OsseoPrint3D Technology for Personalized, Precision Craniomaxillofacial Defect Repair

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Abstract

Craniomaxillofacial (CMF) anomalies can arise from congenital malformations, traumas, and surgical excisions related to cancer. CMF repair can often lead to formidable challenges for overall health and CMF growth and development, due to unfavorable surgical outcomes. OsseoPrint 3D (OP3D) technology endeavors to enable chair-side, patient-tailored methodologies for efficient, precision repair and regeneration of CMF tissues. The study described here utilizes a rabbit mandibular defect repair model to validate OP3D technology for efficient regeneration of hard tissue CMF defects. Bilateral 10mm partial mandibular defects were created in each rabbit using a trephine drill with irrigation, leaving the lingual wall intact. Cylindrical Polydioxanone scaffolds, measuring 10mm in diameter and 5mm in length, were fabricated using the OP3D Technology and printer. Next, a scaffold was place into each rabbit mandible defect, the entire defect was

filled with DynaBlast® Demineralized Bone Matrix with Cancellous Bone, and the soft tissue was sutured closed in layers. Two rabbits (4 implants) were used for each of 4 time points - 5, 70, 90, and 180 days. At each time point, hemi-mandibles containing implants were harvested and fixed in formalin for 48 hours prior to Micro-CT analysis. Next, rabbit mandibles were decalcified, paraffin embedded, and serial sectioned for histological and immunohistochemical analysis. Abundant new bone formation within the scaffold pores was detected in 70-day implants. At later times, scaffolds appeared further degraded, and newly formed bone appeared similar in morphology to natural bone, exhibiting a lattice-like network of trabeculae. Significant bone regeneration was observed by 6-months, validating OP3D scaffolds for effective precision repair of CMF defects. This study presents the first evaluation of bone regeneration using OP3D scaffolds in a clinically relevant animal model, marking a significant milestone in the advancement of this technology.

Keywords: Craniomaxillofacial defects, Bone regeneration, 3-D printing,

1. Introduction

Craniomaxillofacial (CMF) defects, arising from various congenital abnormalities, injuries, and cancer resections, can significantly jeopardize CMF aesthetics and function, ultimately negatively impacting the overall quality of patients' lives [1]. Bone graft therapy, the conventional approach for CMF defect repair, presents challenges due to the limited availability of autografts, the risk of disease transmission and immune rejection with allografts and xenografts, and the high cost, long duration of and frequent need for multiple complex surgical procedures [2]. The unique anatomical features of craniofacial bones make successful structural and functional CMF defect repair even more challenging [3]. Given these limitations, there is growing interest in developing alternative techniques for more effective CMF bone regeneration.

Various fabrication techniques have been developed to form three-dimensional (3D) constructs for bone regeneration by combining supporting scaffolding materials and biochemical cues, such as 3D printing, electrospinning, and freeze-drying technology [4]. Among these technologies, 3D printing is gaining popularity based on the ability to rapidly assemble various biomaterials through a layer-by-layer approach, providing precise control of size, shape and added growth factors [5]. Compared to traditional manufacturing techniques, 3D printing offers the ability to fabricate personalized scaffold constructs tailored to patient-specific needs and complex anatomical structures. 3D printing is a powerful production technique in the field of biomedical devices. Presently, the United States Food and Drug Administration (FDA), the organization responsible for the approval of medical devices, evaluates 3D-printed devices under the same guidelines used for traditionally manufactured products, mainly based on safety, efficacy, and quality [6].

Based on bioink dispensing mechanisms, 3D bioprinting technology comprises three main categories of printing systems: inkjet printers; laser-assisted printers; and microextrusion printers [7]. Inkjet printers use a printer head to deposit biomaterials by thermal, microvalve, or acoustic forces onto a collecting surface [8]. Laser-assisted printers use lasers to generate pressures that propel biomaterials onto a collector substrate [9]. Microextrusion printers use pneumatic or mechanical dispensing systems to extrude continuous struts, rather than droplets, to create 3D scaffolds [10].

OsseoPrint 3D (OP3D) technology aims to provide precision, personalized approaches for effective CMF tissue repair and regeneration. OP3D has developed a proprietary platform to design 3D printed, patient-matched bone scaffolds for fast and effective bone reconstruction surgeries. Figure 1 depicts OP3D's technology workflow. First, a 3D scan of the patient is used to compile a 3D computer-aided design (CAD) model from which the bone defect can be defined and used to create a patient-specific scaffold model. After validating the design, the model is sent to a bench-side 3D printer to print the scaffold aseptically in 30 minutes. Once printed, the scaffold can be filled with bone grafting material and implanted into the patient.

