Journal of Dentistry and Oral Sciences

ISSN: 2582-3736
Sousa LLL, et al., 2022-J Dent Oral Sci
**Case Study**

# Polylactic Acid Printed Scaffold on Collagen Membrane: Physical, Chemical Analysis and the Report of 2 Clinical Cases

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Receiving Date: 08-01-2022

Accepted Date: 08-18-2022

Published Date: 08-24-2022



## Abstract

The bovine collagen membrane LUMINA-COAT (Reg.ANVISA nº80522420002) of the company CRITÉRIA BIOMATERIAS LTDA, membrane basically composed of type 1 collagen, biocompatible and sterile for medicine and dentistry use, had one of its surfaces coated with a polylactic acid (PLA) sheet. Sterile and previously shaped for adjustment on grafted bone site, this device was applied in 2 clinical cases of bone regeneration in the function of guided tissue regeneration barrier.

**Keywords:** Collagen RTG membrane, Scaffold; Polylactic acid; Dentistry; Tissue Regeneration.

## Introduction

The LUMINA COAT collagen membrane has been extensively studied since its entry in the market in 2011. It characterizes itself for its spongy highly porous structure, obtained by the process of lyophilization of the type 1 collagen solution coming from the bovine bone structure, which physical-chemical characterization from LUMINA COAT is presented by the purity with total lack of heavy metals and by its essence as extra-cellular matrix [1,2].

Extensive clinical-surgical cases of dental bone regeneration and correction utilizing the LUMINA COAT barrier, involve techniques in sinus trans fixation [3], severe alveolar resorption by occlusal trauma [4], vertical treatment of the alveolar ridge [5], repositioning of grafted implants [6], horizontal technique of bone thickness increase [7], parendodontic surgery [8], remotion of migrated implant on the maxillary sinus [9], anti-microbial therapy for bone regeneration [10], etc. The polylactic acid (PLA, PDLA, PLLA or


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PDLA/PLLA) is being well known as a biocompatible and resorbable polymer of elevated perspective to compose surgical devices aimed at tissue regeneration [11,12]. PLA applications such as tissue regeneration barriers also have been observed with elevated frequency and commercial products in the market [13,14]. Comparative studies of cases utilizing RTG barriers of PLA against natural resorbable barriers [15-17] or non-resorbable synthetic barriers [17-20] show there are no disadvantages regarding the clinical-surgical process or the bone increase obtained. The combination of the RTG collagen membrane with a PLA scaffold is innovative and beneficial, since it conciliates the hydrophilic capability of the collagen enabling vessels formation by the cellular activity in its porous structure with the capability of the PLA scaffold formation, where both materials are biocompatible and resorbable fully enabling neo-osseous formation in the wanted profile 29.

## Materials and methods

### Lumina-coat barrier

The LUMINA-COAT collagen membrane (Reg. ANVISA nº80522420002) was supplied by the company CRITÉRIA BIOMATERIAIS and is characterized by its structure as shown in Figure 1, in vitro test of cytotoxicity as shown in Figure 2 and its composition according to Figure 3.

### PLA scaffold

The PLA scaffold was printed in a fused deposition modeling (FDM) 3D printer by the company CONSULMAT and can be characterized by its structure as shown in Figure 4, in vitro test of cytotoxicity as shown in Figure 5, in vitro test of genotoxicity as shown in Figure 6, and its characterization by FTIR as polylactic acid in Figure 7.

### Lumina-coat barrier with PLA scaffold

The PLA scaffold was fixed on the LUMINA-COAT barrier by the company Consultant according to the protocol established by the authors, meaning, fully coverage of the barrier's surface and can be seen in Figure 8. The LUMINA-COAT barriers with PLA coverage utilized in the cases hereby presented were vacuum sealed in Bisphenol free polyethylene packaging, inside Medstéril envelope and sterilized in exposure to 60Co in 25kGy (Figure 9,10).

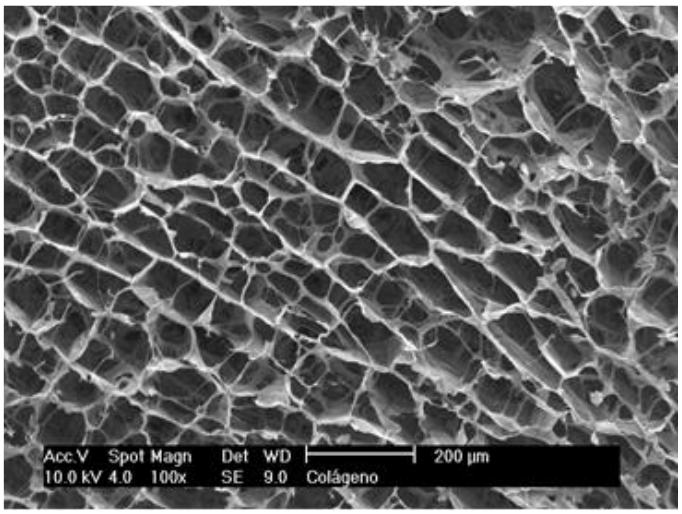


**Figure 1:** Spongy structure of the LUMINA-COAT collagen membrane (500X).


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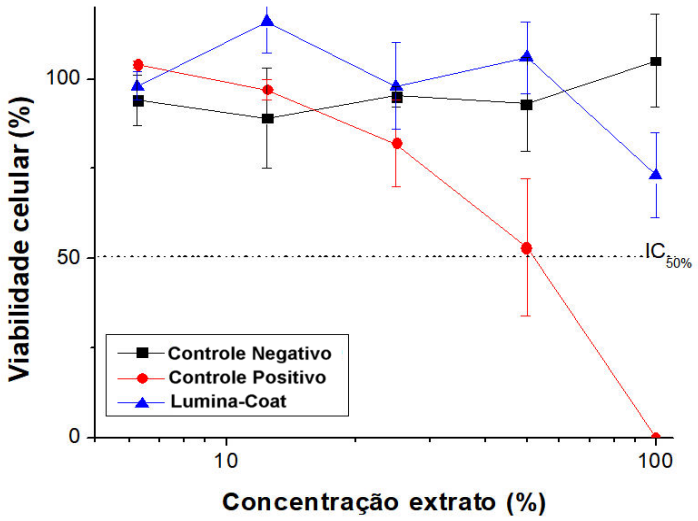


