

IMMEDIATE IMPLANT PLACEMENT BY USING BONE-ALBUMIN ALLOGRAFT AND CONCENTRATED GROWTH FACTORS (CGFS): PRELIMINARY RESULTS OF A PILOT STUDY

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SUMMARY

The provision of structural base and soft tissue support in dental implantology still remains a complex task. In the present study a new technique was introduced, which employs Bone-Albumin allograft and Concentrated Growth Factors (CGFs) integrated in a 1-stage immediate implant placement. The allograft is transformed from cubic to cylindrical shape by means of bone cutting forceps, followed by central osteotomy for screwing the implant within the allograft. The implant/graft combination promotes graft union, increases dental stability and minimizes graft resorption. The successful outcome of the proposed technique is evaluated by means of clinical and radiographic data. In conclusion, the proposed method provides a successful immediate dental implantation in terms of osseo-integration, stability and aesthetic results.

Key words: bone-albumin allograft, concentrated growth factors, immediate implant placement.

Introduction

Implant primary stability is considered as the key factor towards successful dental implantation (1, 10). It can be simulated to implant anchoring inside the bone. The implant site must be surrounded by both hard and soft tissues that are compatible to each other, to succeed in the rapid osseointegration of the inserted implant. The amount and nature of these additional factors, especially in areas with large bone deficiency, play an important role towards more stable and aesthetic results for the patient (11, 18).

The aim of the present study was to evaluate a new surgical technique for immediate dental implantation employing Bone-Albumin allograft (OrthoSera Products, Austria) and Concentrated Growth Factors (CGF), by means of a precise and evaluated protocol.

Materials and methods

A 32-year-old man referred persistent pain at tooth area #22. Following a clinical and radiographic (panoramic scans) evaluation, a tooth



Figure 1
(a) Pre-operative clinical image area #22, (b) clinical image of fractured tooth after extraction.

fracture (Figure 1a) in this area was determined as the cause of the pain. After consultation with the dentist conducting this study, the patient chose the tooth extraction and surgical implant solution by means of immediate implant placement with simultaneous bone allograft (Cancellous block) and CGF employment. He was informed of the requirements for participation in this case study and written informed consent was obtained.

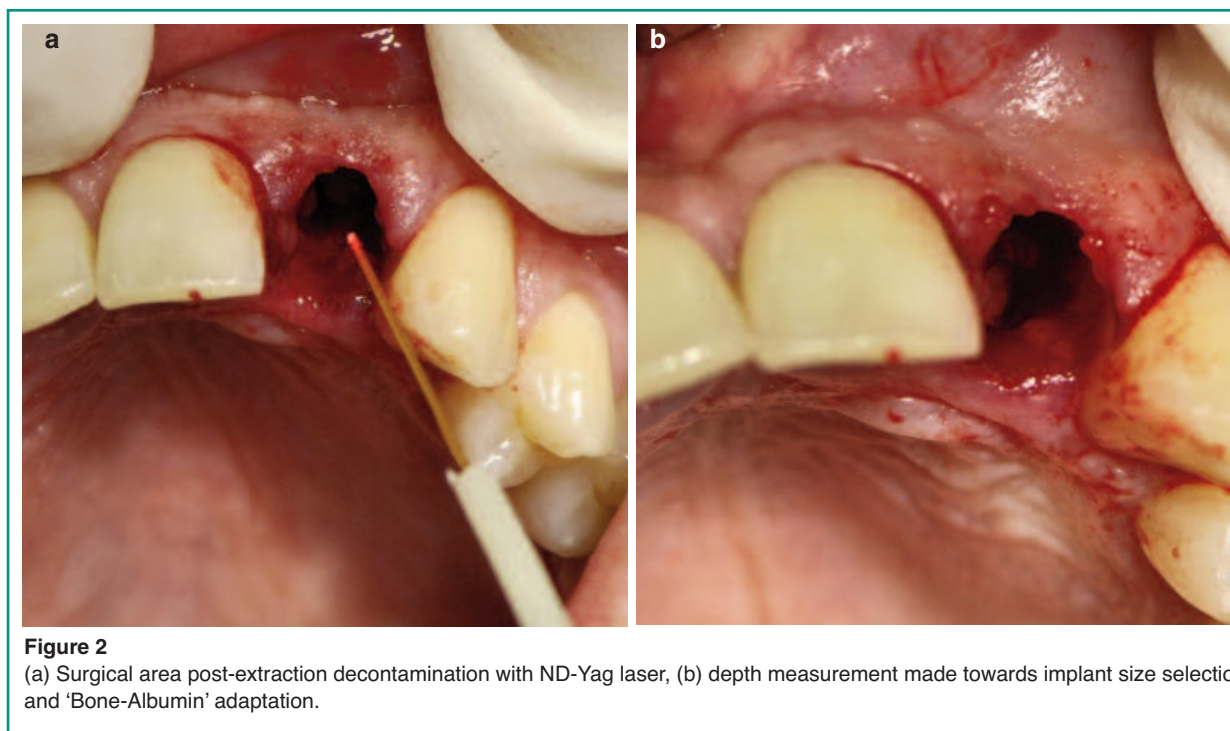
Providine-iodine solution (Betadine) has been employed extra-orally for microbial disinfection of the surgical site. The fractured tooth extraction (Figure 1b) and dental implantation was performed a-traumatically under local anesthesia (2% lidocaine solution, epinephrine 1:100 000). Preoperative and postoperative antibiotic was administrated orally (Augmentin) every 8 hours from surgery day until 7 days later. Additional decontamination within the post-extraction surgical area was employed using ND-Yag laser followed by betadine and normal saline wash (Figure 2a). Additional visual inspection at the surgical area and surrounding bone was followed to ensure successful atraumatic removal. Any soft tissue remnants in the extraction socket were carefully removed to ensure complete removal of all contaminated tissues. Depth meas-

