DOI: 10.21608/adjc.2024.281215.1517 Advanced Dental Journal
Volume 7 (2025) | Issue 2 | Pages 273-280 ©Faculty of Dentistry-Cairo University

Original Article

Clinical assessment of bone marrow aspirate concentrate (BMAC) on 3D printed Polycaprolactone (PCL) scaffold for ridge augmentation in aesthetic zone: Clinical case series

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Submitted:02-04-2024
Accepted: 16-04-2024

Abstract

Aim: This study aimed to assess the soft tissue healing of intraoral wound following horizontal ridge augmentation in the aesthetic zone using 3D printed patient specific polycaprolactone (PCL) scaffolds loaded with bone marrow aspirate concentrate (BMAC).

Subjects and methods : Five patients who underwent horizontal ridge augmentation were included. They received 3D printed patient specific PCL scaffolds loaded with BMAC – aspirated from the anterior iliac crest . The assessment of the soft tissue closure and healing pattern of the intraoral wound was done using Landry wound healing index at the day of surgery then after 1 week, 2 weeks, 1 month, 3 months and 6 months postoperatively

Results: All the patients in the study showed uneventful intraoral wound healing with no signs of infection or wound dehiscence at the six time-points of assessment.

Conclusion: The soft tissue response to PCL was excellent showing the biocompatibility of the material used.

Keywords: Soft tissue, Healing, PCL, BMAC, ridge augmentation, 3D printing

Introduction

Bone defects may occur as a result of prolonged edentulism, trauma, infection, or periodontal diseases and they often necessitate hard and soft tissue rehabilitation. Deficient alveolar ridges require bone augmentation before insertion of the dental implants. The aesthetic zone is a challenging area for dental implant placement due to the aesthetic impact and the need for appealing grafting to ensure proper bone-soft tissue ratio.

Many techniques were introduced for horizontal as well as vertical ridge augmentation such as guided bone

regeneration (GBR), onlay bone blocks, ridge splitting or distraction osteogenesis **(Sánchez-Sánchez et al., 2021)**

Four major principles -so called "PASS"- are said to improve regenerative prognosis when properly followed.; these are primary closure of the wounds to provide a protected environment from microorganisms and mechanical stresses, angiogenesis to encourage new bone formation, space to prevent the collapse of the biomaterial and integrity of the blood clot. These requirements ensure better outcomes and decrease the incidence of possible complication **(Wang and Boyapati, 2006)**

Wound healing is fundamental for success of any surgical procedure; it includes stages of hemostasis, inflammation, proliferation and finally maturation.

Bone tissue engineering (BTE) aims to fabricate materials that are more effective than autogenous bone grafts and allografts. The goal is to produce materials that can be inserted in a bone defect, and then remodeled using the recipient patient's own cells. In order to maximize bone regenerative potential during BTE there must be an osteoconductive scaffold, osteogenic progenitor cells and osteoinductive growth factors which results in neo angiogenesis, osteogenesis and the resorption of the scaffold to replace the damaged or diseased bone. **(Khazaal and Helman, 2022).**

BMA (bone marrow aspirate) is an injectable bone marrow extract that can be used without modification, cultured for cell growth, or is minimally modified, concentrated into bone marrow aspirate concentrate (BMAC) through density gradient centrifugation. This process eliminates red blood cells, granulocytes, immature myeloid precursors, and platelets. BMA can be harvested from a variety of sites, including:the anterior and posterior iliac crests , the ilium , the proximal humerus, the proximal tibia , the distal femur , the distal tibia , the sternum , the mandible , and the calcaneum **(Hernigou et al., 2014)**

BMAC has been extensively studied in orthopedics, particularly for nonunion, surgical augmentation, osteonecrosis, osseous and cartilage deficiencies **(Hoogervorst *et al.*, 2022)**

In craniofacial surgery, BMAC has also been used in conjunction with other biomaterials, or with other bone grafts, to treat atrophic and vertical bone defects in the jawbone **(Hergemöller *et al.*, 2022)**

