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Case Report

Minimally Invasive "IPG-Det Technique" with Autologous CGF and Human Umbilical Cord Blood Derived Mesenchymal Stem Cells towards Posterior Atrophic Maxilla Reconstruction - Case Report

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Abstract

Rehabilitation of maxilla in Implant Dentistry, especially in posterior edentulism is primarily treated with large and painful surgical approaches due to residual ridge atrophy and maxillary sinus pneumatization. Sinus Floor Elevation (SFE), especially in its "lateral version" is considered as the "gold-standard" for over four decades, despite the well documented post-operative morbidity and complications. As an alternative, the novel protocol named "IPG-DET Technique" minimally invasive and equally safe-efficient, promotes sinus membrane intentional perforation secured by healing and augmentative potential of autologous concentrated growth factors and CD34-Stem Cells Matrix.

This study further investigates the efficiency and healing process of "IPG-DET Technique" amplified by Mesenchymal Stem Cells clinical use, derived from human Umbilical Cord Blood for posterior atrophic maxilla reconstruction. Preliminary results have shown safe and inductive bone regeneration within sinus cavity. All implants loaded, 4 months after implant placement, showed high primary stability until final fixed prosthetic rehabilitation.

Keywords: *Human Umbilical Cord Blood Stem Cells; Bone Augmentation; IPG-DET Technique; Sinus Membrane Intentional Perforation; CD34 -Stem Cells*

Abbreviations

SFE: Sinus Floor Elevation; CBEs: Cord-blood-derived embryonic-like stem cells; CGF: Concentrated Growth Factors; CD34+: CD34- stem cells; UC-MSCs: Umbilical Cord Mesenchymal Stem Cells; WJ-MSCs: Wharton's Jelly Mesenchymal Stem Cells; LPCGF: Liquid Phase of the Concentrated Growth Factors; CBCT: Cone Beam Computed Tomography

Introduction

Sinus Floor Elevation (SFE) procedure is considered a well-established treatment protocol for posterior maxilla implant rehabilitation especially in difficult cases of severe bone resorption. However, high incidence of maxillary sinusitis between 8 and 20% of treated patients [1] along with many other intra- and post-operative complications (i.e., Schneiderian membrane perforation in 23.5% - 41% of cases), [2], evidencing invasive SFE as medium or high morbidity (mostly in lateral window than transcrestal approach) procedure for patients.

In recent years, alternative non-invasive protocols have been proposed, due to biotechnology development and introduction of autologous Blood Growth Factors in Regenerative Dentistry, such as PRF, CGF [3], etc. "IPG-DET Technique" [4-6] introduces implant placement within sinus, exploiting intentional Schneiderian membrane perforation combined with autologous concentrated growth factors (CGF with stem cells CD34+) with/or not allograft, in order to augment the height of bone structure in a very safe, simple and efficient manner. "IPG-DET Technique" has been established as a novel minimally invasive approach without the usual drawbacks and complications [7] and can be considered as reliable alternative to the widespread SFE procedure.

Recently, development of Biogenetics or Genetic Engineering has given medical community the prospect of novel regenerative Bio-materials employment such as human Umbilical Cord Blood (UCB) derived Mesenchymal Stem Cells (MSCs). Cord-blood-derived embryonic-like stem cells (CBEs) [8] are perinatal MSCs also exist in placenta umbilical cord, Wharton's jelly (WJ-MSCs) and amniotic membrane. CBEs are considered as the wider range stem cells source globally and the most promising therapeutic choice towards tissue regeneration. They possess superior attributes (enhanced proliferative capacity, no teratomas formation) to already employed adult MSCs (i.e., BM-MSCs, AD-MSCs, etc.) [9].

Throughout this study, the efficiency of "IPG-DET Technique" augmented with Mesenchymal Stem Cells (MSCs) derived from human Umbilical Cord Blood (UCB) towards posterior atrophic maxilla rehabilitation is investigated. The enhanced therapeutic properties of MSCs can lead to Regenerative Dentistry evolution the next decade.