urement was then employed for implant size selection and Bone-Albumin adaptation according to the corresponding extraction socket dimensions (Figure 2b).

CGF with Stem Cells Cd34+ Preparation

Initially, blood was pinched from the patient with eight sterile tubes of 9ml volume. Then immediate centrifugation was performed with Medifuge Benchtop centrifuge device (Silfradent srl, St. Sofia, Italy) for approximately 14 minutes. Blood fractionation derived from centrifugation from top to bottom (Figure 3a) comprises:

1. Upper component that contains Serum (blood plasma without fibrinogen and coagulation factors).
2. Interim components that consists of a large and dense polymerized fibrin buffy coat divided in two distinct intermediate sub-components: (a) the upper Platelet Poor Plasma (PPP), and (b) the lower fibrin rich gel with aggregated platelets and concentrated growth factors (CGF).



3. The lower component which is the red blood cell portion (RBC).

The majority of growth factors and stem cells CD34+ are mainly aggregated between CGF and the upper 3-4 mm of red blood cells (RBC) portion (Figure 3b). It has been shown that CD34 positive cells exist on both layers recording a higher number in the CGF layer due to the CGF network composition in which the cells are trapped (15-25) CGF and CD34+ are isolated from the red corpuscles employing scissors (Figure 3c) to derive the CGF-CD34+ matrix (Figure 3d).

Bone-Albumin (Cancellous Block) Preparation and modification

The Bone-Albumin allograft employed in this study is depicted in Figure 4a. Its dimensions were 10x10x20 mm. A series of implant drills

with increasing diameters was subsequently utilized to modify the graft's central osteotomies according to the final implant diameter to be placed in the center of the allograft. The drill diameters from lower to higher were: 2.1 mm, 3 mm, 3.5 mm and 4 mm. The Bone-Albumin graft dimensions were carefully transformed from cubic to cylindrical by means of bone cutting forceps, for an optimum insertion in the osteotomy site (Figure 4c). The osteotomies previously carried out corresponded to an possible placement of Duravit EV 4 mm L. 14 implant with aggressive treatments (B&B Dental Italy) (Figure 4d). The transformed 'Cancellous 2 Allograft' C2A and the implant were individually embedded and immersed into the Liquid Phase of the Concentrated Growth Factors (LPCGF) to form an autologous "bioactive" membrane on their surfaces and to enhance the osseointegration procedure. LPCGF was derived by squeezing the CGF-CD34+ matrix with the CGF-forceps (Silfradent, Italy) (Figure 4d) (10).