Scaffolds with critical physical, chemical, and biological properties are designed to create a suitable environment to promote cell adhesion, proliferation, angiogenesis, and growth factors (GF) transport. Also, scaffolds should contain interconnected porosity, where the pore size is at least 100 μm in diameter in order to permit the diffusion of nutrients and oxygen necessary for cell survival **(Rouwkema et al., 2008).** However, studies have found that pore sizes ranging from 200 to 350 μm are optimal for in-growth of bone tissue **(C. M. Murphy et al., 2010).** Finally, the scaffolds should be bioresorbable , having the ability to degrade over time in vivo allowing for the growth of the new bone tissue. Polycaprolactone (PCL) is a linear polyester which is one of the most used materials in the fabrication of scaffolds. It is known for its biocompatibility and slow degradation rate, as well as its less acidic breakdown products.The slow biodegradation of PCL provides time for the bone to remodel and can be adjusted to change the biodegradation rate of the polymer. However, its low hydrophilicity and bioactivity limit its application in the biomedical field. A combination of the PCL matrix and bioactive inorganic particulates as fillers offers a potential solution to address these drawbacks. **(Yang et al., 2021)**

We therefore propose to assess soft tissue healing and mucosal biocompatibility of PCL following lateral ridge augmentation

using patient specific 3D printed PCL scaffolds loaded with BMAC in anterior maxilla.

Subjects and methods

This study was performed in the outpatient clinic of the department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University, informed consent was obtained from all the patients included in the study.

The included patients required horizontal ridge augmentation of aesthetic zone (maxillary anterior and premolar areas) following extraction of one or two teeth with age range between 18 and 35 years and ASA physical status I and II , while exclusion criteria included smoking, untreated periodontal disease and uncontrolled systemic diseases.

The presurgical phase included obtaining thorough medical and dental history , clinical and radiographic examination of the patients (CBCT to assess bony dimensions and start the virtual design of the scaffold)

The virtual surgical simulation phase included virtual designing of the scaffolds using specialized softwares for surgical planning [1] and 3D modelling [2] where PCL scaffolds were virtually designed to complement the shape of the bony defects (Fig.(1)) , 3D printed by fused deposition modeling(FDM) 3D printer[3] (Fig.(2)) and sterilized using low temperature hydrogen peroxide plasma sterilization.[4]

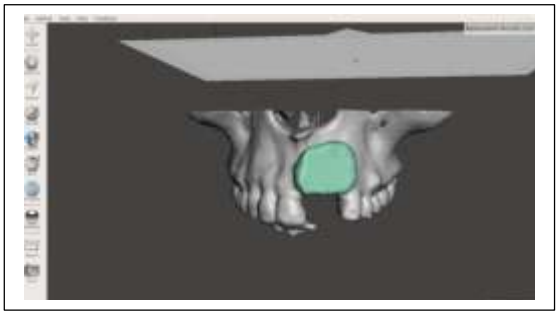
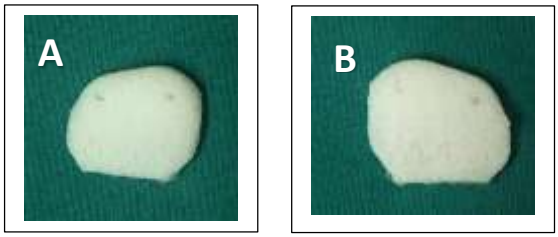


Fig.(1): 3D model of maxilla with the PCL scaffold design that complements the defect



Fig(2): 3D Printed PCL scaffold

A. Front View B. Rear view

The surgical phase included bone marrow aspiration from the anterior iliac crest region with subsequent concentration via density gradient centrifugation to obtain BMAC.(Fig.(3))

Fig.(3): The concentrated nucleated cell layer of BMAC

[1] Materialize mimics , Materialize NV , Belgium
[2] Meshmixer, Autodesk Inc.,USA
[3] Prusa i3 MK3S , Prusa Reseach a.s., Prague, Czech Republic.
[4] Sterrad™ , Advanced sterilization products, INC., USA

The PCL scaffold was soaked in a kidney dish containing BMAC nucleated cells for 5 minutes and for further seeding, the cells were injected in the scaffold using a 3cm plastic syringe(Fig.(4)). Then the scaffold was fixed in the bony defect area with microscrews apically(Fig.(5)).The intraoral wound was sutured by monfilament non resorbable sutures.

