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Guided Bone Regeneration in Implant Dentistry: Basic Principle, Progress over 35 Years and recent Research Activities

Daniel Buser[1,2], Istvan Urban[3], Alberto Monje[4,5,6],

Marcel Kunrath[7,8], Christer Dahlin[7,9]

[1] School of Dental Medicine, University of Bern, Bern, Switzerland

[2] Centre for Implantology Buser & Frei, Bern-Bümpliz, Switzerland

[3] Department of Periodontology and Oral Medicine, University of Michigan, Ann Arbor, Michigan, USA

[4] Department of Periodontology, UIC Barcelona, Barcelona, Spain

[5] Division of Periodontology, CICOM-Monje, Badajoz, Spain

[6] Department of Periodontology, University of Bern, Bern, Switzerland

[7] Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy , University of Gothenburg, P.O. Box 412, SE 405 30 Göteborg, Sweden.

[8] Department of Dentistry, School of Health and Life Sciences, Pontifical Catholic, University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

[9] Department of Oral, Maxillofacial Surgery and Research and Development, NU-Hospital Organisation, Trollhättan, Sweden.

Corresponding author: Daniel Buser - danbuser@mac.com

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Abstract

Bone augmentation procedures are frequent today in implant patients, since an implant should be circumferentially anchored in bone at completion of bone healing to have a good long-term stability. The best documented surgical technique to achieve this goal is guided bone regeneration (GBR) utililizing barrier membranes in combination with bone fillers. This clinical review paper reflects 35 years of development and progress with GBR. In the 1990s, GBR was developed by defining the indications for GBR, examining various barrier membranes, bone grafts and bone substitutes. Complications were identified and reduced by modifications of the surgical technique. Today, the selection criteria for various surgical approaches are much better understood, i.e. in post-extraction implant placement. In the majority of patients, biodegradable collagen membranes are used, mainly for horizontal bone augmentation, whereas bio-inert PTFE membranes are preferred for vertical ridge augmentation. The leading surgeons are using a composite graft with autogenous bone chips to accelerate bone formation, in combination with a low-substitution bone filer to better maintain the augmented bone volume over time. In addition, major efforts have been made since the millenium change to reduce surgical trauma and patient morbidity as much as possible. At the end, some open questions related to GBR are discussed.

Conflict of Interest Statement

The authors do not declare any conflict of interest related to this manuscript.

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1. Introduction

The concept of osseointegration laid the basis for modern implant dentistry and a few years ago, it celebrated its 50th birthday.[1] Using osseointegrated dental implants, dental medicine made tremendous progress in the past 50 year for the rehabilitation of fully and partially edentulous patients. This progress was initiated by fundamental experimental studies performed by two research teams in Europe, the team of Prof. P.I. Brånemark from the University of Gothenburg|Sweden, and the team of Prof. André Schroeder from the University of Bern|Switzerland.[2,3] They described the phenomenon of ossointegration with a direct bone-to-implant contact. Both groups published later further papers of high relevance.[4,5] This review paper summarized the knowledge of the 1980s and early 1990s concerning this phenomenon of osseointegration.[6]

In the early developing phase, osseointegrated implants were primarily utilized in fully edentulous patients to stabilize full dentures or fixed dental prostheses. Promising results were reported in retrospective studies by various groups.[7-10] Encouraged by this promising experience, clinicians started to increasingly utilize osseointegrated implants also in partially edentulous patients, and first reports with promising short-term results were published in the late 1980's and early 1990's.[11-14] In the mid 1990s, partially edentulous patients, presenting with single tooth gaps, distal extensions situations or extended edentulous spaces, became a

more common indication for implant therapy in daily practice, and they clearly dominate daily implant practice today, in particular in industrialized countries with a high standard of dental medicine.[15,16]

Concerning the surgical aspects of implant therapy, there are several key-factors for the achievement of successful implants with a good long-term stability.[17] The present clinical review paper will primarily discuss the most relevant key-factor, the circumferential anchorage of the implant in bone after completion of bone healing. It must be kept in mind that implants in the 1970s and 1980s were primarily inserted in healed sites of the jaw, where tooth extractions had taken place several months or even years earlier. Clinical studies have already shown in the 1980s and 1990s that osseointegrated implants without the presence of a buccal bone wall at the time of implant placement had an increased rate of soft tissue complications and/or a compromised long-term prognosis.[18,19] Both studies proposed that potential implant sites with insufficient bone volume should either be considered local contraindications for implant therapy or should

be locally augmented with an appropriate bone augmentation procedure to regenerate the local bone deficiency.

In the 1980s and early 1990s, several new surgical techniques were presented to augment local bone deficiencies in the alveolar ridge. The goal was to overcome these local contraindications for implant therapy. The published papers reported on vertical ridge augmentation using autogenous block grafts from the iliac crest in extremely atrophied jaws,[20,21] sinus floor elevation procedures in the posterior maxilla,[22,23] lateral ridge augmentation using autogenous onlay grafts,[24,25] or split-crest techniques, among them alveolar extension plasty.[26,27]

In the late 1980s, the first experimental studies were published with barrier membranes, at that time called Guided Tissue Regeneration (GTR) or osteopromotion membrane technique.[28-30] Later, a new term with Guided Bone Regeneration (GBR) was created,[31] which is mainly utilized ever since.

2. Development Phase of Guided Bone Regeneration

2.1. Background with Guided Tissue Regeneration (GTR)

The development of GBR utilizing barrier membranes for implant patients was clearly triggered by the clinical application of GTR for periodontal regeneration. GTR was first developed in the early 1980's.[32,33] In the initial studies, Millipore filters were utilized, which also have been tested already in the late 1950s and 1960s for the regeneration of bone defects in experimental studies.[34-36] However, these experimental studies had no impact on the development of new surgical techniques to regenerate localized defects in the jaws, since the potential of this membrane application was probably not recognized at that time.

The first two papers on GTR created at lot of interest and triggered increased research activities in the mid to late 1980s.[37-40] In these early studies, expanded polytetrafluoroethylene (ePTFE) membranes have been utilized, which is a bioinert membrane and became the standard membrane for GTR and GBR procedures during the development phase of both techniques.

The early experimental studies on GBR applied ePTFE membranes only to cover the defects, without the presence of bone grafts or bone substitute materials underneath the membranes.[28-30] The initial study utilized an established "critical size model", 5 mm defects in the mandible of rats with one side serving as test site and the contra-lateral serving as control. Complete bone fill could be noted at 6 weeks in membrane protected defects while little or no bone formation was noted in control sites. In another experimental study, machined titanium implants were placed in the tibia of rabbits leaving several threads exposed. Membranes were placed covering the exposed area leading to a complete bone fill with direct

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contact between the newly formed bone and the titanium surface in the exposed threaded area in contrast to the controls.

In the classical experimental study by Schenk et al. (1994),[41] the bone healing pattern was described in a comprehensive manner confirming the original hypothesis of GBR, that membrane coverage of bone defects creates a suitable environment, often called secluded space for bone regeneration. The suggested effect of membrane placement was considered as following: *(1)* physical support of the overlying soft tissues, thus preventing the collapse of the space necessary for bone formation; *(2)* creation of a space filled with a blood clot into which osteogenic cells can migrate, and protection of the granulation tissue and the delicate vascular network during the organization of the hematoma; *(3)* exclusion of competing non-osteogenic cells originating from the overlying soft tissues, thus preventing scar tissue formation; and *(4)* possible local accumulation of growth factors and other bone-promoting substances. The latter effect has raised renewed interest and will be discussed later in this article. This study also clearly showed that bone formation originated from the bony walls defining the defect, and that bone regeneration followed the well-known pattern of bone development and growth with different maturation steps.[41,42] First comes the fast formation of immature bone with well vascularized woven bone, followed by the deposition of lamellar bone, followed by bone remodeling.

2.2. First clinical experience with GBR and observed complications

In the late 1980s, the first implant patients were treated with the goal to regenerate peri-implant bone defects. At the University of Bern, the arrival of Professor Sture Nyman in early 1988 initiated this development, and the first implant surgery with a GBR procedure was performed in October 1988 with Professor Sture Nyman as surgical assistant. 18 months later, the first two patients were presented as a case report.[43] In the development phase, ePTFE membranes were exclusively utilized, either with a simultaneous or with a staged approach, primarily for horizontal bone augmentation. The simultaneous GBR approach was used for immediate implant placement in post-extraction sites to regenerate peri-implant bone defects,[44,45] or for implants with crestal dehiscence type defects.[46,47] The staged approach was used in patients with healed sites and significant horizontal bone atrophy requiring a horizontal bone augmentation first with the GBR technique alone, and implant placement in a second surgical procedure after 6-9 months of healing.[48] A few years after, the first attempts were also made to use the GBR technique for vertical ridge augmentation.[49,50]

During the initial development phase, several complications were observed with these various GBR approaches, and the surgical procedures were modified to improve the predictability of successful regen-

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erative outcomes. The most frequent complication was a disturbed wound healing with a soft tissue dehiscence leading to a membrane exposure and often to a subsequent infection.[51-54] To avoid this, a lateral incision technique with a bevelled cut was proposed to reduce the risk for soft tissue dehiscences.[55-57] Another frequently seen complication was the collapse of the membrane, which reduced the volume of the secluded space and thus, the amount of regenerated tissue underneath the membrane. In consequence, membrane fixation and supporting screws were developed to fix and stabilize the hydrophobic ePTFE membrane to the underlying bone and, when needed, to support the membrane like tent poles with supporting screws.[55] As alternative to supporting screws, bone fillers such as autografts or allografts were also recommended to physically support the membrane and reduce the risk of membrane collapse.[55,57,58]

Another option to avoid membrane collapse was the utilization of titanium reinforced e-PTFE membranes, which were introduced in the 1990s as well.[59,60]

2.3. Examination of different barrier membranes

In 1993, an expert meeting took place in Sedona/Arizona to discuss the potential and limitations of the GBR technique used in daily practice after 5 years of clinical experience.[61] The experts agreed that the GBR technique – based on the utilization of ePTFE membranes – had the two above mentioned weaknesses and shortcomings. In addition, the need for a second flap procedure for membrane removal in every patient was considered a clear disadvantage. The experts agreed that the GBR procedure should be simplified and the rate of complications reduced by using biodegradable membranes.

The trend for biodegradable membranes was also initiated in the field of GTR in the early 1990s (62,63). At the same time, during the 1990s, numerous animal studies were performed examining different biodegradable membranes for GBR procedures as well.[64-70] In these studies, two different groups of biodegradable membranes were examined.[71]

- Polymeric membranes made of polylactic or polyglycolic acid
- Collagen membranes produced from various animal sources

Parallel to these pre-clinical studies, clinicians started to use biodegradable membranes in patients. The first published clinical reports were predominantly testing collagen membranes.[72-74]

Today, biodegradable, collagen membranes are preferred in the majority of patients, since they are relatively easy to apply during surgery due to their hydrophilic properties. When soaked in blood, they become adhesive and can be easily put into place without the routine use of fixation pins or tacks. In addition, they are characterized by a low complication rate in case of a soft tissue dehiscence.[72-74] The most significant clinical advantage is that collagen membranes do not require a second open-flap procedure

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for membrane removal in contrast to bioinert, ePTFE membranes. This clearly reduces the morbidity for the patient. However, although these collagen membranes have demonstrated excellent biocompatibility in several experimental studies, they have a short barrier function and a tendency to collapse because of their soft characteristics.[69,75-77] Thus, collagen membranes need to be supported by appropriate bone fillers to avoid membrane collapse.

On the other hand, bionert ePTFE membranes or their successor membranes are still used in demanding defect sites such as for vertical ridge augmentation, which will be discussed in a separate paragraph.[78,79]

2.4. Examination of different bone grafts and bone substitutes

Besides the selection of an appropriate barrier membrane, the selection of appropriate bone fillers for GBR procedures is at least as important for the regenerative outcome of GBR procedures. As already mentioned, allograft particles or autograft chips have been used in the beginning, but primarily from a mechanical point of view to avoid membrane collapse. In the mid 1990's, a first pre-clinical study by Buser et al.[80] examined various bone fillers using standardized bone defects in the mandibular angle of miniature pigs covered by ePTFE membranes. The study showed that particulate bone fillers have two different biological characteristics relevant for GBR procedures: (a) their osteogenic and osteoconductive potential, and (b) their substitution rate during healing and bone remodeling. Significantly more new bone was formed in defects filled with locally harvested autograft chips, as compared to demineralized freeze-dried bone allograft (DFDBA), a synthetic ß-tricalciumphosphate (ß-TCP), and coral-derived hydroxy-apatite (HA). This osteogenic stimulation was attributed by the authors with the favorable bone-to-volume ratio of cortical bone chips and obviously excellent osteoconductive properties of these chips as documented by a high percentage of woven bone formation (87% at 4 weeks) directly deposited to the surface of the applied bone chips. In addition, it was speculated that the release of growth factors would be involved in this process. On the other hand, coral-derived HA particles showed the lowest substitution rate, whereas autograft chips and ß-TCP particles were resorbed very quickly. Based on this first study, a series of pre-clinical studies conducted by Jensen et al.[82-84] examining a wide range of additional bone fillers in the same animal model such as deproteinized bovine bone mineral (DBBM), synthetic biphasic calcium phosphate (BCP), and others. These studies all confirmed the first observation that autograft chips always showed the highest rate of new bone formation during early healing, whereas DBBM as a bovine HA bone filler had the lowest substitution rate over time when compared with all other bone fillers tested in these

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studies. The volume percent of DBBM particles remained around 25 to 30% up to one year of follow-up in this miniature pig model. A similar low resorption rate showed BCP particles, which is a synthetic HA/TCP combination, when the tested material had a high content of HA (60 or 80%). The histological analysis showed that osteoclasts were obviously not able to resorb DBBM and BCP particles during the remodeling process as they did with all other bone fillers examined. Later, the low substitution rate of DBBM has been confirmed in a clinical study with 10 patients and 12 implants.[84] The histologic section has shown good integration of DBBM particles in vascularized bone and a mean volume percentage of 32% in these 12 biopsies. This is a fascinating area of pre-clinical, translational research. A detailed and interesting overview has been written by Bosshardt et al.[85] in the recently published GBR Book (3rd edition).

Several research groups concluded that it is beneficial to combine two different bone fillers with synergistic properties. Firstly, autologous bone chips as osteogenic bone filler to stimulate the speed and volume of new bone formation during the early healing phase, hereby speeding up the healing process which helps to reduce the necessary healing period. Secondly, DBBM particles with a low substitution rate utilized for volume stability to maintain the created volume of newly formed bone over time during the ongoing bone remodeling. This synergistic combination is called «Composite Graft», and clinically used either as a 2-layer or as a mixed composite graft. This biologically inspired concept has been documented with clinical and radiographic studies for GBR procedures, either for horizontal or vertical ridge augmentation using the GBR technique.[79,86-88] A typical case report with early implant placement with simultaneous GBR for horizontal contour augmentation is shown in Figs. 1a-z.

3. Routine Application and Fine-tuning Phase of GBR since the early 2000's

Since the millennium change, several aspects helped to improve the GBR technique further. The main objectives during this routine application and fine-tuning phase were as follows:

1. Improve the regenerative outcome of GBR and reduce the complication rate
2. Reduce the morbidity for the patients
3. Reduce the healing periods for the patients

Major improvements could be made based on new developments in the imaging technique and a much better understanding of tissue biology.

3.1. The CBCT technique as the new 3D radiographic methodology

The development of the Cone Beam Computed Tomography (CBCT) started in the late 1990's and represents one of the most important developments in implant dentistry in the past 25 years. [89] This new 3D radiographic technique allowed cross-sectional imaging with a much better image quality and a clear reduction in radiation exposure for the patient, when compared with the computed tomography technology

used for dentistry in the 1990's.[90,91] The CBCT technique allows cross-sectional imaging not only for the pre-operative examination of patients, but also for the follow-up documentation of bone augmentation procedures, and for radiographic studies to evaluate the bone anatomy in the jaws.[92-94] In addition, CBCT is also one of the basic techniques necessary for the utilization of digital technology, including computer-assisted implant surgery (CAIS) in patients.[95,96]

3.2. The characterization of the defect morphology

As mentioned above, the most important advantage of CBCT is the detailed pre-operative examination of patients, since it helps to assess the exact local bone anatomy in potential implant sites. As already discussed, the bone walls defining the bone defect are the origin of bone formation, since they offer on the one hand access to trabecular bone as source of precursor cells of angioblasts and osteoblasts, both very important cells for new bone formation. For this reason, it is generally recommended to perforate a cortical bone surface to open the bone marrow.[49,55-57,78] On the other hand, the anatomic configuration of existing bone walls is an important factor for the stabilization of applied bone fillers in the augmentation area.

The most important difference is between a horizontal and a vertical bone defect, requiring a horizontal or vertical bone augmentation procedure, respectively. Vertical bone defects are probably the most difficult for the surgeon, since it is always a 1-wall defect and the stabilization of applied bone fillers is challenging. Fortunately, these defects are rather rare and must be operated by master surgeons. Today, vertical bone augmentation is mostly done with ePTFE or PTFE membrane, often reinforced with titanium, and the hydrophobic membrane must be stabilized with tacks or miniscrews.[50,79]

Horizontal bone defects are much more diverse, and we differentiate between 3-wall, 2-wall and 1-wall defects.[97] The best situation for a successful regenerative outcome is a contained 3-wall defect, which are present in fresh extraction sites with fully intact socket walls following extraction. When the inclusion criteria for immediate implant placement are met,[98,99] this surgical procedure does not require a GBR technique anymore, since immediate implant placement is mainly done flapless today with an internal grafting.[100] 2-wall defects can vary a lot and we differentiate the 3-dimensional defect extension in a corono-apical, mesio-distal and oro-facial direction.[97] The corono-apical or vertical dimension of the defect is not important, since the bone regenerates in a 2-wall defect from the lateral bone walls. The mesio-distal extension, however, is an important parameter as documented on immediate implants placed with simultaneous GBR.[101] The authors found at the 1-year examination for extraction sites with an U- and UU-shaped defect a significantly higher frequency and magnitude of facial gingival recession (>1.5 mm) when compared with V-shaped defects. The third and last defect dimension is the oro-facial defect depth, mostly on

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the buccal aspect. A deep defect between 1-3 mm from the buccal bone wall to the exposed implant surface is much more favorable, since large areas of bone walls are available for bone formation. In addition, such a configuration helps to stabilize applied bone fillers. In these sites, the stabilization of membrane with tacks is not required (97). In shallow defects (<1mm) or when the exposed implant surface is even slightly outside the alveolar ridge, the situation is much more demanding for implant placement with simultaneous GBR. In such situations, fixation of the membrane with pins or tacks is advisable to stabilize applied bone fillers. This procedure is often called sausage technique.[102,103]

Horizontal 1-wall defects are most often healed ridges with significant horizontal ridge atrophy, most often on the buccal aspect. In such cases, a staged approach has been recommended. The utilization of autogenous block grafts covered by a barrier membrane is also a well document bone augmentation technique.[104-106] The autogenous bone block is fixed with a screw, and hence, the required stability in the augmentation area is easily achieved. However, this approach requires the harvesting of a block graft from the chin or the retromolar area, which adds additional morbidity for the patients. An alternative is again the sausage technique utilizing a mixture of autologous bone chips mixed with DBBM particles, covered by a tack-fixated collagen membrane.[102,107]

3.3. Improved knowledge of tissue biology in post-extraction sites

The progress in this field was initiated in the early 2000 by fundamental studies on bone alterations in post-extraction sites performed by the group of Lindhe and coworkers in Gothenburg/Sweden. In the beginning, a series of experimental studies in beagle dogs helped to understand the concept of bundle bone resorption in post extraction sites.[108-110] These studies showed a quick resorption of the buccal bone wall following extraction with a vertical reduction of more than 2 mm. It was speculated that a thin buccal bone wall is mainly composed of bundle bone and gets resorbed post extraction due to avascular necrosis. This histological observation was confirmed in a clinical study using the CBCT technique.[111] Sites with a thin buccal bone wall (< 1mm) demonstrated a median vertical bone reduction of 7.4 mm after 8 weeks of healing, whereas sites with a thick buccal wall (≥ 1mm) had one of only 1.1 mm. This improved biologic knowledge was fundamental for the definition of selection criteria used in post-extraction implant placement discussed at the 4th ITI Consensus Conference in 2013.[98,99]

3.4. The technique of socket grafting for alveolar ridge preservation

In the mid 2000s, the group of Araujo & Lindhe[112] showed in a first pre-clinical study that socket grafting following tooth extraction with DBBM was able to significantly slow down the observed ridge alterations post extraction. The goal of socket grafting is *Alveolar Ridge Preservation* (ARP), which is the term utilized

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today, although the ridge volume cannot be fully preserved with socket grafting. The study has triggered a lot of research activites in the past 15 years by testing different grafting materials and different surgical techniques with various clinical applications.[113-119] These studies and systematic reviews all showed that socket grafting does not stop bundle bone resorption, but it is an effective therapy to reduce the dimensional changes of the alveolar ridge that normally takes place after tooth extraction. It is estimated that the ridge volume reduction is reduced to about 10-15% when ARP is utilized. A recent systematic review also showed that the reduction in ridge volume following socket grafting is less pronounced in extraction sockets with a thick buccal wall, when compared to extraction sites with a thin buccal wall.[120]

In the past 10 years, socket grafting for ARP has become a valuable treatment option for clinicians in post-extraction implant placement,[99] and is increasingly utilized by our group in posterior implant sites, i.e. in elderly patients when surgical procedures with a low morbidity are clearly preferred. In such situations, socket grafting often allows flapless implant placement 4 to 6 months later using computer-assisted implant surgery.[121] Hence, when socket grafting with ARP is applied, this approach reduces the necessity of GBR[122] in particular in posterior implant sites, when the esthetic outcome is not the first priority of therapy.

