Chapter 7

Hard tissue engineering

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1. Abstract

Hard tissues in CMF include bone, cartilage and teeth. Hard tissue engineering is carried out in patients who do not benefit from traditional methods, such as free flaps, to reconstruct lost tissue. The defect might have been caused by tumor ablation, trauma or it can be congenital, such as cleft lip and palate.

In craniomaxillofacial (CMF) hard tissue reconstruction, two important principles need to be taken into account. The form and function must be restored together with good esthetic outcome. The CMF skeleton is very complex structure and, hence, symmetry, volume, shape and bone continuity must be restored. If cartilage in the temporomandibular joint (condylar dead and/or disc) is missing, ideally that should be reconstructed as well. Engineering missing teeth remains currently a challenge. Soft tissue engineering, which sometimes is needed simultaneously to cover the hard tissue reconstruct, is discussed in Chapter 5.

Optimal result in CMF tissue engineering will enable several functions like mimics, mastication, swallowing, and articulation. The reconstruction should be considered as marriage of both aesthetic and reconstructive objectives.

Hard tissue engineering is usually carried out from mesenchymal stem cells (bone marrow or adipose tissue derived), together with biomaterials and regulating factors, such as growth factors. 3D printing will most likely be used in the future to produce even more accurate form for the reconstruct.

2. Introduction

Bone is the most common hard tissue to have been tissue engineered. There are several studies on tissue engineered bone, both experimental as well as clinical. Usually, mesenchymal cells and biomaterials are being used, sometimes combined with regulating factors. Recently, some studies on the use of gene therapy together with cell therapy have been published. These are, naturally, still far from being used clinically.

3. Experimental studies

In preclinical studies, regeneration of many tissues in the oral and maxillofacial have been studied. Of these, teeth, salivary glands and nerves have not yet been explored in clinical applications.

3.1.Teeth

Dental stem cells are a minor population of mesenchymal stem cells. Because they can differentiate to dental tissues, they have been considered to be a promising source of stem cells for tooth regeneration (1) (Yi 2018).

To regenerate teeth, many different types of cells are needed to ensblr dental pulp regeneration, dentino- and amelogenesis (2,3,4) (Dissanayka and Chang 2017, Wang et al. 2017, Hu et al. 2017).

Already in 2010, Bakopoulou et al (5) were able to show that dental pulp and apical papilla stem cells from extracted wisdom teeth had both osteogenic as well as odontogenic properties with very active migratory and mineralization potential. Numerous types of stem cells have been isolated from dental tissue, such as dental pulp stem cells (DPSCs), stem cells isolated from human pulp of exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs), and dental follicle cells (DFCs). All these cells can regenerate the tissue of tooth. (6) (Zhai 2018) Adipogenic potential, however, related to all MSCs, was worse than with bone marrow derived stem cells (7)(Gronthos 2000).

Research and clinical application of dental stem cells have reached several breakthroughs. Nevertheless, we are still a long way from routine clinical use. There are several technical problems and mechanisms to stimulate and use these cells need to be studied further. Maybe, if development is rapid and genetic modification is possible, their use might be wide: low morbidity in acquiring the cells and no rejection when autologous cells are being used. (6) (Zhai 2018)

In the future, it might be possible to regenerate the root of a tooth, but at the moment it seems impossible to guide the shape and color of the crown of a tooth. Hence, dental regeneration at least at the moments, cannot replace dental implants with prosthetic crowns or other prosthodontic methods.

3.2.Cartilage

Cartilage is a difficult tissue to engineer as cells often tend to continue differentiation towards bone. Liou recently published a study, where they concluded that of the regulating factors, platelet rich plasma does not promote formation of hyaline cartilage in the knee. (8) (Liou 2018) This is probably true also in the hyaline cartilage of temporomandibular joint (TMJ), which is considered to be one of the most complicated joints in the body due to its movements (combination of hinge and sliding motions). However, the disc in TMJ is not hyaline cartilage but fibrocartilage, which contains 66-80% water. The solid part is made of collagen, elastin, glycosaminoglycans (GAG), proteoglycans, and cells (fibrocytic, fibroblastic, fibrochondrocytic, and chondrocytic phenotypes). The distribution of cells and extracellular matrix (ECM) components vary among the different zones of the TMJ disc according to their contribution to the overall function of the disc (9) (https anastasiouslab).

However, tissue engineering continues to emerge as a promising option to repair or replace the diseased tissues of TMJ. This can be done either with scaffolds or even scaffold-free. Stimulation of cell growth and differentiation can be done in bioreactors by applying, for example hydrostatic pressure, dynamic loading, rotation or perfusion. However, as there are only a limited number of published studies, the effectiveness of a bioreactor in engineering fibrocartilage is still uncertain. (10) (Aruaei 2016).

When engineering TMJ disc, several hydrogels and polymers as well as biological materials have been used alone or with growth factors. (11,12,13, 14,15) (Ahtiainen,

Detamore, Almarza, Brown, Legemate). The biggest challenge seems to be maintaining the size and shape during regeneration. (16) (Detamore 2003)

In cartilage tissue engineering, committed chondrocytes, dermal fibroblasts, ESCs and MSCs have been used. Based on the results and availability of cells, MSCs seem to be a viable choice for this application. The regenerated condylar cartilage in the joint will have to bear large contact area strains and stresses. It must also allow growth of functional tissue by providing appropriate cell-scaffold interactions (17) (Seo and Na 2011).

To enable long term survival of cells inside the scaffold, the scaffold must be either porous or woven. These properties will challenge the appropriate strength of the scaffold. In oral and maxillofacial surgery, the need for cartilage is usually in the TMJ.

Mäenpää with her coworkers (2010) (18) studied the regeneration of TMJ discs in rabbits. The bilayer scaffold disc comprised of a non-woven mat of resorbable PLA and a PLA membrane plate. AT-MSCs were seeded in the discs and cultured in parallel in control and chondrogenic medium for six weeks. Relative expression of the genes, aggrecan, type I collagen and type II collagen, normally present in the TMJ disc extracellular matrix, increased in the discs in the chondrogenic medium. They concluded that the PLA discs seeded with AT-MSCs have potential in the development of a tissue-engineered TMJ disc. The same group later used these discs in in ten rabbit TMJs. The original TMJ disc was bilaterally removed and the AT-MSC-seeded PLA disc was used to replace the removed original disc on one side. On the contralateral side, the cell-seeded PLA disc was pretreated in chondrogenic differentiation media. Fig 1a-c Unfortunately, the cone beam computed tomography and histology showed, that most of the discs had dislocated and caused sclerotic changes and condylar hypertrophy in the joints. The pretreated discs seemed to function slightly better than the non-pretreated discs. No signs of foreign body reaction, infection or inflammation could be seen. Fig 2 a-f The authors concluded that better disc design and fixation technique might lead to better results (11) (Ahtiainen et al. 2013).

To be able to regenerate mandibular condyle, it must be realized that both the bone and the cartilage must be produced and bound together. Chondrocytes and osteoblasts can be harvested or differentiated from above mentioned many sources: The properties of the

scaffold needed is different for bone and for cartilage. The growth factors used need to differ as well. However, if this could be safely and predictably performed, this approach would give great relief to patients suffering from major TMJ disorders and diseases. (19) (Wang and Detamore 2007)

Nasal cartilage has also been a target for tissue engineering. Chang et al. (20) (2007) used autologous chondrocytes injected in fibrin glue to rabbits´ dorsal nasal bones. The histological result was identical to that of normal auricular cartilage. The concentration of fibrinogen and thrombin as well as chondrocytes play a crucial role in the formation of the cartilage. If cartilage cells are not available, bone marrow as well as umbilical cord derived stem cells have been studied. The umbilical cord derived cells seemed to produce more type I collagen and aggregan compared to bone marrow derived cells, a finding which warrants further studies also in a sandwich-type construct for osteochondral reconstruction (21,22) (Wang et al. 2009, Wang et al. 2011).

In condylar fibrocartilage engineering, scaffold-based engineering is a better solution than scaffold-free engineering. It is important to realize that in TMJ condylar fibrocartilage tissue engineering there often is a need of both cartilage and bone. This construct most likely needs two different scaffolds, one per each tissue. Many polymers and cells have been used for this purpose (10, 19, 23) (Aruaey, Alhadlaq, wang).

In scaffold-free approach, costal chondrocytes seemed to work better than TMJ disc cells. This was evident also when costal chondrocytes were co-cultured with dermal fibroblasts – they had superior morphological and biochemical qualities compared to TMJ disc or auricular cartilage cells. In scaffold-free constructs, passaged costal chondrocytes seem to be the most viable alternative for TMJ disc reconstruction (10) (Aruaey).

3.3. Bone

Bone is the most common hard tissue to have been tissue engineered. There are several studies on tissue engineered bone, both experimental as well as clinical. Usually, mesenchymal cells and biomaterials are being used, sometimes combined with regulating factors. Recently, some studies on the use of gene therapy together with cell therapy have been published. These are, naturally, still far from being used clinically.

Skin fibroblasts (Parrilla et al, 2010) ex vivo with a replication-defective adenoviral vector, carrying the LIM mineralization protein-3, and adsorbed on a hydroxyapatite/collagen scaffold were used in Wistar rats with full thickness defect in the mandible. They concluded that the gene therapy accelerated bone formation but more studies with larger animals were needed before clinical trials.(24)

Zhang et al (2018) (25) constructed alveolar bone in rhesus monkeys by using 3D-printed bioactive glass together with chitosan loaded with NELL1 gene. The plasmid of the gene (pDNA-NALL1) was loaded in chitosan, which was then combined with bone marrow mesenchymal cells. This mixture was composited with bioactive glass. They reconstructed the alveolar defect (10x10x5 mm) with this composite and finally concluded that the NELL1 gene played a promotional role in healing.

At the moment, naturally, gene therapy – based bone tissue endineering is still far from being used clinically.

4. Clinical work

There are two main objectives in maxillofacial reconstruction: surgery should provide form and function of oromaxillofacial area. As facial skeleton has a very complex structure, and reconstruction should restore volume, shape, bone continuity and symmetry of bone skeleton. On the other hand, soft and hard tissues in this area enable several functions like articulation, mimics, mastication, swallowing and breathing. When the reconstruction is carried out, esthetic and reconstructive aims need to be met.

Clinically, the applications have been mainly in bone regeneration as well as in epithelial defect repair. Currently, the aim is also to avoid all animal-derived materials and replace them with synthetic or human-derived materials, such as recombinant human BMP (rhBMP) and human serum.