Case Report and Materials and Methods

Patient information

The diagnostic and surgical procedure employed within this study comprise at first, visual diagnosis by means of Orthopantomogram (OPG) and Cone Beam Computed Tomography (CBCT) Images followed by patient's medical history acquisition. Then, consulting between our team and patients is held to decide the optimum solution. All four (4) patients (3 men and 1 woman) with a mean age of 51.4 years and ages ranging from 38 to 68 years, participated within the study suffered with severe atrophy in posterior regions. All patients have chosen "IPG-DET" combined with CGF protocol [3] and Mesenchymal Stem Cells (UC-MSCs) as surgical implant solution. A total of six (6) implants TC-R 4,2 x 10 mm (MultySystem, Lissone (MB) Italy) were placed. The Helsinki Declaration ethical guidelines were followed, and a written informed consent was obtained from each patient participating in the study.

"IPG-DET" Technique and CGF Protocol

Initially, patient's drawn blood is centrifugated with Medifuge MF200 (Silfradent, Italy) in 9 ml sterile tubes. After centrifugation, from the 3 blood fractions produced, the middle one is selected, containing fibrin-rich gel with platelets, concentrated growth factors (CGF) and stem cells CD34+.

Osteotomy is performed with the following drills: a) Pilot drill 2,3 mm, b) First drill 2,55, c) Trimming drill 2,85 [optional followed by (MultySystem, Lissone (MB) Italy) bone compactor - expanders (MultySystem, Lissone (MB) Italy] until intentionally perforation of

Schneiderian membrane. Drilling procedure must be performed during copious irrigation with saline solution at room temperature in order to avoid overheating, that could eventually cause tissue damage, and to maintain perfect visibility on the operating field. To this purpose, the control unit of surgical micro-motor rotation must be able to reach 300-400 rpm to avoid necrosis of the bone, which would deteriorate osseointegration. In addition, torque must be 50 Ncm for drilling and 35 - 40 Ncm for fixture screwing (Figure 1).