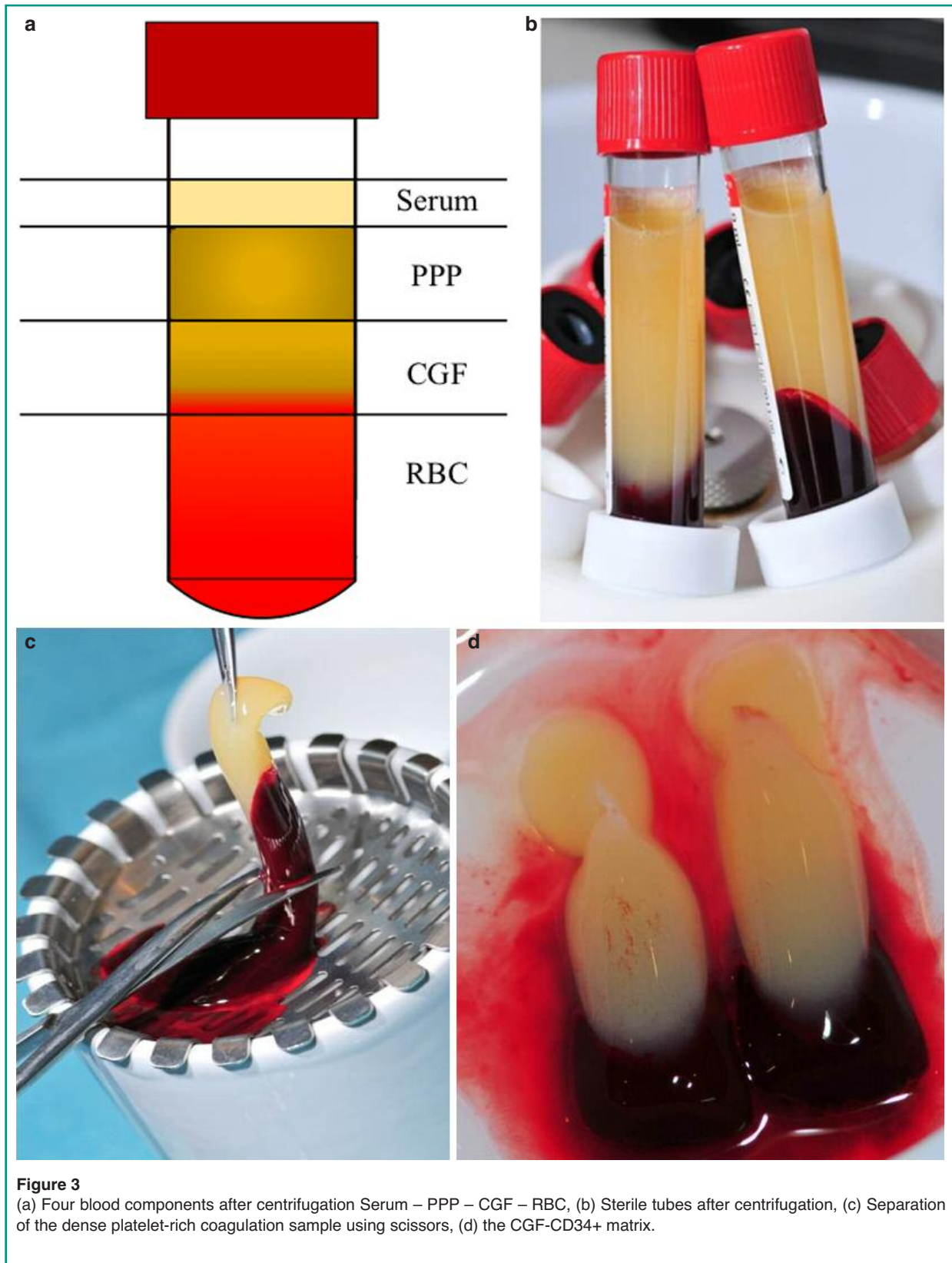