**Figure 2:** Cytotoxicity of the RTG LUMINA-COAT barrier.

| Elementos | RG 2356/13<br>LC 001 - Original<br>Teor (%) |
|---|---|
| Cl | 1,0±0,1 |
| S | 0,20±0,01 |
| Na | 0,17±0,02 |
| Si | <0,09 |
| Ca | 0.008±0,002 |
| P | 0.005±0,001 |
| Fe | 0.003±0,001 |
| Ni | 0.002±0,001 |
| K | <0.004 |
| Zn | <0.002 |
| $C_{12}H_{21}O_7N_3$ | 98,6±0,1 |

**Figure 3:** Chemical analysis of the LUMINA-COAT.

**Figure 4:** PLA scaffold structure (3X).


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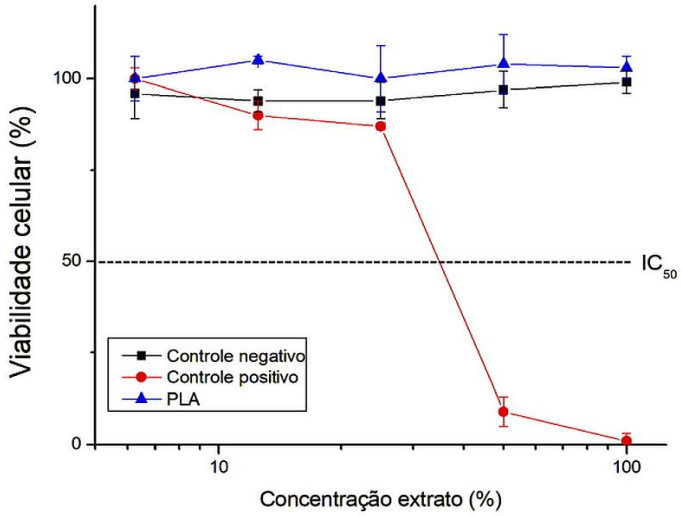


**Figure 5:** Cytotoxicity of the PLA Scaffold.

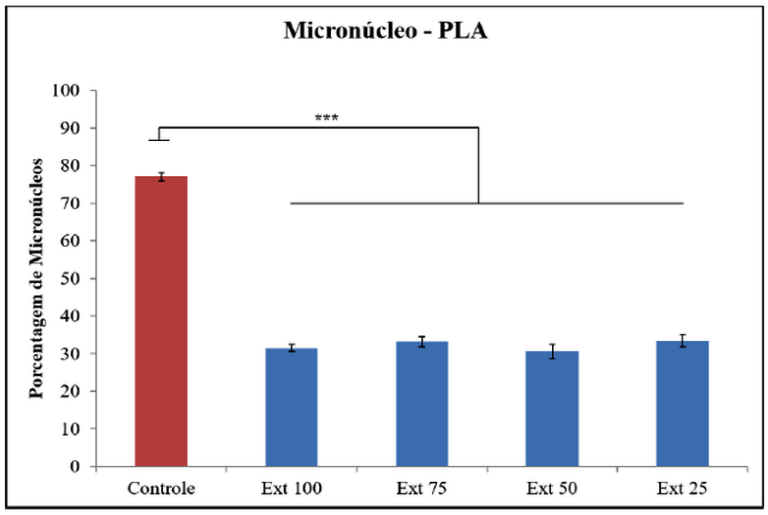


**Figure 6:** Genotoxicity of the PLA Scaffold.

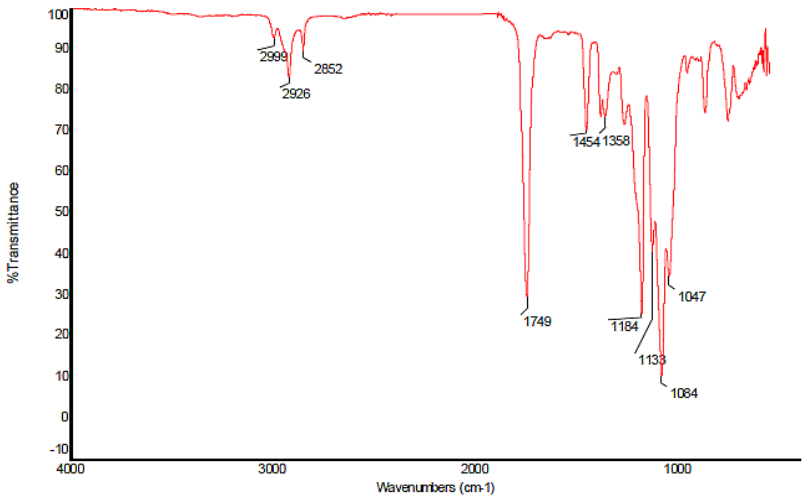


**Figure 7:** FTIR spectrum of the PLA scaffold.


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**Figure 8:** PLA scaffold placed on LUMINA-COAT barrier (3X).