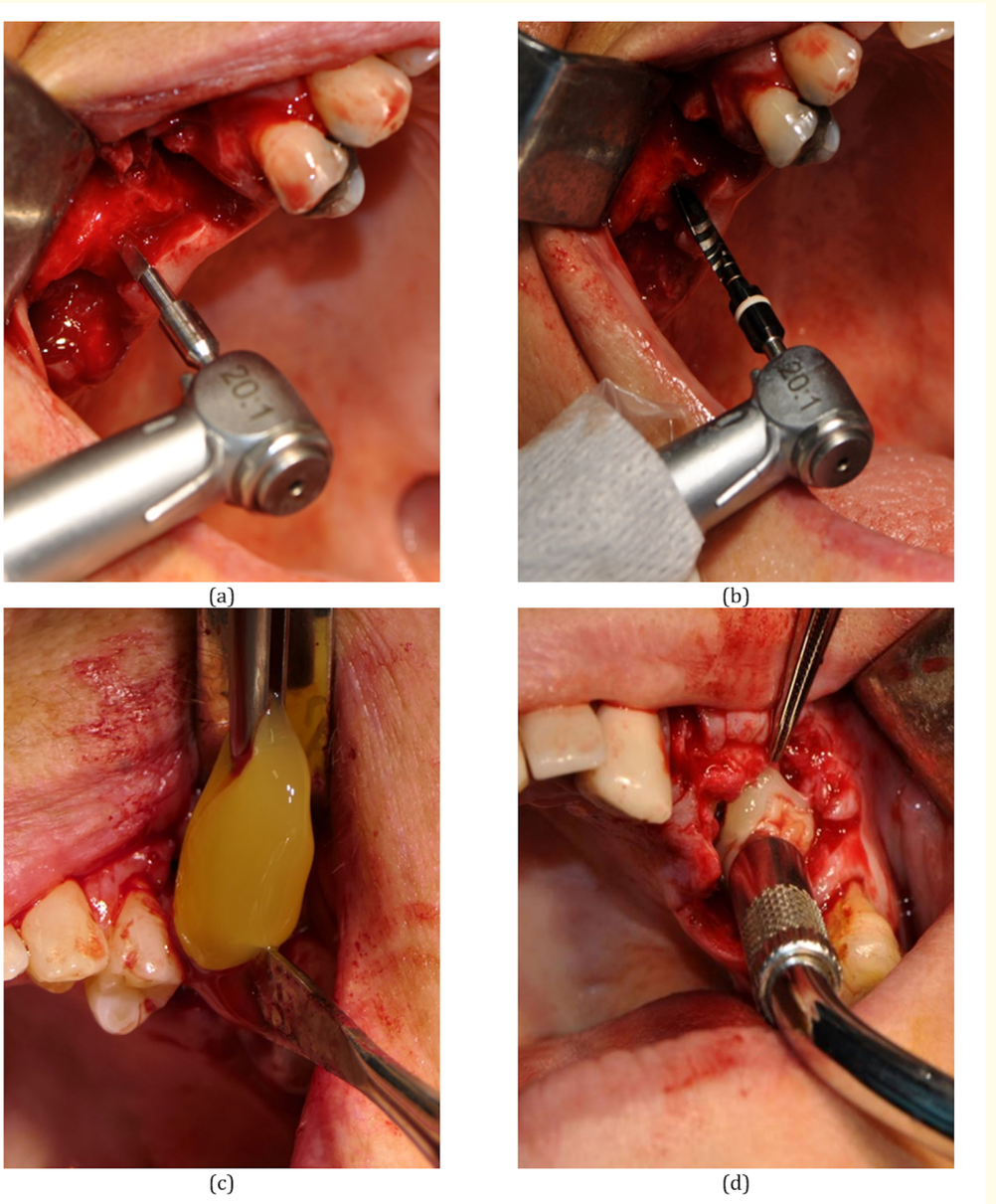


Figure 1: *Drilling osteotomy with (a) Pilot drill 2,3 mm, (b) First drill 2,55 and CGF fibrin-rich gel placement into the sinus cavity prior implant insertion(c) and CGF (d) forwarding into the sinus with a special osteotome.*

MSCs protocol

Mesenchymal Stem Cells (Invitra-DX Dental CBSC-cord blood stem cells Suspension™, Invitrx Therapeutics, Inc, USA) are maintained in original packaging (1 cc) at -˚80^{0}C temperature so they can be delivered and used at the operating room 2 hours before. Preparation instructions are as follows:

- Product must be removed from cold maintenance by opening the carton.
- The frozen vial is then removed from the packaging and must NOT BE PLACED on the sterile field.
- Once defrosted (within assistant's hands with sterile gloves), a sterile syringe must be employed to aseptically remove all the vial contents for insertion into the surgical field.
- Invitra-DX Dental CBSC Suspension™ is now ready to be employed (Figure 2).

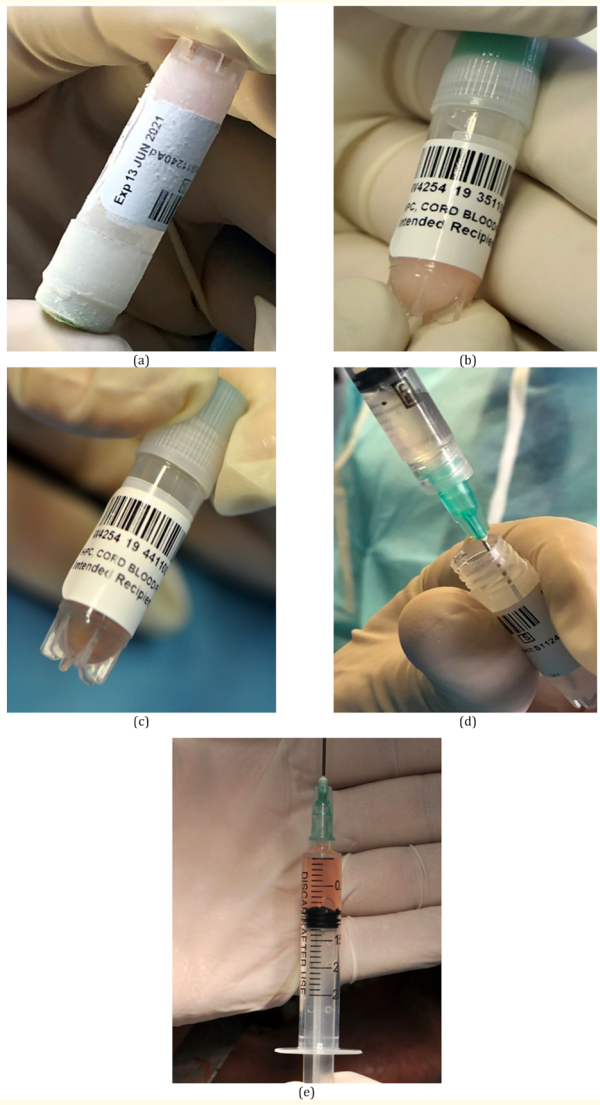


Figure 2: *(a) Invitra-DX Dental CBSC SuspensionTM removed from its original packaging (1 cc) at -80˚C, (b) thawed - pink color content (c) liquid form at room temperature, (d) Vial removal by a 3 ml sterile syringe and (e) Invitra-DX Dental CBSC SuspensionTM - injectable form, ready to use.*