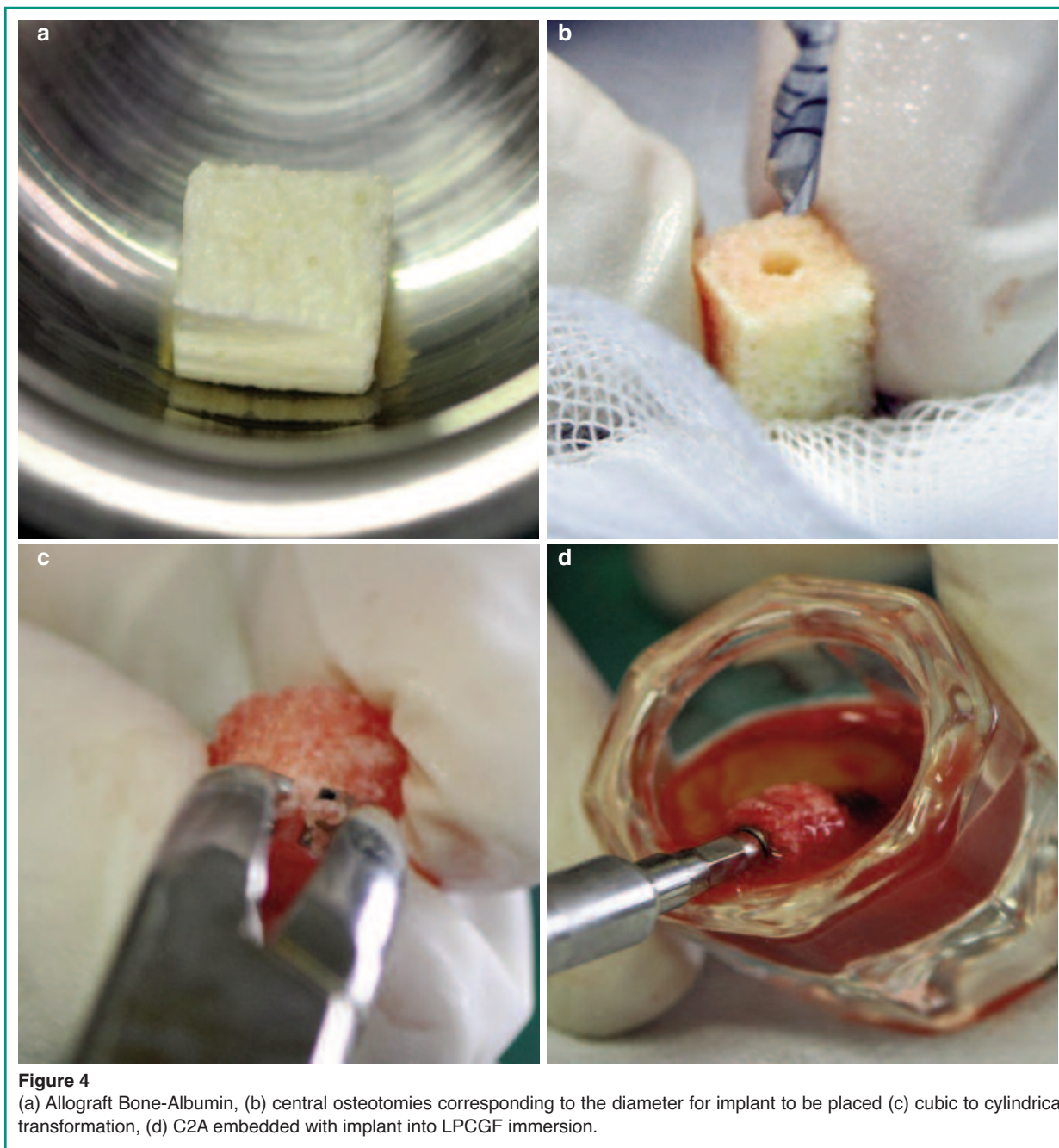