The study described here was designed to obtain initial observations relating to the handleability of the OP3D manufactured bone implants and their ability to promote alveolar bone regeneration in a rabbit mandible CMF defect repair model. In addition, this study analyzed bone regeneration within the defect, host tissue response to the scaffolding materials, and scaffold degradation for up to 6 months. As such, this study will provide valuable guidance for the further development of an OP3D bench-side 3D-printer device to facilitate customized, patient-specific scaffolds for CMF bone regeneration.

2. Materials and Methods

2.1 Scaffold Fabrication

The implants used in this study were manufactured at OsseoPrint's laboratory located within the NJ-State BioSciene Center in North Brunswick, NJ, an incubator facility that provides a clean and safe environment for OsseoPrint's lab activities and implant manufacturing. Polymer specifications were provided by EVONIK, who was contracted to custom-create a powder blend of RESOMER X206S, abbreviated as PDO. Melted polymer of the choice is extruded from a uniformly and steadily heated syringe through the 200 microns nozzle by e precision-controlled motion of the locking plunger, thus providing a uniform flow of the continuous polymer bead. The extruded bead is laid over and chemically adheres to the previously accrued layers of solidified polymer struts. First layer is extruded over the specially designed build-plate assuring reliable adhesion of the bead to the plate itself while providing easy detaching of the final details by the sharp knife. Build-plate is secured on the XY movable table which precisely controlled motion provides the extrusion of each layer according to a rectilinear snaky pattern consisting of long runs across the shape of the implant which runs separated by about 700 microns center-to-center and connected by the short runs along the outer perimeter of the implant. Long runs of each consecutive layer are perpendicular to those of the previous one, thus providing strong mechanical properties and featuring an open interconnected voids between the spatial structure of the straight struts highly permissible to fluids and cell transportation.)

Prefabricated scaffolds were created by an extrusion process using a pre-mixed blend of PDO and 20% (w/w) beta-tricalcium phosphate (b-TCP), packaged individually and sterilized using Ethylene Oxide (ETO). The packages were shipped to Dr. Yelick's lab at Tufts University, Boston, MA and stored at -20°C until use. Initially, three scaffold sizes were printed (8 mm, 9 mm, 10 mm diameter, all 6 mm height) and tested for proper fit in a 10 mm diameter rabbit cadaver jaw defect. The 10mm diameter scaffold fit the best and was selected for use in the *in vivo* study.

2.2 Rabbit Surgeries

All animal experiments were conducted using Tufts University Institutional Animal Care and Use Committee (IACUC) approved protocols, as previously described by us (**Figure 2**) [11]. Briefly, bilateral 10 mm circular defects were created in the left and right mandible of each rabbit using a trephine drill under continuous irrigation. The buccal bone wall, tooth roots, and any remaining bone fragments were carefully removed, leaving the lingual bone intact. Next, a OP3D scaffold was placed into each defect, followed by gently injecting ~0.5 mL DynaBlast® Demineralized Bone Matrix with Cancellous Bone (KeystoneDentalGroup, Burlington, MA) to fill the defect site. Soft tissues were then sutured closed and the animals were maintained at the Tufts University animal facility. Immediately before harvest, the rabbit jaws were perfused with formalin to ensure sufficient fixation of jaw tissues. Two animals each were harvested after 5 days, 70 days, 3 months, and 6 months of implantation.

2.3 Sample analyses

Microcomputed Tomography (Micro-CT) analyses. Micro-CT analyses were conducted on each hemimandible and implant using a Skyscan 1176 imager (Bruker MicroCT, Billerica, MA, United States) and set parameters of 100 kV, 100 A, Al-Cu filter, 0.3 rotation step over 180° and pixel size 9 µm, together with two bone mineral density (BMD) phantoms with BMD values of 0.25 and 0.75 g/cm^{-3}. The Micro-CT data was then reconstructed to rebuild acquisition datasets using NRecon software (Bruker Micro-CT).

