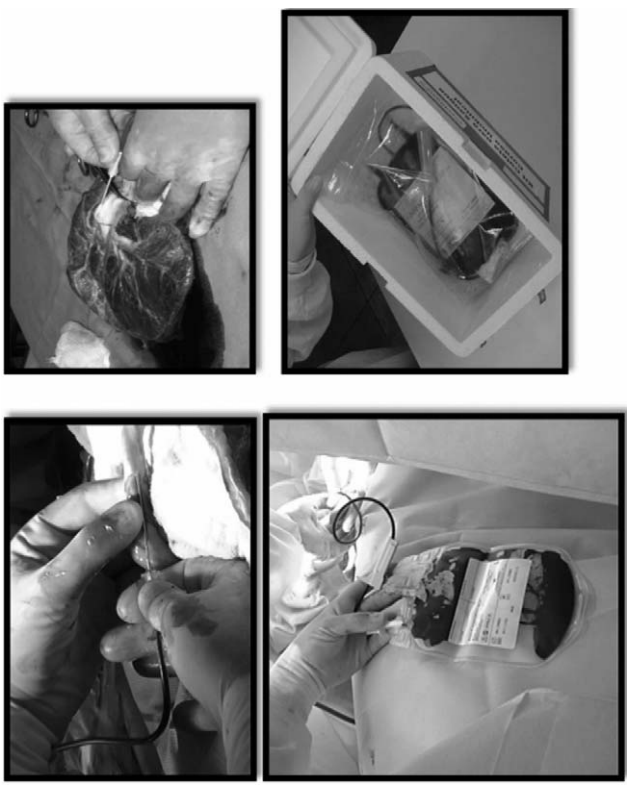

FIGURE 1. Procedure of umbilical cord and placenta blood collection at birth.

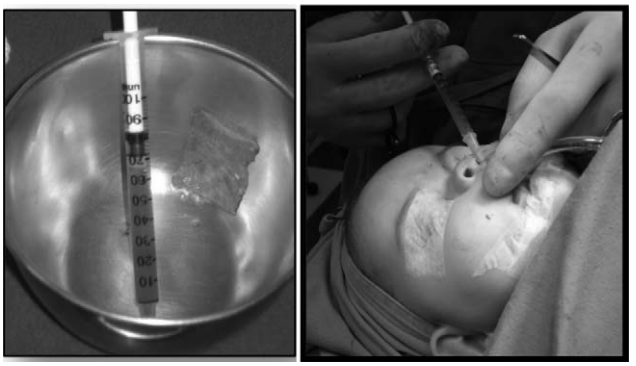

FIGURE 2. Stem cells prepared with Dubelco Modified Eagle Medium solution into a 1 mL syringe. Injection in the lip after cheiloplasty, orbicular muscle, and subcutaneous.

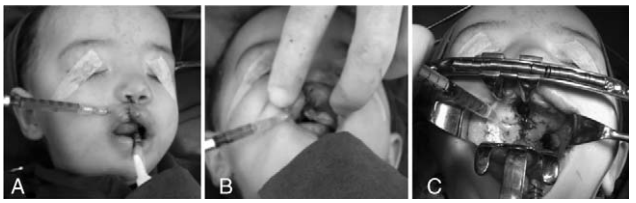

FIGURE 3. Stem cell injection with Dubelco Modified Eagle Medium. (A) Injection into lip subcutaneous. (B) Injection into orbicular muscle. (C) Injection into palate.

in the first 48 hours, lip transitory hypertrophic scar in the first 2 to 6 months, palatal dehiscence, palatal fistula, and fibrosis between soft palate and hard palate at the second surgical time of our protocol. The lip inflammatory process and palatal fibrosis classification are seen in Tables 2 and 3.

The clinical subjective graduation was done with a scale of 1 to 4 (+), which +/4+ has the least clinical evidence and 4+/4+ has the most clinical evidence (Tables 2 and 3).

The statistical analysis was descriptive, with absolute and relative frequencies. There were 2 categorical variable association, for that the exact Fisher test was used for the statistical analysis. It was considered $P < 0.05$.

RESULTS

The time of first surgical procedure was between 4 and 6 months old for all patients. In the cases 3, 7, and 8 of SCG and cases 2, 8, and 9 of CG, an anterior palatoplasty was performed between 6 and 18 months after the first surgery. The clinical aspects and follow-up period in the 2 groups are seen in Table 4.