3.5. Better understanding of the biologic properties of autologous bone chips

As already discussed above, a series of pre-clinical studies have been performedin the 1990s and 2000s to examine the characteristics of bone grafts and bone substitutes. The superiority of autologous bone chips concerning osteogenic properties was striking as shown in histomorphometric analysis. To better understand the biology behind this phenomenon, a series of *in-vitro* studies using cell cultures followed in the 2010s. The studies showed that these bone chips start to quickly release growth factors (GF) within a few minutes when stored in a mixture of blood serum and isotonic NaCl solution (0.9%). The most important GFs detected were TGF-β1 and BMP-2, both potent GF for osteogenesis, and this blood mixture containing GFs has been called *Bone Conditioned Medium (BCM)*.[123-126] It was also interesting to show that BCM is able to biologically activate bone substitute particles and barrier membranes for GBR procedures.[127-129] The BCM concept and the interplay between TGF-β1 and BMP-2 for osteogenesis have been described and summarized in detail by Asparuhova in Chapter 3 of the recent 3rd edition GBR Book (Fig. 2).[130]

4. Recent research activities related to GBR

In recent years, translational research and material innovations in the field of GBR have been ongoing with the goal to further improve GBR procedures for the benefit of patients. This included new developments

concerning barrier membranes, attempts to revisit the principle of GBR and the activities around platelet concentrates.

4.1 Membrane evolution for guided bone regeneration

In recent years, a revival for PTFE membranes can be noted both in the scientific and clinical community. This is largely based on the superior regenerative outcome reported in association with these membranes. The main issue to overcome, apart from an additional removal procedure, has been membrane exposure during healing. It has been reported that a novel dual configuration e-PTFE membrane promotes better protection against bacterial biofilm formation due to the lower bacterial accumulation on the surface in contrast to solid PTFE membranes.[131] Moreover, an increased soft tissue attachment has been reported for the dual configuration ePTFE membrane in contrast to PTFE membranes.[132] In order to improve soft tissue attachment for PTFE membranes, a perforated PTFE membranes has been developed and introduced in the clinical setting.[133]

In an unpublished preclinical study, dense PTFE membranes have been compared with a perforated dPTFE membrane in chronic vertical ridge defects (Urban et. submitted for publication). Similar new bone formation has been observed histologically and radiographically, when comparing the two membrane types. However, significantly higher vascularization and a clear tendency towards bone maturation was observed when the perforated membrane was used. The clinical experience in patients demonstrated similar findings. In a recent publication,[133] 65 sites with vertical bone defects were treated using a tack-stabilized perforated PTFE membrane in combination with a mixed composite graft. The authors experienced a better bone quality after healing when compared with the clinical experience with non-perforated PTFE membranes. However, this was not compared directly in this study. The observed complication rate was 3%, which is comparable with publications of the same group,[133] however less than findings in review papers looking at vertical ridge defects.[79] Future research must now compare perforated vs. non-perforated PTFE membranes for vertical ridge augmentation in well-designed randomized clinical trials to confirm these findings. A typical case report for vertical ridge augmentation with a mixed composite graft and a perforated dPTFE membrabe is shown in Fig. 3a-y.

Currently, membrane technologies are evolving in terms of mechanical properties and topography properties aiming to achieve faster biological responses and significant antibacterial properties. Surface nanotopography applied to membranes for tissue regeneration showed to induce fibroblast orientation and a faster healing time in *in vivo* models.[134] For resorbable membranes, cross-linked collagen structures demonstrated favorable results in a clinical study using a cone beam CT analysis.[135] In addition, polymeric

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membranes revealed the possibilities for incorporation of molecules/drugs accelerating beneficial outcomes in *in vitro* studies that may be translated for future clinical products.[136] Hence, the current target of scientific studies is to understand the molecular activity around membranes with respect to both soft- and hard-tissue in order to create multifunctional actions to improve bone healing, protect bacterial contamination and promote a stable soft-tissue coverage during healing.

4.2. The biological principle of GBR revisited

In recent years, novel findings have implied that the principle of GBR, by utilizing barrier membranes, not only acts as a passive barrier aiming at separating hard and soft tissues, protecting from the invasion of soft-tissue cells but also promotes an environment with wide osteogenic potential under the membrane. The findings implies that membrane application stimulates an active role on molecular events close to the biomaterial. At the first interaction with oral tissues, barrier membranes for guided bone regeneration induce different biological responses by the tissue cells that are dependent on their surface properties (topography, wettability, chemistry, roughness, porosity) and biocompatibility characteristics. It has been reported that these features impact the level of absorbed proteins, and consequently may modulate the host inflammatory responses.[137,138]

A single biomaterial that promotes all the expected biological responses for complete tissue healing still has not been revealed in the market. Therefore, ongoing research is still performed in order to clarify the biological events around the different types of membranes. Recent studies have shown that the molecular events underneath a biodegradable membrane promote and direct molecular cascades rather than only act as a passive biomaterial.[132,139] These events close to the membrane stimulates the migration of specific cells, which promote the expression and the release of pro-osteogenic factors, generating a rapid bone neoformation and remodelling underneath the membrane. Thus, suggesting a direct and more active role of the membrane in the healing process.

Corroborating this idea, studies investigating molecular events during application of non-degradable membranes showed a higher presence of Cbfa1/Runx2 at 6 days of healing and increased expression of osteocalcin at 8 and 10 days in the upper region of the defects, showing relevant molecular upregulated gene-expression associated to bone healing.[138] Additionally, in an experimental study, the application of PTFE membranes induced a significant difference between the gene expression and molecular pathways at 7 days and at 15 days, respectively. A time-dependent impact during the healing of defects with membranes was demonstrated, suggesting an upregulation of inflammatory and immune response at day 7 and a higher expression of bone formation processes at day 15, promoting different

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healing pathways underneath the membranes according to the stage of healing.[140] Clinically, the coverage of periodontal bone defects with PTFE membrane compared to non-covered defects showed upregulation of bone markers such as alkaline phosphatase, osteopontin and BSP underlying the bone defect after 3 weeks.[141]

For resorbable membranes, similar findings were revealed; collagen membranes induced the upregulation of important genes for bone healing at the interface membrane/defect such as osteocalcin, cathepsin K and RANKL compared to analysis without the application of a membrane.[132] Additionally, immunohistochemical investigations demonstrated positive performance for bone proteins (alkaline phosphatase, osteocalcin and osteopontin) close to the lower side of collagen membranes, indicating a possible bioactive action of this specific biomaterial.[142] These results were corroborated by histological and microscopy analyses demonstrating bone formation and mineralization underneath resorbable membranes in *in vivo* models.[143]

The compilation of results described above demonstrates that the membranes play a primary and bioactive role in the process of guided bone regeneration. However, the large variety of membranes available, different protocols for research, different defect sizes evaluated, among other factors make a comprehensive analysis of the data difficult to perform. Therefore, consistent information regarding every type of membrane (non-degradable, resorbable, synthetic) and its impact directly on the surrounding tissues is still scarce. Furthermore, clinical investigations should be associated with pre-clinical evaluation in order to achieve a more complete understanding of the GBR process.

In this context, the use of titanium meshes (customized or not to the alveolar bone defect) has been advocated as adjunctive measure to GBR procedures in the goal of assisting in the achievement of space creation and maintenance. Nonetheless, the stifness supplied by the meshes often leads to its exposure and this can be detrimental for the regenerative outcome since it is associated with subsequent complications such as infection and significant graft resorption. Moreover, these titanium meshes alone without barrier membrane do not fullfil the principle of compartimentalization since there is no cell occlusion effect. Recent studies have shown that using traditional or computer-generated titanium meshes have similar complication rates of about 20%, which is higher than reported outcomes of vertical augmentation with the GBR technique.[133, 144-146]

4.3 Is the PRF technique a barrier membrane?

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Platelet concentrates collected from blood was first introduced by the team of Marx[147,148] reporting on platelet-rich plasma (PRP) roughly 25 years ago. It was based upon the concept that growth factors derived from human blood proteins, such as platelet-derived growth factors (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-beta), insulin-like growth factor (IGF) and epidermal growth factor (EGF), which may support angiogenesis and tissue ingrowth. In fact, platelets are key components affecting hemostasis and blood clot formation during the early phases of healing in tissue regeneration.[149] Moreover, cytokines, chemokines and angiogenic factors including fibroblasts, neutrophils, macrophages and mesenchymal stem cells have been shown to be released by platelets. In the early 2000s, the preclinical examination of PRP did not result in supporting evidence demonstrating ability to stimulate the formation of bone.[150,151] Hence, PRP use has significantly been reduced from daily practice after a few years. A rejuvenation of platelet concentrates was noted roughly 10 years ago with the development of platelet rich fibrin (PRF). It was claimed that the centrifugation strategy including speed and/or the use of anti-coagulants such as $CaCl_2$ dictates the amount, properties and cells containing in the matrix. Accordingly, PRF was developed using a centrifugation protocol that requires 1 cycle or 12 minutes at 2700rpm.[152]

Three dominant components are provided by PRF during tissue regeneration. First, major cell types, including platelets, leukocytes, macrophages, granulocytes, and neutrophils, which are key during immune regulation. In addition, a natural fibrin matrix, which may enhance the manageability and its use for bone regeneration as membrane or to improve the handling/stability of the bone graft. Finally, PRF contains cytokines for rapid proliferation and migration of mesenchymal cells. Moreover, PRF seems to play a role on periosteum behaviour. As such, it was noted that following the use of PRF there is a stimulation of the capacity of blood supply within the periosteum which led to enhanced properties, including thickness and characteristics of the soft tissues over the area presenting bone regeneration.[153]

Concerning the clinical effectiveness of PRF on bone formation in GBR procedures, the most recent systematic reviews are still inconclusive.[149,154,155] The current evidence suggests that PRF offers little or no clear advantage to promote new bone formation as evaluated in various studies on GBR and sinus floor elevation procedures, nor in implant stability and treatment of peri-implantitis. Nevertheless, it is worth noting that up to date, the heterogeneity in study designs and protocols, and the lack of appropriate controls and available information regarding patient selection preclude delivering conclusive statements on its effectiveness. Clinical research must continue to test the effectiveness of PRF in well controlled studies.

Conclusions

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Based on the past 35 years of development and progress with Guided Bone Regeneration (GBR) in implant patients, the following conclusions can be made:

- The development of GBR was initiated in the late 1980s to regenerate local bone defects in implant patients.
- In this early development phase, the bioinert ePTFE membrane was the only membrane utilized with the goal to achieve a horizontal bone augmentation using either a simultaneous or a staged approach.
- Several clinical complications were frequently observed such as soft tissue dehiscences with membranes exposure, often leading to an infection, membrane collapse or insufficient bone regeneration in the membrane protected space.
- In addition, the need for a second flap procedure to remove the non-resorbable membrane was a clear disadvantage, since it increased the morbidity for the patient.
- In the early 1990s, pre-clinical and clinical research was intensified to improve the clinical outcomes and reduce observed complications. This included the examination of bone grafts and bone substitutes, the testing of resorbable barrier membranes, the utilization of membrane fixation screws and tenting poles, and modifications of the incision technique.
- Parallel, the indications for the GBR procedures were expanded to vertical ridge augmentation.
- As result, the utilization of composite grafts, either in 2-layers or mixed, has become the gold standard for GBR procedures, since autologous bone chips serve as osteogenic filler to speed up the formation of bone during early healing, whereas the second filler with a low-substitution rate – such as bovine bone fillers – is used to maintain the augmented bone volume over time. In addition, the bone fillers support the membrane and eliminate the risk for a membrane collapse.
- Today, the surgeon has two groups of barrier membranes available for GBR, the group of resorbable membranes, most often collagen membranes, and the group of non-resorbable, bioinert PTFE membranes.
- Collagen membranes, either non-crosslinked or crosslinked, are used in the majority of patients for the regeneration of small and medium sized defects. Most important advantages are an easy surgical application, a low complication rate during healing, and no need for a second open flap procedure for membrane removal.
- PTFE membranes, expanded or dense, non-perforated or perforated, are primarily utilized for complex and very demanding clinical situations with large bone defects, in particular for vertical ridge aug-

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mentation. They require membrane fixation with screws or tacks, an expanded healing period up to 9 months, and always a second open flap procedure for membrane removal.

- In specific clinical situations, the frequency of GBR procedues has been reduced, since GBR was replaced by other surgical techniques.
- The first one is socket grafting following flapless tooth extraction for alveolar ridge preservation (ARP). ARP has become a valuable surgical protocol in particular in elderly patients to minimize surgical morbidity for patients, but also in posterior implant sites, where the esthetic demand is not first priority. The second one is immediate implant placement with a flapless approach, since bone regeneration is done with an internal grafting.

In summary, GBR is today the most important surgical technique for localized bone augmentation in the field of implant dentistry. The progress made over the past 35 years for horizontal and vertical ridge augmentation is impressive and the documented long-term stability of regenerated bone as well. The local defect anatomy is most important to select the most appropriate bone augmentation procedure, including the selection of appropriate biomaterials. A key element for success is proper case selection based on well defined selection criteria. In addition, it is important to keep an open mind for further progress in this area.

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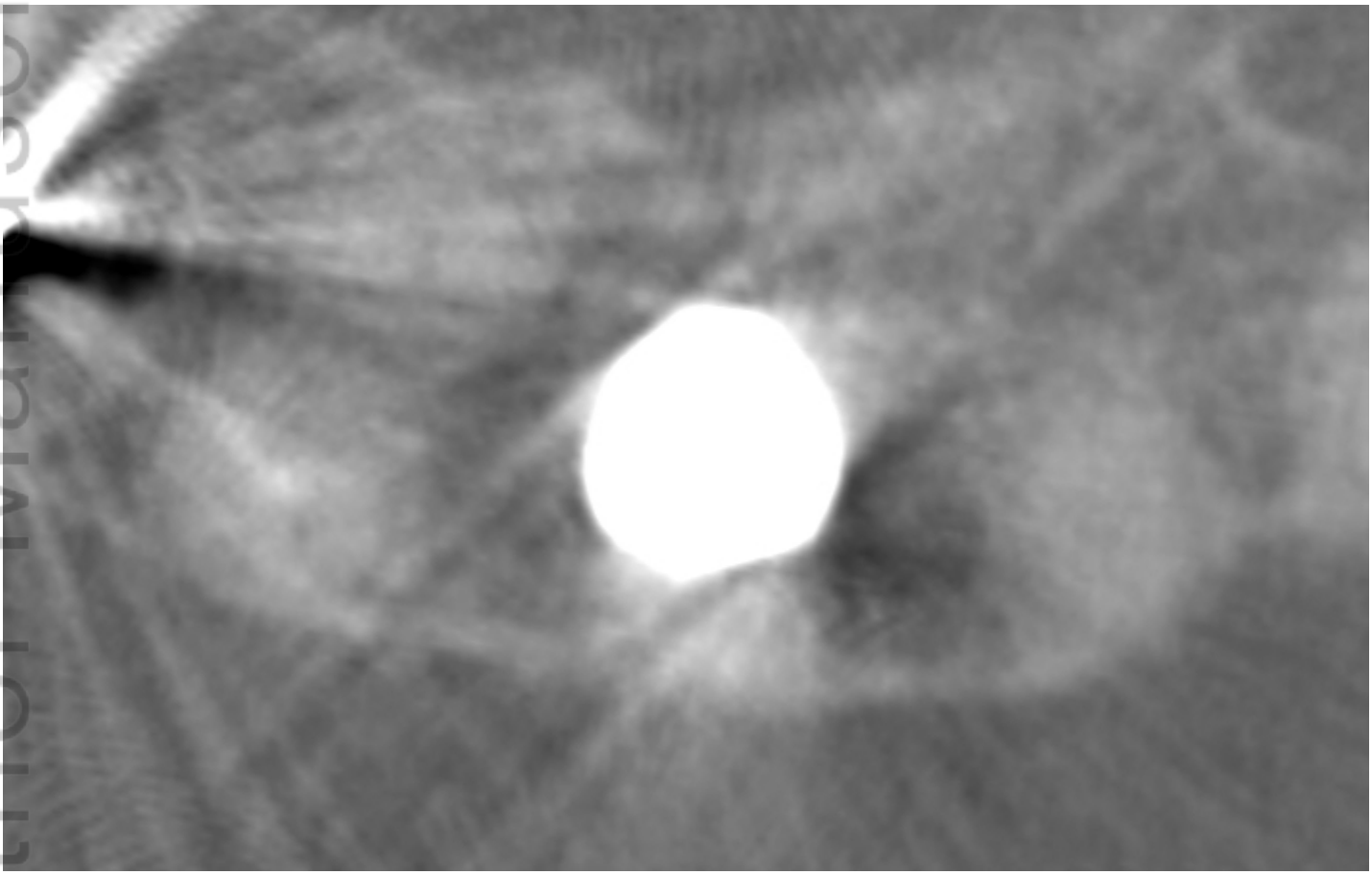


Fig.1z.jpg

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Fig. 1a.jpg

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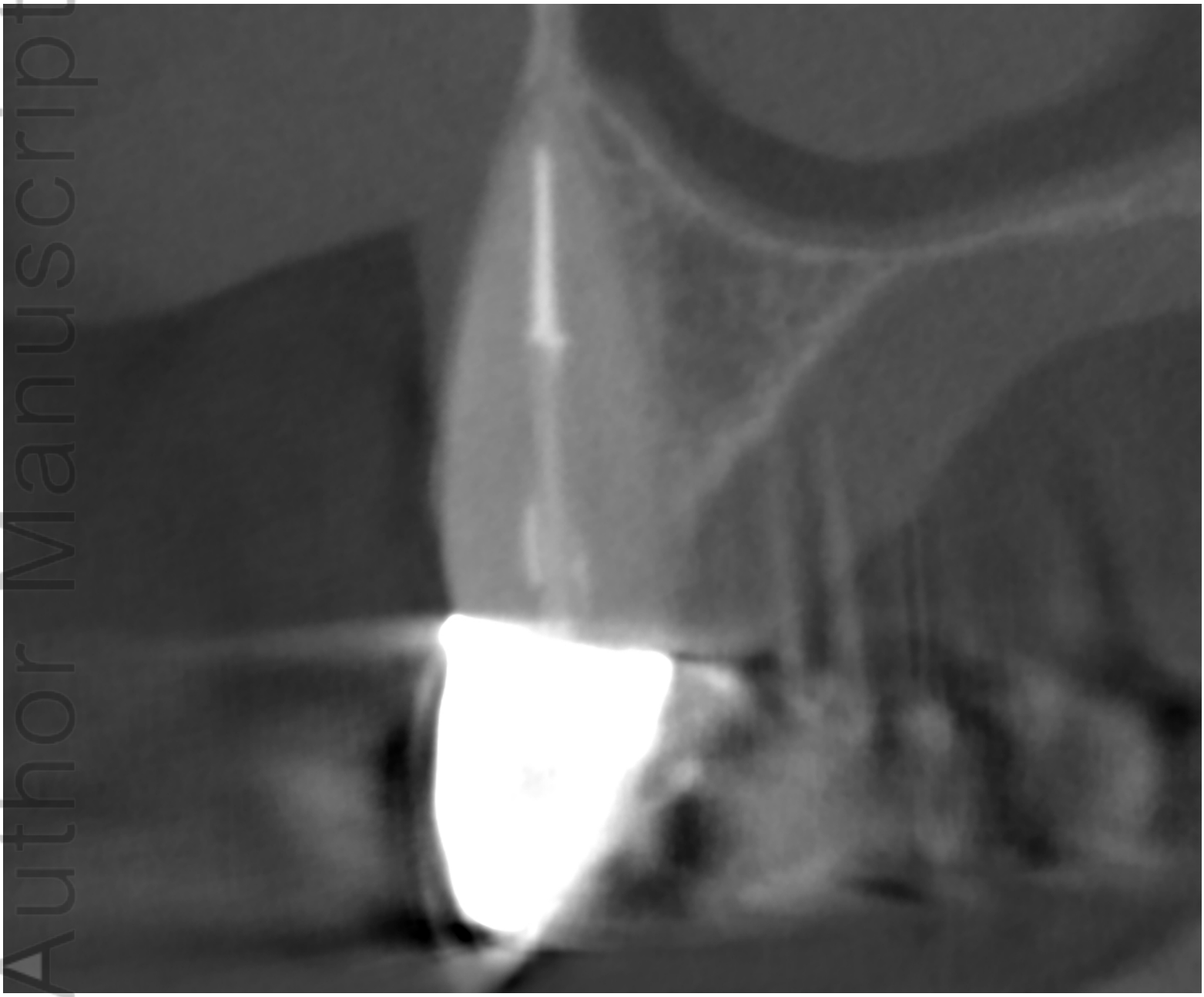