The region of interest (ROI), defined as a full thickness (6 mm), 10 mm diameter cylinder that matched the defect area was further selected and evaluated for new bone formation. Properties of the newly formed bone, including the bone mineral density (BMD), bone volume/tissue volume (BV/TV), trabecular thickness, separation, and number were fully characterized using CTAn (Bruker Micro-CT) and Avizo software (Version 1.6.9.15, ThermoFisher Scientific, Materials & Structural Analysis Division, Hillsboro, OR, United States). Areas of unoperated control mandibles were analyzed as age-matched, natural bone controls.

Paraffin Embedding and . After Micro-CT scanning, harvested mandibles were decalcified in (1:1) 45% Formic Acid:20% Sodium Citrate solution, changed twice per week. Complete demineralization was confirmed by mixing (1:1) demineralization solution with Saturated Ammonium Oxalate and demonstrating no precipitate formation. Demineralized mandibles were then trimmed close to the defect site and prepared for paraffin embedding and sectioning using standard protocols as previously described by us [11]. The samples were serially sectioned at 6-micron widths, mounted onto microscope slides, and prepared for histological staining including Hematoxylin and Eosin (H&E) and Masson's Trichrome staining. Stained slides were analyzed and imaged using Zeiss Axiophot microscope and digital Zeiss Axiocam camera (Carl Zeiss AG, Jena, Germany).

Statistical analysis. A group-to-group comparison of bone density and bone quality from the MicroCT data and image analysis of H&E sections was conducted to quantify bone formation and scaffold degradation. A two-way analysis of variance with Tukey's post hoc analysis was used to compare the bone quality at each time point. P value ≤ 0.05 were indicated as significant differences.

3. Results

3.1 Scaffold observation

3D printed OP3D scaffolds exhibited high porosity, with well-organized and open pore architecture. Macropores within the printed struts showed the pore size range and architecture like trabecular bone (**Figure 3**).

3.2 Post-Surgical Analyses

All eight rabbits exhibited excellent recovery and healing with no signs of inflammation post-surgery. No weight loss or other adverse reactions were observed throughout the entire period.

Five (5) Day Time Point: At 5 days, the micro-CT scan of harvested jaws with implants showed an easily identifiable radiolucent circular defect site (**Figure 4**). Although no new bone is present within the defect at this time point, small radio-opaque bone-like fragments appeared evenly distributed throughout the defect area due to the presence of bone chips in the DynaBlast filler and b-TCP in the implant struts. Histological staining confirmed the Micro-CT observation, with no new bone formation observed (**Figure 5 A**). The scaffold material grids, which appear as bright white speckles under polarized light, were visible throughout the defect area (**Figure 5, A2**), and Dynablast bone chips were present within the pores of the scaffold (**Figure 5, A**). A small amount of immune cell infiltrate, including neutrophils and macrophages, were identified in some of the histological stained sections (**Figure 5, A3**).

Seventy (70) Day Time Point: After 70 days, robust new bone formation within the defect area was identified in all implants (**Figure 4, 5B**). The newly formed bone, primarily identified as large pieces, filled the pores of the implants. No distinct bone formation was observed on the buccal surface of the defect. Scaffolding materials, though less noticeable

compared to 5-day implants, still retain their original grid structure (**Figure 4 and Figure 5, B3**). No inflammatory response was observed at 70 days.

Three-Month Time Point: At 90 days, the defect margins were less distinguishable, and new bone formation was observed along the buccal surface of the implants (**Figure 4**). New bone formed within the defect area showed a more mature morphology, and OP3D scaffold grid struts were less distinguishable. Remaining scaffold and DynaBlast bone chips were occasionally observed (**Figure 5, C2 and C3**).

Six-Month Time Point: A six month time point was chosen based on the fact that dental implants are commonly placed 4 and 6 months after bone grafting in human clinical practice. At 6 months, a lattice-like network of bone marrow and trabecular bone was observed, closely resembling the natural rabbit mandible (**Figure 5D**). Small amounts of remaining scaffold material were observed **(Figure 5, D3,s).** The buccal bone surface appeared largely healed (**Figure 4**).

In summary, the CMF defect margin was clearly identifiable in 5-day Micro-CT and histological sections (**Figure 5A1, A1'**). The defect margins became less distinguishable over time as the bony defect was replaced by newly formed bone (**Figure 5C1, C1'**). The 3D printed scaffold and DynaBlast containing bone chips underwent degradation over time. Remaining scaffold dissolved during histological processing, leaving obvious voids in the sectioned and stained implants. New bone formed initially within the scaffold pores, gradually filling them over time. In the 6-month harvested samples, newly formed bone appeared organized into a lattice-like network of trabeculae with bone marrow-like structures inside, closely resembling that of natural rabbit mandibular bone.

