Personalized Scaffolding Technologies for Alveolar Bone Regenerative Medicine

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Introduction

The key components of tissue engineering encompass proliferating cells, biocompatible scaffolds, biological signals, and an appropriate blood supply. Regenerative medicine in the periodontal and alveolar bone fields follows the general principles of tissue engineering, while possessing several unique aspects. Regenerations around teeth supporting structures require a careful consideration of the future mechanical loading. The dynamic microbial challenges that the newly regenerated tissues constantly face make the pathogen control in periodontal regeneration a critical aspect (**Figure 1A**)(1, 2).

In periodontal tissue engineering, both resident cells and distant stem cell-like progenitors have been intensely studied for both preclinical and clinical applications. Gingival tissue and periodontal ligament (PDL) harbor cells that possess the abilities to restore the damaged periodontal and alveolar bone architectures (3). Gingival fibroblasts have demonstrated potent bone formation properties; PDL cells enhance both bone and ligament repair (4, 5). Mesenchymal stem cells (MSC) derived from various lineages also possess strong properties for bone regenerative medicine (6).

Designing and assembling biocompatible scaffolds to house the oral regenerative cells maximizes the outcomes of tissue engineering. An "ideal" scaffold should contain a porous architecture to withstand a desired mechanical function, while at the same time to allow a mass transport for nutrients and growth factors contributing to the reparative process (7). Advancements in three-dimension (3D) imaging methods and 3D printing technologies make scaffold fabrications much more suitable for personalized reconstruction of complex topographies of periodontal osseous defects (8).

How to incorporate the biological signals on top of cells and scaffolds is another critically appraised topic in tissue engineering. Classically, gene transfer strategies adopt either a viral or non-viral approach. These carriers for biological factors can work directly on cells or indirectly on the scaffolds themselves. When applying directly on scaffold biomaterials, carriers can be covalently immobilized utilizing a reactive polymer coating prepared via chemical vapor deposition (CVD) polymerization of substituted paracyclophanes onto the scaffolds (9). When working on cells, the conventional cell transduction/transfection methods have been widely adopted.

PDL Fiber-Guiding Scaffolds

The topography of scaffolds serves as structural constraints for the tissue regeneration approaches. When being assigned to an optimized surface topography, scaffolds can induce cells adhesion, migration, differentiation, as well as triggers certain molecular pathways, which can have longer lasting impacts on cellular function, beyond the period of regeneration (10).

A critical component of the scaffolds' topography is the specific architecture design. The concept of fiber-guiding scaffold originated from the utilizations of the pillars, which can induce a spontaneous alignment of PDLs. Park and co-workers designed a mold-casted polymeric scaffolds with "pillars" that ran perpendicularly against the tooth root in vivo (11). The fiber-guiding design allowed the PDL cells and associated fibers to grow in an oriented fashion, mimicking how the Sharpey's fibers can connect the bundle bone to the cementum. The fiber-guiding scaffold also ensures a high customization, as a pre-surgical computed tomography (CT) scan would acquire the defect's anatomical data for fabricating the scaffolds (11). Building upon these efforts, Pilipchuk and colleagues included surface topographical cues on the fiber-guiding scaffolds to enhance cell attachment and growth. A Micro-patterned polycarprolactone (PCL) film containing pillars with micro-grooves featuring specific depths and widths considerably increased the fraction of the aligned cells, surface treatments, and enhanced cellular adhesions (12). PCL is a hydroplytically degradable polymer that has been approved by the Food and Drug Administration. More recently, the micro-patterned fiber-guiding scaffolds were tested in an alveolar fenestration animal model (35). Additionally, the novel CVD surface treatment method was utilized to permit the

immobilizations of the adenoviral gene therapy vectors. The results have demonstrated strong potential of the combined strategies of fiber-guiding scaffolds and gene therapy in restoring the lost periodontium (**Figure 1B**).