4.1. Cartilage

Our own research group has used tissue engineering to produce cartilage to the nasal septum. The two operations, in which a resorbable Chronos® sheet was seeded with patients' own ASCs, were successful. However, one of the patients continued her nose

picking with artificial nails and after the initial healing period the graft was lost. (Sandor et al. 2014). (26)

4.2. Bone

Bone transplants are the second most used tissues in clinical work after blood transfusions (Shegarfi and Reikeras 2009) (27). However, if autologous bone is used, usually another surgical site required which causes more morbidity to the patient as well as extends the length of the operation. Bone banks provide solution for this is some cases as allogeneic bone can quite safely be used even though there is a small risk of immunologic reactions and disease transfer. Bone grafts usually resorb partly, hence, in oral and maxillofacial area it might in some cases be difficult to predict how much bone needs to be transplanted.

4.2.1. Sinus lift

Sinus lift is one of the most common procedures to enable placement of dental implants in the edentulous maxilla. Traditionally it is carried out by using autologous bone harvested in the craniomaxillofacial skeleton or iliac crest. However, it was one of the first application where bone regeneration was attempted by tissue engineering. The used carries for cells and/or growth factors are resorbable fleeces, HA, bovine bone and, naturally, autologous and allogenic bone.

Schimming & Schmeltzheisen (2004) (28) periosteal cells on a resorbable (polyglactin 910 combined with polydioxanone) fleece in 27 patients for augmentation of edentulous posterior maxilla. They used Good Manufacturing Practice (GMP) -class expanded periosteal cells from mandibular angle and the fleece was soaked with cell suspension. Bovine thrombin in FBS was used to seal the cells in the fleece. Cells were cultured for nearly 2 months after which they were transplanted in the sinus floor. One patient had to be dropped out due to an infection. In 18 patients the result was excellent, however an unsuccessful result was seen in 8 patients (30%) needing further supplementary autologous bone transplantation.

Meijer et al. (2008) (29) sinus floors or walls prior to dental implant insertion in six patients. BM-MSCc were harvested from iliac crest and cultured for a week on porous HA in an osteogenic culture medium, containing also xenogeneic materials such as

FBS. The cells were then transplanted and the augmentation effect studied 4 months after augmentation. Of the 11 biopsies taken, bone formation was observed only in 3 patients (50%). It can be speculated, that inadequate vascular supply might have been the reason for failures.

4.2.2. Other small local defects

In oral and maxillofacial surgery, large bone defects, caused by cysts, are often filled with autologous bone, bovine bone or synthetic materials such as hydroxyapatite (HA), ß-TCP or BAG. In a study published by Stoor et al. (2017a) (30) 21 bony cavities in 20 patients were filled with BAG S53P4, some even in the presence of infection (65%). The authors state that the use of this material provides and infection-free and reliable bone regeneration. When cells have been used, autogenous osteoblasts seeded in biomaterials have shown to be an excellent choice to fill these defects compared to iliac crest bone grafts (Pradel et al. 2006) (31). Unfortunately, the need for a GMP-class facility to produce the tissue engineered filling materials is very labor-intensive and not very cost effective hindering their use to become more widespread. According to current legislation, iliac crest bone graft can be obtained simultaneously during the same operation when the cyst is removed, hence lowering the costs markedly.

4.2.3. Continuity defects

Continuity defects are caused mainly due to tumor ablation of trauma. The can include only the bone, sometimes teeth and some soft tissue. If the soft tissue coverage and blood supply to it is adequate, it is possible to tissue-engineer the transplant directly in the defect site. However, if there is a major loss of soft tissue, the construct needs to be transplanted first to an ectopic site and after maturation transplanted again to the defect site either as a microvascular flap or a pedicled flap.

4.2.3.1. On-site regeneration

On 2011, Zétola (32) and his coworkers published a report of mandible defect repair using recombinant human bone morphogenetic protein-2 associated with collagen sponge, autogenous bone chips and synthetic hydroxyapatite and β -TCP blocks. The mandible continuity defect was due to ameloblastoma resection, and an indirect technique was executed. The titanium reconstruction plate and titanium scaffold filled with the above-mentioned combination were implanted into the

defect area. The collagen with rhBMP-2 was superposed above the open titanium mesh to allow muscle cells and periosteum to migrate to the defect area. After the follow-up of 7 months, the patient had stable occlusion and mandible. The control CT showed good bone formation directed to the center of the defect. The authors concluded that the reported reconstruction technique gave a satisfactory result with less invasive surgery and with minimum morbidity. However, studies with larger number of patients are required to indicate the treatment modality as a routine in cases of bone continuity defects

The largest number of patients with using autologous stem cells in 13 consecutive cases of cranio-maxillofacial hard-tissue reconstruction has been published by Sandor and coworkers (2014) (26).Clean room expanded (according to ATMP principles) autologous adipose-derived stem cells (ASCs) were used with biodegradable material (beta-TCP or bioactive glass). In some cases, recombinant human bone morphogenetic protein-2 was also used.

The group reported on reconstruction of defects at four anatomically different sites: cranial bone (5), frontal sinus (3), mandible (3), and nasal septum (2). In the mandible, continuity defect repair was carried out using computer aided surgical planning and AT-MSCs. If scaffolds were needed, titanium mesh or β-TCP sheet was used. The patients were followed between up to 51 months. Two patients with mandibular reconstruction received a total number of seven dental implants later, which are being loaded in masticatory function. The authors concluded that although results are promising, however, further research is needed with animal studies and long-term human series. This view is supported by other research groups, too.

In the mandible, continuity defect repair was carried out using computer-aided surgical planning and ASCs. After resection of ameloblastomas, in all three cases, the defect was repaired with ASCs, β -TCP granules, rhBMP-2 and indirect custom-made titanium scaffold. The patients were followed between 27 and 51 months. In all three patients, the healing was uneventful. Two patients received a total number of seven dental implants later, which are being loaded in masticatory function. The authors concluded that although results are promising, further research is needed with animal studies and long-term human series. This view is supported by other

research groups, too. (Figure 3. a-g)

Matsuo et al. (33) used indirect technique in mandible defect repair. After surgical simulation, a PLLA patient-specific mesh tray was manually prepared and filled with hydroxyapatite. Intraoperatively, particulate cancellous bone marrow was harvested and with platelet-rich plasma (PRP) placed into the tray. Two patients with mandible defects were included to the study. The follow-up was 28 and 33 months. One of the patients received dental implants after 10 months of the initial surgery. The heterotopic bone was macroscopically well formed. The CT evaluation showed good bone quality and the screws used to attach the resorbable mesh tray did not hinder placement of dental implants. However, the authors concluded that there are several limitations to the trial.

Stoor et al. (2017b) (34) used direct CAD – CAM technique and tissue engineering to repair mandibular defects in 14 patients immediately at the time of ablation surgery. Most of the patients had squamous cell carcinoma or ameloblastoma. The surgery was simulated and patient specific implant (PSI) designed on virtual model. The PSI was a combination of scaffold and reconstruction plate with screw holes. The virtual PSI was manufactured using Arcam electron beam melting technology to solid titanium PSI. During the surgery, PSIs were filled with β -TCP granules (ChronOS granules 1.4–2.8 mm, Synthes, Oberdorf, Switzerland) and with autologous cancellous bone chips harvested from iliac crest.

With ameloblastoma and drug-induced osteonecrosis cases, BMP-2 (Inductos®) soaked in a sponge was placed to cover the cage to improve the bone formation. The scaffold was filled with β-TCP and autologous bone. In four patients with ameloblastoma or drug induced osteonecrosis cases BMP-2 soaked in a sponge was placed to cover the cage to improve the bone formation. Finally, PSI was covered with collagen membrane or sponge (10 patients) and either radial for arm or anterolateral thigh (ALT) microvascular flap (12 patients). The follow up was between 9 – 24 months.

The overall recovery of the patients was favorable considering how demanding the patients were. Nine patients had an uneventful recovery. The facial appearance with respect to symmetry and continuity of the mandible was obtained. Three patients had a

major complication. Major dehiscence through the mucosa and/or microvascular flap leads to infection, and the PSI is needed to be removed. In these patients, the mandible was re-reconstructed with a deep circumflex iliac artery (DCIA) composite microvascular flap. The authors concluded that PSI combined with tissue engineering seems to be a promising solution for treatment of patients demanding large reconstruction of the mandible. Extreme caution should be exercised to avoid soft tissue injury or dehiscence during the surgery and follow-up.

On 2011, Zétola (32) and his coworkers published a case report of mandibular defect repair using recombinant human bone morphogenetic protein-2 associated with collagen sponge, autogenous bone chips and synthetic hydroxyapatite and β -TCP blocks. The mandible continuity defect was due to ameloblastoma resection, and an indirect technique was executed. The titanium reconstruction plate and titanium scaffold filled with the above-mentioned combination were implanted into the defect area. The collagen with rhBMP-2 was superposed above the open titanium mesh to allow muscle cells and periosteum to migrate to the defect area. After the follow-up of 7 months, the patient had stable occlusion and mandible. The control CT showed good bone formation directed to the centre of the defect. The authors concluded that the reported reconstruction technique gave a satisfactory result with less invasive surgery and with minimum morbidity. However, studies with larger number of patients are required to indicate the treatment modality as a routine in cases of bone continuity defects.

In 2015, Park et al. (35) a case study, where a large continuity defect after resection of ameloblastoma in the angle of the mandible was reconstructed with iliac bone and autologous BM-MSCs. The iliac bone served as a scaffold, fixed with titanium plates and screws, with cancellous bone removed. The gap was then filled with cultured BM-MSCs and fibrin glue covered with collagen membranes. Later three dental implants were placed in the graft resulting in uneventful healing.

It is noteworthy, that all these reports can be estimated having been successful due to sufficient coverage of the regenerate with vascular soft tissue enabling oxygen and nutrient supply to the healing area.

4.2.3.2. Ectopic prefabrication

One of the first clinical papers was published by Warnke and coworkers in 2004 (36). They reconstructed a mandibular continuity defect using vascularized custom-made bone flap with indirect technique in which patient's CT data was uploaded to CAD software and the defect reconstructed in the mandible was virtually simulated. A virtual implant to repair the defect was designed and converted into solid 3D Teflon replica, which was used as a model when manually shaping titanium mesh around it. The shaped mesh was filled with bovine bone mineral blocks combined with growth factor rhBMP-7, bovine collagen type-1 and autologous iliac crest bone marrow. The filled mesh was implanted into patient's back muscle (latissimus dorsi). A microvascular flap was raised 7 weeks later and 4 weeks after the implantation the patient was able to use her mandible and was satisfied with the aesthetic outcome. The authors concluded that ectopic bone formation is possible and causes less burden to the patient compared to conventional bone grafts.