3.3 Bone Quantitative Observations

Hard tissue within the defect area was quantified by bone density and bone BV/TV using μCT image analyses. Trabecular thickness, separation, and number were quantified to evaluate the maturity of the newly formed bone (**Figure 6).** Statistically different mean

bone density was observed between 5 and 70 days ($p = 0.03$), 5 days and 3 months ($p=0.03$), and 5 days and 6 months ($p = 0.001$), and in mean BV/TV at 5 days versus 70 days ($p < 0.001$), 5 days versus 3 months ($p = 0.001$), and 5 days versus 6 months ($p < 0.001$). Trabecular thickness and separation at 5 days also showed significant differences as compared to all other time points. With respect to trabecular number, statistically significant differences were observed between 5 days and 3 months ($p = 0.02$), but not between any other times tested. All parameters measured, including bone density, bone BV/TV volume, and trabecular thickness, separation, and number as compared to natural rabbit jawbone showed significance to all of the time points.

4. Discussion

Scaffold design is an important consideration for successful CMF hard tissue regeneration, including the ability to direct osteogenic biofunctionalization, provide sufficient mechanical support during healing, and facilitate soft tissue integration over the scaffold. A variety of materials have been studied for CMF bone regeneration. Titanium scaffolding is widely used in clinical settings to repair craniofacial bone defects, especially the cranial vault and in load-bearing areas, due to its excellent biocompatibility, osteointegration and mechanical properties [12, 13]. 3D-printed titanium can incorporate porosity into the scaffold to provide better bone-scaffold integration [14]. Biopolymers such as Polycaprolactone (PCL) and PLGA, widely used scaffolding materials for clinical repair of CMF boney defects, are valued for their high degree of tunability, biocompatibility, and mechanical strength.

Customized 3D-printed PCL scaffolds have been used for effective repair of complex CMF defects in humans, where patient-specific 3D-printed PCL scaffolds were fabricated using computer-aided design and placed into the post-ablative maxillary defects of three patients [15]. Improved facial morphology was achieved, even up to two years post-implantation. However, tissue ingrowth primarily consisted of soft tissue, with minimal bony in-growth [15]. Calcium phosphate compounds (mainly β-TCP) are widely used for CMF bone repair due to their excellent biocompatibility, osteoconductive

properties, and similarity to natural bone [16]. 3D-printed Calcium phosphate ceramic scaffolds have also been tested for skeletal bone regeneration in an adult rabbit, critical-sized, mandibular defect model [17], where regenerated bone achieved similar properties to the native bone after 8 weeks. Clinical studies using 3D-printed bioactive ceramics achieved successful CMF bone repair, although the brittle nature of ceramics has limited their broader application [18, 19].

Based on these observations, we employed tyrosine-derived polycarbonate (TyrPCs) polymer formulations, developed by the Kohn Laboratory at Rutgers University, for a rabbit CMF defect repair study [20]. Based on prior studies demonstrating that among the TyrPC family-derived porous scaffolds, the E1001(1k) scaffold provided the best support for bone regeneration, especially when enhanced with calcium phosphate [21, 22], we collaborated with the Kohn lab to explore the potential use of E1001(1k) for CMF repair. Our collaborative studies first demonstrated that hDPSC-seeded E1001(1k)-beta-tricalcium phosphate (bTCP) scaffolds effectively directed robust bone formation in a critical-sized rat ramus defect repair model [23]. We then tested E1001(1k) scaffolds in a medium-sized rabbit mandible defect repair model, demonstrating that E1001(1K)-bTCP scaffolds seeded with hDPSCs and HUVECs facilitated rabbit critical-sized mandible defect repair [11]. The Kohn laboratory further modified E1001(1k)-bTCP scaffolds for improved bone regeneration, by replacing bTCP with dicalcium phosphate dihydrate (DCPD, CaHPO4·2H2O) [24, 25], demonstrating comparable biocompatibility and enhanced bone formation [26]. Finally, we demonstrated that the E1001(1K)-DCPD scaffold, TyroFill, containing an embedded titanium implant, formed significant new bone around the titanium implants, particularly in hDPSCs and HUVEC-seeded TyroFill constructs, indicating the potential utility of TyroFill scaffolds for coordinated CMF bone and dental implant repair [27].