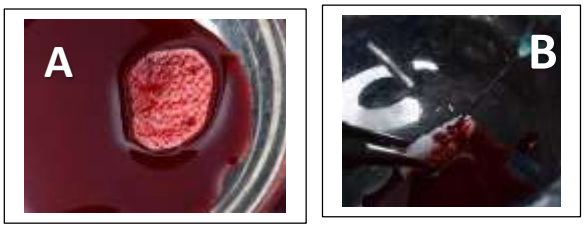


Fig.(4): Seeding of the BMAC on the PCL scaffold

A. By soaking the scaffold in BMAC

B. B. By injecting BMAC into the scaffold

Fig. (5): PCL scaffold fixed in place using microscrews

Postoperatively, instructions included strict oral hygiene instructions ; regular use of antiseptic mouthwash, soft tooth brush and warm saline rinse 3 times daily for 1 week starting from the day after surgery in addition to soft diet for the first 48 hours and avoidance of dairy products for the first 72 hours, the medications prescribed were Amoxicillin/Clavulanic acid 1g q12h for 5 days, Metronidazole 500 mg q8h for 5 days, Diclofenac potassium 50 mg q8h for 3 days, Chlorhexidine Gluconate 0.1% mouth wash q8h for 10 days .

After 10 days, the sutures of the intraoral wound were removed.

The assessment of the soft tissue closure of the intraoral surgical site was conducted at six-time points; immediately after suturing of the wound then after one week , two weeks, one month , three months and six months postopratively.

The assessment of the soft tissue healing process at the surgical site was done by using the Landry wound healing index. These were done at the day of surgery (T0) (Fig.6), after one week (T1), two weeks (T2) (Fig.7), one month (T3) (Fig.8), three months (T4) and six months(T5)(Fig.9) where the healing pattern is classified into five categories: 1. very poor, 2. poor, 3. good, 4. very good and 5. excellent according to soft tissue colour whether red or pink, the amount of bleeding in response to palpation, presence or absence of pus, granulation tissue presence and integrity of the wound margins and exposure of underlying connective tissue. **(Alrayyes et al., 2022).**

At the six months time point (T5), a postoperative CT was done to assess the bone formation at the augmented site using a surgical planning software [5] by superimposition of the 6 months postoperative CT on the preoperative CBCT to detect the amount of new bone formation.

Fig.(6): Intraoral wound after suturing (T0)

[5] Blue sky plan, Blue Sky Bio, USA

Fig.(7): Wound healing after two weeks (T2)

Fig. (8): Wound healing after one month (T3)

Fig(4): Wound healing after one month

Fig.(9): Wound healing after six months (T5)
A. Front view
B. Occlusal view

Results

A total of five patients were included in this study (three females and two males) with an age of range 18-35 years mean of which was 26.5 years.

All the patients in the study showed uneventful wound healing with no signs of infection or wound dehiscence. mild postoperative edema was noted, it resolved completely by following the postoperative instructions by the seventh day postoperatively.

The soft tissue response to PCL was excellent ie, no soft tissue inflammation or dehiscence occurred in any of the included patients within our follow up period..

The mean values of healing index scores recorded at six time-points showed uneventful proper healing of the intraoral wounds over time. (table 1)

The 6 months postoperative CT showed no evidence of bone formation, and after superimposition of the 6 months postoperative CT over the immediate postoperative CBCT, no changes in the bone width were noticed as shown in Fig. (10)

Table 1: Mean and standard deviation values of Landry healing index scores at six time points; immediate(T0), after one week(T1), two weeks(T2), one month(T3), three months(T4) and six months(T5).

Time-point	Mean value of Healing index score	Standard Deviation
T0	2.6	0.54
T1	3.4	0.54
T2	3.8	0.44
T3	4.8	0.44
T4	5	0
T5	5	0

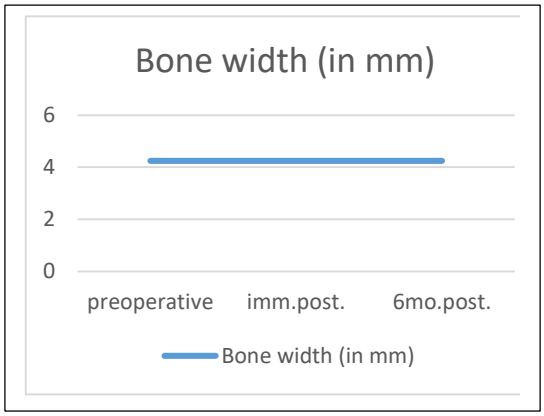


Fig.(10): A line chart showing change in preoperative, immediate postoperative and 6 months postoperative bone width in millimeters .