Figure 3

(a) Four blood components after centrifugation Serum – PPP – CGF – RBC, (b) Sterile tubes after centrifugation, (c) Separation of the dense platelet-rich coagulation sample using scissors, (d) the CGF-CD34+ matrix.



Implant surgery

The CGF matrix, created in the previous process of blood centrifugation, was placed within the osteotomy site by means of a fibrin injector (Silfradent-Italy), which proved to be a great tool

for the swift insertion of the fibrin gel block (Figure 5a, b). The CGF matrix release liquid growth factors within the site prior to implant/C2A placement. Additional CGF-CD34+ gel is applied to fill any observed gaps found between C2A and the implant (Figure 5c). The cylindrically transformed C2A together with

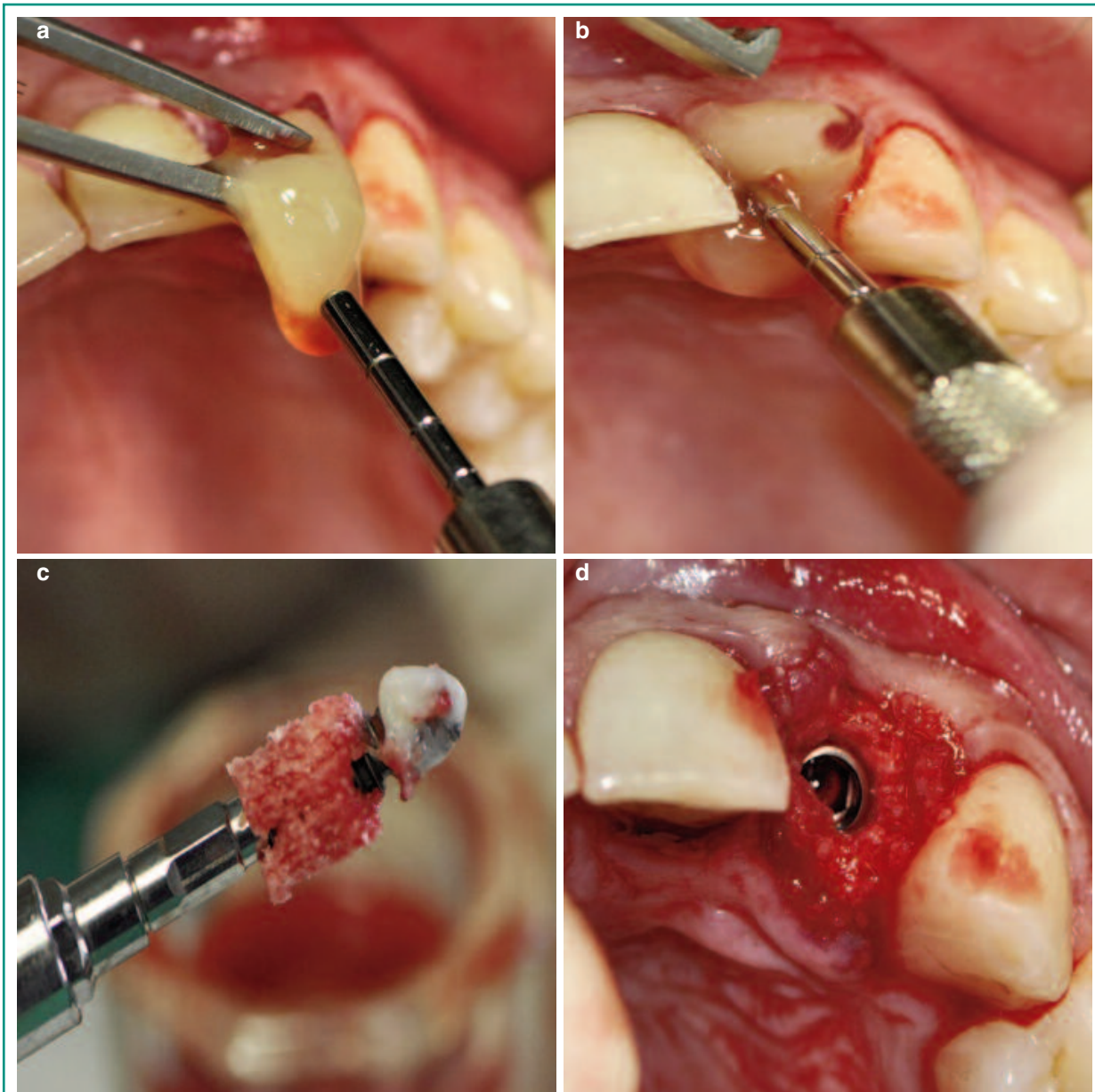


Figure 5
(a), (b) CGF matrix insertion within osteotomy site with fibrin injector, (c) implant/graft gaps coverage with CGF-CD34+ gel (d) Implant/C2A placement into extraction socket.

the implant were then fitted in the extraction socket. Minor contour adjustments were necessary to ensure adaptation and stability (Figure 5d).

A CGF biological membrane is then prepared

with the special Silfradent pincers (Figure 6a) and sutured in order to seal the operation site by means of 3/0 surgical sutures (Figure 6b). This procedure provides continued soft tissue support and minimizes postoperative edema.



Figure 6
(a) CGF Biological membrane preparation with the special Silfradent pincers, (b) CGF matrix insertion within osteotomy site CGF biological membrane, (c) surgical suture within the operation site to seal it.

Results

The implant is osseointegrated. At the follow up panoramic radiograph after six (6) months the improvement in the overall alveolar bone height and surrounding soft tissue in the operation site is apparent (Figure 7). No signs of infection were observed around the implant's bone interface.