In addition to the pillar and groove designs that constitute the core components of fiber-guiding scaffolds, lessons learned from neuron regeneration have shed light on many intraluminal scaffold designs that may benefit periodontal tissue regeneration. Ahn et al tethered carbon nanotubules (CNTs) onto glass conduits to guide the regeneration of transected sciatic nerves in vivo (13). As a result of the nanoarchitecture, neurites grew longer and with more directional outgrowth in vitro (13). CNTs were well-suited for neuronal regeneration, as they themselves are electrically conductive, which may favorably influence the regenerative mechanisms. CNTs have been studied in bone regeneration. Li et al reported that CNTs promoted the osteogenic differentiations of the adipose-derived mesenchymal stem cells by inducing multiple bone forming proteins (14). Currently, there have been no reports on the utilizations of CNTs in PDL regeneration. However, the electrically conductive nature with the addition of tubules mechanical property could be promising in ligamentous tissue regeneration.

Therefore, periodontal tissue engineering strategies should consider the fiber guidance design. Architectures can be chosen to guide tissues in a desired orientation, whether directional as for PDL or non-directional as for bone. Topographical cues should dial up (or down) the appropriate cellular processes, such as enhancing mineralization to encourage osteogenesis. And non-physical cues, such as magnetism or conductivity, can be paired to tissue types (such as bone or nerves) for which they desirably intervene on regenerative mechanisms.

Image-based scaffolds to adapt to periodontal and oral topography

As technology is advancing, 3D bioprinting enables fabrication of a customized biomedical tool with a precise shape and function based on individual-specific anatomical data (15). In image-based personalized tissue engineering, 3D printing is used for making a scaffold via multi-step processes; 1. Image acquisition; 2. Image processing with DICOM conversion and STL file generation; 3. Image post-processing: 3D reconstruction and 3D CAD modeling; and 4. Rapid prototyping: 3D printing and custom model fabrication (**Figure 2**).

3D Image acquisition

Precise data acquisition for the accurate production of personalized scaffolds is done using CT or magnetic resonance imaging (MRI) image datasets. Scaffolds are produced based on computer-aided design/ computer-aided manufacturing (CAD/CAM). This entails generating the scaffold, on macro, micro, and nanoscopic levels of architectural design which matches its proper function (16). Patients' image data help to create the exterior shape of the scaffold and optimize the internal porous structure providing a balance between load bearing and biological factors. On the one hand, a microporous structure is needed to ensure adequate drug delivery or if not, the structure may compromise the function of mechanical load bearing. These characteristics should be considered quantitively, resulting in the final mechanical properties at least partially resembling those of the native tissues, if not entirely (7, 17).

Image processing for scaffold fabrication

Technology is advancing to provide more attention to simulating the intricate internal and external tissue structures. There is a demand to develop imaging techniques that facilitate better understanding of structure and function, as well as enhance reproducibility of the scaffold. DICOM CT images are segmented and utilized in image-based computer aided design to create porous anatomic structures. Image based-computer designs and models are suited to produce anatomic implants and scaffolds (18). Hollister et al developed an image-based hierarchical design method, where the scaffold was designed with a local image voxel database and assigned with varying voxel densities. The porous scaffold design could follow either periodic or random geometric pore structures. In a separate voxel image dataset, the anatomical defect was assigned with voxel densities as well. Finally, the scaffold database can be integrated with image database of the anatomic defect to create the final tissue engineering scaffold design (18). This scaffold designing approach allows an increased flexibility in design; therefore, facilitates the use of a wide range of materials of different properties. The challenge of the scaffold design, on the other hand, necessitates the emergence of topology optimization, which is essentially an algorithm facilitating desirable scaffold properties (16). In addition, to better visualize and mimic the anatomical structure, post-processing of the images is usually performed. The most common tools used are volume rendering and multi-planar reconstructions. Combining different segmentation

algorithms provide greater accuracy in determining the region of interest for detailed and comprehensive analysis (19). After post-processing, 3D reconstruction and CAD modeling strive to demonstrate conceptual behavior of tissues, incorporating treatment outcomes (20). It blends in the complex macro- and micro-structure of bone into one unique conformity. Additionally, it accommodates with principal stress on tissues, simulating different dynamic states of tissues. This shows a clear advancement in treatment planning in interdisciplinary dental fields (21).