Discussion

Wound healing is a multifactorial process which is crucial in prognosis of any surgical procedure , the aim of this study was to evaluate the soft tissue healing following a novel technique of ridge augmentation using a bone tissue engineering concept in aesthetic zone which comprises PCL as a scaffold material. PCL was chosen due to the advantages it possesses as it is biocompatible with relatively slow biodegradation rate , non-toxic to the BMAC's cell popoulation as well as its degradation byproducts, non-allergic to the patients, good mechanical properties and cost effective , and being biodegradable , this eliminated the need of second surgery to remove it from the augmented site .**(Yang *et al.*, 2021)**. To date, no literature on the use of PCL as a GBR scaffolding material was reported but it was used in conjunction with BMAC in alveolar cleft repair yielding satisfactory results regarding soft tissue healing of palatal mucosa as well as bone formation **(Ahn et al., 2018).** PCL was printed by desktop FDM 3D printer [6]using a specialized 3D printing software [7] the external configuration of the scaffold as well as internal configuration in terms of pore size , shape and interconnections were adjusted ,The pore size ranged from 200µ to 300µ which are found to be optimum for bone tissue in-growth The PCL scaffolds were sterilized using low temperature hydrogen peroxide plasma sterilization to avoid melting of the scaffold owing to the low melting point of the material. **(Dai et al., 2015).** Secondly, fixation of the scaffold in the recipient site was done by the micro-screws to prevent micro-movement that could cause infection which would adversely affect the soft tissue healing and subsequent bone formation.

Postoperative follow up was done in close intervals to check for any wound dehiscence or infection in the recipient site. Uneventful healing was observed in all patients with absence of any signs of wound dehiscence or infection indicating biocompatibility of PCL which was enhanced with BMAC loaded on it.

The soft tissue response to PCL was compared to that of other materials used in GBR such as titanium meshes and non resorbable PTFE membranes where the main drawback of non-resorbable membranes is their stiffness which may cause soft tissue dehiscence resulting in membrane exposure and subsequent infection. In addition, a second surgery is needed to remove non-resorbable membranes, leading to patient discomfort and higher economic burden. Similarly, the major drawback of titanium mesh is increased wound dehiscence and difficulty in soft-tissue coverage maintenance which leads to infection.**(von Arx et al., 1996)**

Shadow of the micro-screws fixing the scaffold appeared through the soft tissue of two patients about 3 months postoperatively due to thin gingival biotype of those patients, a finding that didn't affect the wound healing or prognosis of those cases but it was worth mentioning.

[6] Prusa i3 MK3S , Prusa Reseach a.s., Prague, Czech Republic

[7] Ultimaker Cura, Ultimaker, Utrecht, Netherlands

The assessment of the soft tissue healing process at the surgical site was done by using the Landry wound healing index which was chosen for such assessment due to the incorporation of several parameters of healing in the index leading to realistic presentation of the wound healing using numerical score of each healing parameter **(Alrayyes et al., 2022).**

Conclusion

Polycaprolactone is a biocompatible material which is a good candidate for bone tissue engineering procedures. Our results showed excellent soft tissue biocompatibility which may be attributed to the addition of BMAC as supported in other reports of additives on PCL that increased hydrophilicity. The well healed mucosal coverage allows for future implant placement with a decent soft tissue collar and good esthetic outcome which is crucial in the aesthetic zone . We therefore believe the PCL/ BMAC combination should be put in further research trials to further assess its clinical applicability.

Acknowledgment

The authors would like to thank eng. Ahmed Kidwani and eng. Mohamed El-Sayed for their technical assistance in 3D printing process which facilitated completion of this study as anticipated.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics

This study protocol was approved by the ethical committee of the Faculty of Dentistry- Cairo university on: 27.7.2021 approval number: 15-7-21 .

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