The stem cell quantity injected into the surgical area is presented into Table 4.

The amount of mononuclear hematopoietic cells injected was 19.92×10^6 in average (Table 5) (Fig. 4).

The result analysis was based on clinical examination and photographies. We analyzed inflammatory process of lip soft tissue, transitory hypertrophy scar, palate dehiscence, and palate fistula presence observed in Tables 6 and 7.

There were no surgical complications at Stem Cell Group and all patients presented good scar aspect and little inflammatory process at the postoperative follow-up. None of them presented dehiscence (Figs. 5–7).

Statistically there was an association of the lip classification between the groups. The SCG presented higher percentage of "negative" classification (−) in soft tissue inflammatory process (P=0.001), transitory hypertrophy scar ($P < 0.001$), fibrosis between hard and soft palate ($P < 0.001$).

According to the palate evaluation there was no statistical difference between the groups ($P = 0.471$).

The group with stem cells injection presented less postoperative complications and fibrosis.

Facial tomography was done in the first patient to evaluate the osseous development. It was done 2 years of postoperative. The tomography result demonstrated a maxillary alignment, the alveolar cleft became smaller. However, it was no evidence of osseous development.

DISCUSSION

The lip and palate cleft treatment is always a challenge in multidisciplinary specialties to reach better results. The surgical protocol applied in the patients of Stem Cell Group and Control Group was similar and followed the conventional technique of the professional group.[10]

Nowadays, other technologies as bioengineering and tissue engineering are growing and been developed in medical procedures.[7,9,11]

Experimental studies with the use of stem cells already demonstrated important results of regenerative, neovascular, anti-inflammatory and tissue neoformation.[4,5,8,12]

This study had the purpose to demonstrate the advantages of stem cell into tissue healing at patients with lip and palate cleft. The surgical protocol was used in all lip and palate cleft patients studied, part of them had stem cells injection and part had only the conventional treatment done, considered as control group.[10]

It was imagined the possibility of increasing the results quality acting in the inflammatory process, muscular function, osteogenesis

TABLE 2. Parameters Used in Clinical Analysis of Lip Inflammatory Process.

Parameter Graduation	Aspect Used to Graduate	Aspect Description
+ Absence	Visual aspect	Same color of lip tissue and outside skin/without lip deformation
	Finger Sensitivity	Same consistency of lip tissue and outside skin
+ Very small	Visual aspect	Same color of lip tissue and outside skin/without lip deformation
	Finger Sensitivity	Slight increase in consistency in the surgical area
++ Small	Visual aspect	Same color and small brightness/without lip deformation
	Finger Sensitivity	Small increase in consistency in the surgical area
+++ Mild	Visual aspect	Same color and brightness/ Small lip deformation
	Finger Sensitivity	Mild increase in consistency in the surgical area
++++ Intense	Visual aspect	High brightness and small pallor
	Finger Sensitivity	High increase in consistency in the surgical area

TABLE 3. Parameters Used in Clinical Analysis of Palatal Fibrosis.

Parameter Graduation	Aspect Used to Graduate	Aspect Description
+ Absence	Visual aspect Finger sensitivity	Same color of palatal tissue around/same consistency of palatal tissue around
+ Very small	Visual aspect Finger sensitivity	Small pallor Slightly increased consistency
++ Small	Visual aspect Finger sensitivity	Pallor Slightly increased consistency
+++ Mild	Visual aspect Finger sensitivity	Whitish Mild increased consistency
++++ Intense	Visual aspect Finger sensitivity	Very whitish Very increased consistency and "frozen" with necessity of surgical removal

process, and scar healing after the injection of stem cells. No clinical data (for cleft lip and palate) were found in the literature.

In two experimental studies with rabbits to reconstruct craniofacial bone at where stem cells were used, from adipose tissue[1] and bone,[12] the results demonstrated the real possibility of utility of the stem cells in treatment of cleft lip and palate and encouraged us to move this research.

de Mendonça Costa et al[5] in an experimental study demonstrated more mature bone formation using stem cells in rats cranial defects.

Bueno et al[4] used orbicular oris muscle in rats and demonstrated reduction of granulation tissue and bone formation. Ramazanzadeh et al[13] demonstrated, in an experimental study, the effectiveness of the stem cells in the orbicularis oral muscle function.

Brock et al[12] did an experimental study using platelet-rich plasma and mesenchymal stem cell from adipose tissue and had more mature bone formation with the group that used bone fragments, platelet-rich plasma, and stem cels.