The neovascularization with prompt recovery of nutrition is considered to be a key issue of bone regeneration. Kokemueller et al. (37) reported a clinical case of craniomaxillary defect that was reconstructed with the combination of autologous iliac crest bone marrow, β -TCP and rhBMP-2 in titanium scaffold. The reason for the defect was chronic osteomyelitis. The patient suffered from several other comorbidities as well.

They designed and produced β-TCP cylinder that had central passage with the diameter of 7mm. The for blood-soaked cylinders were implanted into latissimus dorsi muscle. Perforator vessels were placed into central passage to enhance capillary growth. After 6 months, the flap with heterotopic bone was raised and transferred to oral and maxillofacial (OMF) defect and covered with titanium mesh. The mesh was fixed with titanium screws. There was no complication during the follow up of 1 year. The authors conclude that the use of autologous bone marrow and β-TCP block with central vessels to repair OMF bone defects is reliable and well tolerated. Furthermore, most of the donor site morbidity can be avoided with this technique. The research group performed also experimental studies with same protocol and the results confirmed the clinical achievements.

Mesimäki and coworkers (38) reported a successful hemimaxillary bone and soft tissue defect reconstruction using microvascular flap with heterotopic bone in the year 2009. The male patient had a hemimaxillary defect due to recurrence and resection of a large keratocyst. The patient did not tolerate removable obturator prosthesis. The repair was decided to execute using heterotopic bone flap. The construct consisted of ß-TCP as scaffold material seeded with patient's autologous adipose-derived stem cells expanded in a GMP-class laboratory and commercially available growth factor BMP-2. The material was the inserted into a titanium mesh preformed to fit the size and shape of the defect. The construct was first implanted into the patient's rectus abdominis muscle, where it was let to mature. Figure 5.

After 8 months, the construct together with the surrounding muscle was transplanted using microvascular technique (TRAM-flap) to the site of the defect and connected with titanium plates and screws to the adjacent bones. The anastomosis of flap recipient vessels was performed to neck vessels and flap was fixated with titanium plates. After uneventful healing of one year, four dental implants were inserted into the regenerated "neobone" and finally a fixed bridge was used to reconstruct the masticatory function.

The histological samples obtained at the time of fixture operation confirmed normal bone tissue in heterotopic bone area. The follow-up has been uneventful for a decade, only some small pieces of titanium mesh have had to be removed as they have protruded through the thin oral mucosa (unpublished results).

The same group performed similar reconstruction to a male patient after total maxillary defect caused by ablation of a large squamous cell carcinoma (SCC). The combination of AT-MSCs, β-TCP granules, rhBMP-2 in polylactide scaffold was implanted into the ALT-flap and shaping of a resorbable polylactic scaffold was done with computer aided design, with indirect technique. At the same time, the titanium patient-specific (PS) reconstruction plate to fixate the future 'neomaxilla' was designed and manufactured using direct computer technique and laser rapid prototyping. After a maturation of 7 months, the microvascular ALT flap with heterotopic bone was raised and placed into the area of resected maxilla. The orientation and fixation of the flap were secured with PS reconstruction plate. After eventful healing of 5 months, the dental implants were placed and occlusion was established with removable prosthesis (not yet published) (Fig. 16.5).

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4. Challenges

Despite many promising studies, many theoretical mechanisms need to be studied before wide clinical use. The effectiveness as well as safety are of utmost importance.

One big challenge is radiotherapy or chemoradiotherapy. The regenerate cannot tolerate radiotherapy and after radiotherapy the implantation of the regenerate can be very challenging due to shrinkage of the tissues as well as poor vasculature in the area. However, we have successfully implanted large microvascular regenerate to a patient who had lost nearly whole maxilla due to a large squamous cell carcinoma and had receiver chemoradiotherapy after the operation. The key finding here was the microvascular blood supply and that the new soft tissues in the maxilla were in the same composite graft (Unpublished results).

Real prospective clinical studies, needing huge amounts of money and resources, need to be arranged before this treatment method will be widely accepted. The price of the reconstruction must also go down, which can be done by off-the-shelf products and scaling up the production. However, for a researcher, there is still many aspects to be studied.