Continuing "IPG-DET Technique" and CGF and MSCs Protocols, one or two CGF matrices are inserted through the osteotomy site and membrane perforation into the sinus, employing a fibrin injector (Silfradent, Italy), before each implant placement (TC-R Biphasic implants Ø 3,7 - Ø 4,2 x10 mm, MultySystem, Italy).

Each fixture is then immersed at first, into the LPCGF (liquid phase concentrated growth factors) in order to achieve creation of a "bio-energetic membrane" around the fixture and afterwards into the Invitra-DX Dental CBSC Suspension™, before implantation.

Each implant is inserted within osteotomy (1 mm subcrestally) by Extralong Screwdriver Handle (MultySystem, Italy), in a controlled manner. Then the implant sealing screw placement is performed followed by Invitra-DX Dental CBSC Suspension™ residue injection around surgical field and implants and finally the CGF membrane onto the implant placement and suturing. A minimum healing period of four (4) months prior to assess osseointegration and implants loading is considered as prerequisite for a successful procedure (Figure 3).

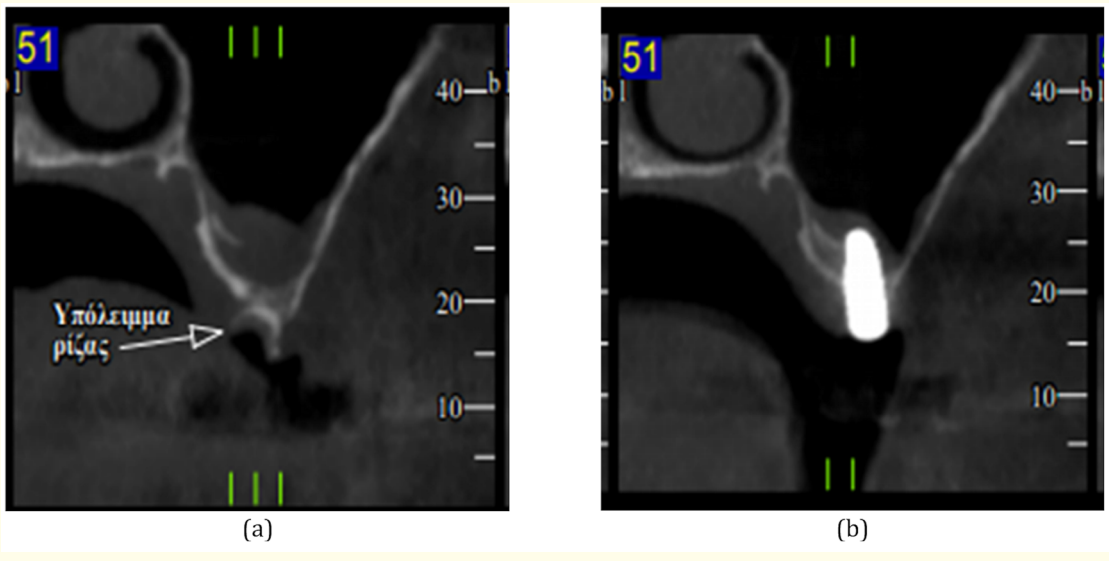
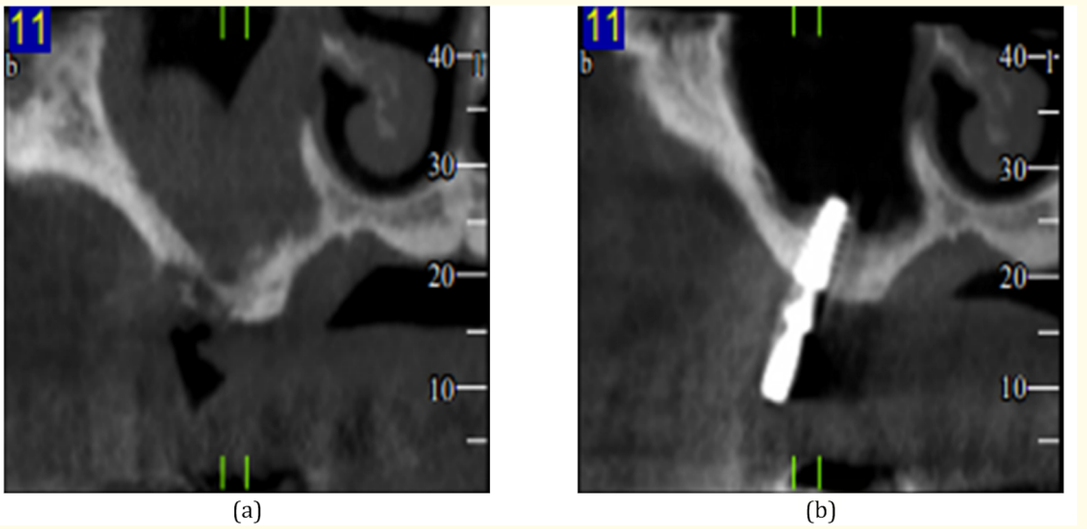