Spiekman et al[14] reviewed the power of adipose-derived stromal cells in treatment of fibrotic scars trough effects in extracellular matrix remodeling, angiogenesis and modulation of inflammatory process. This study showed evidences of how the stem cells would benefit postoperative results.[14]

At the beginning of this study, it had a high cost to work with stem cells; however, after 10 years of the study it was noticed that the cost decreased 4-fold. Other negative aspect was the absence of other studies to be used as clinical guidelines to give us the assurance of no maleficence to the patients.

these reasons it was opted for a limited number of patients in this study. The groups had only 9 patients each, a small sample number. This technique did not intend to cause any harm to the children and not interfere into the patient's craniofacial deformity treatment.

A long time of follow-up was defined to make sure that there will not be any patient maleficence and, second, to observe the clinical results in the soft and hard tissues operated.

At clinical researches the possibility of using stem cells in cleft lip and palate was cited initially by Hibi et al in alveolar cleft to restore bone. Other authors published good results with stem cells in the alveolar bone.[7,15,16]

Khojasteh et al[16] in a literature review of the use of stem cell in alveolar cleft defects treatment concluded that there is no sufficient evidence of the treatment efficacy with tissue engineering, they talked about the necessity of well-designed controlled studies to be compared.

Ten years ago, when the present study started, the only evidence was that clinical trials with autologous stem cells did not cause damage to the patients and there was no rejection. However, it was not known if the outcomes could be advantageous to the patients.

The protocol used for collection, freezing, and thawing the stem cells followed the one from Brazil Cryogenic Center and this protocol was unable to supply our necessities because the protocols were made to a single use of the stem cells and in the cleft lip and palate treatment it is necessary to perform more than 1 surgical time. The protocol to stem cell storage to patients with cleft lip and palate was modified and the freezing was done into many storage bottles.

The plasticity of frozen stem cells is preserved (Matsuo et al), and this investigation suggested the possibility of using artificial bone grafting with the use of mesenchymal stem cells.[17]

The multiple storage in many bottles allowed us to use the stem cells in all surgical procedures and became pattern after the first case. Other problem was found with the stem cells injection.

The first use was done by direct infusion and it was observed that this option was good for the lip, but inappropriate for the palate, as the liquid with stem cells leaked through the palatal sutures.

The lip and palate require different techniques to apply stem cells: in the lip the direct infusion was appropriate. In the palate, it was first used hyaluronic acid as a matrix without success because the hyaluronic acid overflows from the surgical location. Second, the stem cell was mixed with washed powder collagen and it demonstrated to be a more efficient method to maintain the stem cells in the palate and to produce a hemostatic effect.

In these cases, with the use of stem cell into the posterior palate, there was no bone formation, but when the second surgery to close the hard palate was performed, it was observed by the surgeon that there was less amount of fibrotic tissue in all cases, particularly better in the last cases, which used a higher amount of stem cells and had a well-established protocol to use it in the palatal surgery.

TABLE 4. Patients Description of Age, Gender, Procedure, Stem Cell Number, and Time of Follow-Up.

Case Number	Gender	Age in First Procedure	Stem Cell Injection Place	Stem Cell Quantity ($\times 10^4$)	Age in Second Procedure	Stem Cell Injection Place	Stem Cell Quantity ($\times 10^4$)	Time of Follow-Up
1	Female	5 mo	Lip	3.37	—			5 y
2	Female	4 mo	Lip	1.36	—			4 y
3	Male	5 mo	Lip + Palate	5.60	11 mo	Under mucoperiosteal flap	5.60	8 y
4	Female	5 mo	Lip	10.60	—			2 y
5	Male	6 mo	Lip	6.02	—			2 y
6	Male	5 mo	Lip	9.35	—			1 year
7	Female	6 mo	Lip + Palate	19.80	14 mo	Under mucoperiosteal flap	19.80	3 y
8	Male	5 mo	Lip + Palate	45.00	12 mo	Under mucoperiosteal flap	45.00	10 y
9	Female	5 mo	Lip + Palate	23.11	—			1 year

TABLE 5. Number of Mononuclear Cells and Number of Stem Cells Injected in the Patient Surgical Procedure in 1 mL.