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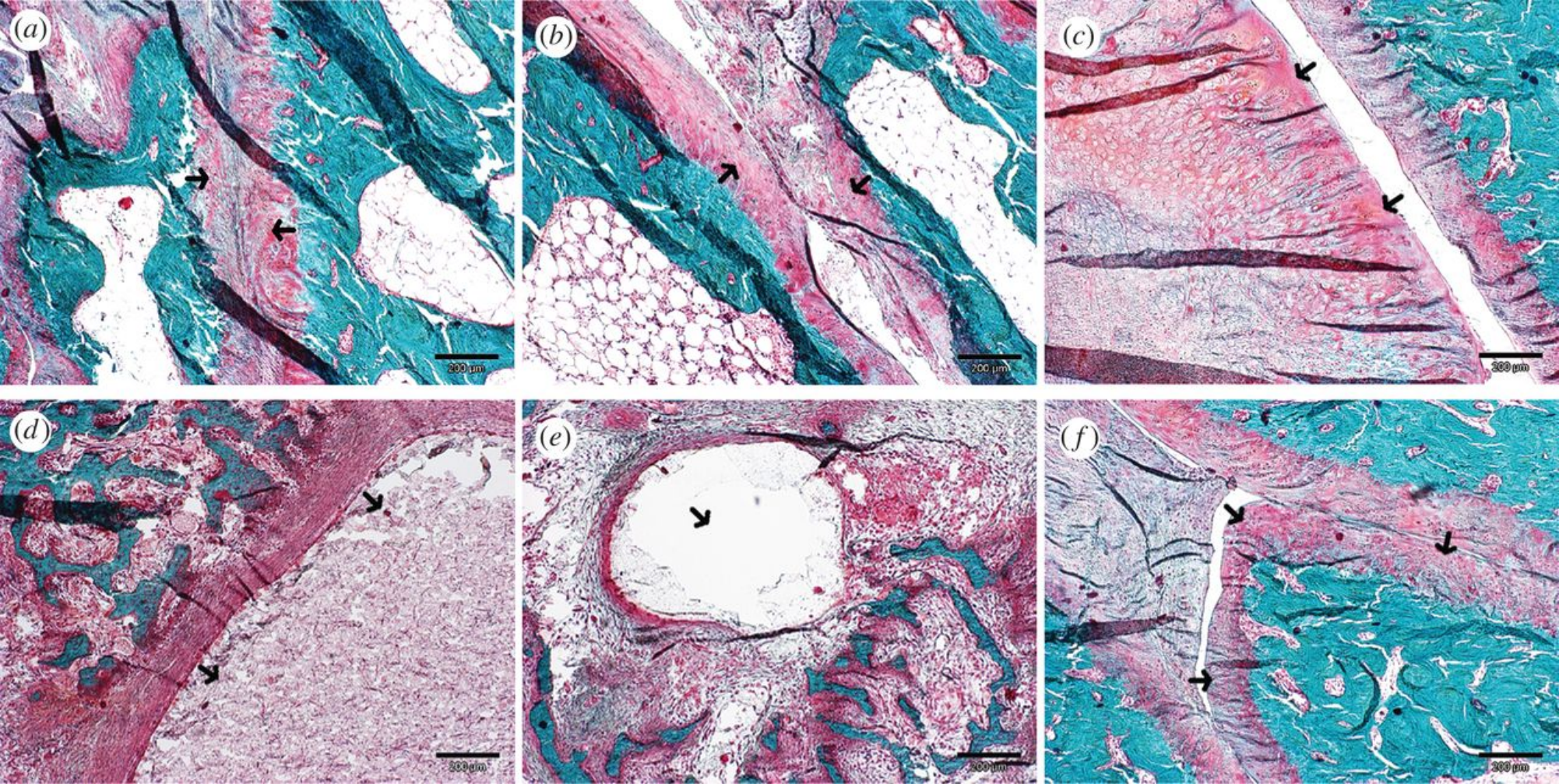
(a)
200 µm
(b)
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(d)
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(e)
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(f)
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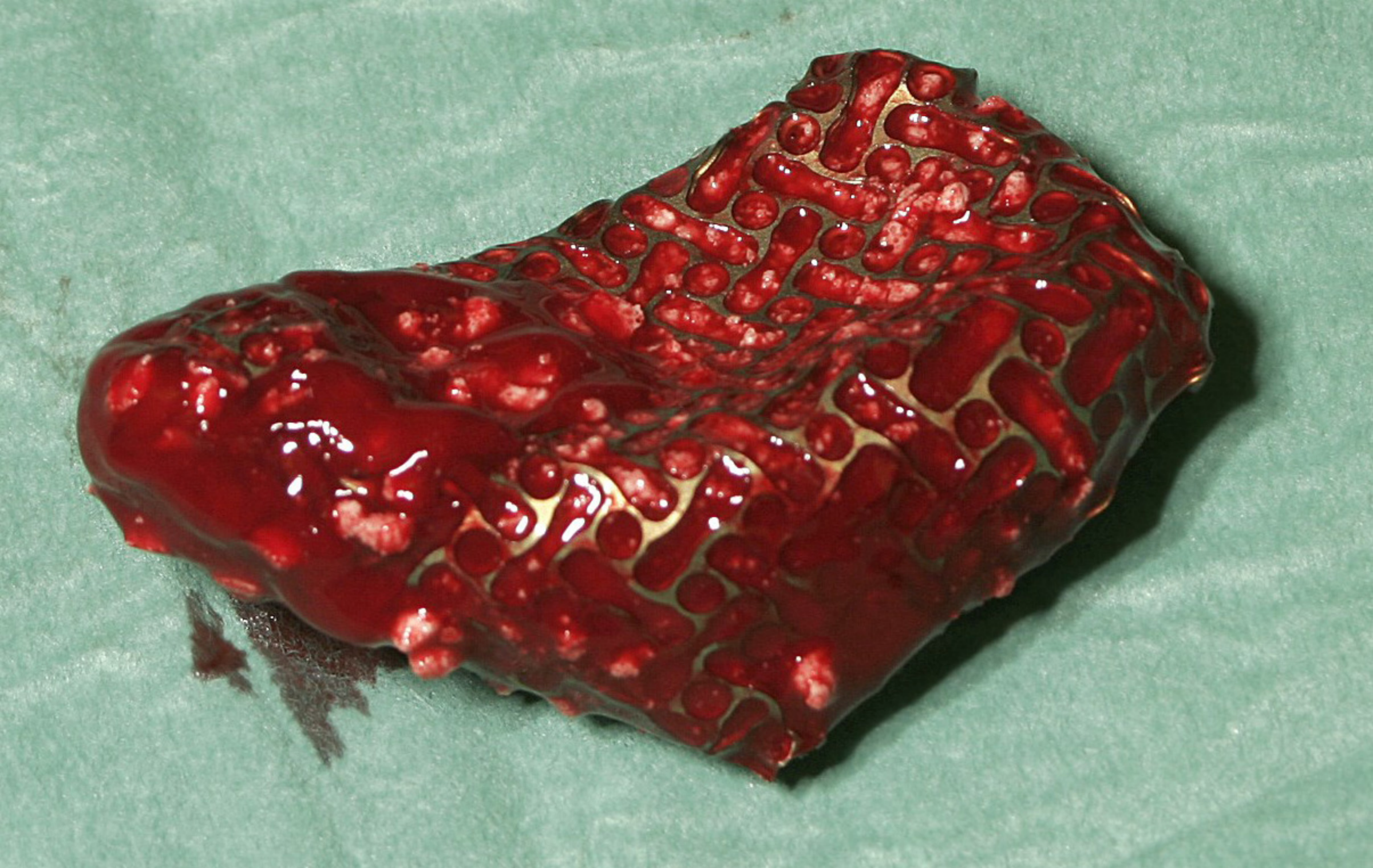
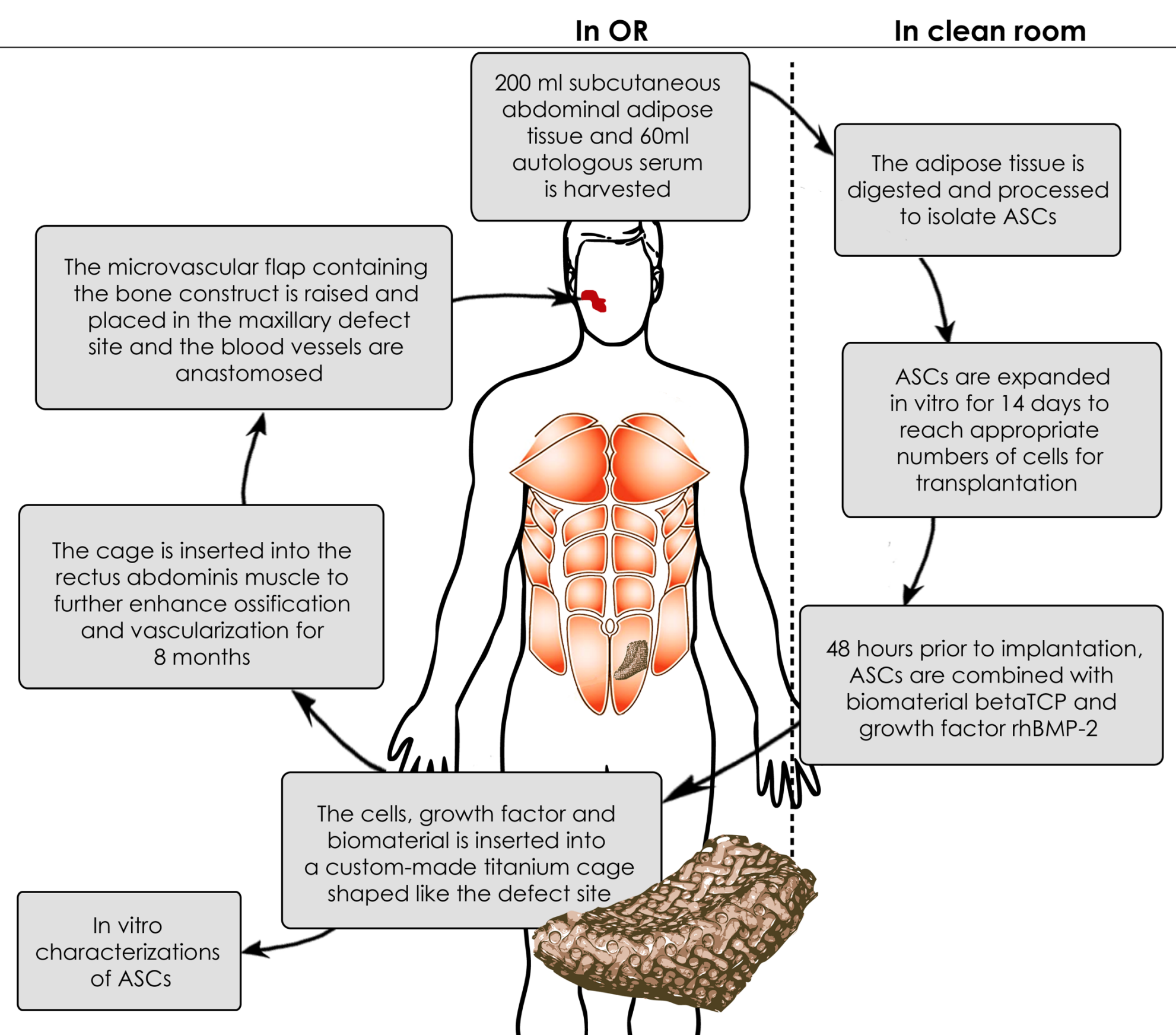
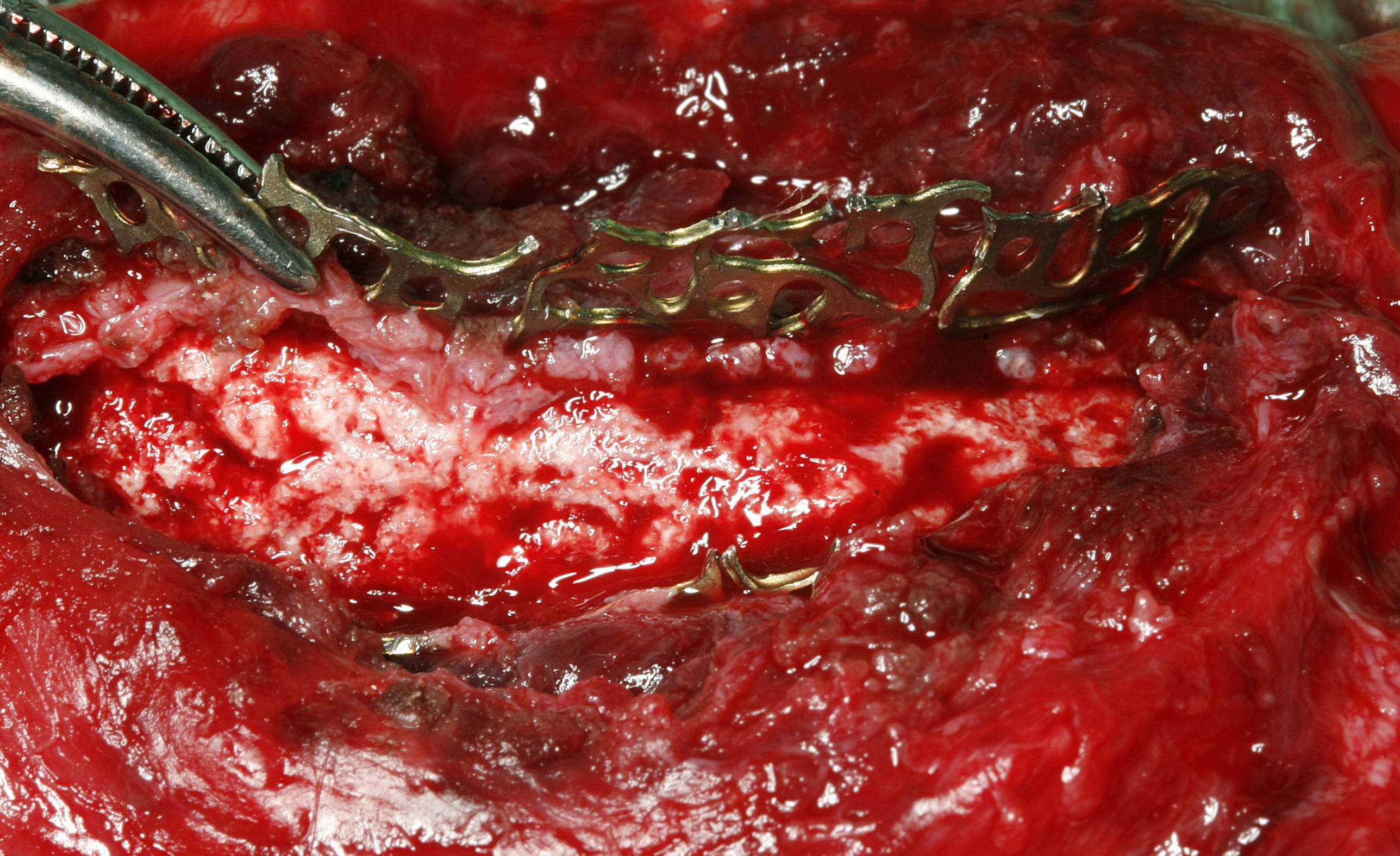
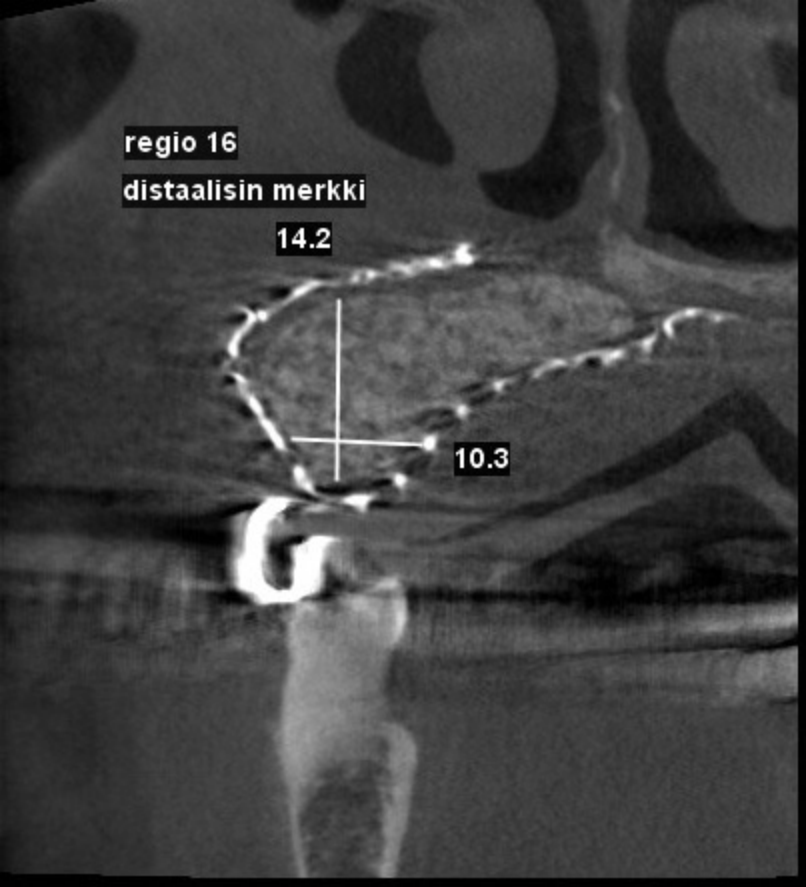