The postoperative (6 months) panoramic radiograph (down) depicts the increased crestal bone height gained by the utilization of C2A and CGF around the implant. In the same radiograph the new generated bone surrounding the cervical part of the implant is also shown. The high albumin concentration, that comprises the Cancellous Block combined with the regenerative properties of CGF, provides the ability of stem cells to grow and therefore the growth of the new tis-

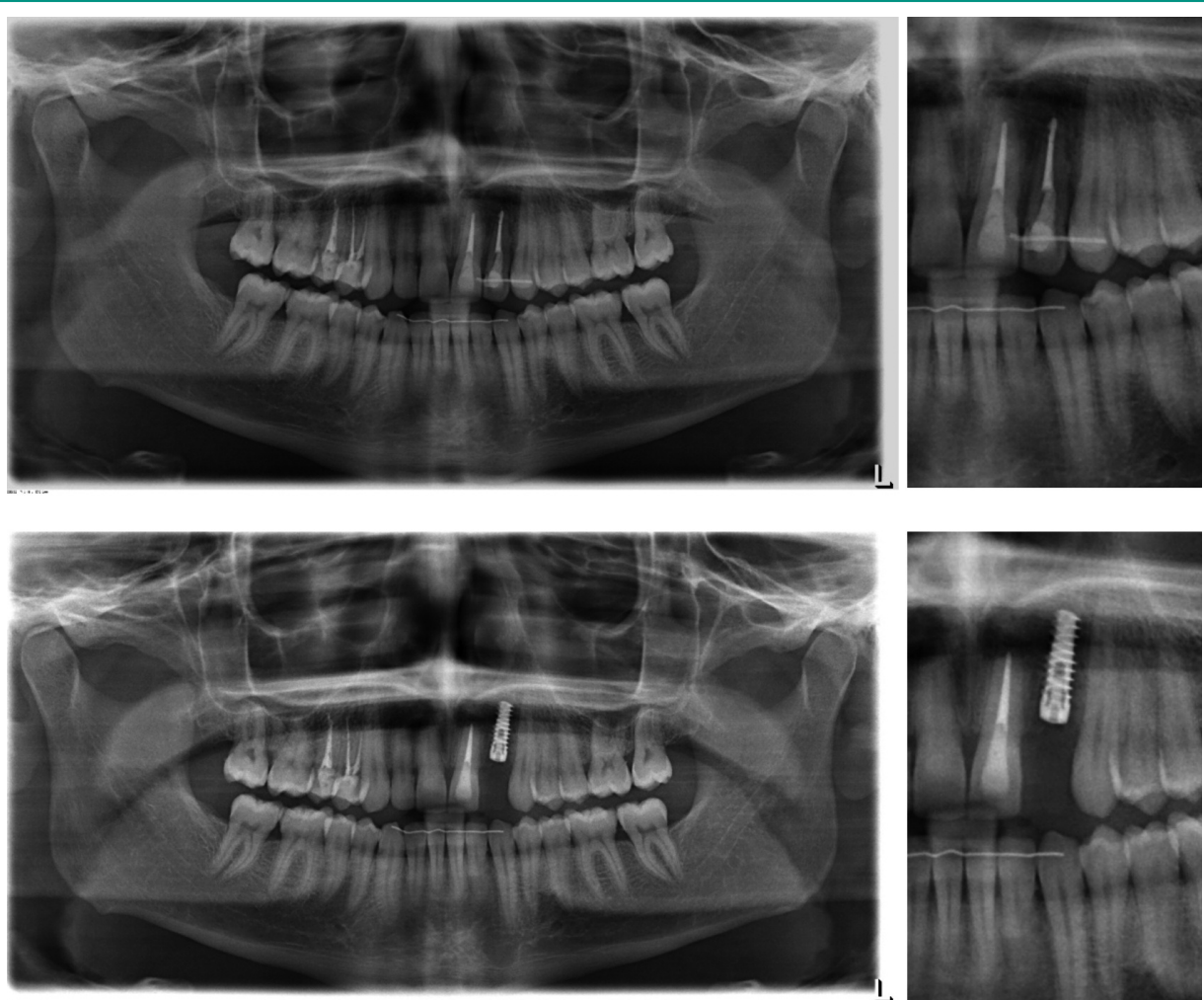


Figure 7
(a) Preoperative panoramic radiograph (up) showing fractured tooth at area #22, (b) postoperative (6 months) panoramic radiograph (down).

sue, as well. In addition, the special properties of Bone-Albumin allograft induce the restructuring procedure filling the whole cavity and slowly forming new bone tissue around the graft/implant.