Fig. 1b.jpg

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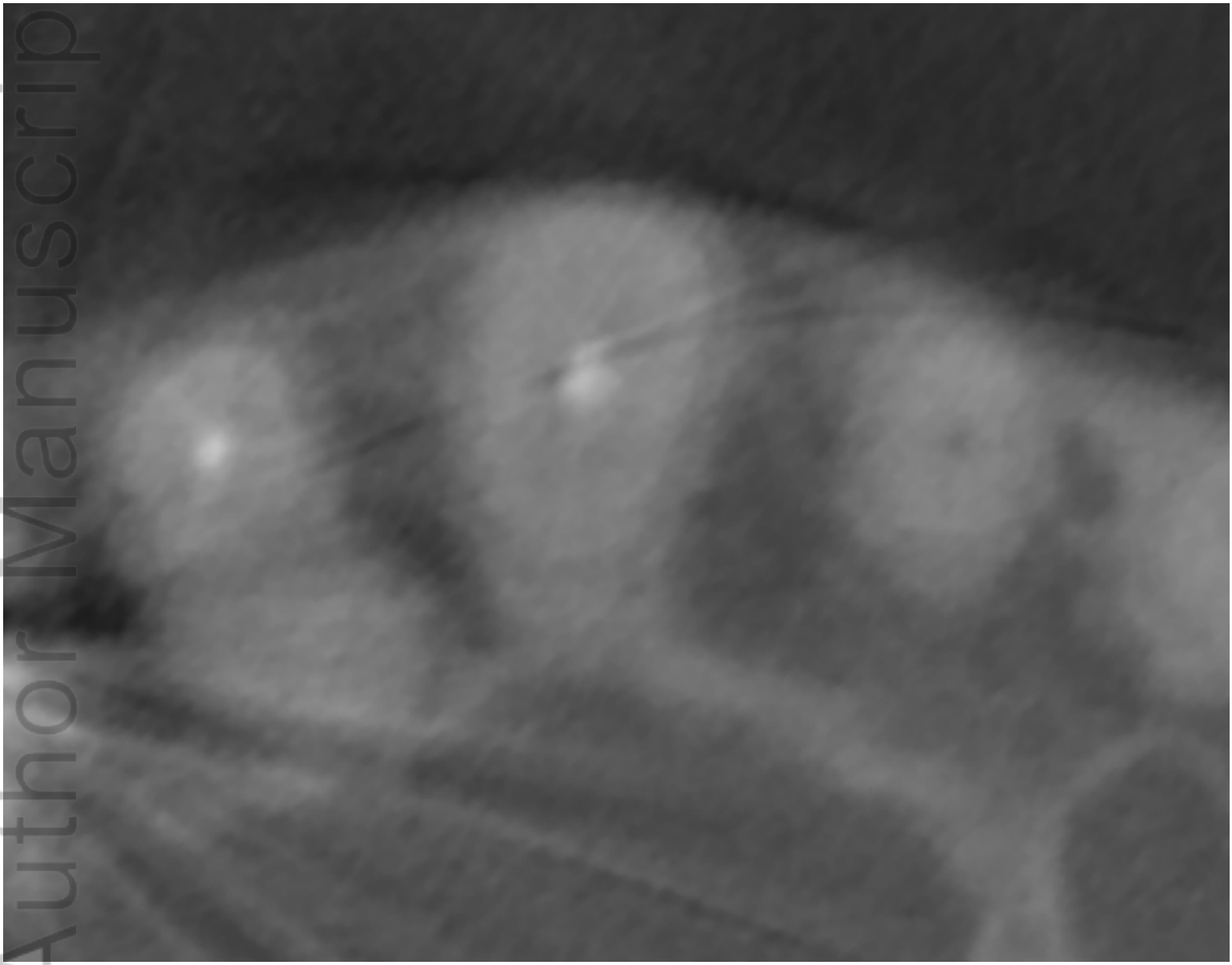


Fig. 1c.jpg

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Fig. 1d.jpg

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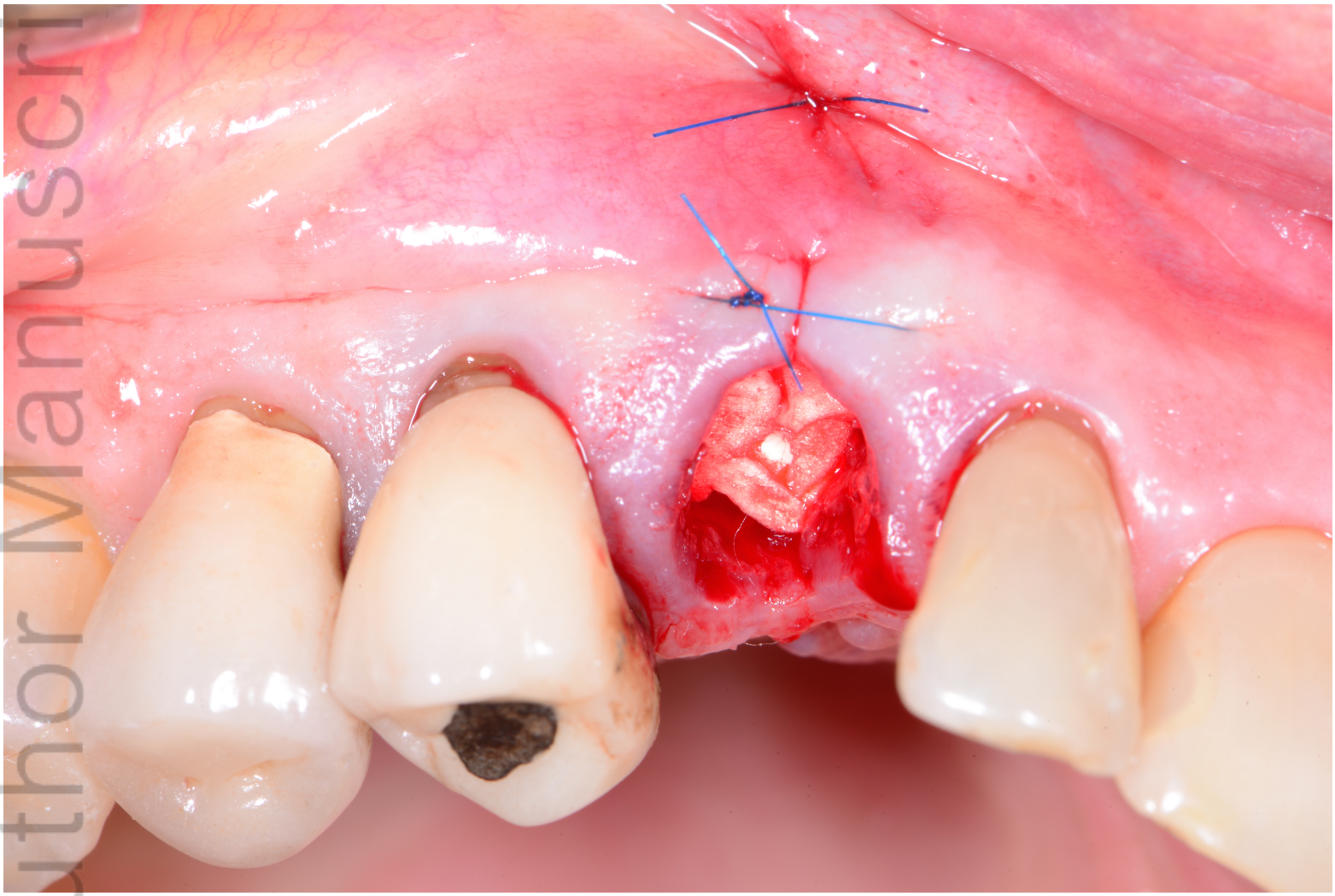


Fig. 1e.jpg

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Fig. 1f.jpg

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Fig. 1g.jpg

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Fig. 1h.jpg

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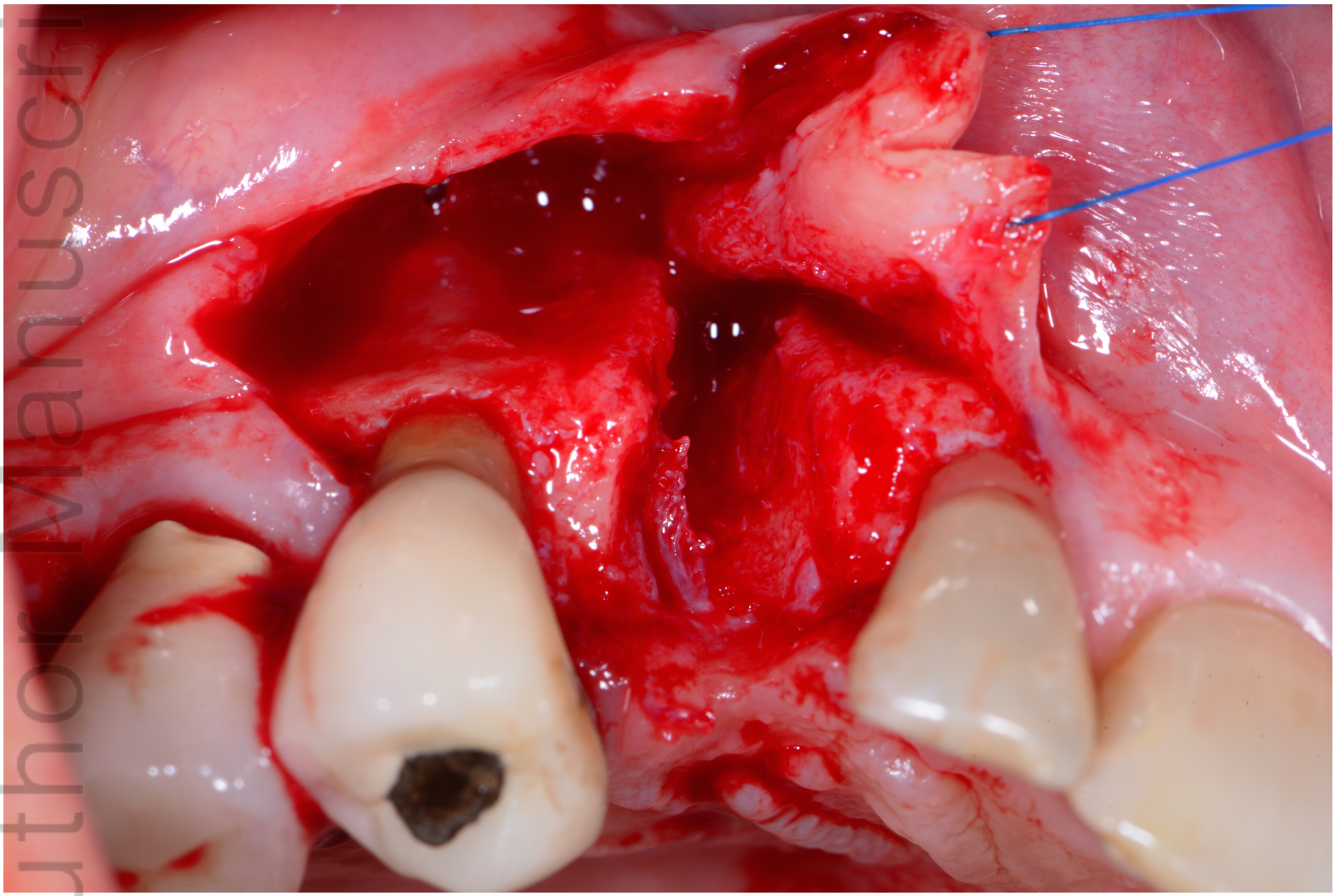


Fig. 1i.jpg

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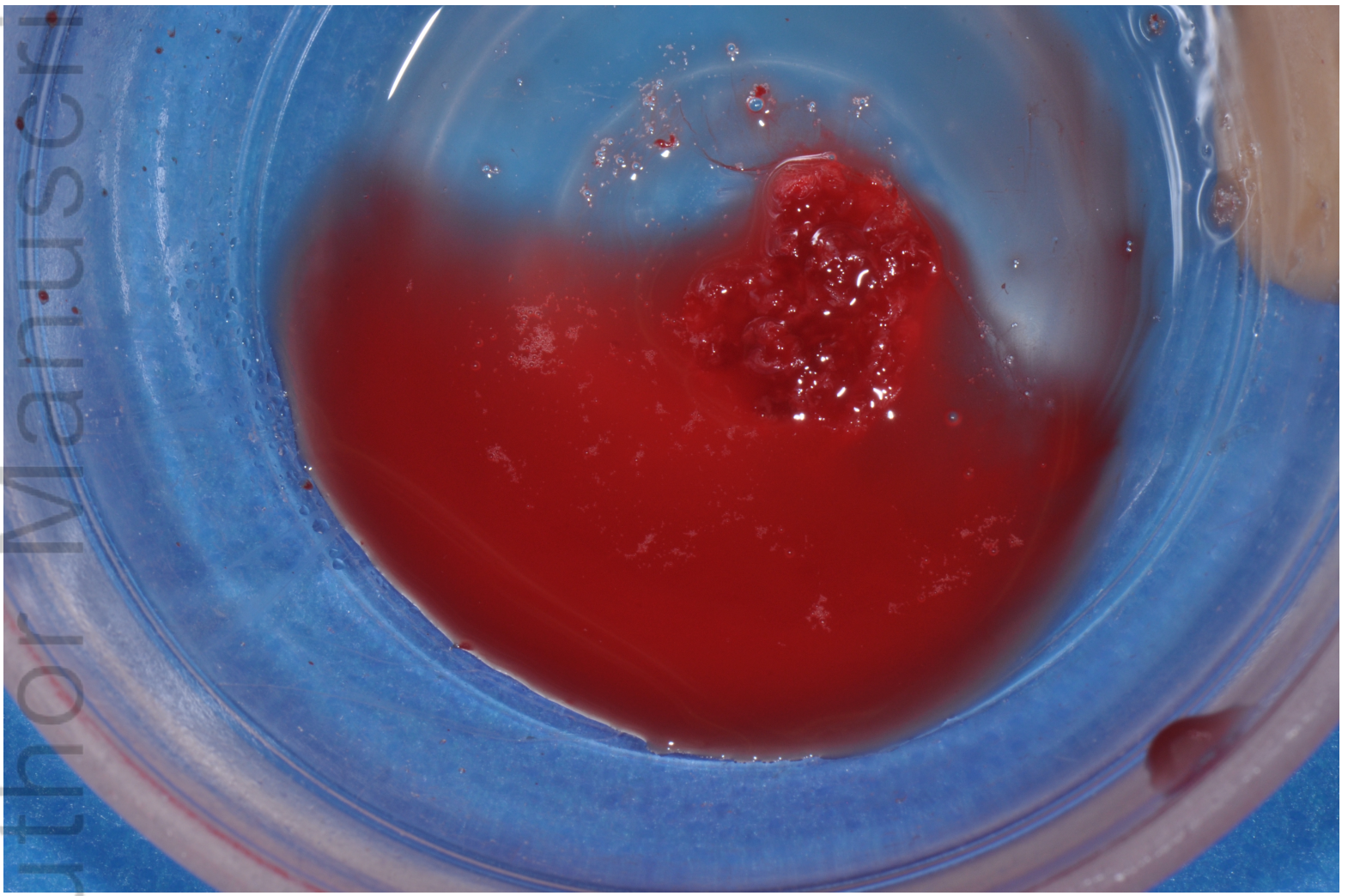


Fig. 1j.jpg

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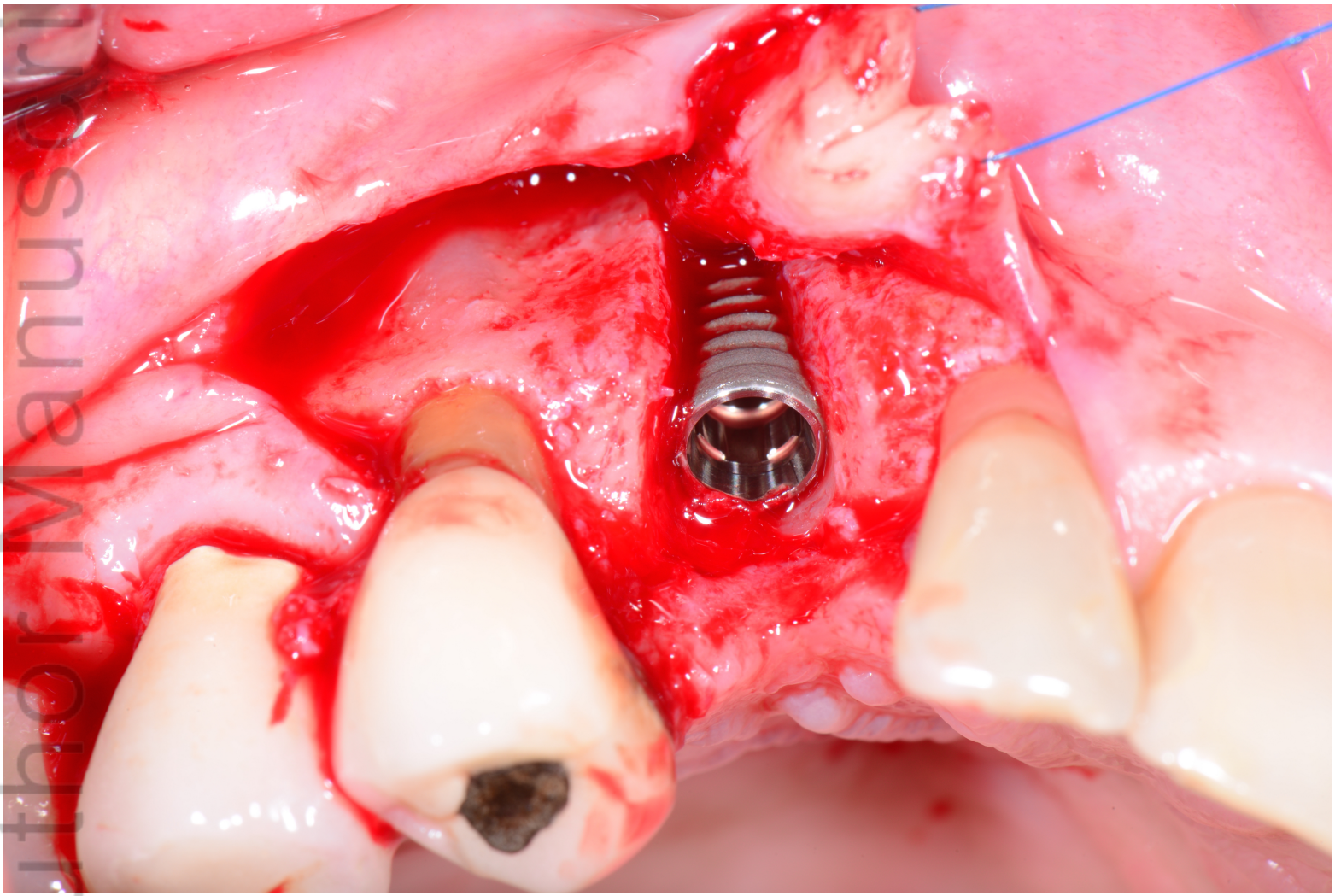