In OR
In clean room
200 ml subcutaneous abdominal adipose tissue and 60ml autologous serum is harvested
The adipose tissue is digested and processed to isolate ASCs
ASCs are expanded in vitro for 14 days to reach appropriate numbers of cells for transplantation
48 hours prior to implantation, ASCs are combined with biomaterial betaTCP and growth factor rhBMP-2
The cells, growth factor and biomaterial is inserted into a custom-made titanium cage shaped like the defect site
The cage is inserted into the rectus abdominis muscle to further enhance ossification and vascularization for 8 months
The microvascular flap containing the bone construct is raised and placed in the maxillary defect site and the blood vessels are anastomosed
In vitro characterizations of ASCs

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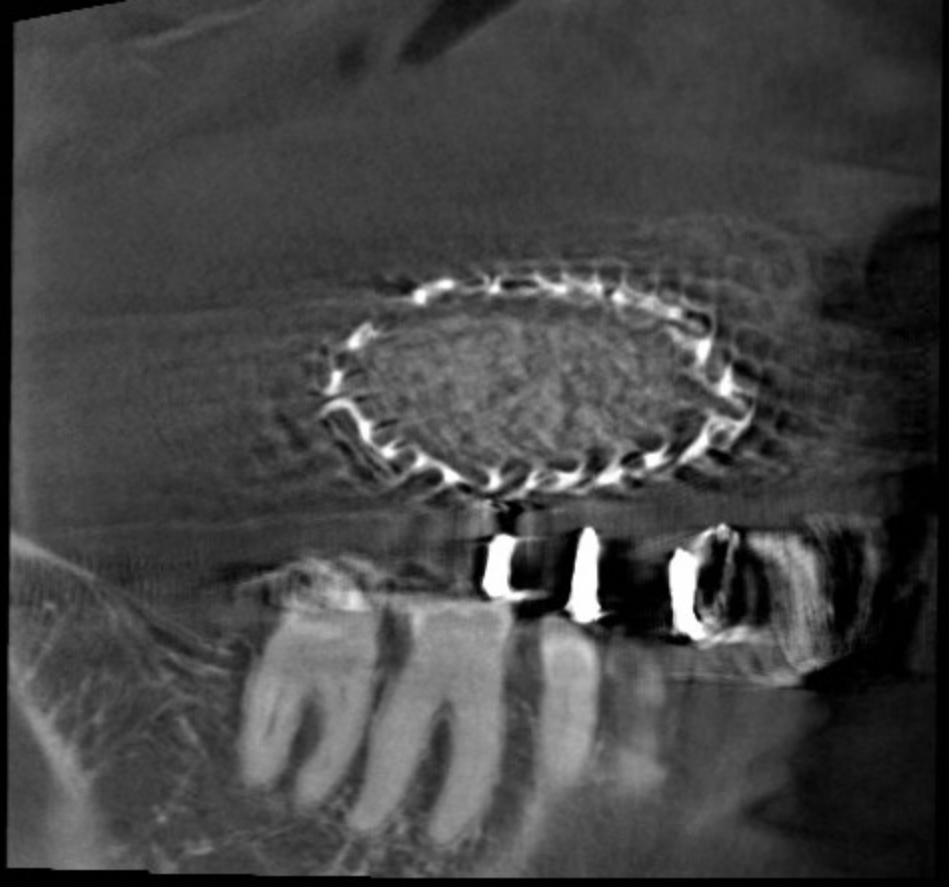
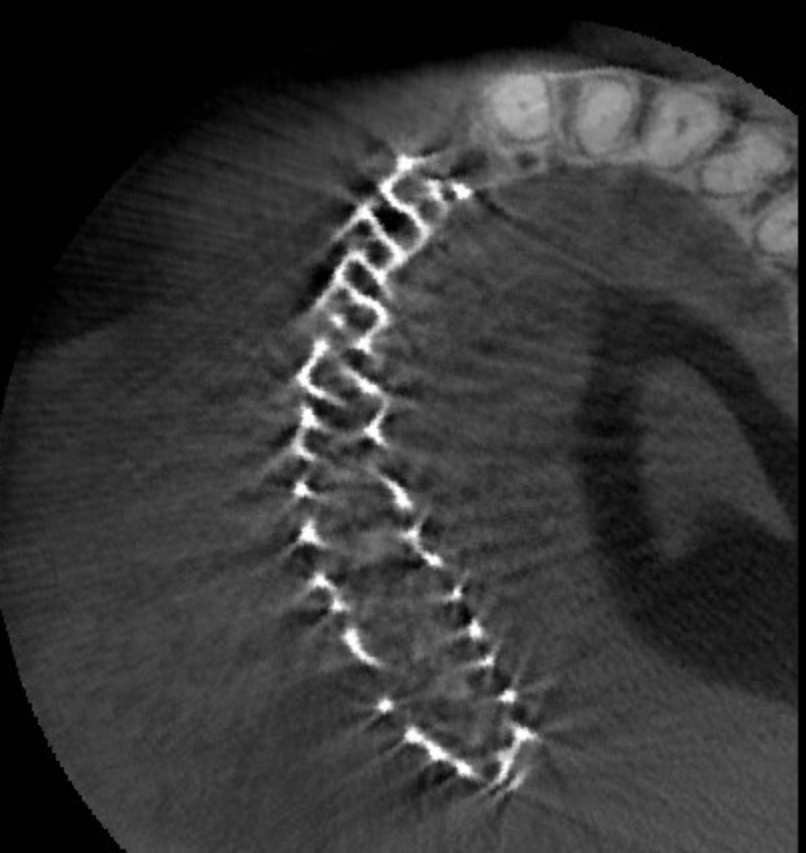
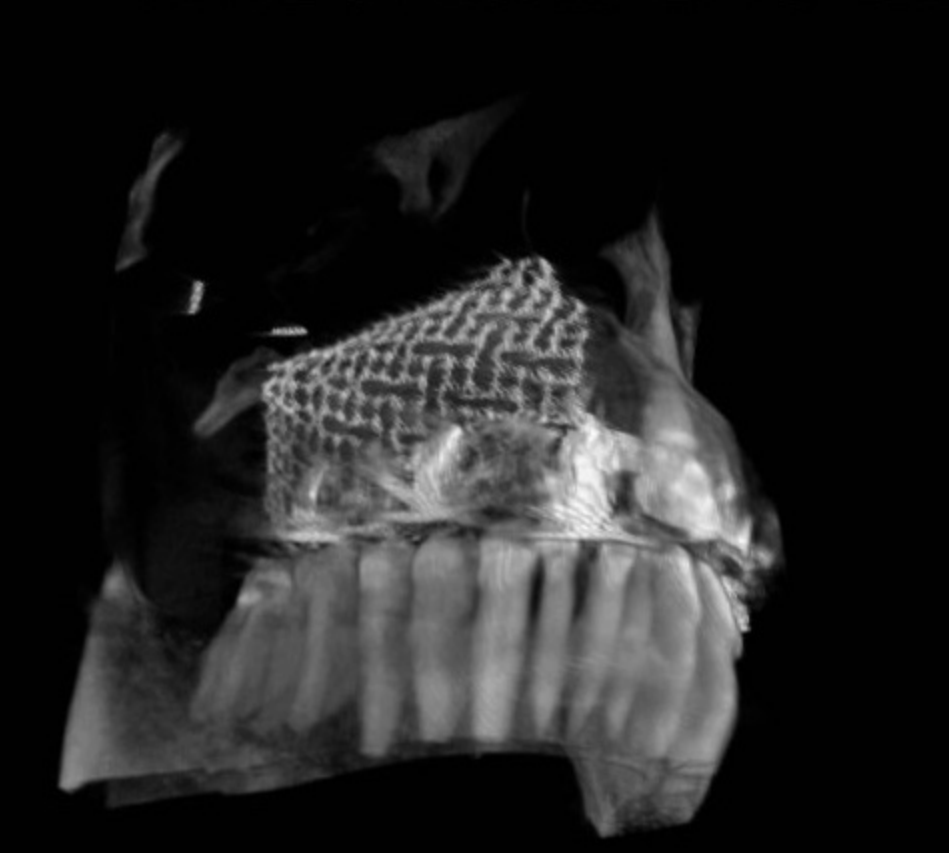
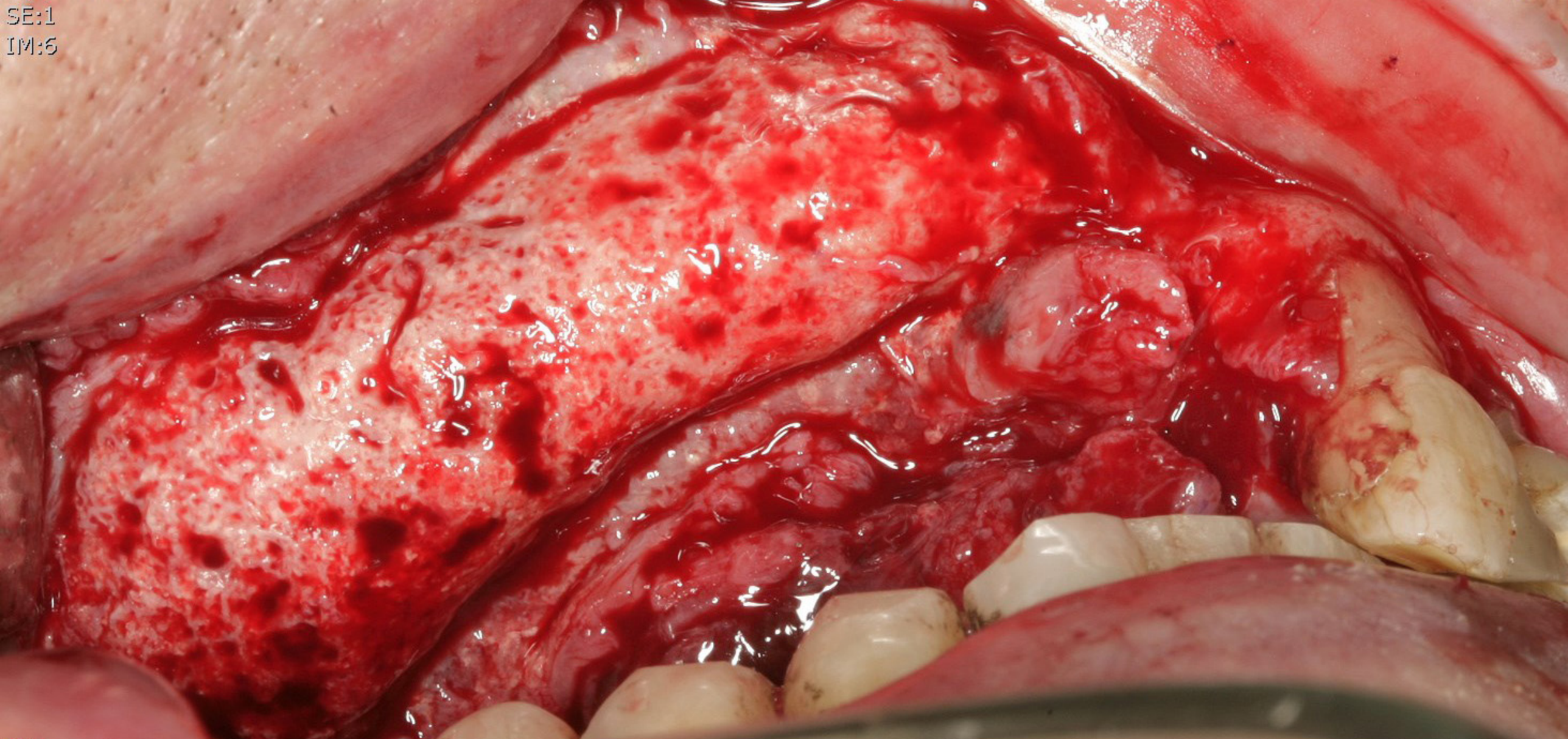
14.2
10.3