Scaffold Fabrication

Solid freeform fabrication (SFF) method enables to produce individual-specific 3D scaffolds (15). SFF method makes a 3D scaffold controlling the materials or parameters based on CAD/CAM. This technique permits the following applications in tissue engineering: fused deposition modeling (FDM), stereolithography (SL), selective laser sintering (SLS), and 3D bio-printing (22). Meticulous planning is improved by rapid prototyping techniques, whereby models could be simulated rapidly prior to actual fabrication of materials or implementation of regenerative methods. Solid freeform fabrication (SFF) and rapid prototyping (RP) necessitates improvement in imaging and printing techniques. With this technology, prototyping is done prior to material manufacturing. It is also referred to as layer-by-layer fabrication. 3D treatment planning is quite useful in cases of periodontal regeneration. Van Dyke and co-workers demonstrated that nano-proresolving medicines designed for treatment of inflammation-induced bone loss showed strong potential in vivo (23). SEM images facilitate the quantification of collagen fibers as well as marking their orientation. The complexity in regeneration is marked by the various types of tissues as well as their adaptability to their function, simulating the entire native architecture of newly repaired tissues.

Clinical applications of personalized scaffolding technology for periodontal and alveolar bone engineering

Given the complexity of osseous structures requiring new blood formation, new technologies are employing microvascular techniques to enhance the reconstruction of large defects. For example, 2D microcapillaries are stacked forming blood circulation to ensuring optimum regeneration. Tissue fabrication is further investigated by deposition of materials with live cells, i.e., via bioprinting. With the advent of computer

tomography and sequential sectioning, a clear process line is developed marked by pre-processing for the first stage of tissue or organ printing (24). Organ printing uses the principle of cellular self-assembly into tissues, as dictated by developmental biology (25). There are various clinical applications of personalized scaffolding technology in periodontal tissue and alveolar bone engineering as described below.

Periodontal tissue regeneration

3D printed scaffolding technology has been developed in the attempt to regenerate the periodontal tissues surrounding natural teeth. Scaffolds that are designed by computer-based technologies precisely fill the region-specific defect space, creating an optimal environment for cellular growth and angiogenesis. Rasperini et al reported the first clinical application of personalized scaffolding in periodontal tissue regeneration (26). A customized scaffold was 3D-printed to adapt to an osseous defect of a cuspid, using a prototype model according to the CBCT scan. The scaffolding materials used were slowly degrading PCL/4% HA. Prior to delivery to the patient's defect, the scaffold was immersed in platelet-derived growth factor BB as an FDA-approved biologic. At 14 months, a biopsy of the scaffold construct was evaluated and it was noted that while the scaffoled resorbed (~25% by this time), there was evidence on new connective tissue and bone formation in the construct. This case suggests that more slowly resorbing biomaterials may not be appropriate for periodontal repair. Subsequent periodontal applications have included a combination on PCL/PLGA blends that will drive a more rapid rate of matrix resorption in vivo (11) (27) (28). Currently, models are being improved using personalized scaffolding approaches to treat challenging furcation defects where wound stability and space maintenance is crucial (**Figure 3**). A 3D-printed scaffold made from an equitable ratio of PCL and PLGA has also exhibited an ability to carry therapeutic genes that benefits bone regeneration. This novel cell-based gene delivery with personalized scaffolding has showed promising effects on periodontal engineering.

Craniofacial reconstruction

Personalized scaffolding technology utilizing 3D-imaging and 3D-printing has contributed greatly new prototype biomaterials for craniofacial reconstruction. Conventionally, dental and skeletal relationships are analyzed through wax-ups, 2D radiographs, photographs and articulators that are time consuming and cumbersome. In

many complex cases, such as facial asymmetry, skeletal movements in the relation with the complex are difficult to be established and analyzed using traditional 2D approaches (29). In these cases, 3D imaging builds a platform in which dental and skeletal features are documented accurately to permit a precise diagnostic system and hence benefits the efficiency of treatment planning (30).

During diagnostic work-up, 3D imaging enables an integration of dental, skeletal and soft tissue relationships through their movements at the cranial base, providing relevant anatomical features. Furthermore, using 3D printing, skeletal components can be simulated and translated into physical models through the customized fabrication of appliances, which could serve as a surgical splint and/or a stainless-steel arch wire. During the treatment planning phase, orthodontists and oral surgeons can utilize these appliances to review the relevant anatomical movements and discuss the detailed surgical procedures and anticipated surgical end point (21). This approach helps to reduce the surgical time and fulfill the esthetic demands.