Patient	Number of Mononuclear Cells ($\times 10^6$)	Number of Stem Cells ($\times 10^4$)
1	13.6	3.37
2	5.5	1.36
3	11.2	5.60
4	11.4	10.60
5	12.1	6.02
6	18.7	9.35
7	18.1	19.80
8	37.2	45.00
9	48.5	23.11

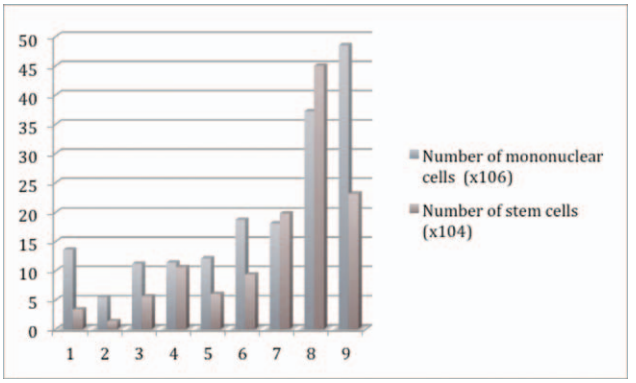

FIGURE 4. Demonstration of the mononuclear cells and stem cells number variance in all 9 patients of Stem Cell Group. It did not present statistical difference between mononuclear cell and stem cell.

The mean total nucleated hematopoietic cell number used was 19.92×10^6. It was used CD34 to express the stem cells. It is a glycosylated transmembrane protein, considered a marker for blood progenitor cell.

The mean number of stem cell used was 9.76×10^4. The ideal number of stem cells was not established yet and more researches will be necessary to do it. Actually, it is possible to amplify the number of the stem cells, technique that was impossible to be done in the present study but can be an option into future researches.

After the challenges of clinical injection of stem cells, it was needed to find clinical parameters to evaluate the results as described in the Method as it had not a histological parameter to demonstrate the tissue response to stem cell. The fibrous area between the soft and hard palate, which is usually resected in the anterior palatoplasty, could be useful to be sent for histological analyses and provide a more objective evidence of the inflammatory process in palatal tissue.

TABLE 6. Result Analysis of the Patients Submitted to Surgical Procedure Associated With Stem Cell Injection (Group 1).

Group 1 Stem Cell	Lip: Soft Tissue Inflammatory Process	Lip: Transitory Hypertrophy Scar	Palate: Dehiscence	Palate: Fistula	Fibrosis Between Hard and Soft Palate (Second Palatal Surgery)
Patient 1	–	–	–	–	
Patient 2	+	+	–	–	
Patient 3	–	+	–	–	–
Patient 4	–	–	–	-	
Patient 5	–	+	–	–	
Patient 6	+	–	–	–	
Patient 7	–	–	–	–	–
Patient 8	–	–	–	–	+
Patient 9	–	–	–	–	

The fibrosis between hard and soft palate was evaluated only in 3 cases submitted to the second surgical procedure.

TABLE 7. Result Analysis of the Patients Submitted to Conventional Surgical Procedure (Group 2).

Group 2 Control	Lip: Soft Tissue Inflammatory Process	Lip: Transitory Hypertrophy Scar	Palate: Dehiscence	Palate: Fistula	Fibrosis Between Hard and Soft Palate (Second Palatal Surgery)
Patient 1	+	++	–	–	
Patient 2	+++	++++	–	–	++
Patient 3	++	++	+	–	
Patient 4	+++	+++	–	–	
Patient 5	+	+++	–	–	
Patient 6	+++	++++	–	–	
Patient 7	+++	++	+	–	
Patient 8	++++	+++	–	–	++
Patient 9	+++	+++	–	–	+++

The fibrosis between hard and soft palate was evaluated only in 3 cases submitted to the second surgical procedure.

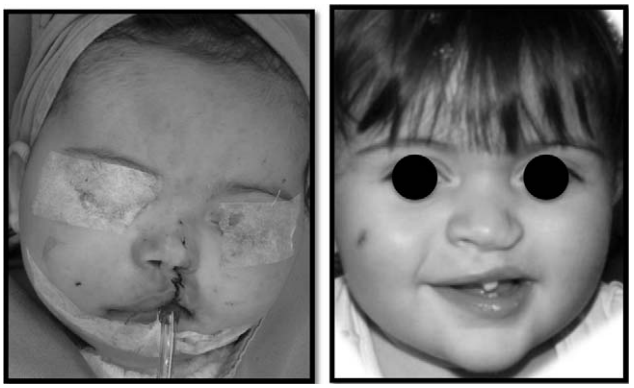

FIGURE 5. Case 1: Immediate postoperative of cheiloplasty and after 2 years and 7 months of follow-up. Classified as soft tissue inflammatory process: −, transitory hypertrophy scar: −.