**Figure 9:** Sterile packaging from LUMINA-COAT with PLA scaffold.

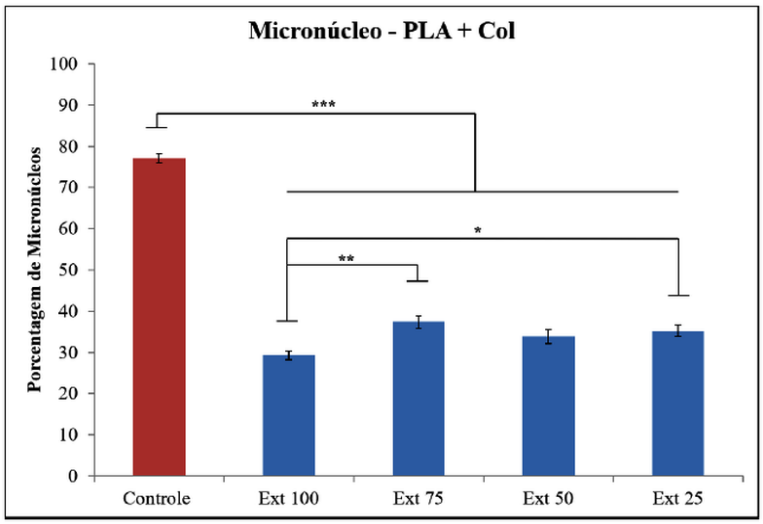


**Figure 10:** LUMINA-COAT genotoxicity with PLA scaffold.


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## Clinical cases

### Case 1

Patient LRM, 57 years old, normotensive and reactive, arrived at the clinic reporting compromise and symptomatology in several implants of the maxilla, installed by another professional, with peri-implant compromise, out of adequate standard positioning, implants and cover totally inserted in the maxillary sinus with compromise on the left and right, still having major functional and aesthetic compromise (Figure 11). Protocol of request of complementary exams was carried and no alteration was observed. After primary analysis in orthopantomography, several options of treatment plans were presented to the patient, which the patient opted for the explantation of all implants, followed by bilateral sinusotomy of the maxillary sinus, regularization of the ridge and longitudinal planning and new installation of implants in All-on-Four maxillary technique [21].

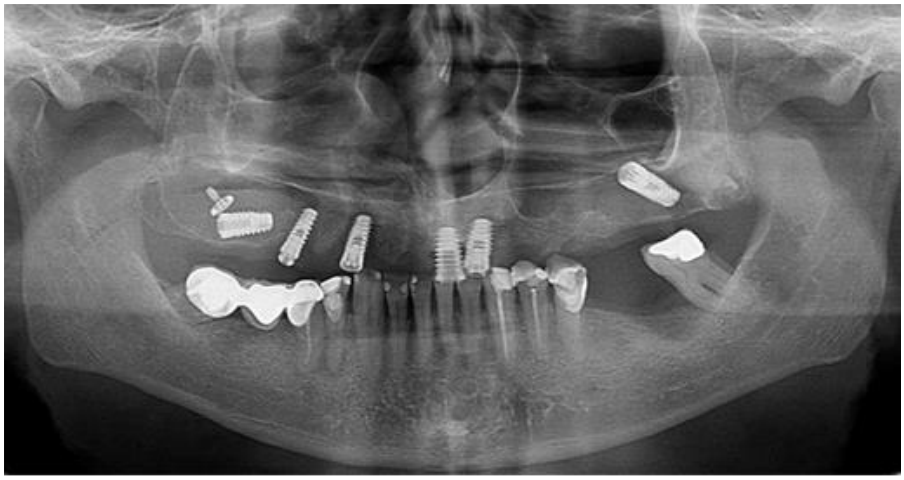

**Figure 11:** Primary orthopantomography, highlighting the installed implants previously compromised, demonstrating positioning failure, and introduction in maxillary sinus left and right.

Among several possibilities in the planning of the dental implants, the All-On-Four technique [21], proposing the use of 4 (four) dental implants distributed in the most precise manner in equidistance following the Roy polygon principle [22], for a better dissipation of the masticating loads for the protein rehabilitation support was elected mainly for the fact of the possibility of the surgical procedure occur outside the compromised area by the buccosinusal communication occurred by the loss and introduction of posterior implants which by chance were inserted in this region. 2g amoxicillin and 4g of dexamethasone were prescribed 1hour before the surgical procedure. Extraoral antisepsis was performed with chlorhexidine 2% and intraoral with chlorhexidine 0.12%. Anesthetic infiltrating technique was performed in the entire maxillary region, with anesthetic articain salt 4% (100.000:1 dilution). Incisions in the area of the elements 18 extended to 28 were performed, with the incision made in the ridge and, followed by diffusion and peeling of the vestibular and palatal tissue, maintaining an observation in the most complex anatomic structures. Implants explantations were performed in the approximations of the 11,14,16,17 with the Cover removal of the implants 21 and 27, irrigating with sodium


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chloride solution at 0.9% using Retrievers in the implants 11,14,16,21. Compromised right maxillary sinus access curetted, partially removing the compromised sinus membrane and liberating straight access to the removal of the implant and component in the region of the 17, following the same approach in the region of the 27, curetting and removing partially the sinus membrane on the left side of the maxillary sinus, by the access predicted by the inherent buccosinusal communication and subsequent removal of the totally lost and compromised implant. Preventively, the decontamination was made with low intensity rinses with saline solution at 0.9% of sodium chloride, associated with 500mg of amoxicillin diluted in the solution. After the structure readjustment, the process of tissue healing was initiated, inserting 3 (three) fibrin rich platelet membranes on each side (Figure 12).