Alveolar bone regeneration

Personalized scaffolding technology has been increasingly used in the alveolar ridge reconstruction for the implant site development. 3D-printed diagnostic models, and preoperative templates are widely applied for ridge augmentation in severe vertical and horizontal bony defects (31) (32). Despite the advanced applications of CBCT combined with CAD/CAM fabrications, current 3D printing for alveolar ridge augmentation is limited using printing templates, which might not directly induce bone regeneration as inert biomaterials. Considering its clinical potential in other specialties, future directions suggest applying 3D technology using osseoinductive and osseoconductive biomaterials will better enhance bone and tissue regeneration for vertical and horizontal ridge augmentation supporting implant placement.

In the rehabilitation of partially or fully edentulous patients with a lack of maxillary posterior bone support, sinus floor augmentation is required prior to dental implant placement. An anatomically sinus-specific block graft can be fabricated via 3D technology for bone augmentation has recently been introduced in a clinical trial for lateral sinus augmentation (33). Briefly, the scaffold manufacturing process applies a virtual planning and designing a custom-made scaffold and then a 3D fabrication of the

scaffold is performed using the CAM technique. The customized block graft is created from an original hydroxyapatite (HA) block using a cutting guide also made from 3D image-based analysis and planning.

In summary, utilizing 3D imaging and 3D-printing for fabrication of customized scaffolding technology to regenerate periodontal tissue and alveolar bone is a rapidly growing research field. With continued advancement of novel 3D imaging technologies, broader and more accurate clinical applications are anticipated combining printing with imaging to customized reconstructive scaffolds to repair large bone defects in the jaws.

Future directions

The implementation of personalized or customized regenerative scaffold technologies for periodontal and alveolar ridge regeneration is coming very close to reality in clinical practice. The more routine use of 3D, high-resolution imaging of both soft and hard tissues makes integration with system software more feasible for reconstruction at the bone-tooth, bone-implant interface as well as for larger osseous defects of the alveolar ridge and craniofacial complex. Advances in bioprinting now allow the delivery of cells, extracellular matrices, genes and/or biologic agents onto polymeric, ceramic and natural biomaterials. These technological developments in regenerative medicine create exciting solutions for surgeons and restorative dentists for predictable regenerative therapies to improve patient care (34).

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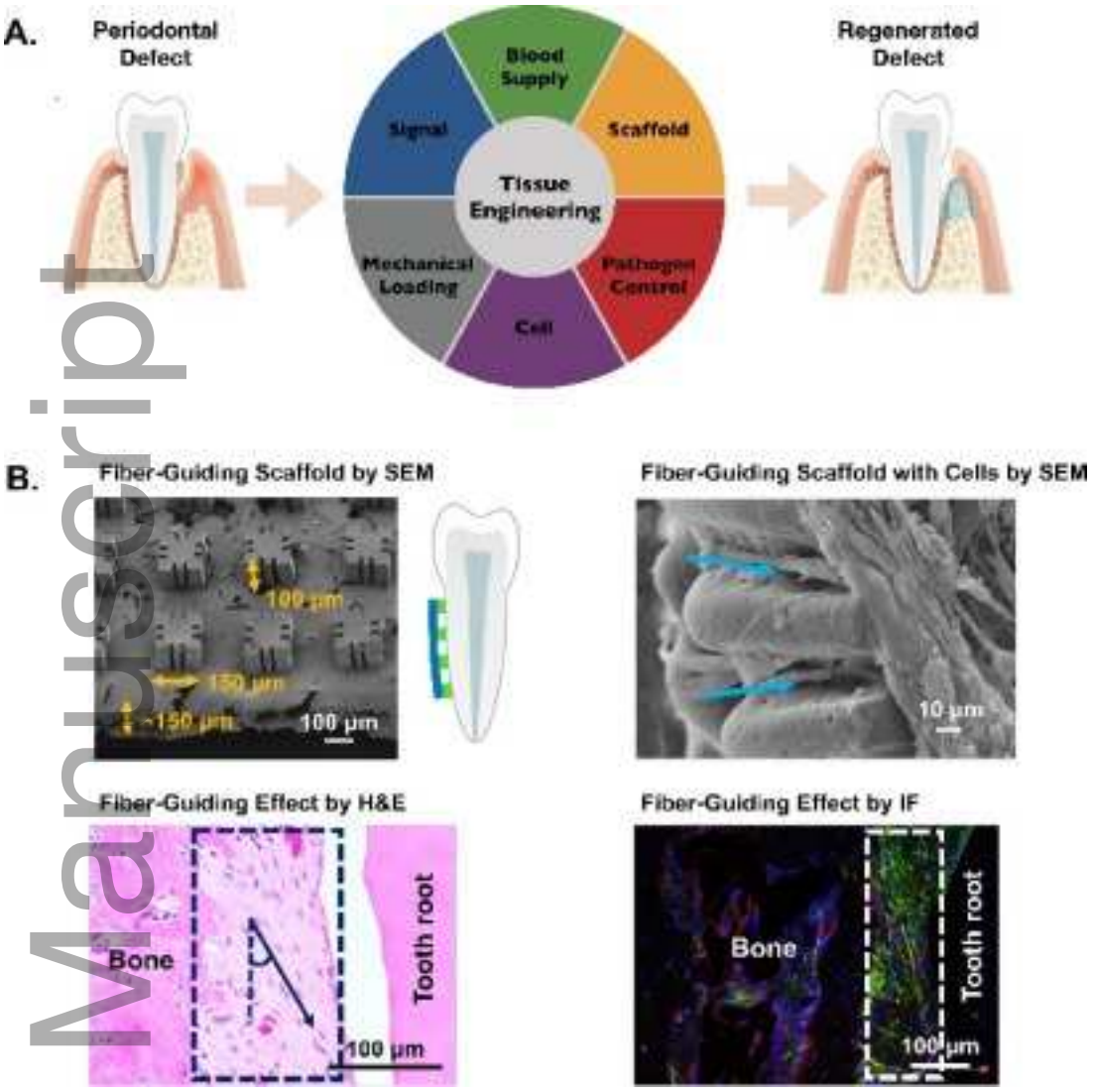
Figure legends