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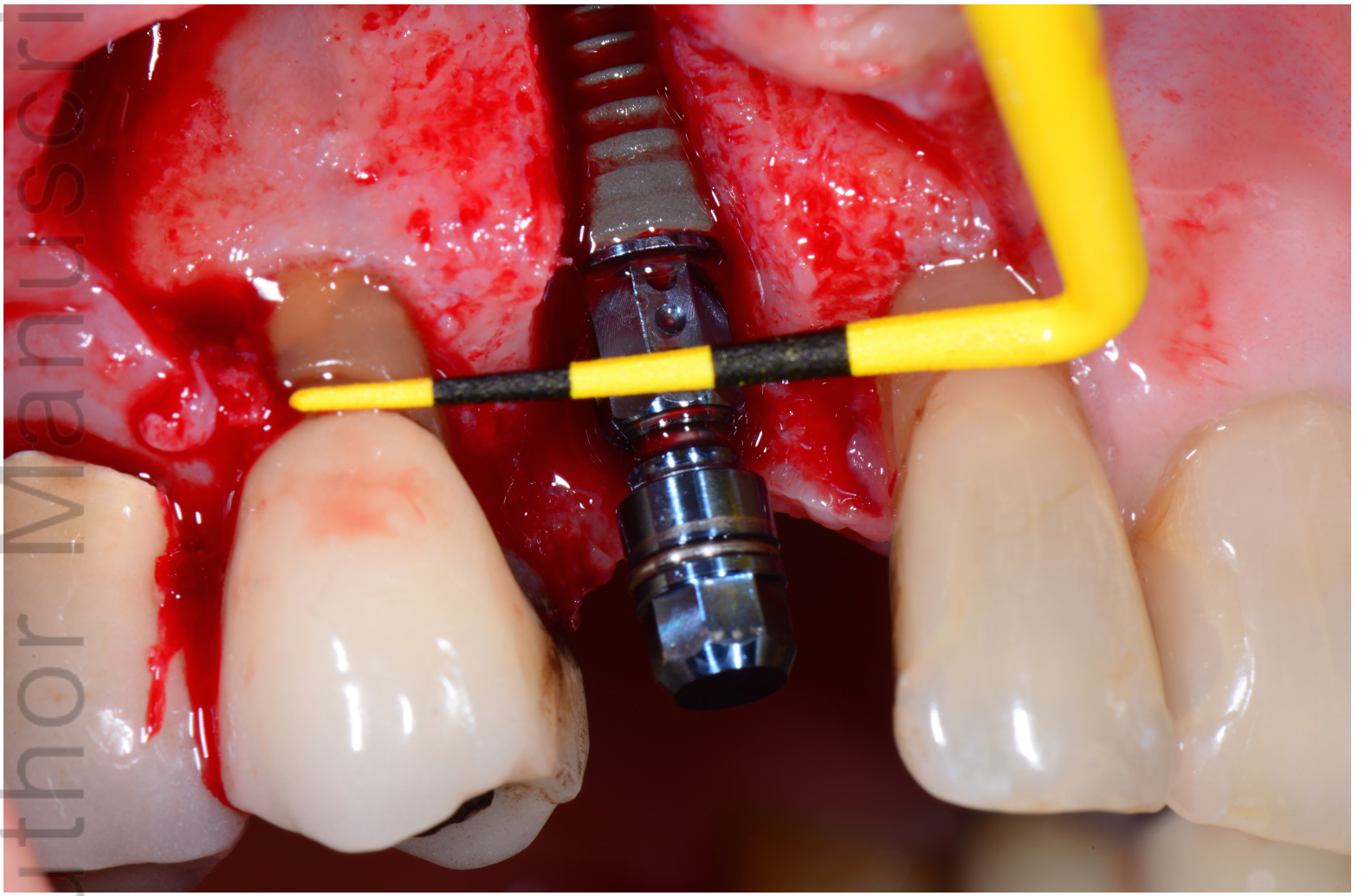


Fig. 1l.jpg

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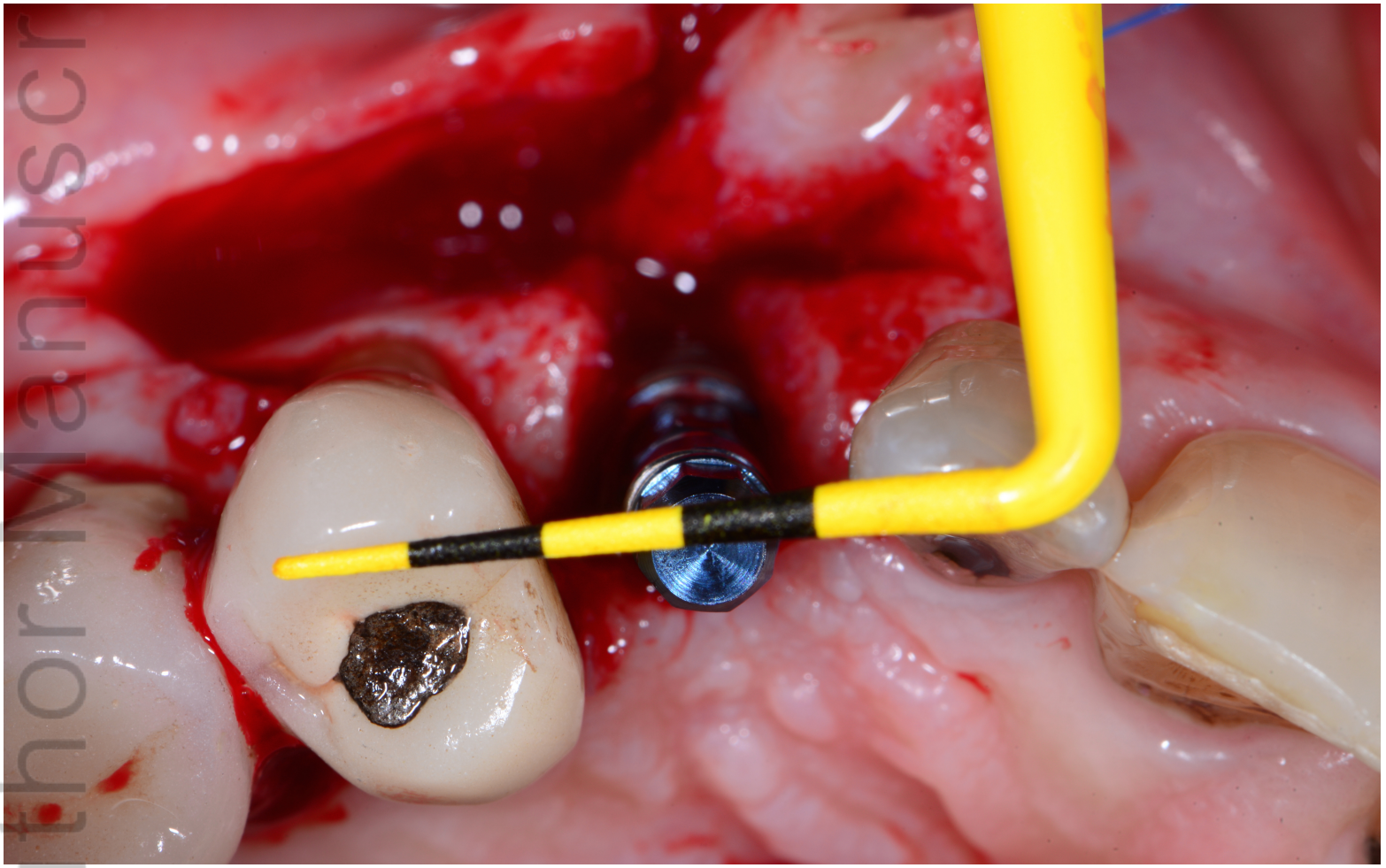


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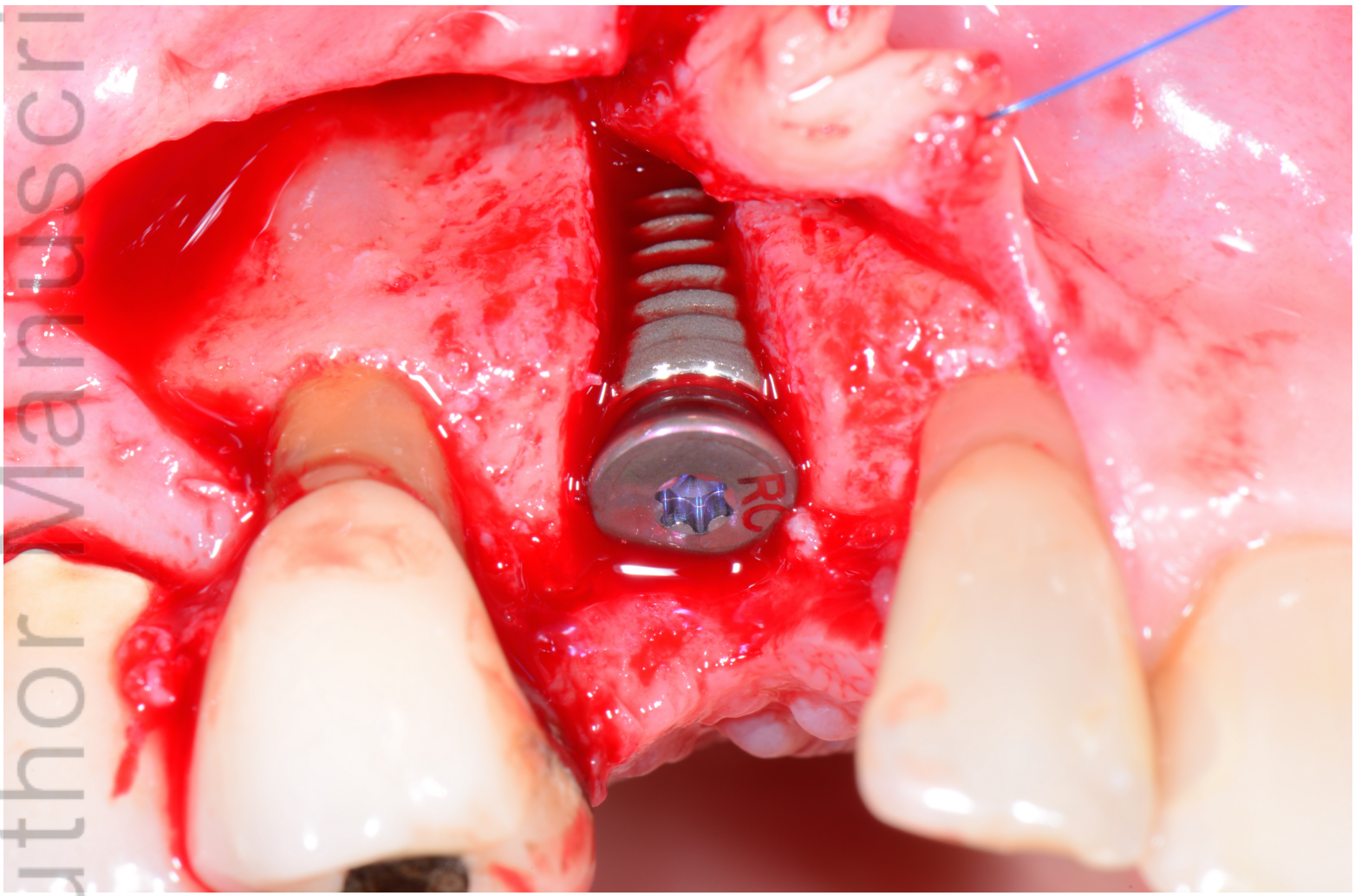


Fig. 1n.jpg

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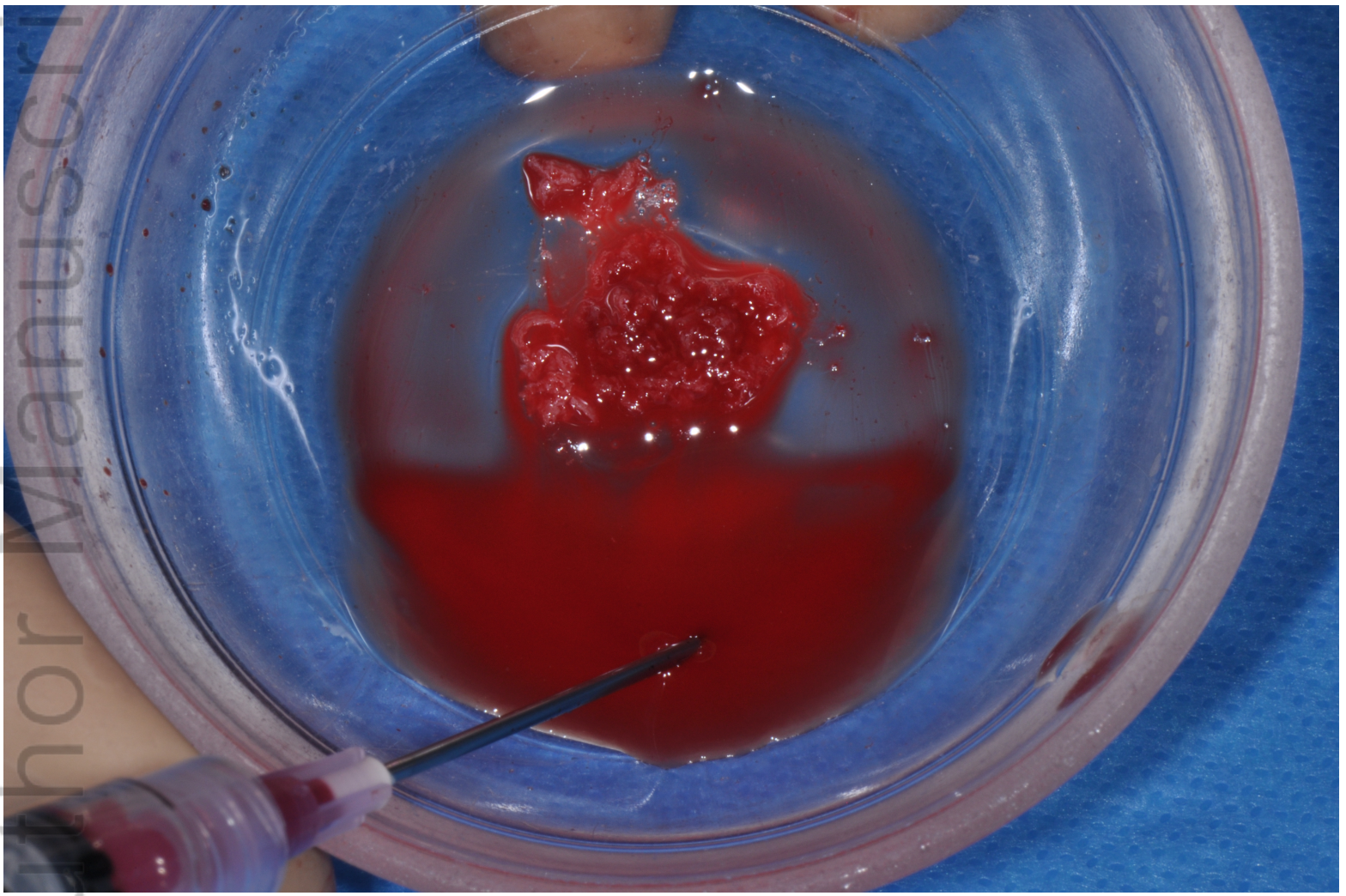


Fig. 1o.jpg

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Fig. 1p.jpg

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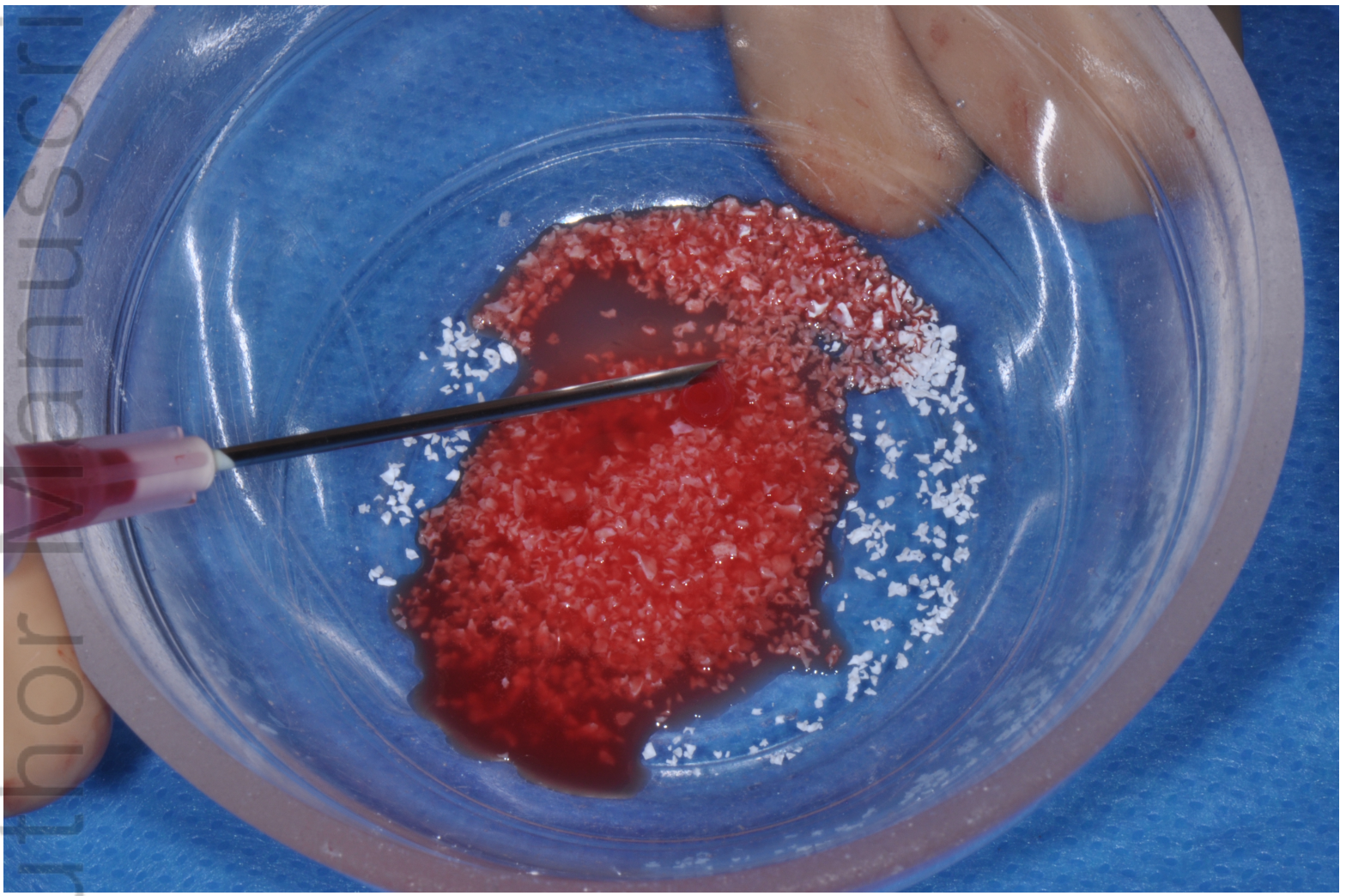