**Figure 12:** Platelet rich fibrin Clot, in post centrifugation phase.

The procedure sequence occurred with the insertion of the Lumina Coat collagen membranes of 1X20X30 reinforced with PLA printed structured and porous scaffold. This membrane presentation was modeled from plastification by heating, performed through the placing of the PLA modified membrane, in a tray filled with saline solution of sodium chloride at 0.9% heated and measured at 60°C temperature in digital thermometer. (Figure 13).

Modeled and packaged to the new membrane manually, according to the shape of the compromised defect on both right and left sides of the maxilla, fixating both membranes with pins inserted on the vestibular and crestal bone walls (Figure 14).

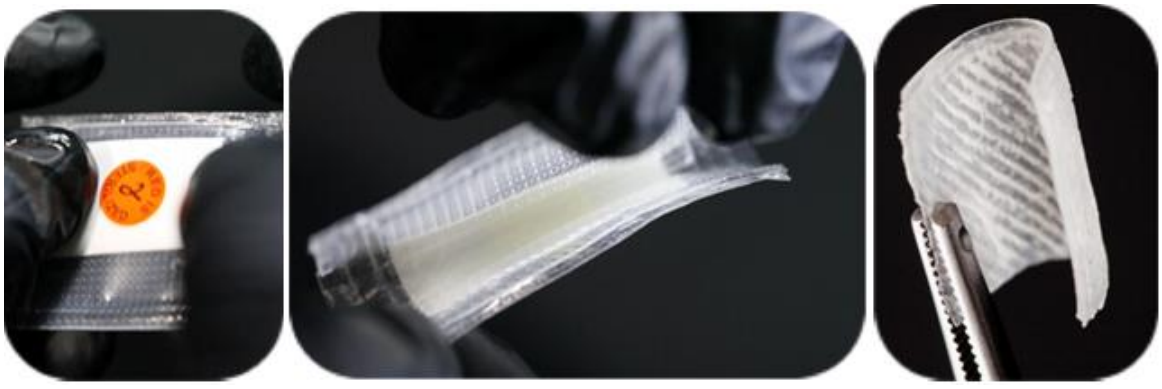

**Figure 13:** Printed PLA barrier on collagen membrane type 1 Lumina Coat of 1X10X20 during the handling before and after plastification in saline solution 0.9% heated at 60°C.


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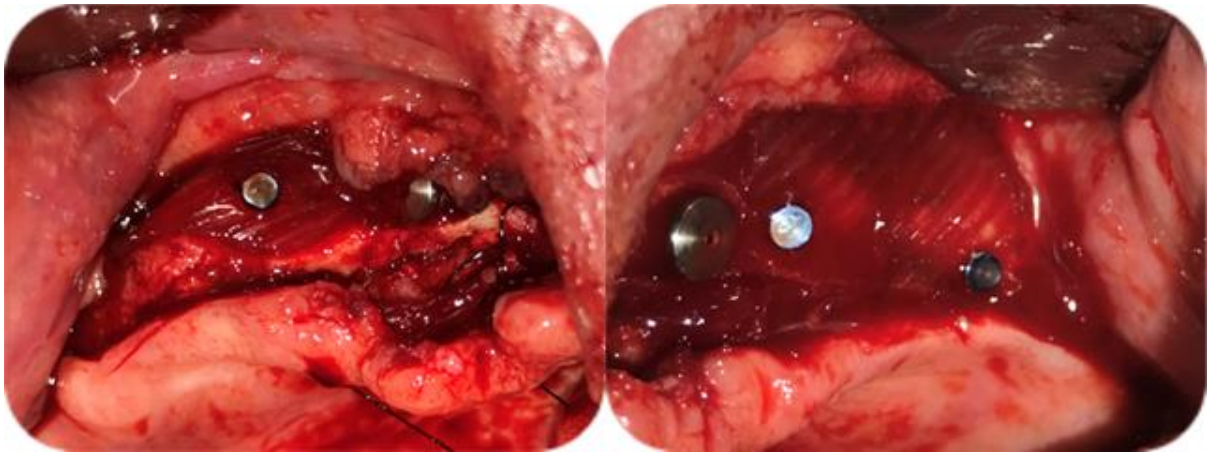

**Figure 14:** Image of the surgery right after the installation of the printed PLA barriers on Lumina Coat type 1 collagen membrane of 1X10X20, locked in position after the plastification using titanium pins fixated to the bone.

The remaining irregular structures after the implants explantation, were curetted with Lucas 85 curette (Quinelato-Rio Claro, SP-Brazil), which proceeded with the longitudinal planing on the alveolar bone ridge remnant from the anterior part of the irregular maxilla, performing then the osteopathy, using a drill of tungsten Maxicut type of medium lamination (Supremo Odonto-São Paulo, SP-Brazil), again abundantly irrigating with saline solution of sodium chloride at 0.9%.

After the removal of the great volume of tissue, the formation of a bone plateau was observed in the farther anterior portion of the mandible. On the prepared pristine bone, we performed the start of the milling, following the protocol proposed by the selected implant manufacturer (Titanium Fix, Taubaté-SP, Brazil) for this procedure, aiming the best three-dimensional positioning of each implant, following the good distribution of the implants. For the milling protocol, with the drill bit 2.0 the milling proceeded in the regions where the future installation of the implants was determined with a rotation of 1200 RPM, next, the helicoidal mill 2.0,2.5, and 2.8mm were utilized and last the countersink mill, the anterior being (11/12 and 21/22) with 11.5mm deep and the posteriors (14/15 and 24/25) with 13mm deep and rotation of 750 RPM (Figure 15).