Discussion

Patients having bilateral gaps on the implant coronal plane or patients with areas having a small crestal bone height can be addressed suc-

cessfully by an immediate implant placement described within this study (12, 15, 18-20). The proposed method based on C2A and CGF provides additional stability of the implant at the crestal region by screwing the implants to the transformed C2A and immersing both in LPCGF prior to the precise fitting within the extraction socket (10-19).

The simultaneous utilization CGF-CD34+ gel and CGF biological membrane enhances the soft tissue regeneration procedure providing a good esthetic result. The Bone-Albumin allograft can be formed into any desired dimension, regard-

less of the dimensions of the extraction socket, to optimize its biomechanical properties. The implant/C2A combination promotes graft union and minimizes graft resorption. In conclusion, the proposed method provides a successful immediate dental implantation in terms of osseointegration, stability and aesthetic results.

References

1. Aghaloo TL, Moy PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? *Int J Oral Maxillofac Implants.* 2007;22(suppl):49-70.
2. John V, De Poi R, Blanchard S. Socket preservation as a precursor of future implant placement: review of the literature and case reports. *Compend Contin Educ Dent.* 2007;28:646-653; quiz 54, 71.
3. Campelo LD, Camara JR. Flapless implant surgery: a 10-year clinical retrospective analysis. *The International Journal of Oral & Maxillofacial Implants.* 2002;17(2):271-276.
4. Georgakopoulos IP, et al. A case-Study of Seven Implants Placed in the Maxillary Sinus With Intentional Schneiderian's Membrane Perforation. *Journal of Implant and Advanced Clinical Dentistry.* 2014;6(1):9-19.
5. Marrelli M, Pujia A, Palmieri F, Gatto R, Falisi G, Gargari M, Caruso S, Apicella D, Rastelli C, Nardi GM, Paduano F, Tatullo M. Innovative approach for the in vitro research on biomedical scaffolds designed and customized with CAD-CAM technology. *Int J Immunopathol Pharmacol.* 2016 Dec;29(4):778-783. Epub 2016 Apr 22.
6. Marrelli M, Falisi G, Apicella A, Apicella D, Amantea M, Cielo A, Bonanome L, Palmieri F, Santacroce L, Giannini S, Di Fabrizio E, Rastelli C, Gargari M, Cuda G, Paduano F, Tatullo M. Behaviour of dental pulp stem cells on different types of innovative mesoporous and nanoporous silicon scaffolds with different functionalizations of the surfaces. *J Biol Regul Homeost Agents.* 2015 Oct-Dec;29(4):991-7.
7. Tatullo M, Marrelli M, Falisi G, Rastelli C, Palmieri F, Gargari M, Zavan B, Paduano F, Benagiano V. Mechanical influence of tissue culture plates and extracellular matrix on mesenchymal stem cell behavior: A topical review. *Int J Immunopathol Pharmacol.* 2016 Mar;29(1):3-8.
8. Cantore S, Ballini A, Mori G, Dibello V, Marrelli M, Mirgaldi R, De Vito D, Tatullo M. Anti-plaque and antimicrobial efficiency of different oral rinses in a 3-day plaque accumulation model. *J Biol Regul Homeost Agents.* 2016 Oct-Dec;30(4):1173-1178.
9. Ballini A, Mastrangelo F, Gastaldi G, Tettamanti L, Bukvic N, Cantore S, Cocco T, Saini R, Desiate A, Gherlone E, Scacco S. Osteogenic differentiation and gene expression of dental pulp stem cells under low-level laser irradiation: a good promise for tissue engineering. *J Biol Regul Homeost Agents.* 2015 Oct-Dec;29(4):813-22.
10. Ballini A, Cantore S, Farronato D, Cirulli N, Inchingolo F, Papa F, Malcangi G, Inchingolo AD, Dipalma G, Sardaro N, Lippolis R, Santacroce L, Coscia MF, Pettini F, De Vito D, Scacco S. Periodontal disease and bone pathogenesis: the crosstalk between cytokines and porphyromonas gingivalis. *J Biol Regul Homeost Agents.* 2015 Apr-Jun;29(2):273-81.
11. Grassi FR, Pappalettere C, Di Comite M, Corsalini M, Mori G, Ballini A, Crincoli V, Pettini F, Rapone B, Boccaccio A. Effect of different irrigating solutions and endodontic sealers on bond strength of the dentin-post interface with and without defects. *Int J Med Sci.* 2012;9(8):642-54. doi: 10.7150/ijms.4998. Epub 2012 Sep 24.
12. Scattarella A, Ballini A, Grassi FR, Carbonara A, Ciccolella F, Dituri A, Nardi GM, Cantore S, Pettini F. Treatment of oroantral fistula with autologous bone graft and application of a non-reabsorbable membrane. *Int J Med Sci.* 2010 Aug 11;7(5):267-71.
13. Ballini A, Tetè S, Scattarella A, Cantore S, Mastrangelo F, Papa F, Nardi GM, Perillo L, Crincoli V, Gherlone E, Grassi FR. The role of anti-cyclic citrullinated peptide antibody in periodontal disease. *Int J Immunopathol Pharmacol.* 2010 Apr-Jun;23(2):677-81.
14. Tatullo M, Falisi G, Amantea M, et al. Dental Pulp Stem Cells and Human Periapical Cyst Mesenchymal Stem Cells In Bone Tissue Regeneration: Comparison Of Basal And Osteogenic Differentiated Gene Expression Of A Newly Discovered Mesenchymal Stem Cell Lineage. *J Biol Regul Homeost Agents.* 2015;29(3):713-8. PMID: 26403412.
15. Tatullo M, Gentile S, Paduano F, et al. Crosstalk between oral and general health status in e-smokers. *Medicine (Baltimore).* 2016 Dec;95(49):e5589. DOI: 10.1097/MD.0000000000005589.
16. Tatullo M, Simone GM, Tarullo F, et al. Antioxidant and Antitumor Activity of a Bioactive Polyphenolic Fraction Isolated from the Brewing Process. *Sci Rep.* 2016;6:36042. DOI: 10.1038/srep36042.
17. Marrelli M, Gentile S, Palmieri F, et al. Correlation between Surgeon's experience, surgery complexity and the alteration of stress related physiological parameters. *PLoS One.* 2014;9(11):e112444. doi: 10.1371/journal.pone.0112444.
18. Tatullo M, Marrelli M, Scacco S, et al. Relationship between oxidative stress and "burning mouth syndrome" in female patients: a scientific hypothesis. *Eur Rev Med Pharmacol Sci.* 2012 Sep;16(9):1218-21. PMID: 23047505.
19. Tatullo M, Marrelli M, Amantea M, et al. Bioimped-