In the study described here, we tested the utility of OP3D 3D printed POD polymer skeleton and DynaBlast® filling for precision CMF mandible repair. In preparation for this study, OP3D evaluated several biodegradable polymers including poly (lactic acid), polycaprolactone, the Evonik RESOMER R203H, RESOMER X206S, and polymer samples provided by PolyMed Inc. Evonik's RESOMER X206S was ultimately selected

as the most promising candidate material. To mitigate the potential release of acidic degradation products from the polymer, OP3D included 20% (w/w) b-TCP in the final composition, which consisted of a polymer skeleton to provide structural support and to maintain the defect space, and internal micropore architecture designed for enhanced cell ingrowth and oxygen and nutrient infiltration, biocompatibility, and cell attachment, proliferation, and differentiation [28].

Our results showed significant vascularization and the absence of necrotic tissue even in the center of the implants, demonstrating that OP3D 3D-printed scaffolds facilitated host cell infiltration and bone formation. No obvious immune response was observed in any of the implants. Only mild inflammation was observed in the 5-day implant sites, likely in response to the surgical procedures and not the implant itself. Lack of inflammation at later time points demonstrated excellent scaffold biocompatibility.

In this study, microCT derived BMD and BV/TV data were used to assess and analyze the quantity and quality of the newly regenerated bone. Morphometric analyses of bone structure, including trabecular thickness, separation, and number, were used to assess the maturity of the regenerated trabecular bone, with more mature bone exhibiting greater trabecular thickness and number, and reduced trabecular separation. Statistical differences were only observed between 5-day results to all three later time points, with no differences observed after 70 days, the commonly used time point to evaluate bone regeneration. Still, BMD and BV/TV demonstrated an increasing trend over time, with a slowed rate of new bone growth. Additionally, the 3 month time point morphometric results showed the highest average trabecular thickness and number, and the lowest average trabecular separation. These results align with our histological observations, indicating that new bone formation began in the pores of the OP3D scaffolds, and was subsequently remodeled into a lattice-like trabecular network closely resembling natural bone, coordinated with scaffold degradation. These results confirm that OP3D scaffolds effectively supported active bone remodeling. Since micro-CT analyses cannot distinguish between newly formed bone, cancellous bone chips present in the DynaBlast filling, and the TCP coating of the OP3D scaffolds, it is likely that the quantitative MicroCT results do not fully reflect new bone formation.

The DynaBlast filler used in this study is an injectable paste, allograft bone grafting product that contains demineralized bone matrix and cancellous bone, that has been extensively tested for sinus elevation surgeries, with a one-year follow-up study on 49 patients confirming successful bone regeneration. [29, 30, 31]. Still, granular bone substitutes present challenges when processing into porous form, and fail to adequately retain tissue volume under loading forces [32]. While CMF defect repair involves the regenerating both boney and soft tissues, soft tissue infiltration can inhibit bone regeneration, and in some cases, collagen membranes are needed to prevent soft tissue ingrowth and promote bone regeneration [33]. In this study, DynaBlast filling was used to fill the scaffold pores and block soft tissue infiltration from the buccal side [34]. As DynaBlast degraded over time, it was replaced with newly formed bone at later time points. Both the scaffold and DynaBlast bone chips degraded over time and were not observed at 6 months in low magnification images. At high magnification, small fragments of remaining scaffold material were visible, however it is reasonable to postulate that residual implant material after 6 months was insignificant.

Although our results confirmed that OP3D 3D scaffolds met the essential requirements for CMF bone repair, certain design modifications might further enhance their performance. First, based on our successful results using E1001(1k) scaffolds exhibiting interconnected macropore and micropore structure, it could be beneficial to revise OP3D scaffold design to increase the interconnection of the micro- and macropores of the scaffolds. Secondly, the high biocompatibility of the scaffolds provides the opportunity to include the addition of cells, such as dental pulp stem cells, to promote bone generation. Furthermore, the additive manufacturing 3D printing technology allows for precise control over the addition of bioactive factors, such as growth factors, to enhance bioactivity and further enable the construction of multiphasic constructs tailored to meet patient-specific needs.