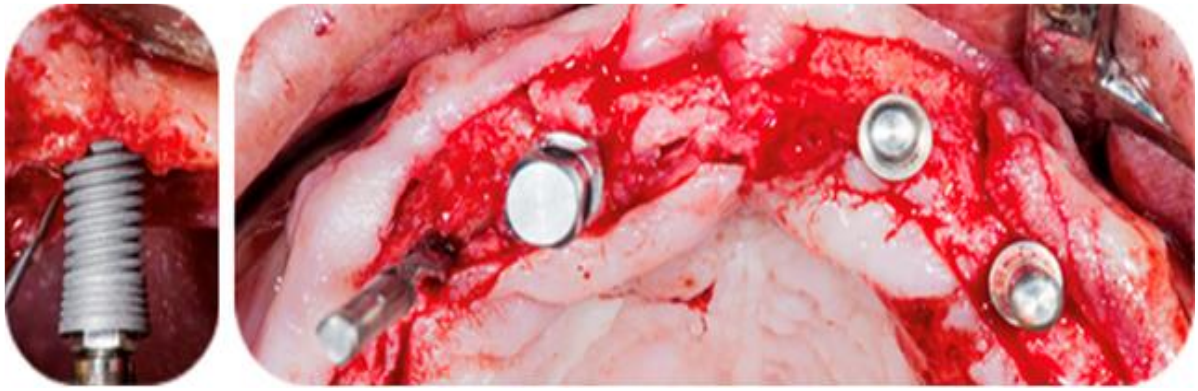

**Figure 15:** Demonstration of the inserted implants after all the process and milling proposed by the manufacturer in the surgical bed.


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Finally, the installation of the 4 cylindrical implants of external hexagon with internal torque were performed (Titanium Fix, Taubaté-SP, Brazil) of 11.5X3.5mm in the anterior region of the 11/12 and 21/12 and implants of 13X3.5mm in the regions of 14/15 and 24/25, where the 35Ncm and 40Ncm lock was obtained in all installed implants (Figure 16). The excessive gingival tissue was readjusted, performing gingivectomy in the anterior region in order to better condition the synthesis, finishing with sutures of regular type for a better locking of the tissue utilizing nylon 4-0 threads (Microsuture, São Paulo-SP, Brazil). After 180 days, new monitoring orthopantomography was taken, where it was possible to observe stability of the reconstructed structures and satisfactory analysis of the clinical aspect, allowing the referral of the patient to the clinical care for the adequate prosthetics attainment (Figure 17).

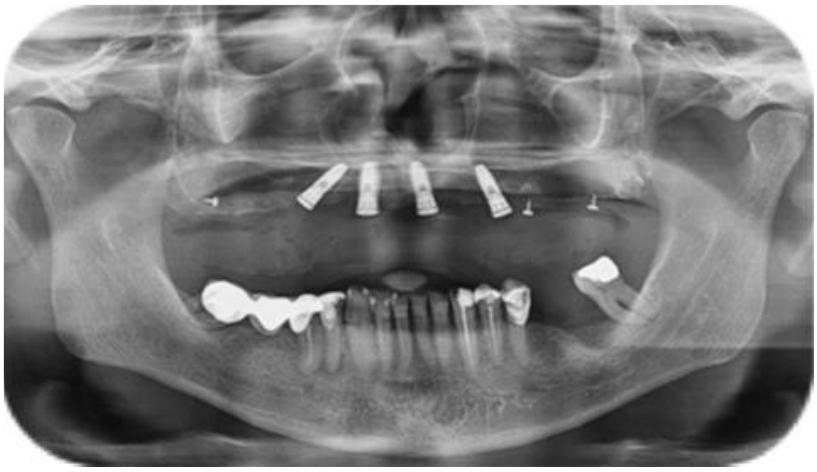

**Figure 16:** Monitoring orthopantomography after 180 days, highlighting the result after the removal of all the implants and repositioning of new implants in All-On-Four technique.

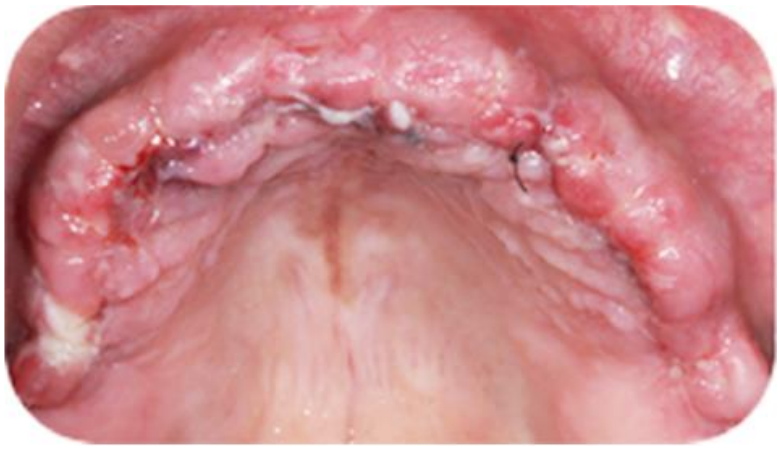

**Figure 17:** Clinical vision 15 days after surgery.

**Case 2**

Patient EGR, 50 years old, normotensive and reactive, arrived at the clinic reporting functional compromise in the region 13 and 14, for the missing elements prematurely lost, influencing the aesthetic stability of the region. Protocol of request of complementary exams was carried and no alteration was observed. After primary analysis through cone bean type CT scan,


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several treatment plans were presented to the patient, which, the patient chose the bone regeneration and immediate installation of implants (Figure 18).