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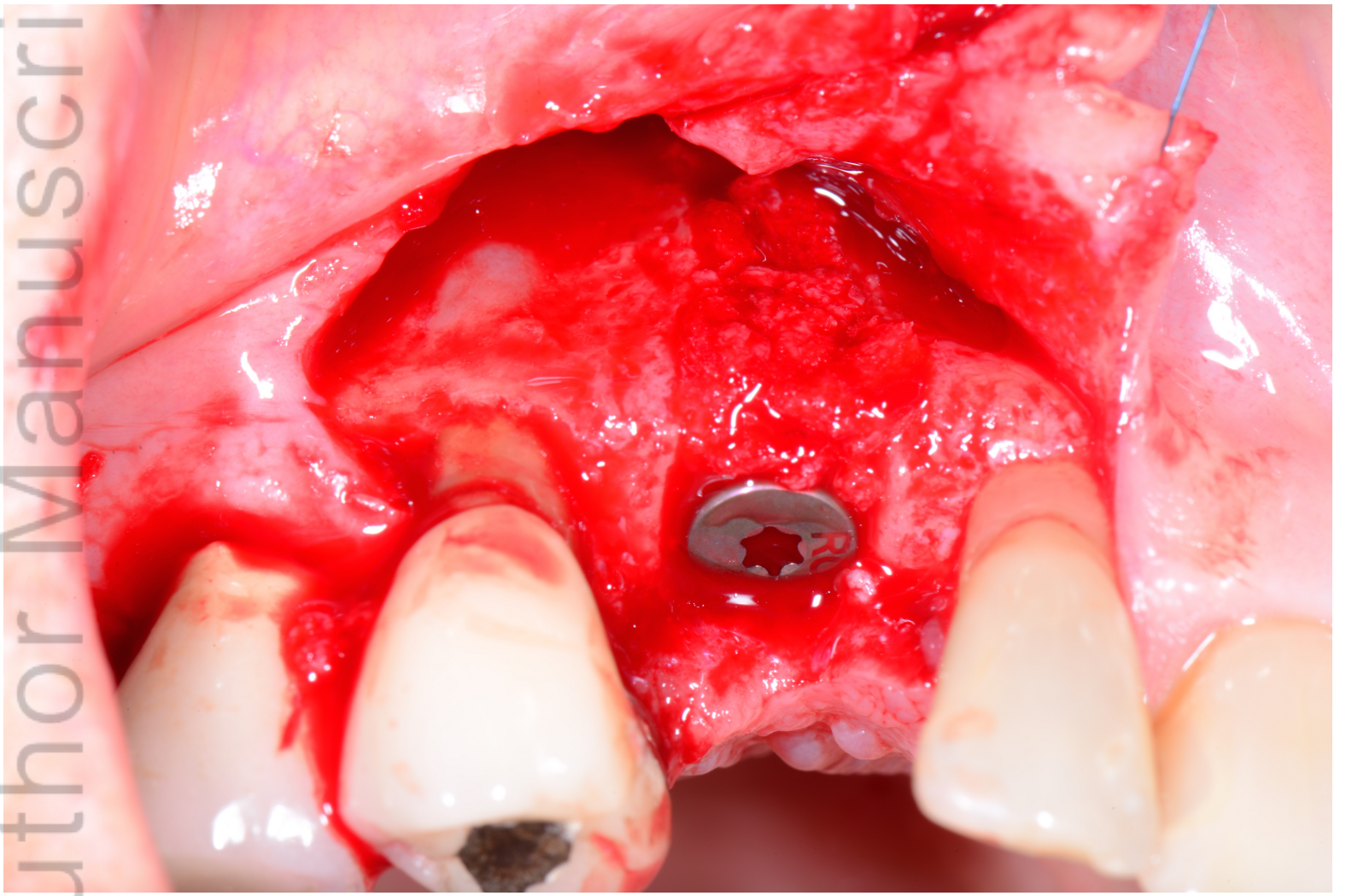


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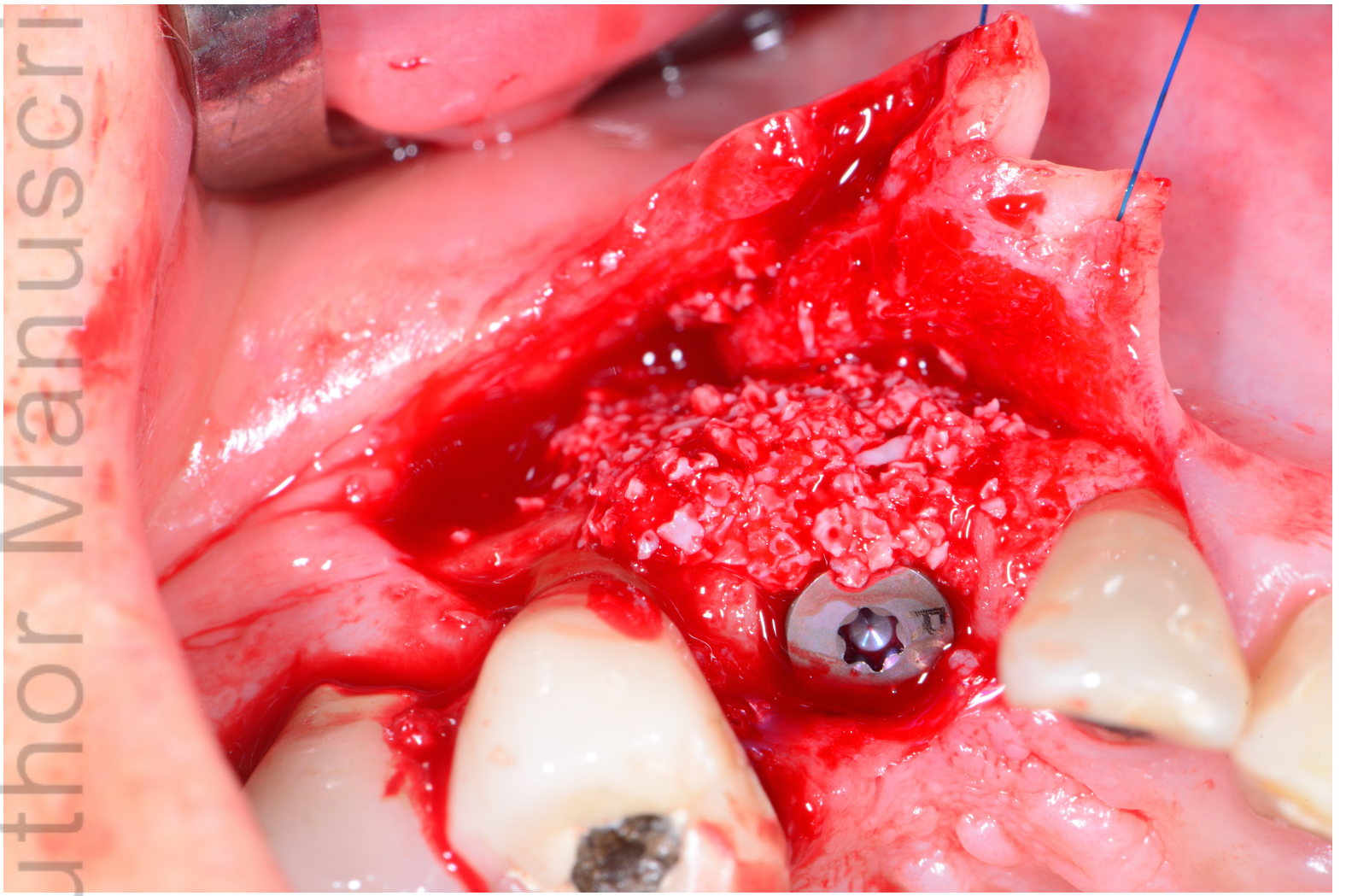


Fig. 1s.jpg

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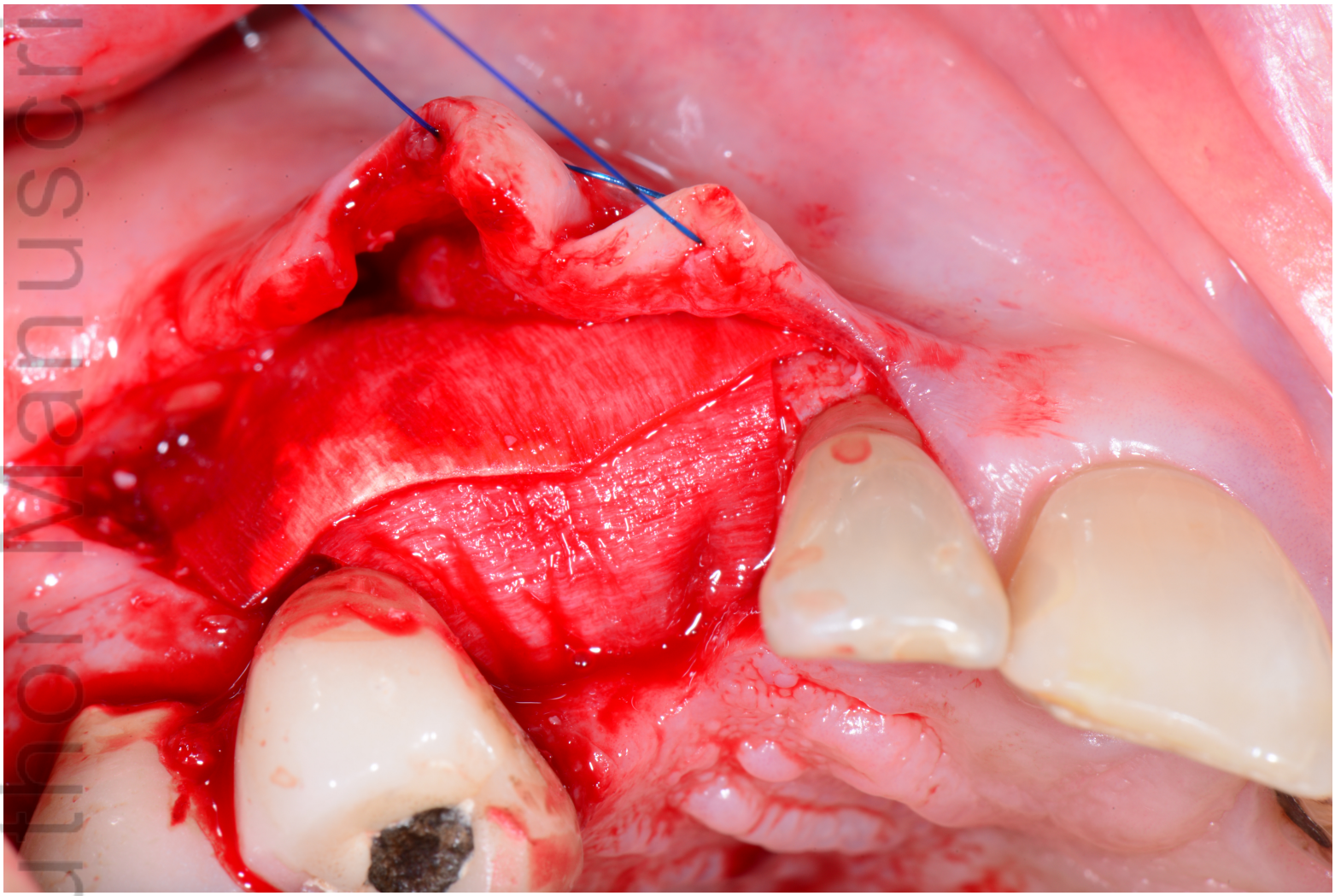


Fig. 1t.jpg

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Fig. 1u.jpg

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Fig. 1v.jpg

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Fig. 1w.jpg

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Fig. 1x.jpg

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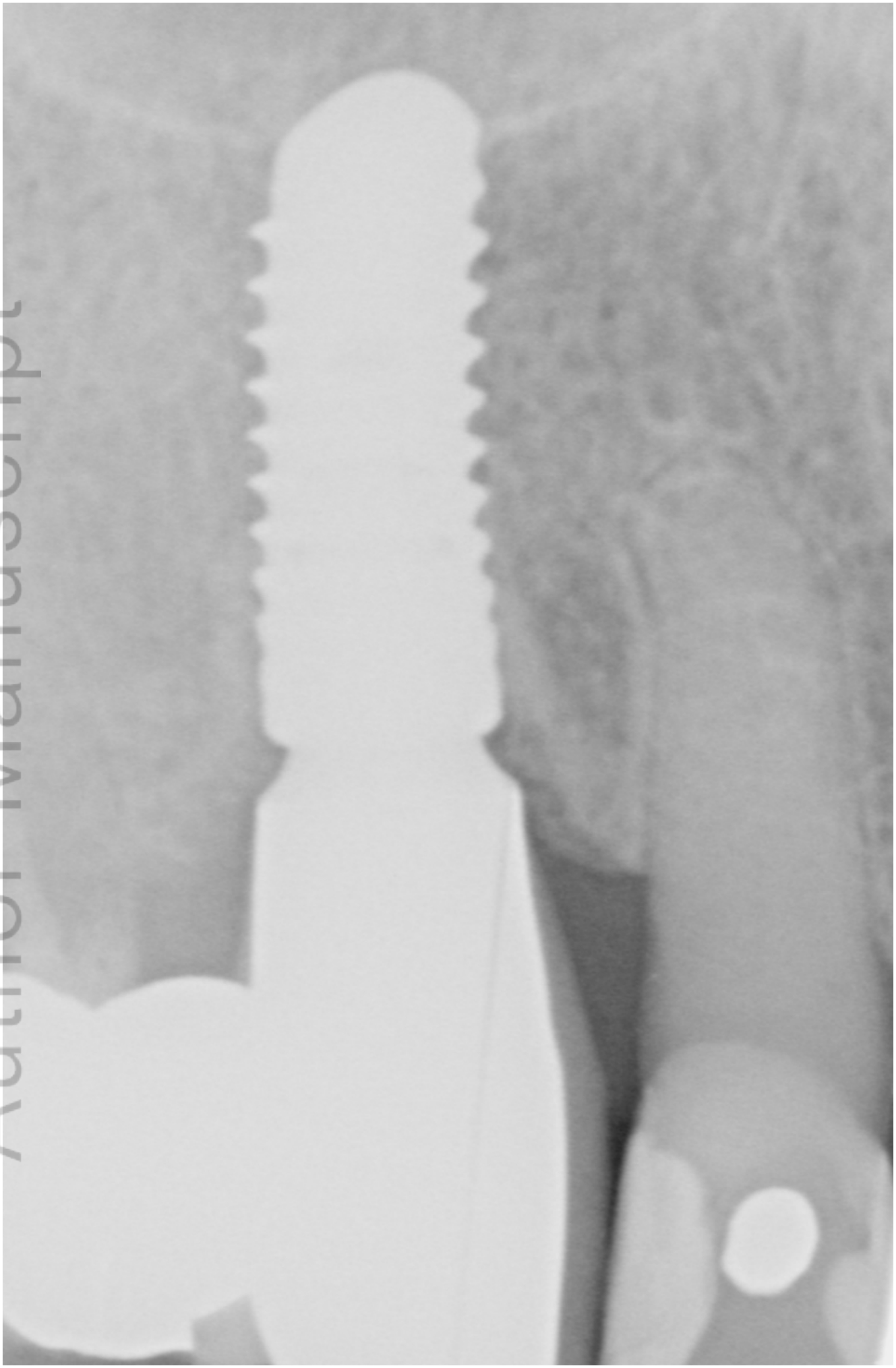


Fig. 1ya.jpg

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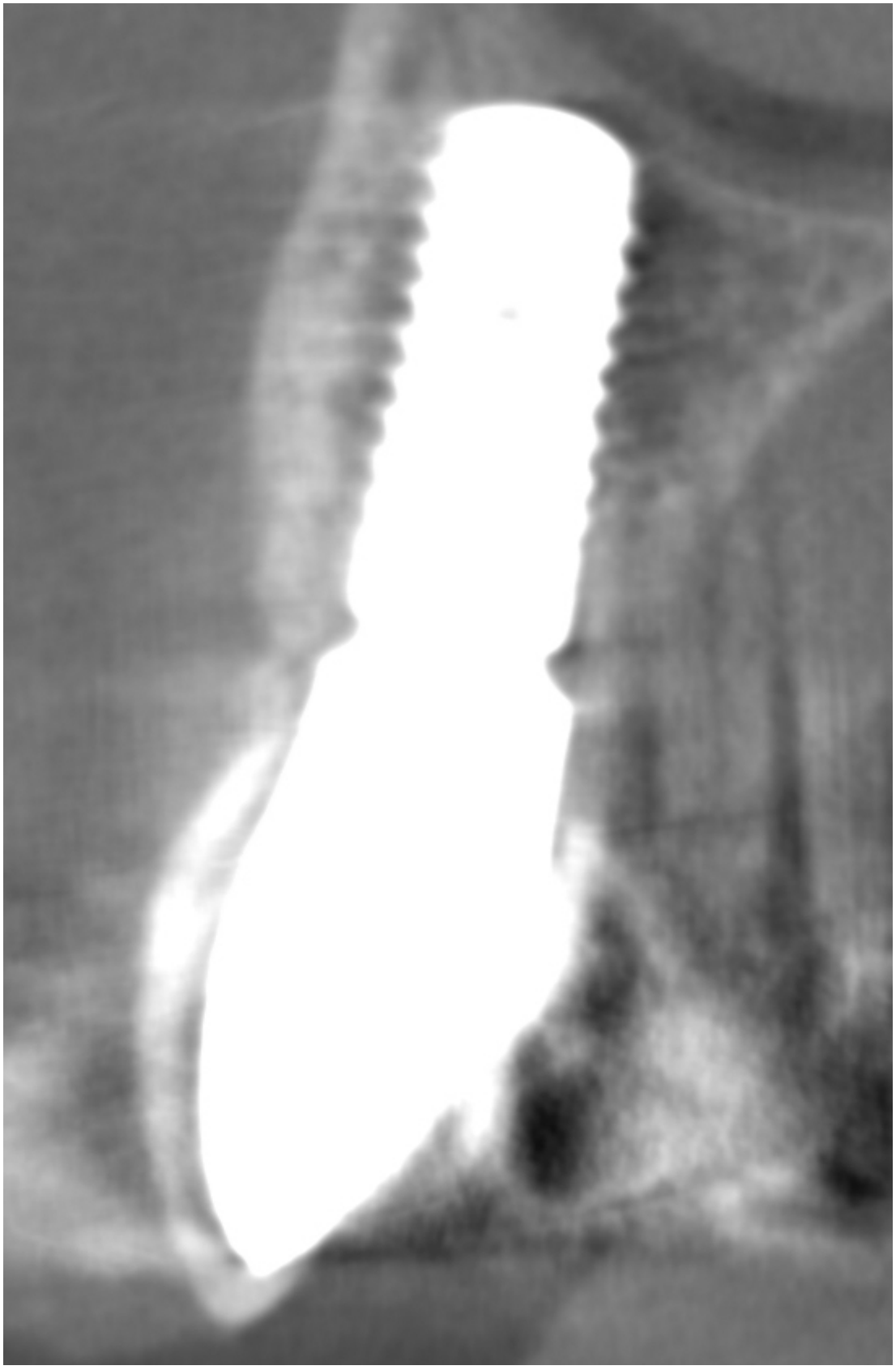
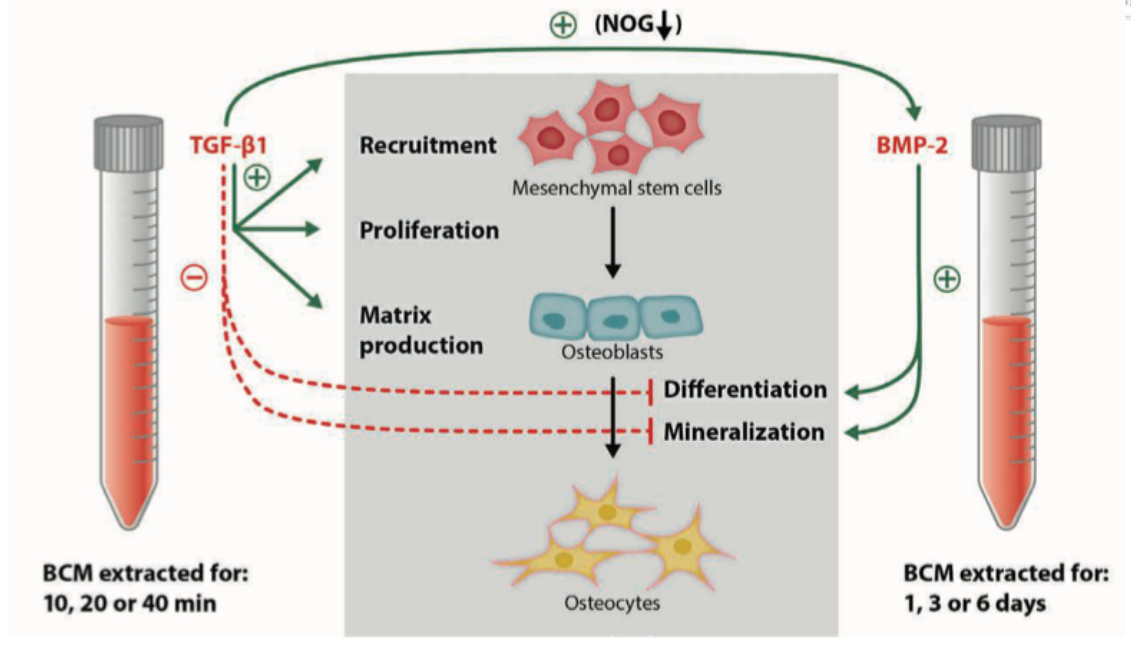