5. Conclusions

In summary, our results demonstrated that OsseoPrint 3D technology can successfully be used to repair CMF defects, showing an increase in bone density and trabecular bone maturity over time. This study marked a significant milestone in the advancement of this technology, as it represents the first evaluation of *in vivo* bone regeneration within OP3D 3D printed scaffolds in a clinically relevant animal model. Commercialization of the OP3D technology would allow orthopedic, dental, cosmetic, and other surgeons to fabricate and implant precision patient-matched bone scaffolds at the point of service without the need for an outside lab or vendor. OP3D chair-side 3D printing technology would allow surgeons to augment, reconstruct and repair boney CMF defects with precision, speed, and safety. Future studies will further validate the use of this technology to repair significant patient-specific CMF defects caused by congenital malformations, trauma, and cancer resection.

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Figure legends

Figure 1. OP3D Prototype Design. The 3D printing digital workflow for OP3D's technology. First, a 3D scan of the patient is performed and used to create a patient-specific scaffold design. Next, a precision scaffold will be 3D printed using our approved materials. Finally, the 3D printed OP3D scaffold can be implanted into the patient.

Figure 2. Surgical Approach. A) Rabbit mandible in vivo animal model. Bilateral cylindrical defects, 10mm diameter and 6mm high, were created in rabbit mandibles as indicated by the black circles. B) The mandibular bone was exposed from the buccal side, and a 10mm circular defect was made using a trephine drill. C) The buccal bone

wall and tooth roots were removed, while the lingual bone was left intact. D) An OP3D implant with DynaBlast filling was placed into each defect.

Figure 3. OP3D scaffold. Light microscopic imaging shows (A) scaffold micropores formed by layer-by-layer printing, and (B) micropores within the struts.

Figure 4. Micro-CT Analyses. The surface (upper panels) and cross-sections through the middle of the implanted scaffolds (lower panel) showed continuous bone formation in implants harvested at 5, 70, 90 and 180 days. Bone formation began in the scaffold micropores and was gradually remodeled to exhibit the morphology of natural rabbit mandibular bone. Buccal bone healing was confirmed by surface imaging of the 6-month samples.

Figure 5. Histological Analysis. Hematoxylin and Eosin (H&E) staining revealed bone formation within the defect area after (A) 5 days , (B) 70 days, (C) 3 months, and (D) 6 months. New bone formation appeared to form within the scaffold pores (A), gradually filling them over time (B). Newly formed bone appeared to be remodeled into a lattice-like network closely resembling natural rabbit jaw bone over time (C, D). The defect margin was clearly identifiable in 5-day harvested samples (A1, A1') and gradually becames less distinguishable over time as the bony defect healed (C1, C1'). Small DynaBlast bone chips are visible within the scaffold pores at 5 days (A2, A2'), and up to 3 months (C3, C3'). Degrading scaffold material can be identified under polarized light up to 6 months (D1, D1') after implantation. Scale bar = 1 mm (A-D), and 50 μm (1~3, 1'~3'). Abbreviations: t=tooth, bc= bone chips, s=scaffolding materials, nb=new bone.

Figure 6. Statistical Analysis of new bone formation within the defect area. A) Bone Density. B) Bone Volume/Tissue Volume (BV/TV). C) Trabecular Number D) Trabecular Thickness. E) Trabecular Separation. (***** $p \leq 0.05$, ****** $0.05 \leq p \leq 0.001$, *** $p \leq 0.001$). Significant differences were also observed between the control and all other time points.

Supplement Figure 1: Masson's Trichrome staining. (A, B, C, D) Masson's Trichrome staining. (A1, A2, A3, B1, B2, B3, C1, C2, C3) higher magnification images. (A1', A2', A3', B1', B2', B3', C1', C2', C3') Polarized light images. Masson's trichrome stained sections confirmed observations from the H&E staining for 5 days (A), 70 days (B), 3 months (C), and 6 months (D). New bone formation appeared in and gradually filled the scaffold pores, and later remodeled into a lattice-like trabecular bone closely resembling natural rabbit jaw bone. Scaffolding material retained their original grid structure until ~70 days. Bone chips (bc) within DynaBlast filling showed similar morphology and color as the natural bone, without any live cells, and underwent degradation over time. Scale bar = 1 mm (A-D), and 50 μm (1~3, 1'~3'). Abbreviations: t=tooth, bc= bone chips, s=scaffolding materials, nb=new bone.

Supplement Figure 2: Histological Analyses of natural rabbit jaw bone and teeth. (A) H&E stained natural rabbit teeth and jaw section. (B) Masson's Trichrome stained natural rabbit teeth and jaw section. Blue indicates dentin, red indicated enamel and soft tissues. Scale bar = 1 mm. Abbreviations: T, Teeth; B, bone; BM, bone marrow,

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