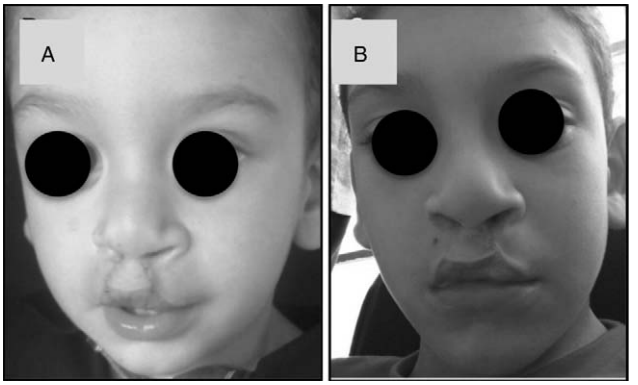

FIGURE 7. (A) Patient with bilateral lip and palate cleft at 1 year of postoperative. (B) Patient at 8 years of follow-up.

There were experimental studies with labeled stem cell with superparamagnetic iron oxide nanoparticles and visualization by magnetic resonance imaging (Jasmin et al).[18] This technique could be able to demonstrate the stem cells into the surgical area, but was not possible to find commercial superparamagnetic iron oxide nanoparticles to be used in this study.

The result analysis was subjective with clinical examination and photos. Tomography was thought to be an appropriate examination to analyze the stem cell activity into bone cleft; however, it did not demonstrate any evidence of osseous development. For this reason this examination was performed only in 1 patient to avoid possible radiation consequences.

The subjective analysis demonstrated that the group of patients with stem cell injection had less inflammatory response at lip soft tissue, less scar hypertrophy, there was no palate fistula or dehiscence and less fibrosis between hard and soft palate at the second palatal surgery. However, there was no evidence of bone neoformation.

The statistical analysis demonstrated significant improvement of the inflammatory process of the lip in Stem Cell Group, different from the control group.

The width of the palate cleft was not different in the 2 groups. The use of palatal plates very early and a constant maintenance of these devices help an adequate palatal development and the use of stem cells could be a favorable factor to rush the process.

To develop bone tissue at the palate gap, it was noticed that only stem cells were not enough. It possibly may happen if the stem cells were associated with other growth factors or regenerative procedures. The use of Bone Morphogenetic Protein-2 and platelet-rich plasma could induce osseous neoformation and reduce the palate cleft.[2,12] Another possibility of improving the results and performing bone formation could be the use of a higher number of stem cells. These possibilities open new pathways to future researches.

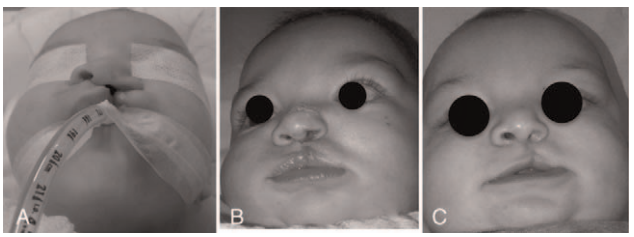

FIGURE 6. Case 3: (A) Preoperatory, (B) 1 week postoperative, (C) 1 month postoperative. Classified as soft tissue inflammatory process: −, transitory hypertrophy scar: +.

The inflammatory result, with less hypertrophy and dehiscence, was the most effective result comparing the 2 groups. The analysis demonstrated statistical difference with better tissue response into the group with stem cell.

This study was designed due to the desire of the authors to achieve better results in the cleft lip and palate treatment. The potential regenerative power of the stem cell stimulated and encouraged to find new methods to be added to the classical surgical techniques and make possible to reach better results with a small number and size of surgeries for cleft patient.

The development of protocols to collect, freeze, thaw, amplify, apply, and observe the outcomes will permit us to open a clinical research line with stem cells and cleft lip and palate surgery. It aims to improve results in soft and hard tissues, with less surgery in a near future, with other sources of stem cells that allow an increase of mesenchymal cells quantity.

At the beginning of the study, 10 years ago, it was not allowed to perform stem cells amplification by the Regulatory Health Institutions. Actually, it is possible to amplify the cells, which lead to a significant increasing number of the stem cells and consequently it should improve the clinical results of these cells use in cleft lip and palate patients.