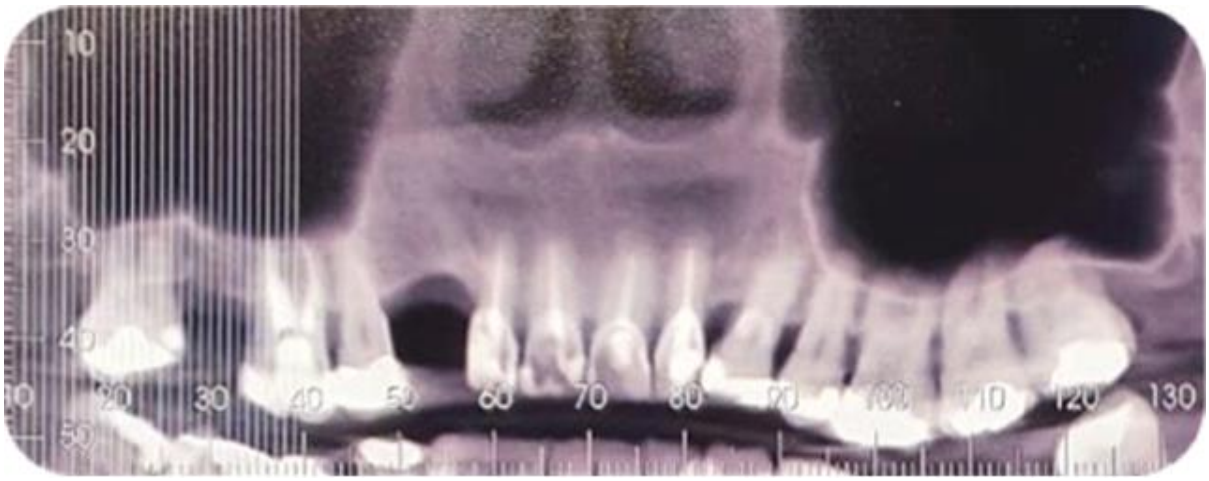

**Figure 18:** Primary orthopantomography, highlighting the bone defect to be reconstructed.

Among the several possibilities in the planning of the dental implants, the technique with the use of individual implants, proposing the use of 2 dental implants distributed in the most precise manner in equidistance, for a better dissipation of the masticating loads for the protein rehabilitation support was elected. 2g amoxicillin and 4g of dexamethasone were prescribed 1 hour before the surgical procedure. Extraoral antisepsis was performed with chlorhexidine 2% and intraoral with chlorhexidine 0.12%. Infiltrating anesthetic technique was applied in the entire alveolar ridge region and buccal fornix, with anesthetic articain salt 4% (100.000:1 dilution). Incisions in the area of the elements 12 extended to 15 were performed, with the incision made in the ridge and, followed by diffusion and peeling of the vestibular and palatal tissue, maintaining an observation in the most complex anatomic structures.

The installation of the implants was performed in the approximations of the 13, 14, irrigating with sodium chloride solution at 0.9%. The entire bone structure was curetted, removing the adjacent soft tissue. After the structure readjustment, the tissue regeneration process was started, creating micro perforations in the vestibular portion to increase basal sanguine support nutrition. Then, the entire area of Lumina Bone Porous granulation large particulate bone graft was covered (Critéria Biomateriais, São Carlos-SP, Brazil) with platelet fibrin rich membranes (Figure 19). The procedure sequence occured with the insertion of the Lumina Coat collagen membranes of 1X20X30(Critéria Biomaterials, São Carlos, SP-Brazil), reinforced with PLA printed structured and porous scaffold. This membrane presentation was modeled from plastification by heating, performed through the placing of the PLA modified membrane, in a tray filled with saline solution of sodium chloride at 0.9% heated and measured at 60°C temperature in digital thermometer. Modeled and conditioned to the new membrane manually, according to the shape of the anatomic defect of the compromised region, fixating it with pins inserted on the vestibular and crestal bone walls (Figure 20).


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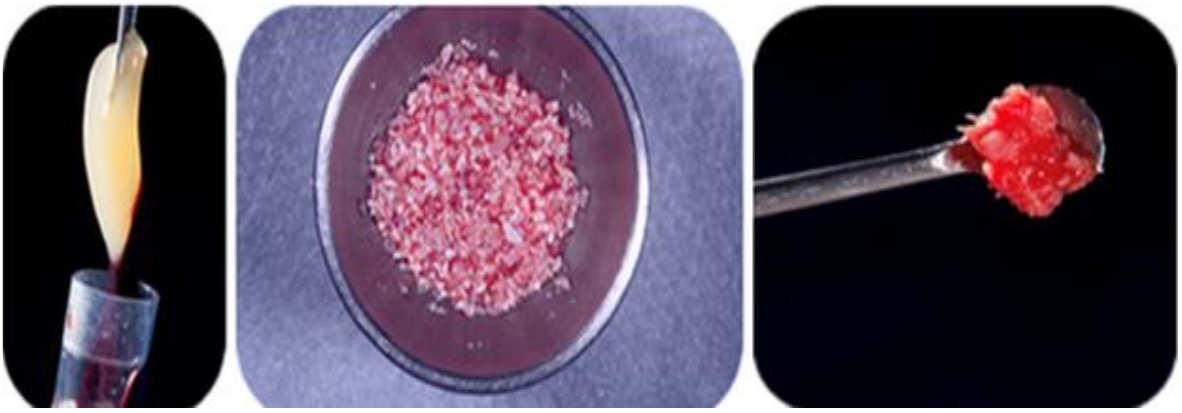

**Figure 19:** Handling of the particulate biomaterial Lumina Bone Porous Granulation Large associated with the serum extracted from the supernatant of the Fibrin Rich Platelet after centrifugation and processing.