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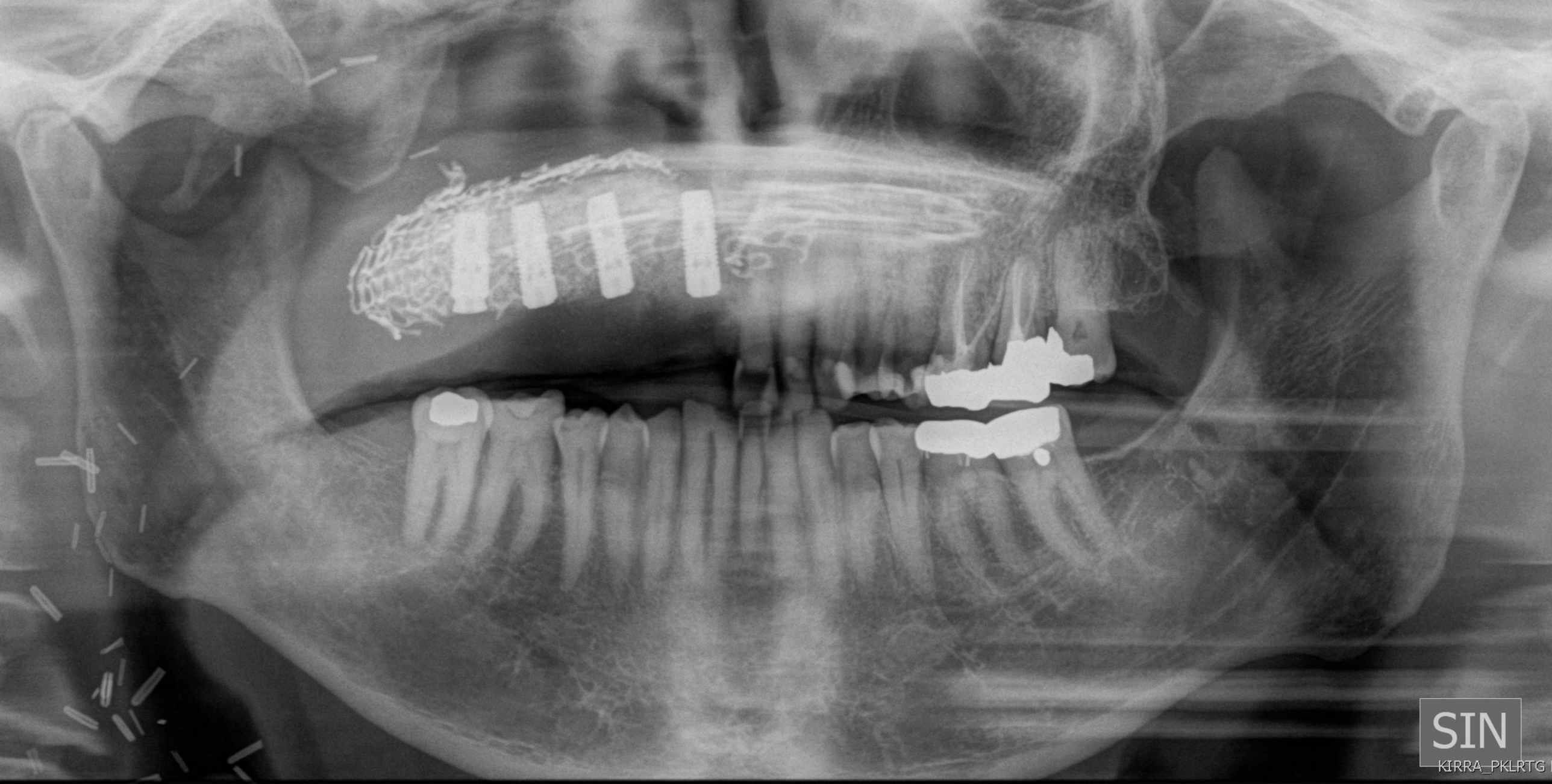
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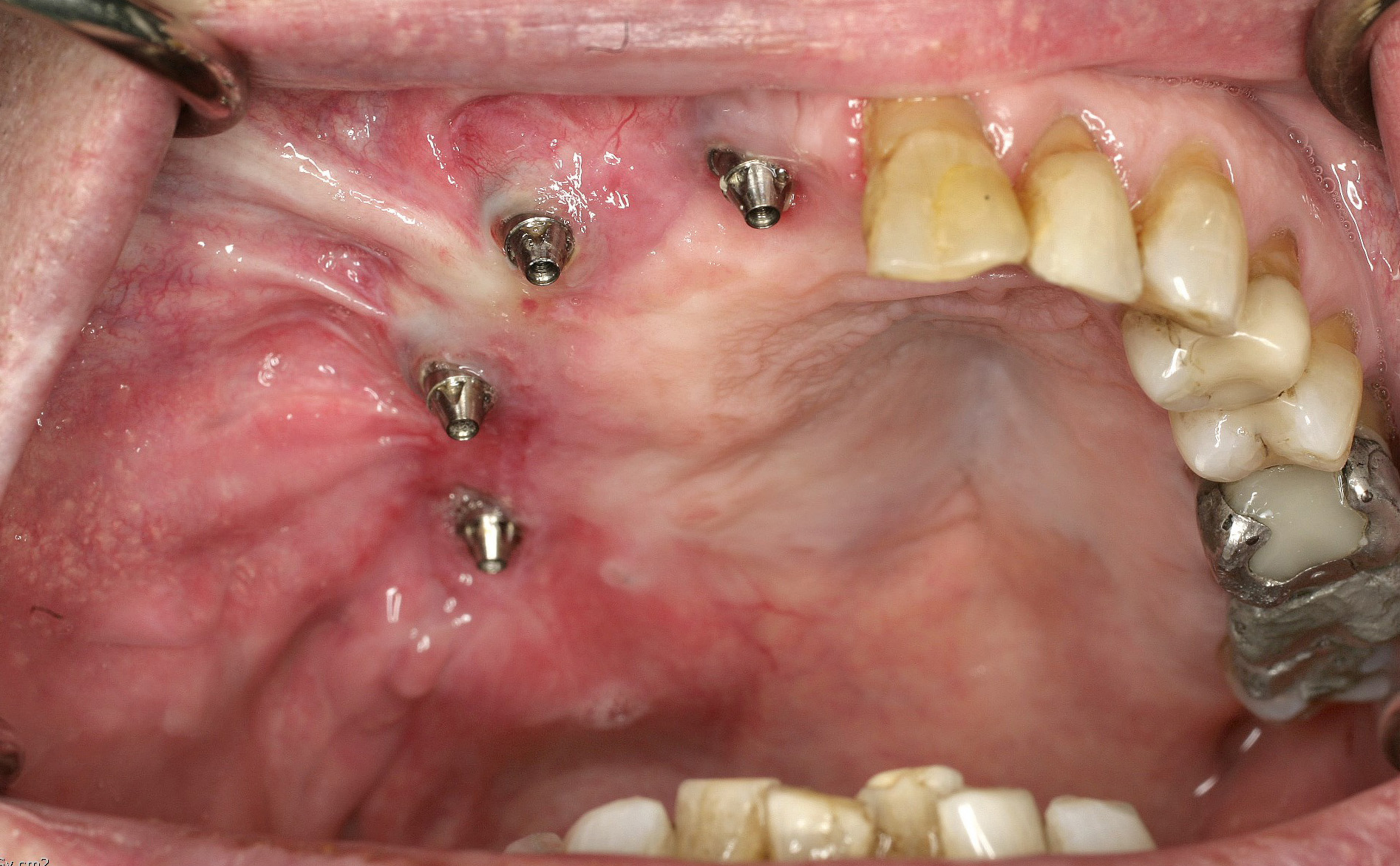