Fig. 1yb.jpg


⊕ (NOG↓)
TGF-β1
⊕
⊖
Recruitment
Mesenchymal stem cells
Proliferation
Matrix production
Osteoblasts
Differentiation
Mineralization
Osteocytes
BMP-2
⊕
BCM extracted for:
10, 20 or 40 min
BCM extracted for:
1, 3 or 6 days

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Fig. 3a.jpg

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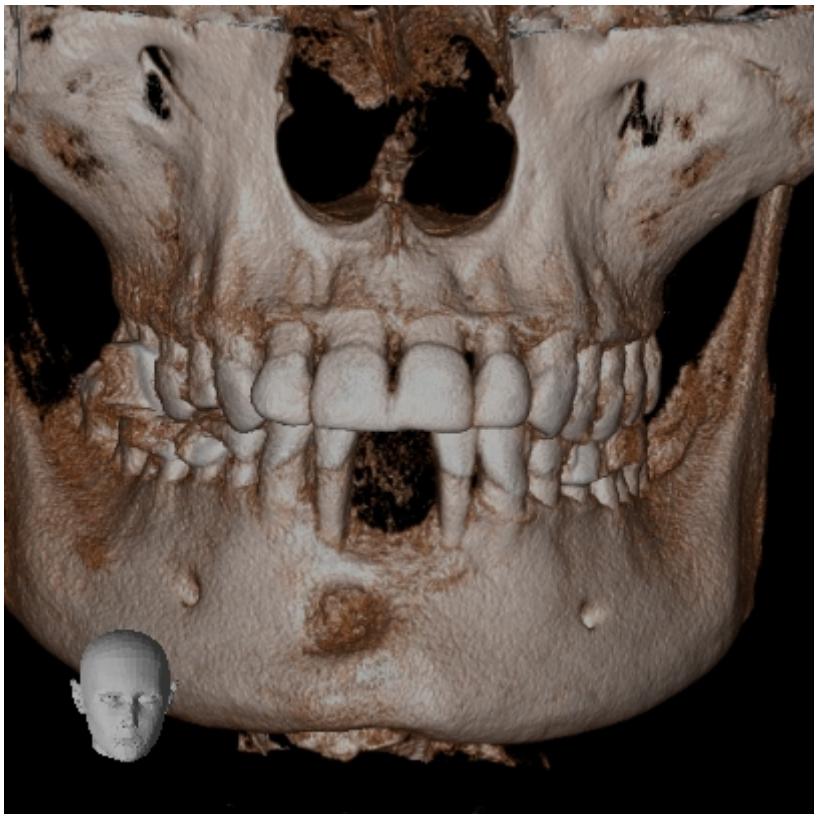


Fig. 3b.jpg

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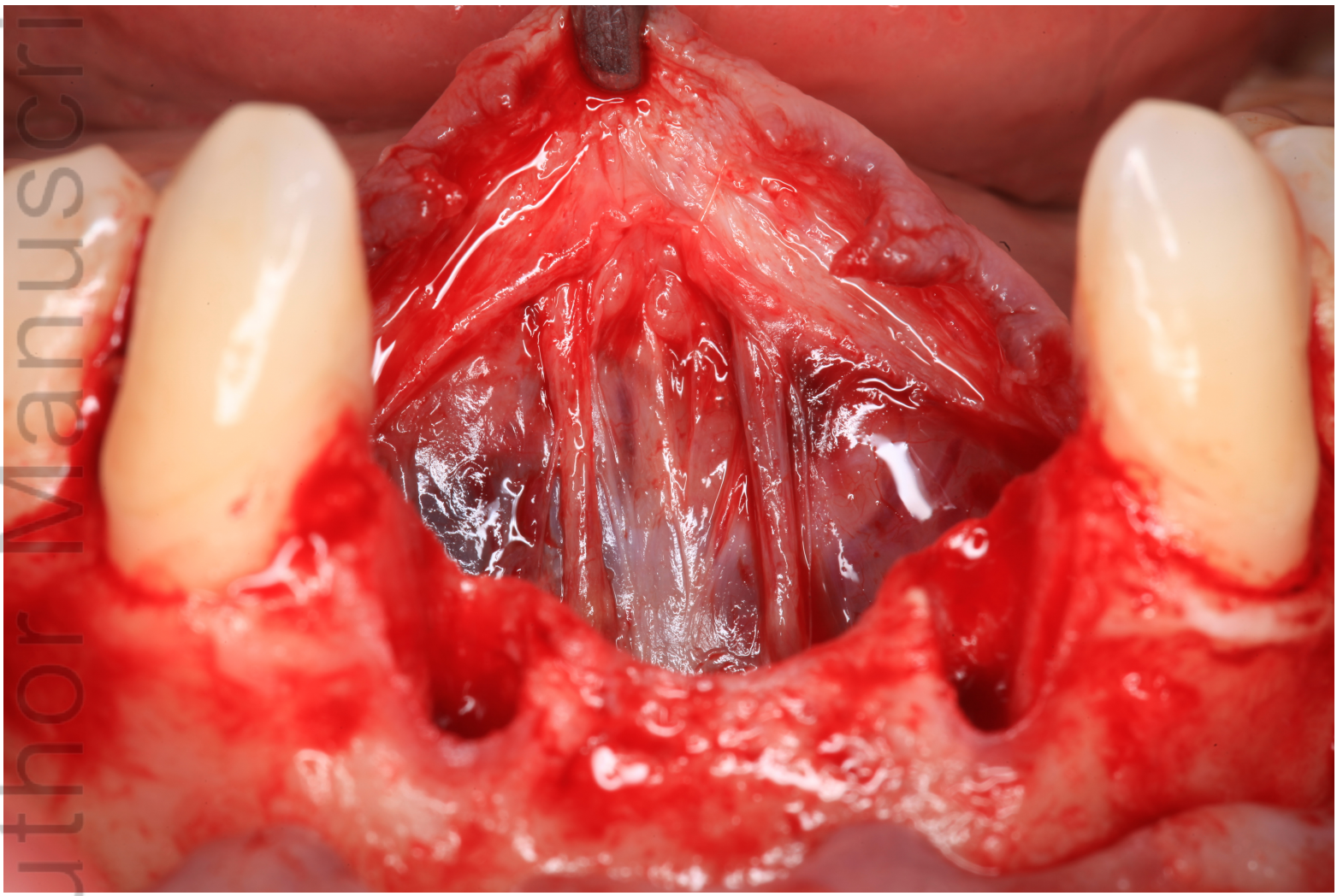


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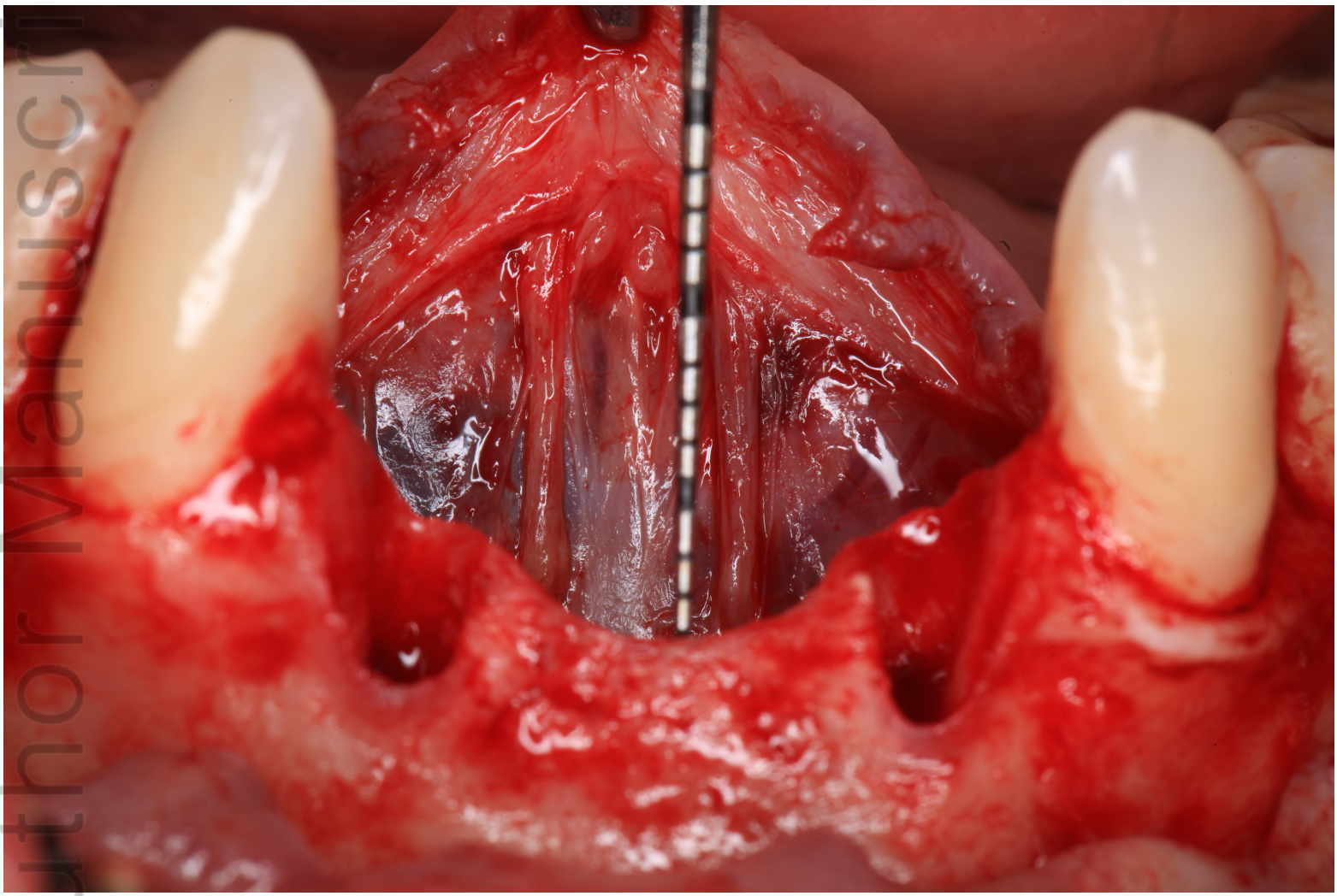


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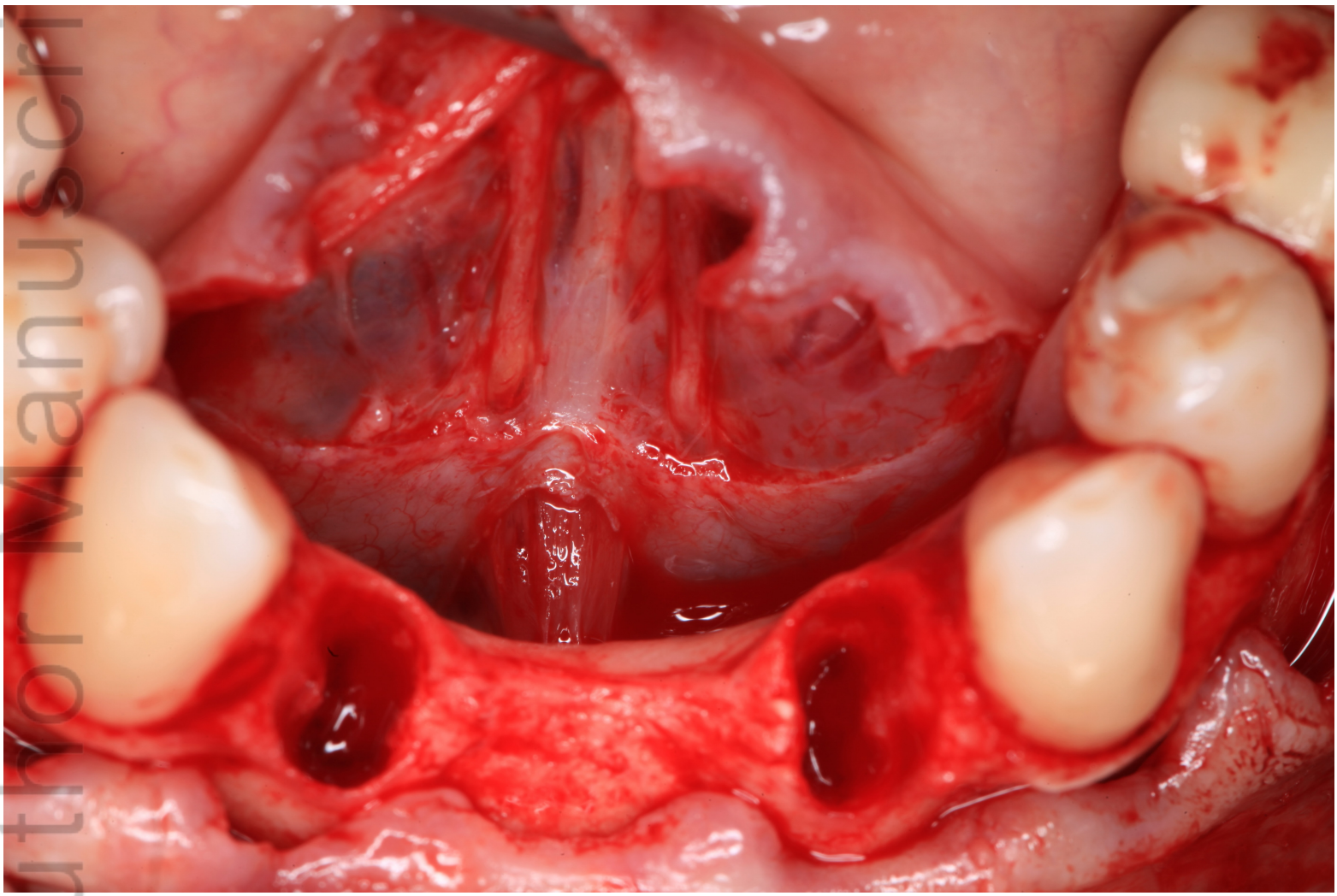


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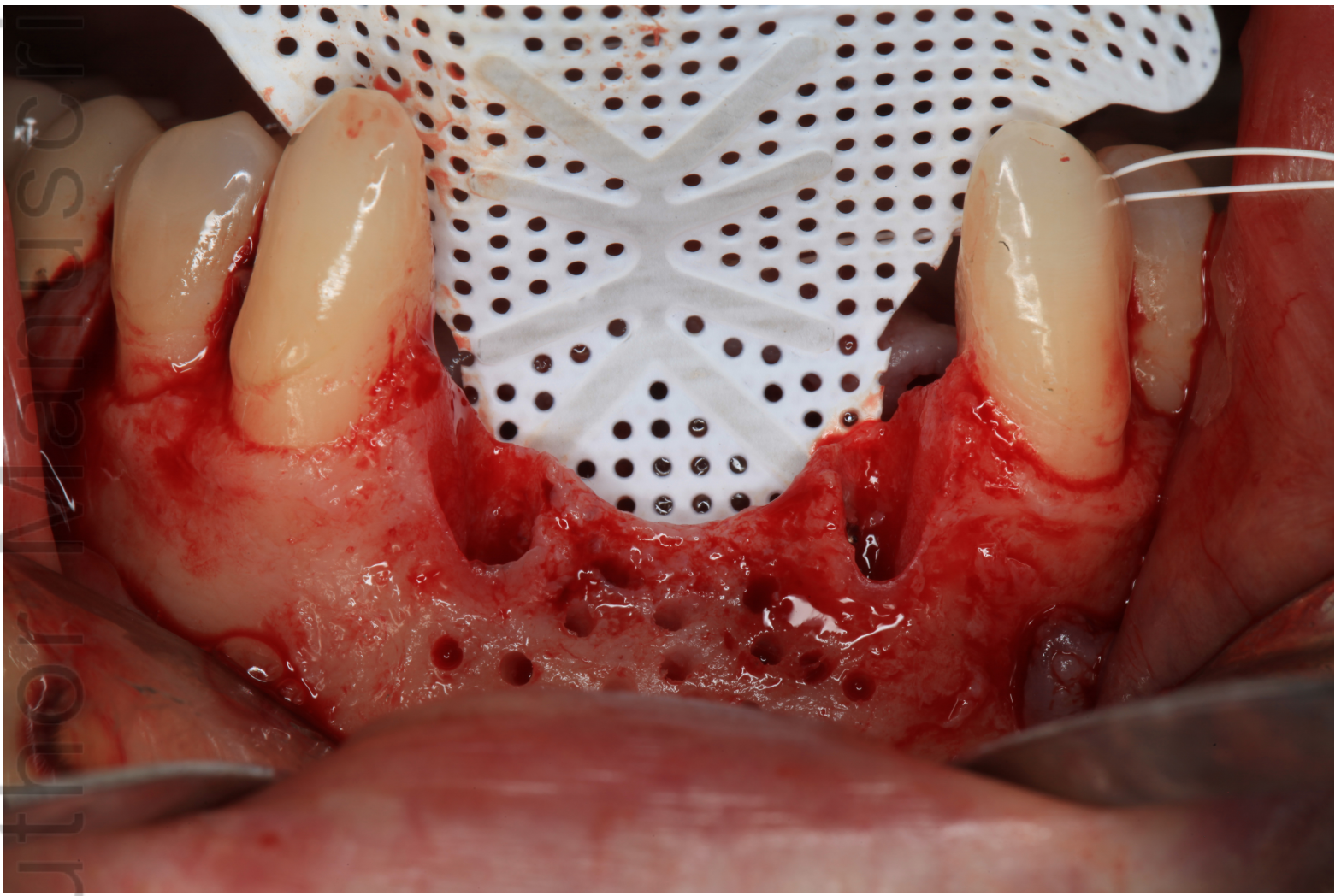


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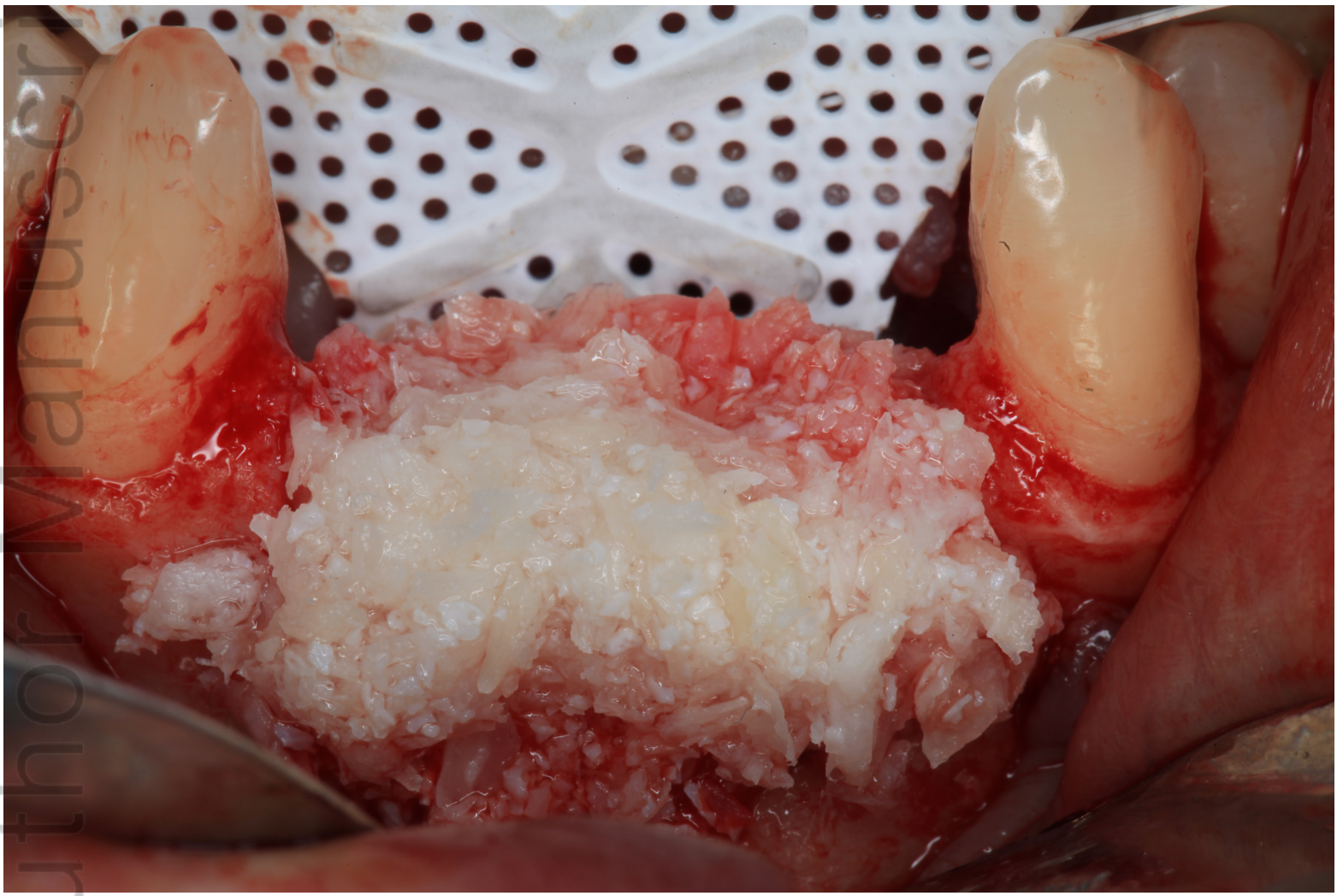