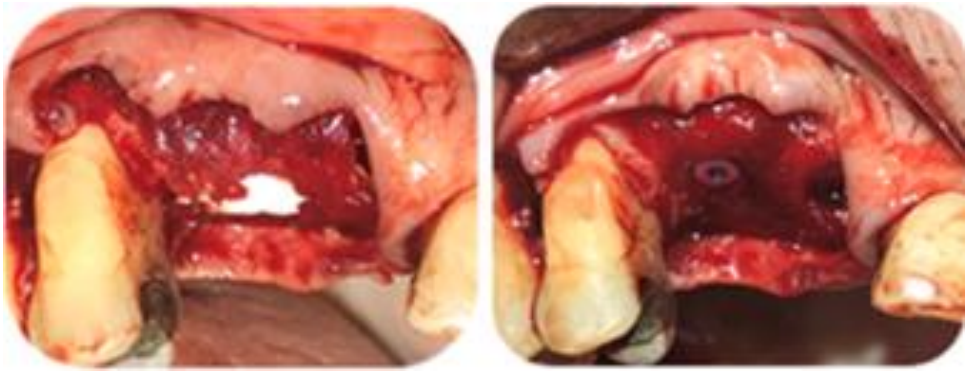

**Figure 20:** Image of the surgery right after the installation of the printed PLA barriers on Lumina Coat type 1 collagen membrane of 1X10X20, locked in positioning after plastification using the implants titanium cover screw for fixation.

On the prepared pristine bone, we performed the start of the milling, following the protocol proposed by the selected implant manufacturer (Nobel Biocare, USA) for this procedure, aiming the best three-dimensional positioning of each implant, and following the good distribution of the implants. For the milling protocol, with the drill bit 2.0 the milling proceeded in the regions where the future installation of the implants was determined with a rotation of 1200 RPM and next, the helicoidal mill 2.0 was utilized, followed by the drill bit of 11.5X3.5mm with 750RPM rotation and 11.5mm deep. Finally, the installation of 2 Replace Conical Connection implants (Nobel Biocare, USA) of 11.5X3.5mm was performed in the anterior region of the 13 and 14, where a 35Ncm lock was obtained in all installed implants. The excessive gingival tissue was readjusted in the anterior region in order to better condition the synthesis. The protocol was finalized with sutures of regular type for a better locking of the tissue utilizing Micropoly 5-0 polypropylene threads (Microsuture, São Paulo-SP, Brazil). After 180 days, new monitoring orthopantomography was performed, where it was possible to observe stability of the reconstructed structures and satisfactory analysis of the clinical aspect, allowing the referral of the patient to the clinical care for the adequate prosthetics attainment (Figure 21).


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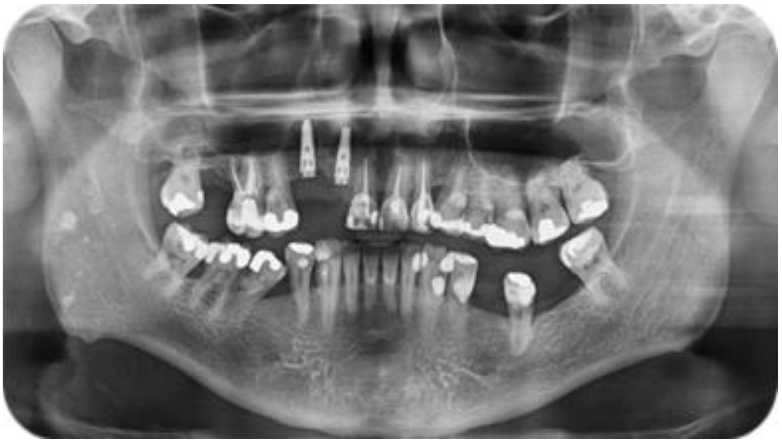

**Figure 21:** Monitoring orthopantomography after 180 days, highlighting the result after the installation.

## Results and discussion

### LUMINA-COAT barrier with PLA scaffold

Synthetic polymers are being studied and increasingly used as implantable biomaterials [23], and the polylactic acid (PLA) places itself as a high potential synthetic polymer due to its good biocompatibility, mechanical resistance, and easy molding. In this regard, PLA scaffold presents itself as a main function to promote a collagen membrane scaffold, once it possesses very low rigidity enabling a greater stability in the grafted bone site, besides creating possibility of proper bone height and width increase protecting the grafted area against the invagination of the soft tissue.

Cytotoxicity assays, performed at IPEN utilizing the neutral red incorporation technique, in NCTC 929 cells from the ATCC tissue bank, determined the degree of cytotoxicity verified from the IC50% index or below for the cellular viability. It can be seen that both components from the LUMINA-COAT barrier with PLA scaffold presented themselves as non-cytotoxic with elevated cellular viability (Figures 2 and 5). Genotoxicity tests were performed at UNIFESP-Santos through micronucleus technique using cell culture (stem CHO-K1) added to mitomycin and different concentrations of extracts. It can be seen that both components from the LUMINA-COAT barrier with PLA scaffold presented themselves as non-genotoxic causing less than 50% of chromosome damage in the cellular stem (Figures 6 and 10). Therefore, through Figures 2,5,6 and 10 it is possible to observe that the PLA scaffold did not infer toxicity increase to the LUMINA-COAT barrier.