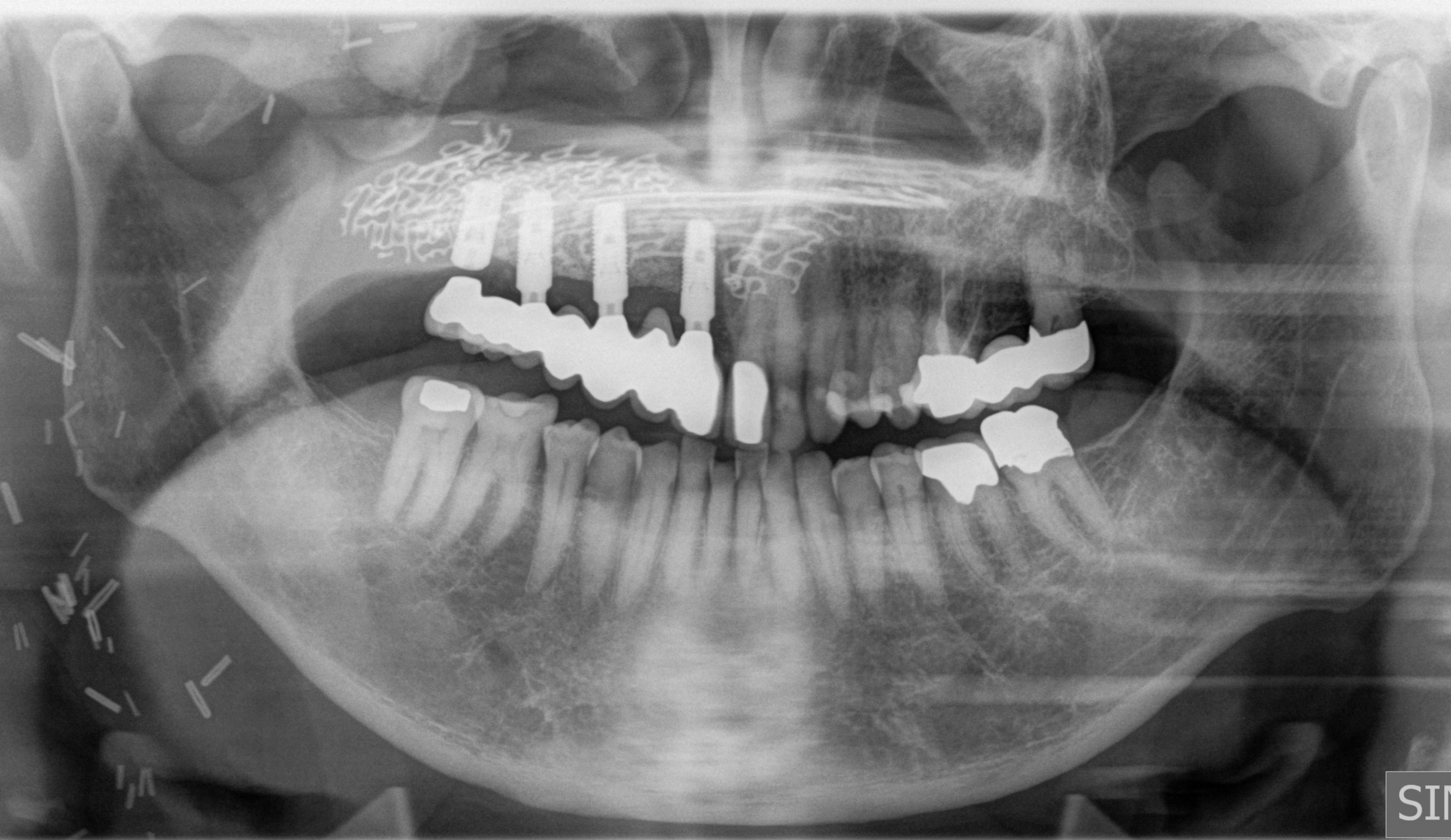

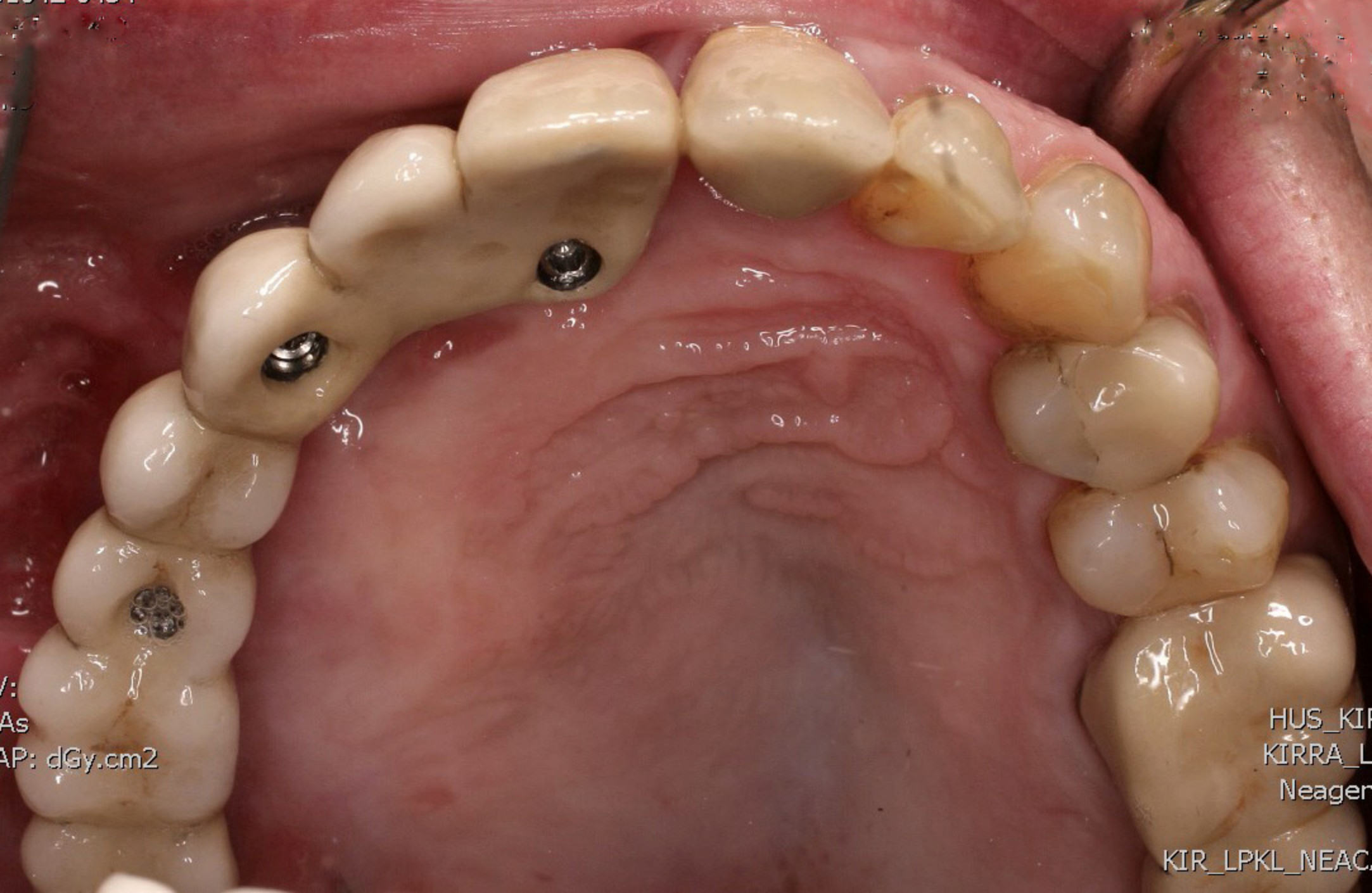
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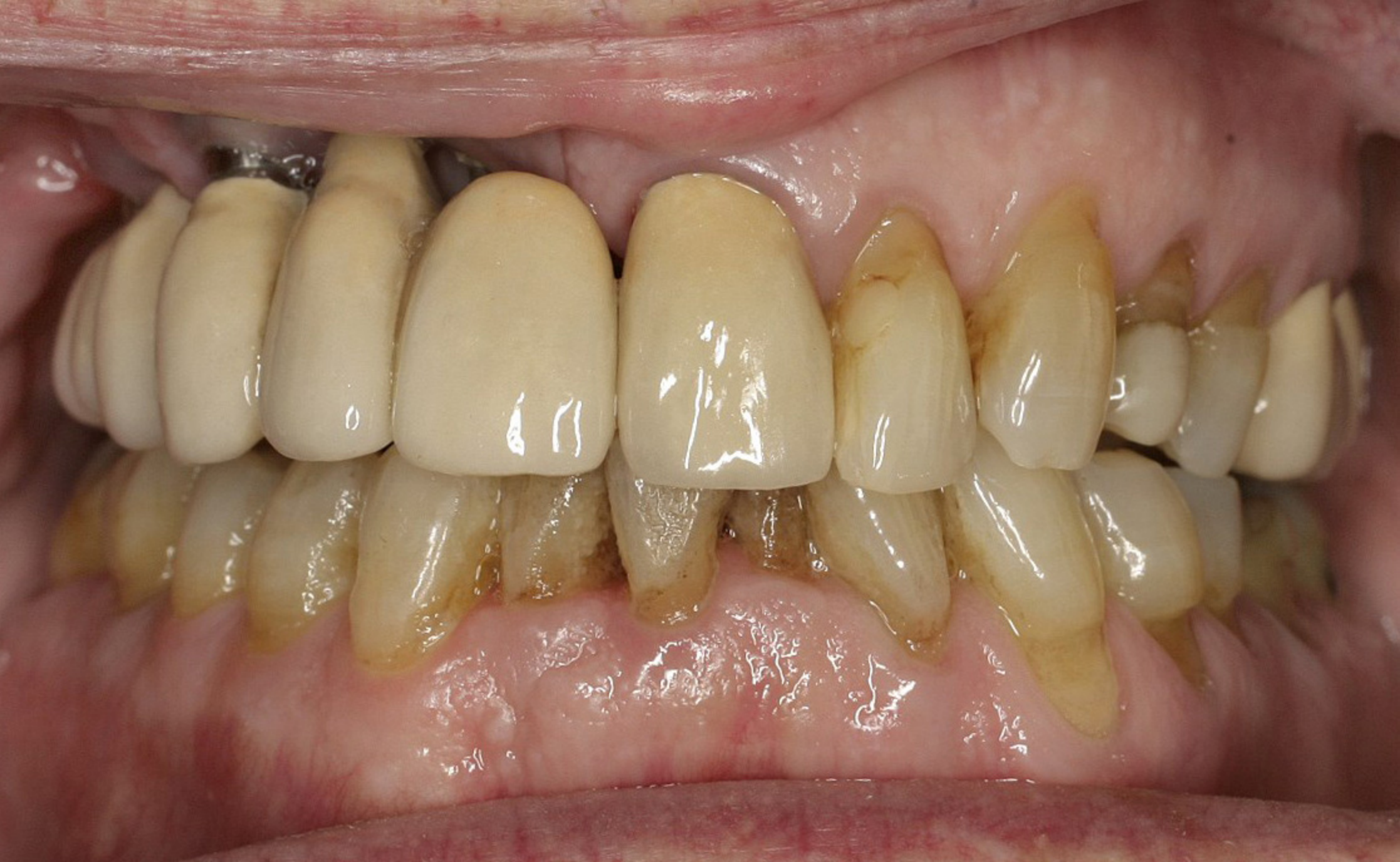
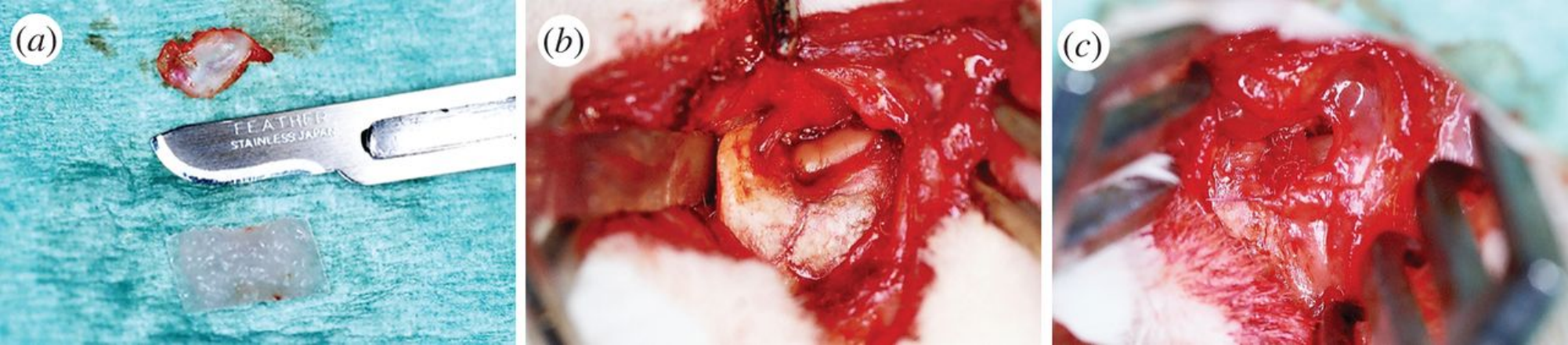
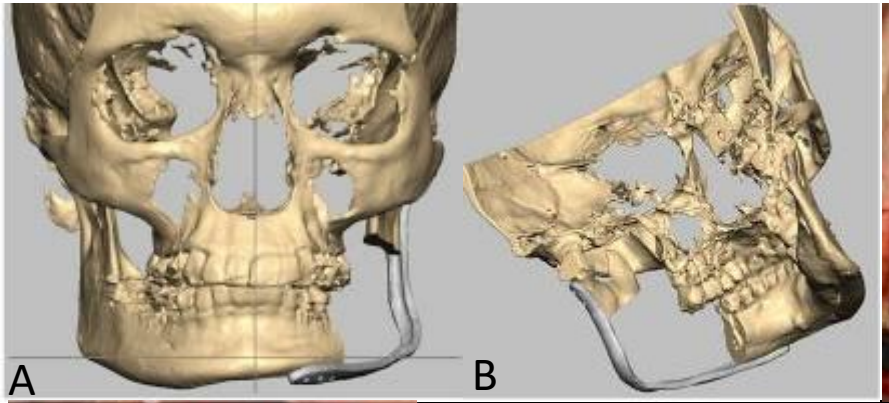
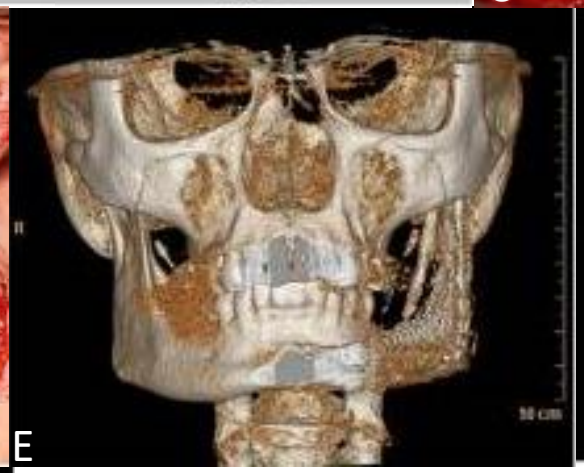