- ance Detection of Oral Lichen Planus Used as Preneoplastic Model. *J Cancer*. 2015;6(10):976-83. doi: 10.7150/jca.11936. eCollection 2015.
20. Inchingolo F, Tatullo M, Abenavoli FM, et al. Non-Hodgkin lymphoma affecting the tongue: unusual intra-oral location. *Head Neck Oncol*. 2011;3:1. doi: 10.1186/1758-3284-3-1.
 21. Inchingolo F, Tatullo M, Abenavoli FM, et al. Non-syndromic multiple supernumerary teeth in a family unit with a normal karyotype: case report. *Int J Med Sci*. 2010;7(6):378-84. doi:10.7150/ijms.7.378.
 22. Aulino P, Costa A, Chiaravalloti E, et al. Muscle extracellular matrix scaffold is a multipotent environment. *Int J Med Sci*. 2015;12(4):336-40. doi: 10.7150/ijms.10761.
 23. Perniconi B, Coletti D, Aulino P, et al. Muscle acellular scaffold as a biomaterial: effects on C2C12 cell differentiation and interaction with the murine host environment. *Front Physiol*. 2014;5:354. doi: 10.3389/fphys.2014.00354.
 24. Paduano F, Marrelli M, Amantea M, et al. Adipose Tissue as a Strategic Source of Mesenchymal Stem Cells in Bone Regeneration: A Topical Review on the Most Promising Craniomaxillofacial Applications. *Int J Mol Sci*. 2017;18(10). doi: 10.3390/ijms18102140.
 25. Inchingolo F, Marrelli M, Annibali S, Cristalli MP, Dipalma G, Inchingolo AD, Palladino A, Inchingolo AM, Gargari M, Tatullo M. Influence of endodontic treatment on systemic oxidative stress. *Int J Med Sci*. 2013 Dec 6;11(1):1-6.

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