Figure 3: *(a) TC-R Implant immersed into Growth Factors to form a "bio-energetic membrane" around it, (b) The Implant ready to enter into the osteotomy, (c) Wider sealing screw placed for better initial stability, notice regular screw size posteriorly in another TC-R Implant (MultySystem), (d) Autologous biological CGF membrane prior it's placement onto the Implant and (e) Injectable Invitra-DX Dental CBSC SuspensionTM infusion around surgical field and Implant (placed using IPG-DET Technique).*

Results

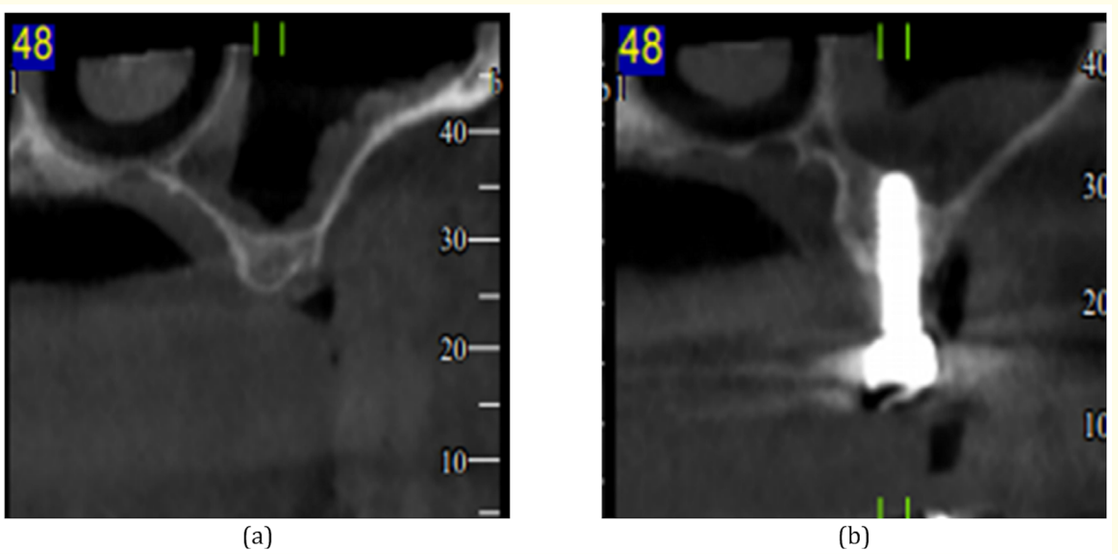
None of the patient's participated within this study presented any complications (intra- and post-operative) into their sinuses such as excessive pain, infection, abscesses, fistula formation, dehiscence, graft exposure, dislocation of graft, bleeding and facial haematoma, implant exposure or displacement, oroantral communication, vertigo etc. [2]. All implants loaded, 4 months after implant placement, showed high primary stability until final fixed prosthetic rehabilitation.

CBCT 3d was employed for the proposed protocol evaluation, immediately after procedure and 4 - 8 months post-op following. All images (Figure 4-6) have showed full osseointegration with no sinus infection or any other pathology.