Regarding the RTG LUMINA-COAT PLA scaffold device involving the result of the resorbable 3D printed PLA scaffold fixation on LUMINA-COAT collagen membrane, it showed very favorable to adjustment and adhesion between the parts not presenting detachment during its shaping on the model of the patient (Figure 22) and also during surgery. The era of oral rehabilitation with implants had its beginning in 1981 with the presentation of results obtained by Bränemark and team (Adell et al. 1981) [24]. At that moment, the success and the predictability of bone integration was proven according to a longitudinal clinical monitoring of fifteen years. In the reports of the cited cases of this study, it is possible to evince the structural need of the bone tissue presence for


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implementation support, as well as it is evident and was excessively exposed to the need of organization of the structural complex composed by the bone and gingival tissue. In this context, the priority in the resolution of the clinical cases that were mainly functional, demonstrated the need of parallel measure, the search for satisfactory aesthetic results, in a large proportion of the cases, were left for a second moment. It is at this moment that the use of adequate barriers, with covering function and guarantee of stability of the bone graft on defect region, is fundamental.

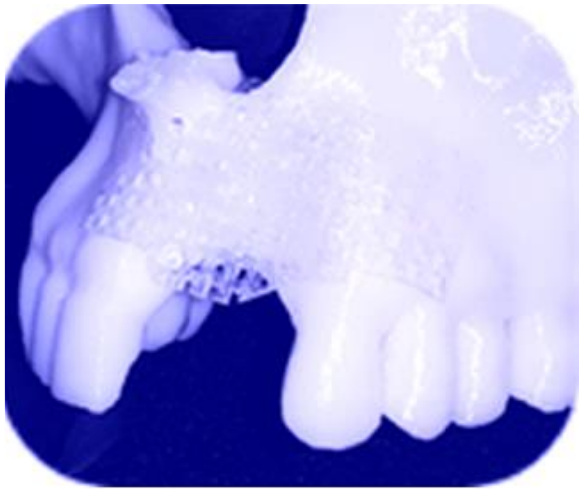

**Figure 22:** PLA barrier, laminated at 60°C, handled in printed biomodel.

However, based on the clinical evidence proposed in this study, it was evident that the use of Osseo integrated implants in the rehabilitation of individuals partially edentulous in previous areas and with unsatisfactory aesthetic resolution has brought up the matter of the aesthetic component in the end result of the implant-supported rehabilitations (Buser & Martin 2004) [25], as well as the reorganization of areas compromised by accidents or severe anatomic damages. With the goal of achieving aesthetic excellence in treatment with Osseo integrated implants it is fundamental that the planning of the clinical case be done with a multidisciplinary vision. Bone regeneration aim to stabilize or correct anatomic deformities, but not always they result in stable aesthetic tissue contours, since they often only follow the imperfections of the underlying bone tissue. That way, in efforts to minimize or eliminate the bone deformities, regenerative mucogingival surgery techniques are being applied in the implantology (Puisys & Linkevicus 2015) [26]. Biodegradable aliphatic thermoplastic polyesters made of lactic acid, are gathering attention from the biomedical field in areas such as sutures, antibiotics release systems, temporary macroscopic implants, and scaffolds for tissue engineering, associated to the fact that this material has as characteristic, its biodegradation, which in this field, due to the reliance on anchorage of most cells in the living tissue and organs, it is essential to combine adequate structures to the proliferating cells, provided to these scaffolds, an adequate superficial interaction and allowing the connection, proliferation, and growth of the cells (Sarassua et al. 2011) [27].

In our study, we use these scaffolds, to readjust the anatomic structures that on the other hand, were compromised, becoming


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possible for the implant installation in severe defects, bypassing the atrophy or destruction of the bone tissue associating with the bovine collagen membranes with PLA, and performing the immediate insertion of dental implants, in a single surgical moment, with implicit intent of the morbidity factor. On the other hand, the fibrin rich platelet and leukocytes (L-PRF) was developed and introduced in the scientific community through the study of professor Choukroun [28] published in 2001 and, since then, has been proposed as a autologous biomaterial which is extracted from the patient's own blood and acts as mechanical barrier and as a biological stimulus that has the capability of modulating the remodeling of the bone and the gum providing better quality to the newly formed tissue.

This biomaterial associated is utilized with bone substitutes of heterogenous origin moisturizing it with the extracted serum from its own processing, as well as including its presentation in membrane, applying the principle of protein release of growth factors present in the plasma, which are important for fulfilling the stimulating chemotactic effects of the osteoblasts, TGF-β important in the promotion of the bone matrix, and PDGF-AB acting in the repairing mechanism, making both processes important in the bone repair and stimulus during its natural degradation, once the PRF is a natural optimized coagulant that can improve the natural healing process. All these properties applied to the tissue healing procedure serving as a base for a prerogative of use of the PLA barrier, allows us to observe advantages in its application in the clinical procedures, as shown in this study [29].

## Conclusions

PLA scaffold on LUMINA-COAT membrane barrier offered the possibility of projecting the anatomic profile of the neo-osseous, through its capability of forming a scaffold with a malleable profile of interest of the surgeon. Clinically, the patients presented characteristic pain symptoms, having evidenced inflammatory process without exacerbation and no apparent additional discomfort, characterized in surgeries of compatible magnitude. During the handling of the Lumina Coat membrane, it was possible to observe advantages regarding the possibility of using printed bio models through the creation of 3D Figures, making the process more agile and precise regarding the adaptation in surgical bed. It was possible to conclude that the Lumina Coat collagen membrane, when added of the PLA printed scaffold, became safer and more stable to the structural maintenance of the defect to be reconstructed, bearing in mind, the ease of handling and adaptation in surgical bed, guaranteeing structural stability for the shaping of the particulate biomaterial increment as well as distancing of the soft tissue in direct contact to the biomaterial, reaching the expectation after 180 days of the formation of the reconstructed tissue and with the advantage of not needing to remove the mechanical barrier, due to its direct resorption, decreasing the degree of morbidity in this sense.

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