(a)
FEATHER
STAINLESS JAPAN
(b)
(c)


A
B


C


D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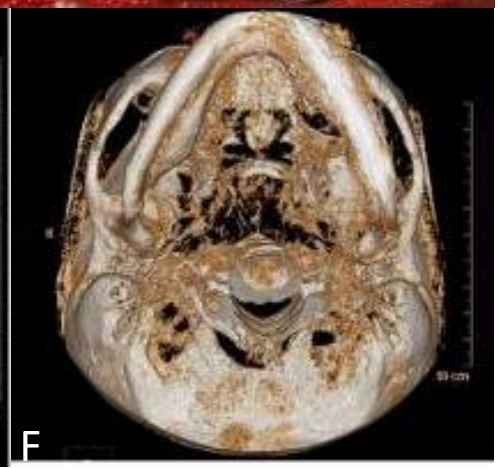
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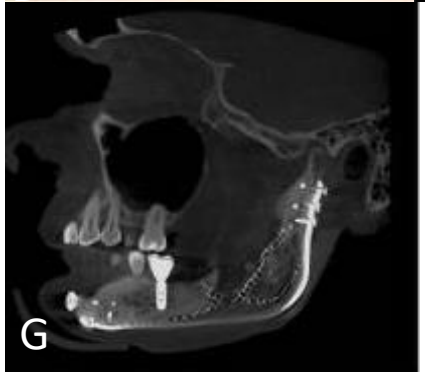
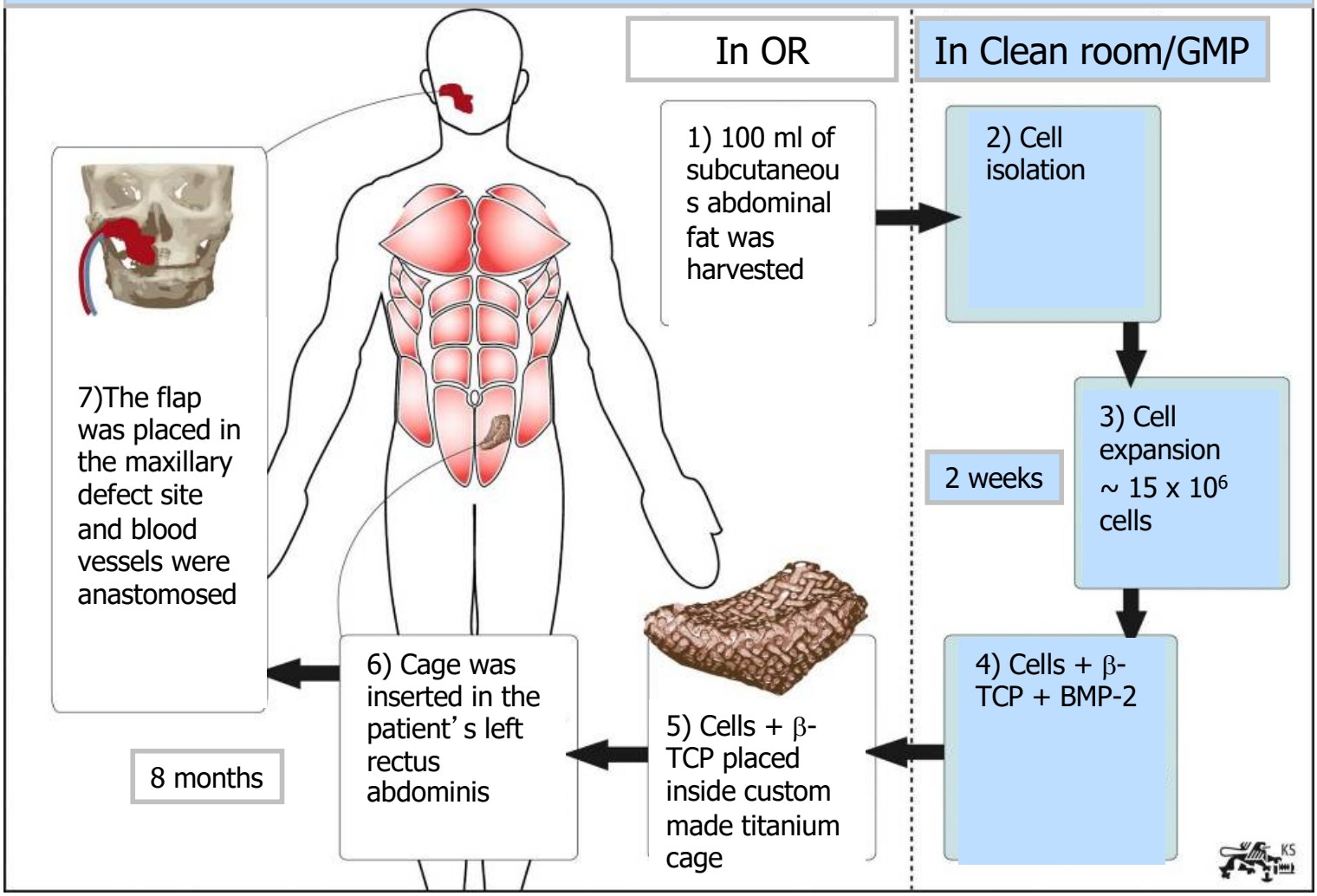

G

Ectopic Bone Formation



Modified from Suomen Kuvalehti