(a) (b)

Figure 4: *CBCT scan before (a) and after rehabilitation (b). Full bone regeneration in height and around Implant.*



(a) (b)

Figure 5: *(a) CBCT Scan pre-operation with minimum residual bone height (1 mm) and excessive inflammatory thickness of the Schneiderian membrane due to root remnant, (b) Same position CBCT scan 4 months post-operation implant placement. Noticeable bone regeneration (8-9 mm) without any more sinus membrane pathology.*

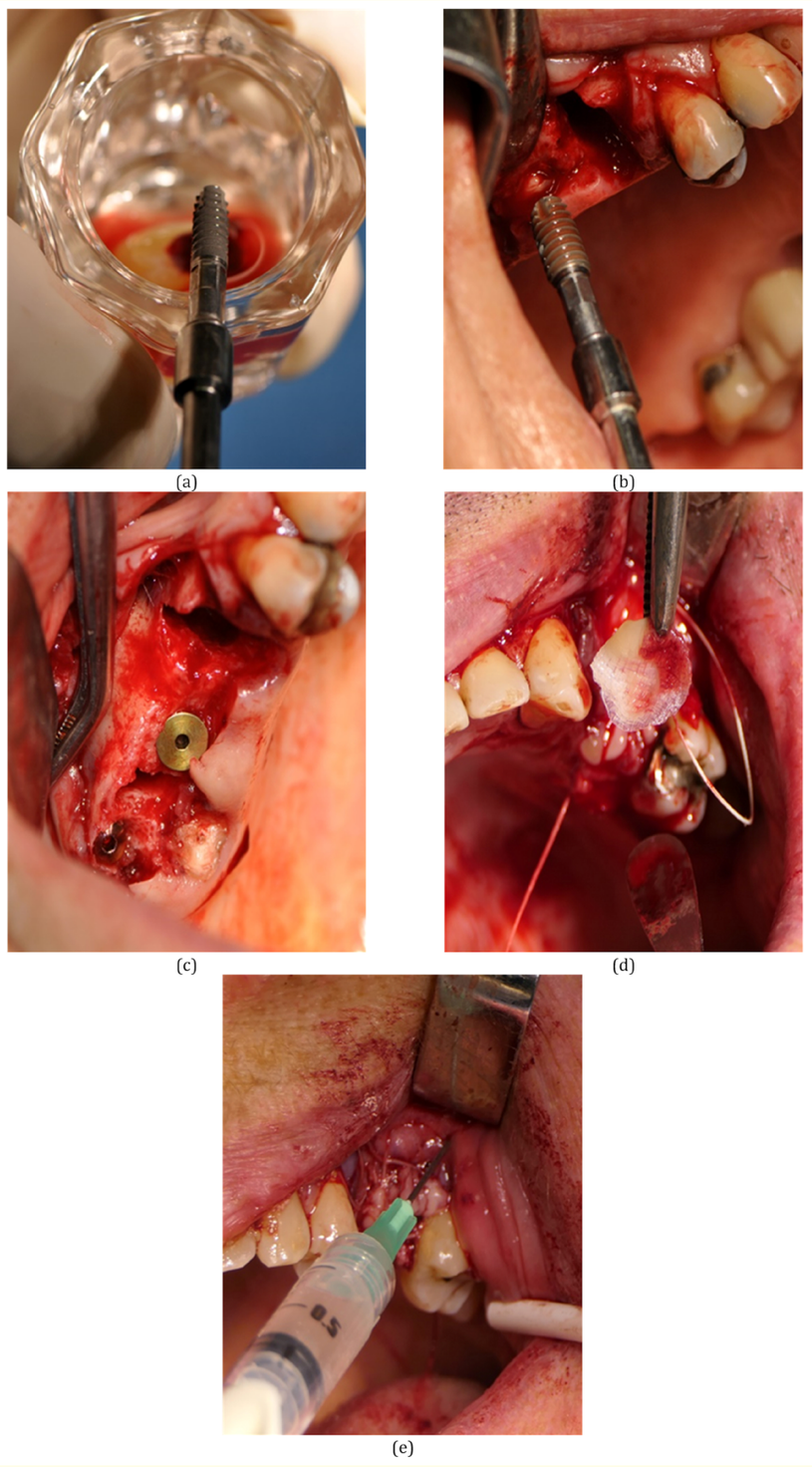


Figure 6: *(a) CBCT scan pre-operation with root remnant and Maxillary Sinus Retention Cyst (MSRC) in the area, (b) Same position CBCT scan 4 months post-op implant placement. Apparent implant osseointegration with bone volume augmentation and the absence of any sinus membrane pathology.*