Other experimental studies demonstrated the improvement of the stem cells in soft tissue including the muscle, this study over used it into subcutaneous and skin tissue achieving better muscular function, lip projection, and better scar appearance.

With these initial clinical observations and a defined clinical protocol, it will be able to proceed with researches looking for improvement at cleft lip and palate patient's results.

CONCLUSION

Our conclusion of this preliminary report is that stem cells in cleft lip and palate surgery decrease the inflammatory process and develop better scars than the regular series. The stem cells used in association with the regular surgical treatment do not cause any damage to the patient.

Besides the clinical conclusion, we developed a clinical protocol to safety use of stem cells, in a high amount of stem cells, with future possibilities to get better results in future clinical series.

REFERENCES

1. Vaccari-Mazzetti MP, Oliveira IS, Miranda-Ferreira R, et al. Qualitative and quantitative analysis of rabbit's fat mesenchymal stem cells. *Acta Cir Bras* 2010;25:24–27
2. Ladeira PRS, Alonso N. Protocols in cleft lip and palate treatment: systematic review. *Plast Surg Int* 2012;2012:562892

3. Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: characteristics and clinical applications. *Folia Histochem Cytobiol* 2006;44:215–230
4. Bueno DF1, Kerkis I, Costa AM, et al. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in cleft lip and/or palate patients. *Tissue Eng Part A* 2009;15:427–435
5. de Mendonça Costa A, Bueno DF, Martins MT, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. *J Craniofac Surg* 2008;19:204–210
6. Valente AMS, Espinosa MM, Silva AN, et al. Characteristics of patient who underwent primary correction of the cleft-lip and palate. *Rev HCPA* 2013;33:32–39
7. Yagi H, Soto-Gutierrez A, Parekkadan B, et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cell Transplant* 2010;19:667–679
8. Raposo-Amaral CR, Kobayashi GS, Almeida AB, et al. Alveolar osseous defect in rat for cell therapy: preliminary report. *Acta Cir Bras* 2010;25:313–317
9. Tanikawa DY, Aguena M, Bueno DF, et al. Fat grafts supplemented with adipose-derived stromal cells in the rehabilitation of patients with craniofacial microsomia. *Plast Reconstr Surg* 2013;132:141–152
10. Vaccari-Mazzetti MP, Roxo CEMB, Brock RS, et al. Unilateral and bilateral cleft lip associated to posterior palatoplasty [in Portuguese]. *Arquivos Catarinenses Medicina* 2009;38:46–48
11. Raposo-Amaral CR, Denadai R, Alonso N. Three dimensional changes of maxilla after secondary alveolar cleft repair: differences between rhBMP-2 and autologous iliac crest bone grafting. *Plast Reconstr Surg Glob Open* 2015;3:e451
12. Brock RS, Viterbo F, Deffune E, et al. Osseous flap of galea and periosteum filled with mesenchymal stem cells, platelet-rich plasma, bone dust, and hyaluronic acid. *J Craniof Surg* 2017;28:1654–1660
13. Ramazanzadeh B, Jahanbin A, Eslami N, et al. Are mesenchymal stem cells implanted in lip defect capable of returning orbicularis oris muscle function? *J Craniofac Surg* 2015;26:1539–1543
14. Spiekman M, van Dongen JA, Willemsen JC, et al. The power of fat and its adipose-derived stromal cells: emerging concepts for fibrotic scar treatment. *J Tissue Eng Regen Med* 2017;11:3220–3235
15. Gladysz D, Hozyasz KK. Stem cell terapyin alveolar cleft reconstruction. *Arch Oral Biol* 2015;60:1517–1532
16. Khojasteh A, Kheiri L, Motamedian SR, et al. Regenerative medicine in the treatment of alveolar cleft defect: a systematic review of the literature. *J Craniomaxillofac Surg* 2015;42:1608–1613
17. Matsuo A, Yamazaki Y, Takase C, et al. Osteogenic potential of cryopreserved human bone marrow-derived mesenchymal stem cells cultured with autologous serum. *J Craniofac Surg* 2008;19:693–700
18. Jasmin ALMT, Nunes HMP, Passipieri JA, et al. Optimized labeling of bone marrow mesenchymal stem cells with superparamagnetic iron oxide nanoparticles and in vivo visualization by magnetic resonance imaging. *J Nanobiotechnol* 2011;9:1–13