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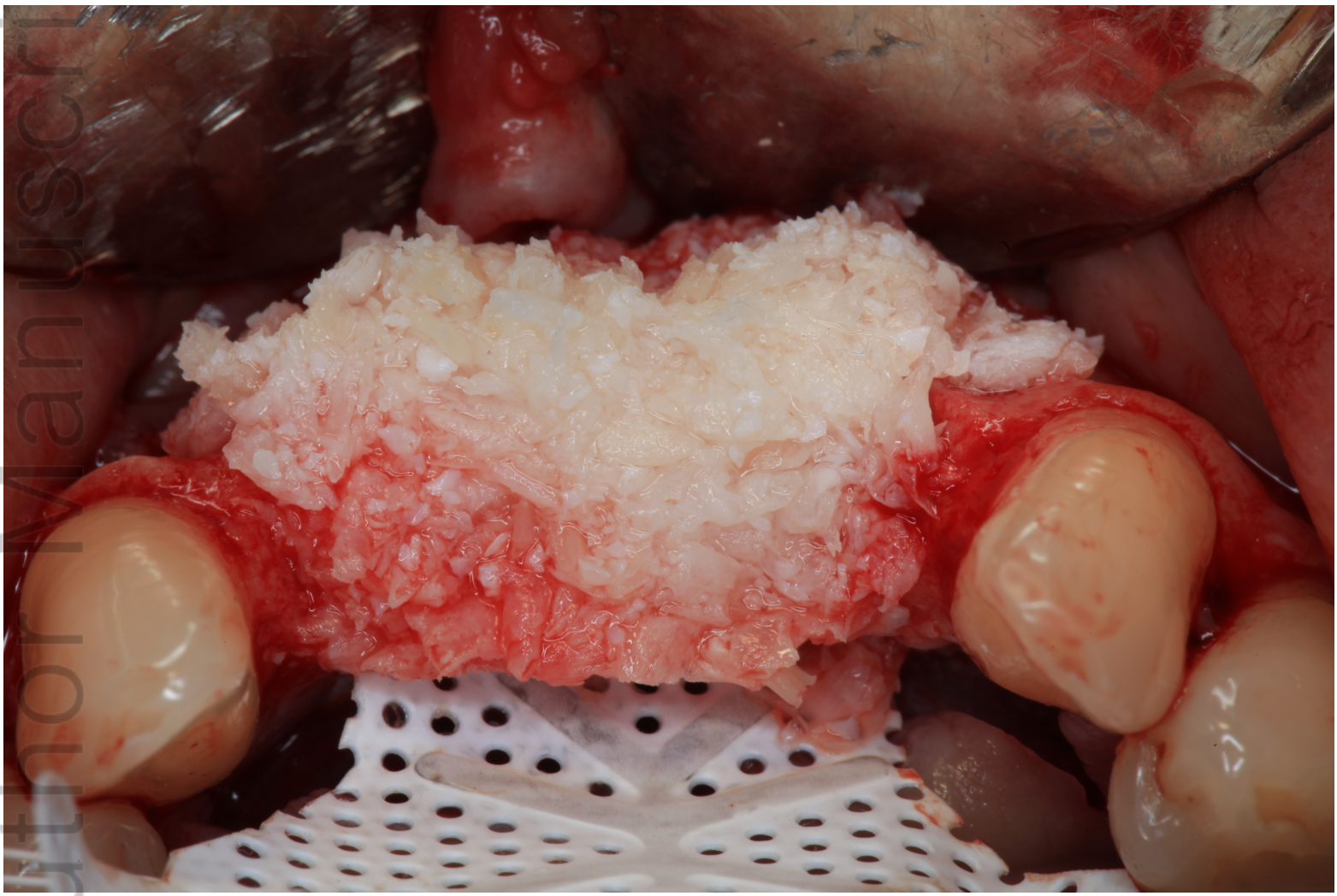


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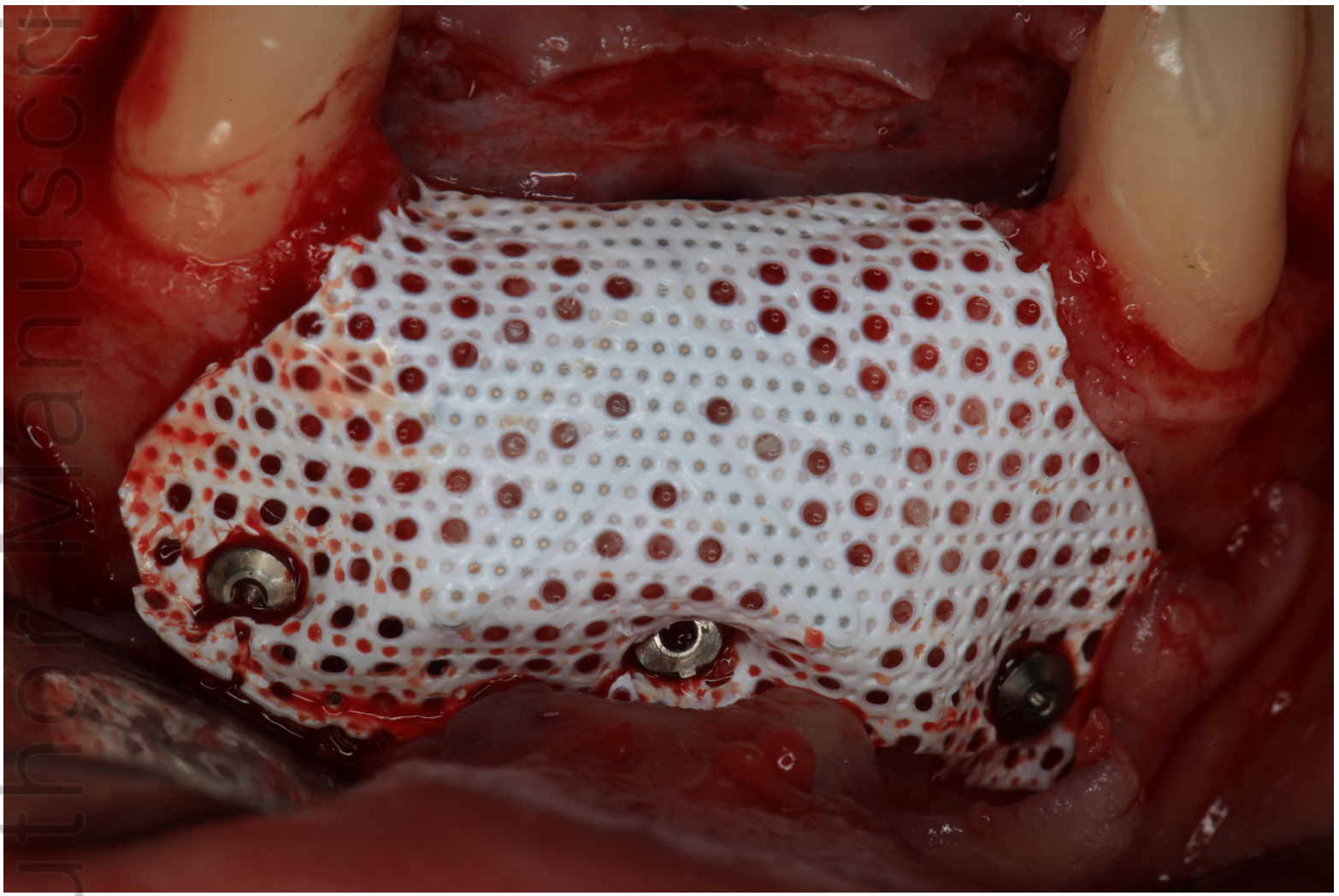


Fig. 3i.jpg

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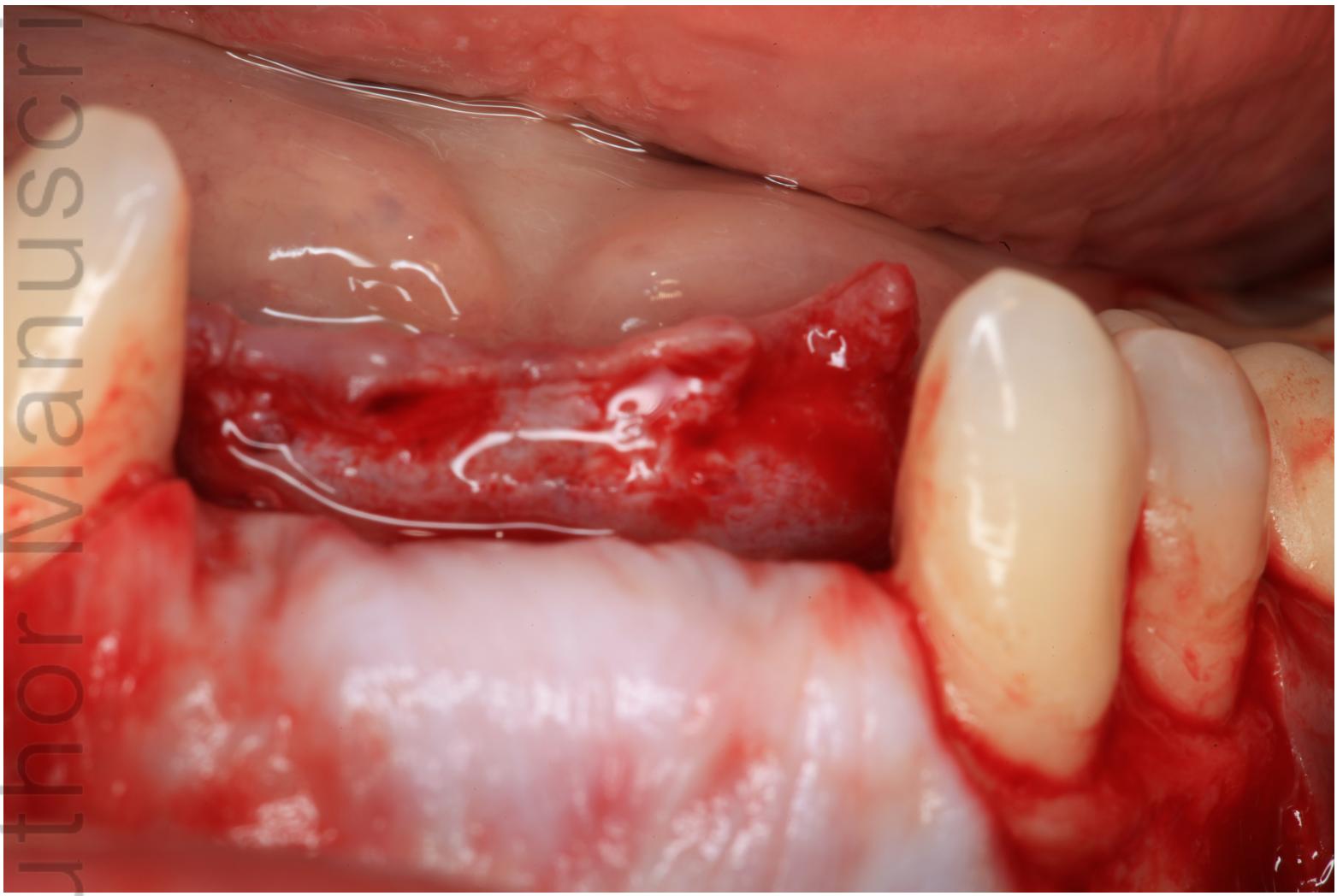


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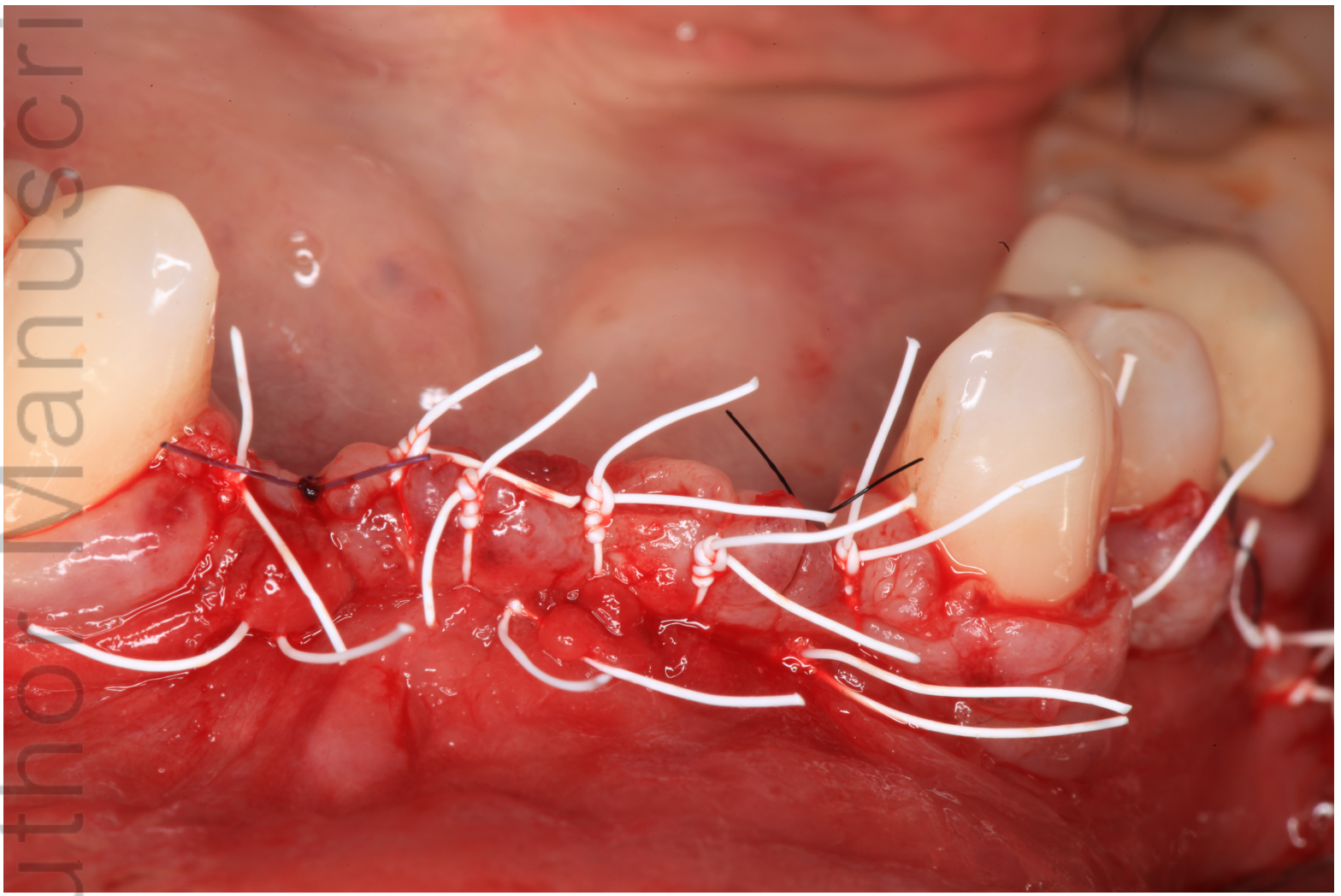


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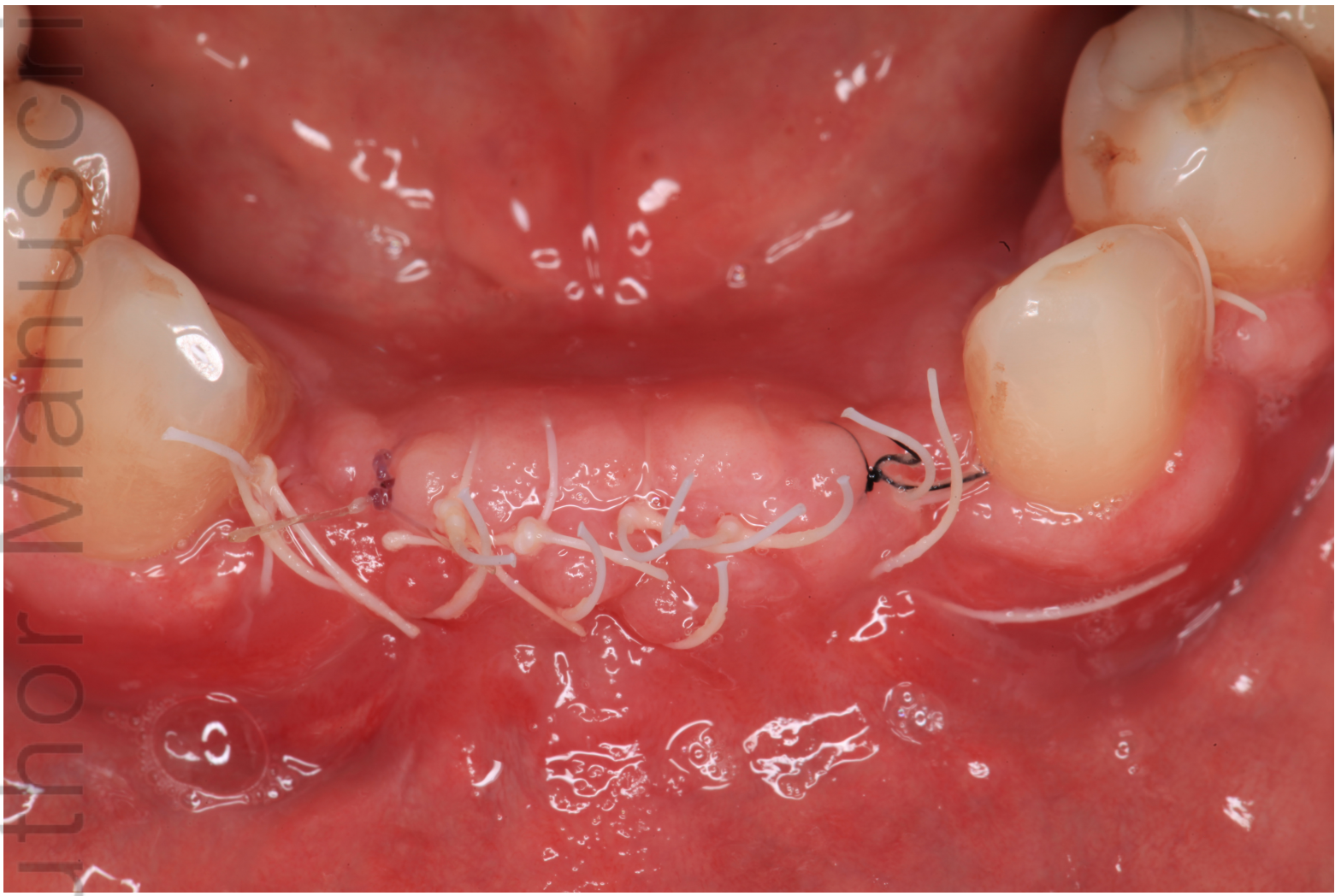