Discussion

Sinus membrane perforation is considered as contradiction in all previously presented SFE methods. Throughout this study and by means of "IPG-DET Technique" combined with Autologous CGF-CD34+ matrix and clinical use of human Umbilical Cord Blood derived MSCs, sinus perforation has been established as significant breakthrough posterior atrophic maxilla rehabilitation. Any post-operation complication is reduced due to healing and anchorage mechanism of autologous CGF and hematopoietic stem cells CD34+ matrix (con-

sisting of enabled platelets with pseudo-legs), enhanced by the regenerative properties of Umbilical Cord Mesenchymal Stem Cells (UC-MSCs).

Since 1998 by the revolutionary and historical scientific published report of a UW-Madison developmental biologist and lead author James A. Thomson and his team, a new chapter opens for Regenerative Medicine as paradigm shift "in human developmental biology, drug discovery, and transplantation medicine" [10]. It is based on human blastocyst-derived, pluripotent cell lines which have still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers [10]. Stem cells are successfully employed against very serious diseases and syndromes [9].

Invitrx Therapeutics has established a quality assurance program by defining standards and outlining the steps necessary to ensure the safety, purity, potency and identity of manufacturing products with haematopoietic potential. Invitra CBSC™ (Cord-Blood-derived Stem Cells) is a minimally engineered cellular allograft suspension derived from human Umbilical Cord Blood (UCB). They utilize a unique procedure towards tissue characteristics and properties preservation. Stem cells were identified in cord blood over 40 years ago and employed regularly for hematopoietic stem cell transplantation. Cord blood comprise a mixed population of cells such as hematopoietic stem cells (HSCs) and multi-purpose, non-hematopoietic stem cells (NHSCs). These cells can self-renew, release growth factors, and further transformed into more specialized cells. They can also be associated with tissue homeostasis, anti-inflammatory responses and antioxidant effects.

Preliminary results in our study, have shown safe and inductive bone regeneration (without any bone grafting material), not only in quantity (in 3-dimension), but also in quality in the sinus cavity, critical prerequisite for implant placement and also osseointegration conditions improvement (Figure 4 and 5). Moreover, simple and ease grafting in sinus by creating a CGF bioactive membrane and blocking the oroantral communication has been demonstrated in all clinical cases. Even in pre-operation sinus-inflammatory situations such as: Maxillary Sinus Retention Cyst (MSRC), sinus polyps and/or localized swelling of the sinus mucosa because of root remnants etc. (Figure 5 and 6), mesenchymal stems cells (MSCs) treatment seems to operate as minimally invasive alternative to old fashion guided bone and tissue regeneration techniques, while in addition, a pain-free postoperative period has been offered to all patients eliminating all drawbacks and rehabilitation period time of SFE.

Embryonic-like stem cells derived from human umbilical cord blood (CBEs) can lead to most of the cell types *in vitro* and potentially *in vivo* [8]. Such stem cells can be found in the wider range stem cells source globally, the human umbilical cord blood [8] and umbilical cord tissue. Contrary to other stem cells derived from adults that retain only part of their ability to differentiate and proliferate (plus the risk of teratoma formation), adult stem cells (ADS) can develop into a limited number of different types of tissues. For this reason, cord-blood-derived embryonic-like stem cells (CBEs) and umbilical cord tissue have a comparative advantage [9]. Tissue Engineering and Regenerative Medicine and Dentistry is considered one of the most growing fields of Biotechnology of the future and can augment bone regeneration in dental implant procedures.

Conclusion

The reliable and well promising "IPG-DET Technique" enhanced by the regenerative properties of autologous CGF-CD34+ and clinical use of Cord Blood Stem Cells, has been proven safe and effective in the limited dataset as an alternative to SFE under regular, or not, conditions. Further studies are required to clarify and determine the positive clinical impact of the proposed innovative combination.

Conflict of Interest

None.

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