Fig. 3l.jpg

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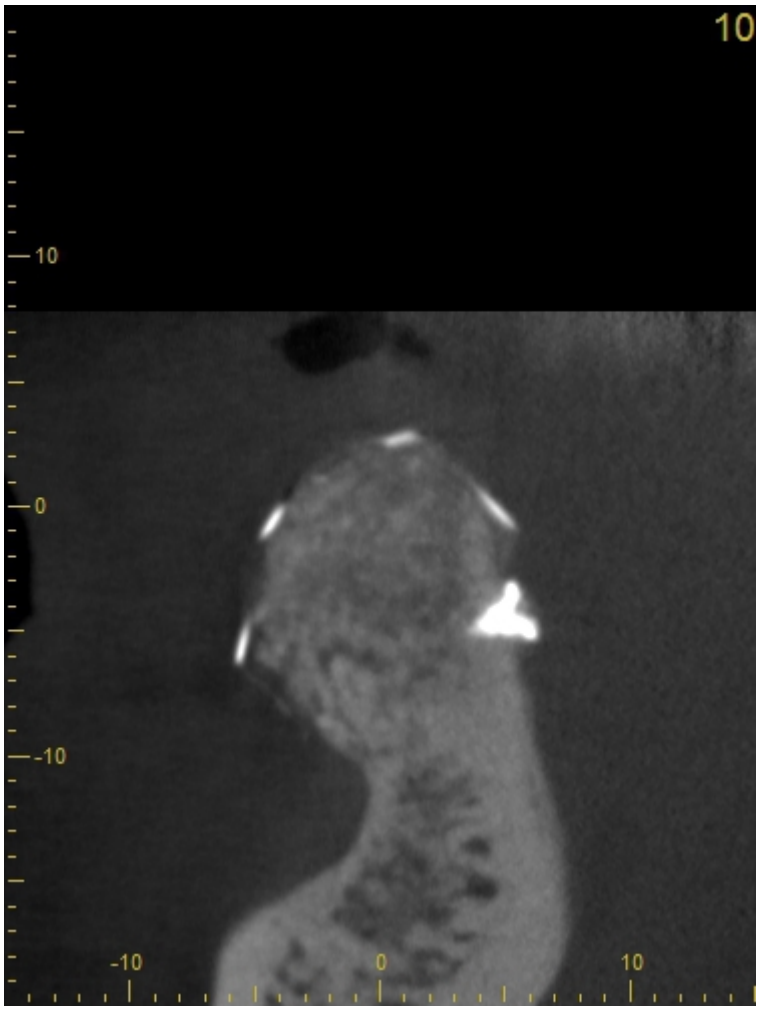


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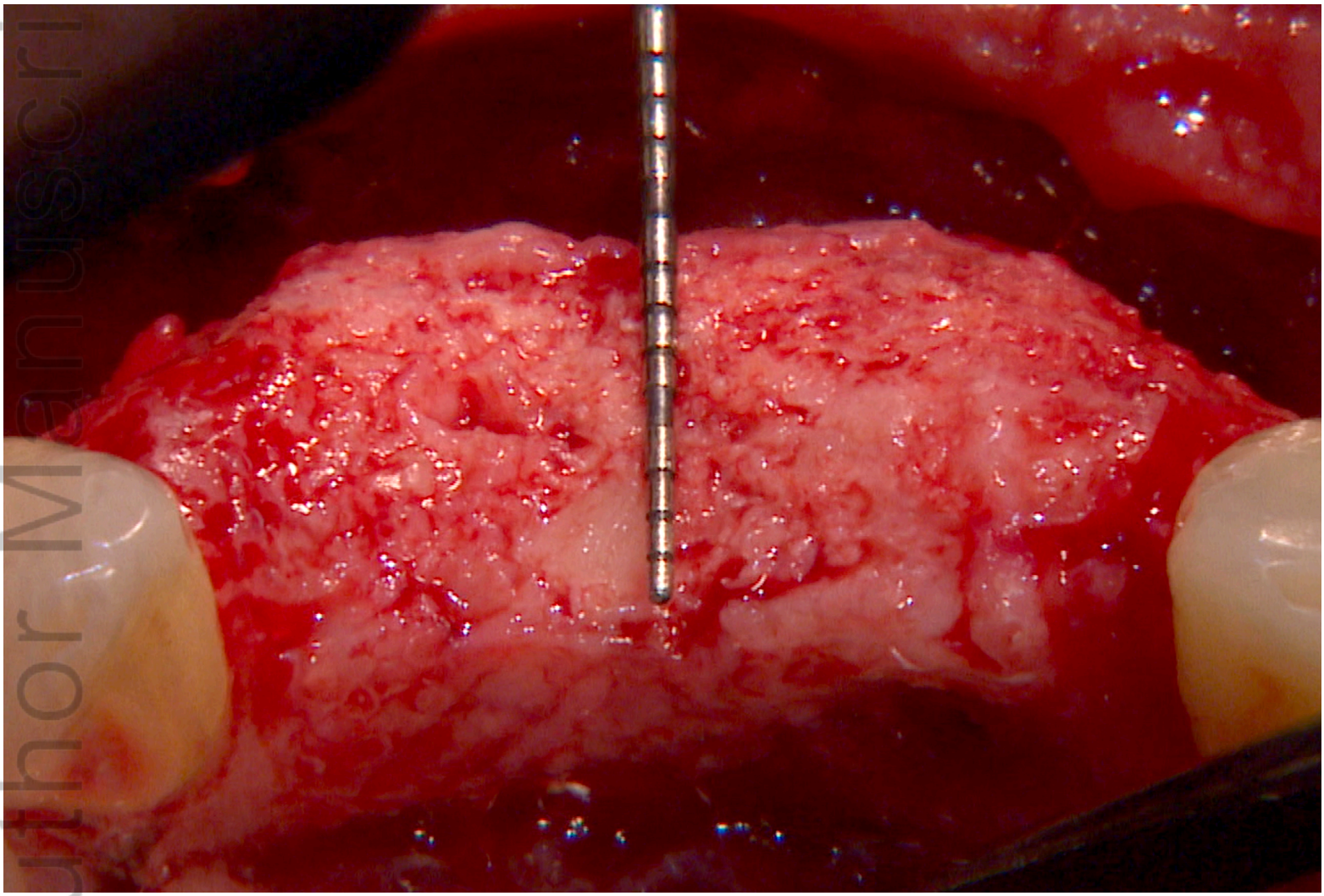


Fig. 3r.png

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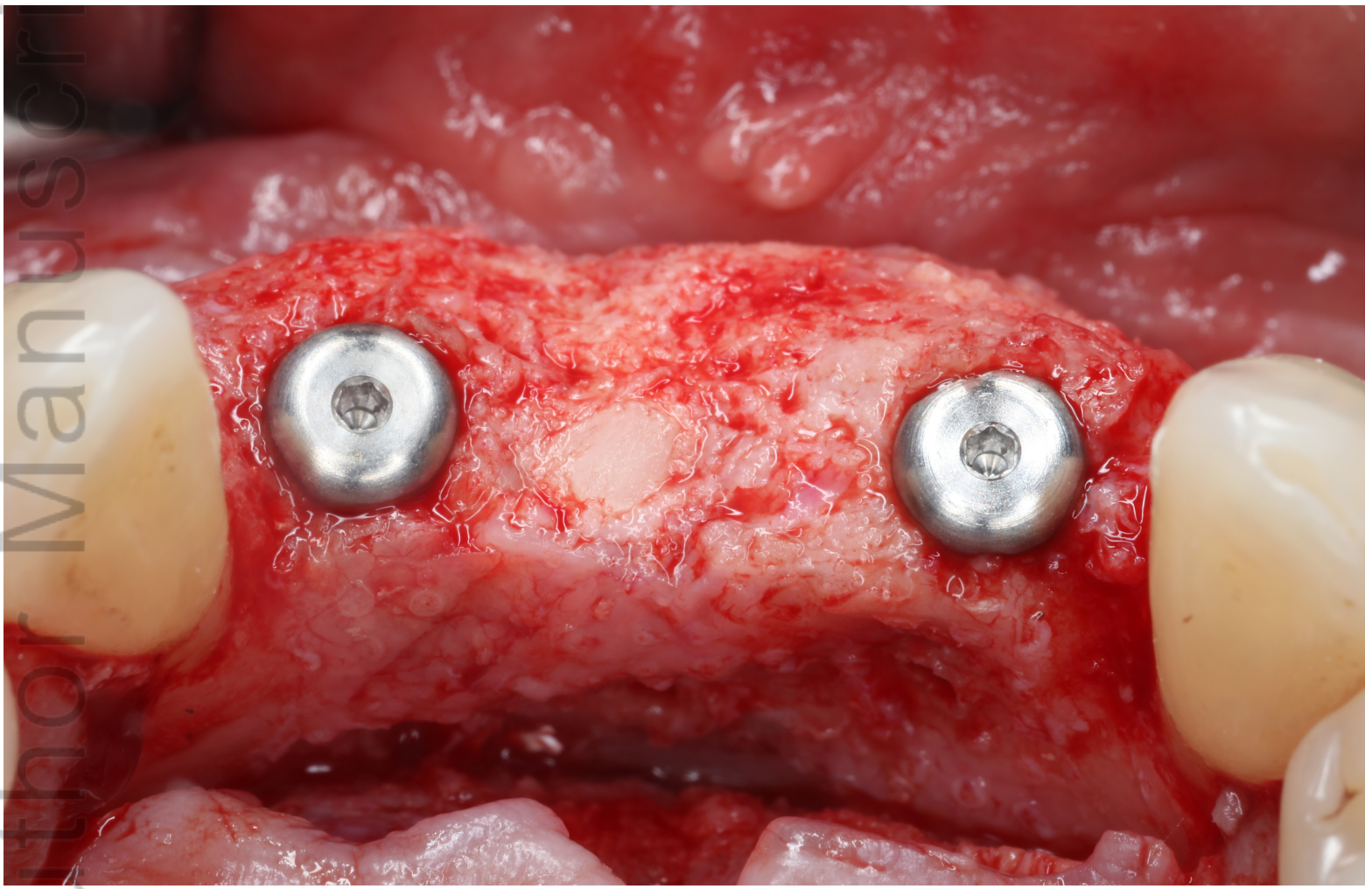


Fig. 3t.jpg

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Fig. 3x.jpg

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Fig. 3y.jpg

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Fig 3m&n

Labial and occlusal view of the site after 9 months of uneventful healing.



same legend as 3m

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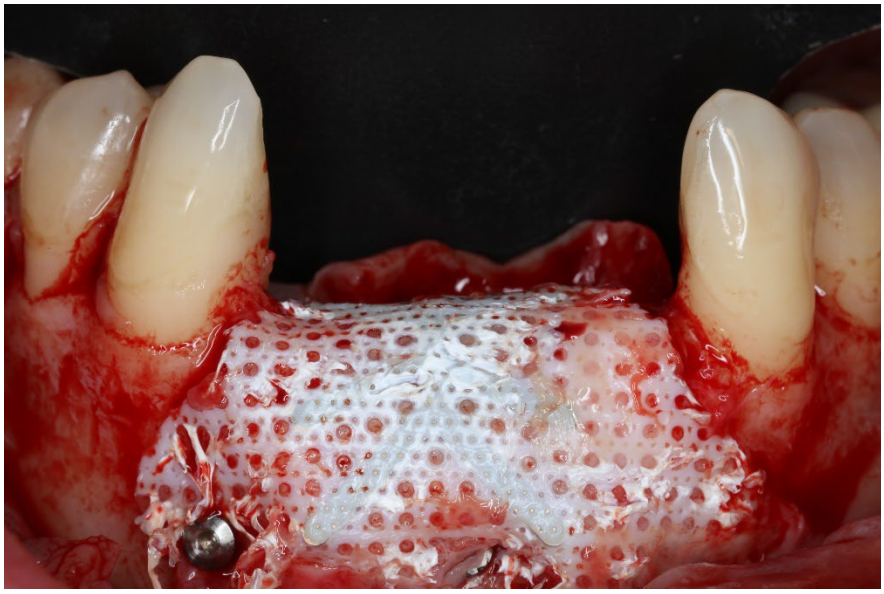


Fig 3p

Labial view of a d-PTFE membrane in place after flap elevation.

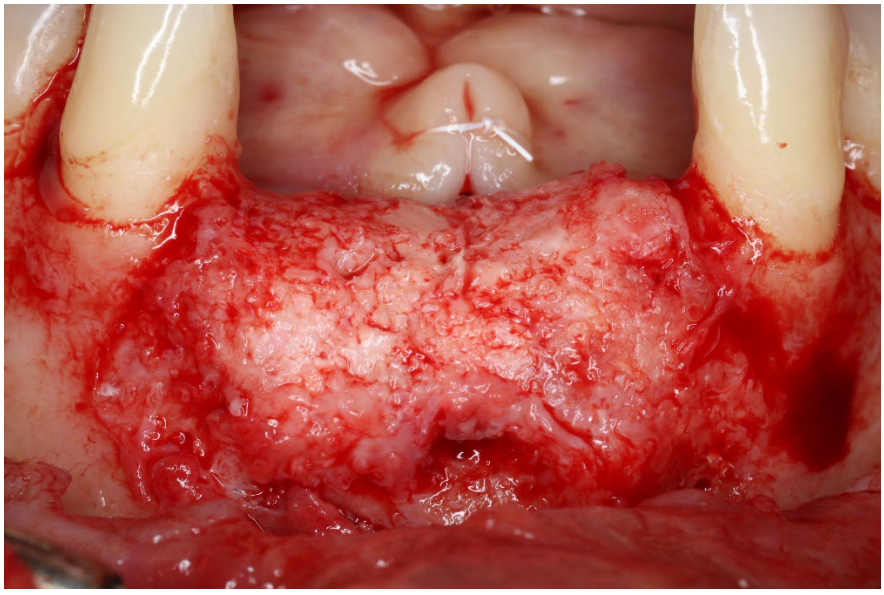


Fig 3q&r

Labial and occlusal views of the regenerated bone.

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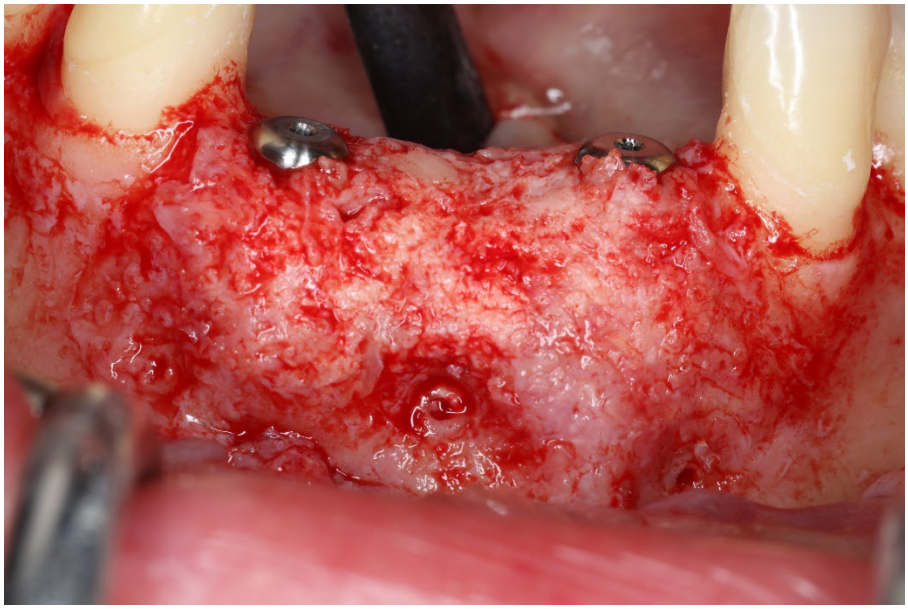


Fig 3S



Fig. 3u - Labial view of the graft (1:1 ratio of autograft chips and DBBM) in place. The bone chips were locally harvested with a bone scraper

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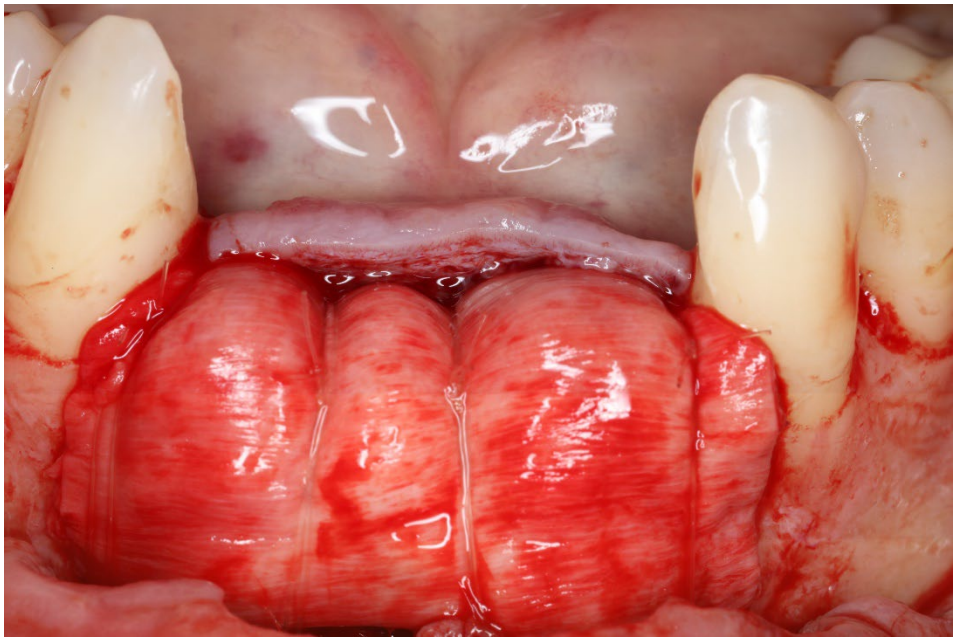


Fig 3v&w

Facial and occlusal views of a native collagen membrane fixated with resorbable sutures.10

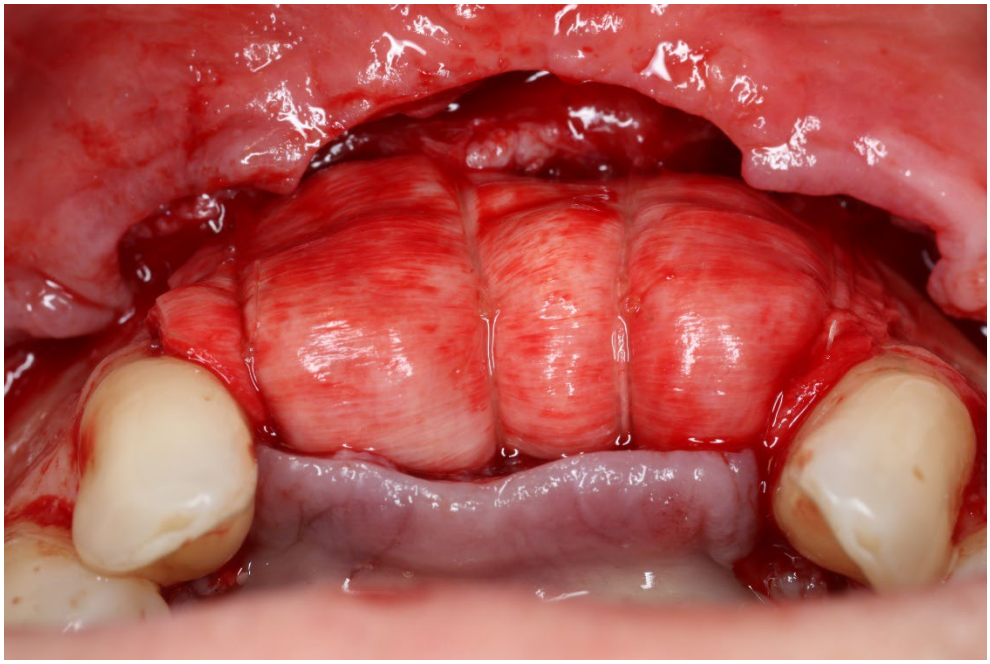


Fig.3w - same legend as fig. 3v