Figure 1. Principles and Application of Periodontal Tissue Bioengineering. A. Components of periodontal tissue engineering. Six factors constitute for successful periodontal tissue engineering: cells, signal, scaffold, mechanical loading, pathogen control, and ideal blood supply. **B. Examples of fiber guidance in periodontal tissue regeneration. On the upper left panel:** SEM image of a micropatterned scaffold with grooves, a topographical cue to guide the aligned formation of new ligamentous tissue**; On the upper middle panel:** an illustration of PDL fiber guiding scaffold (green) and amorphous bone scaffold (blue) against the tooth surface; **on the upper right panel**: SEM image of seeded human PDL cells on micropatterned scaffolds (Blue arrows indicate the alignment of cells along the pillars of the scaffolds and their grooves). **On the lower left panel:** hematoxylin and eosin-stained (H&E) section of regenerated tissues in a rat that received a micropatterned scaffold with immobilized BMP-7 and PDGF-BB genes at a periodontal defect. The box and arrow indicate the regenerated ligamentous tissues and their oblique orientation, respectively. **On the lower right panel:** section of regenerated tissues in the same treatment group, stained for immunofluorescence (IF) with periostin (red), collagen III (green), and DAPI (blue). Periostin and collagen III are biomarkers for the formation of ligamentous tissue, and DAPI indicates cell nuclei. The collagen fibers are obliquely oriented and expressed throughout all the tissue due to the patterning. Adapted from Lin 2010 and Pilipchuk 2018.

Figure 2. Image-based Scaffold design for alveolar bone bioengineering. Step 1: Image acquisition with a cone-beam CT scan for hard tissue and intraoral scan for soft tissue; Step 2: Image pre-processing; the images from step 1 are integrated as DICOM file, then converted to STL file for preparing 3-D printable condition; Step 3: Image

post-processing; 3-D volume visualization for optimization of scaffold shape; Step 4: Rapid prototyping; based on image processing, scaffolds are manufactured by the 3-D printer. Step 5: Clinical application; custom-fit scaffold is applied at the time of reconstructive surgery.

Figure 3. Personalized scaffold technologies for periodontal engineering in furcation defects. Personalized scaffolding process consists of 3 stages: The scaffold can be initially designed based on the tooth and bone morphology taken from a CBCT scanning before treatment. The scaffold construct then is fabricated from a balanced ratio of polymeric biomaterials such as PCL and/or PLGA using 3D printing technology. The scaffolds can be coated with genes for osteogenic factors using chemical vapor deposition (CVD) technology to allow gene vector release after cell attachment to the tissue scaffolds.



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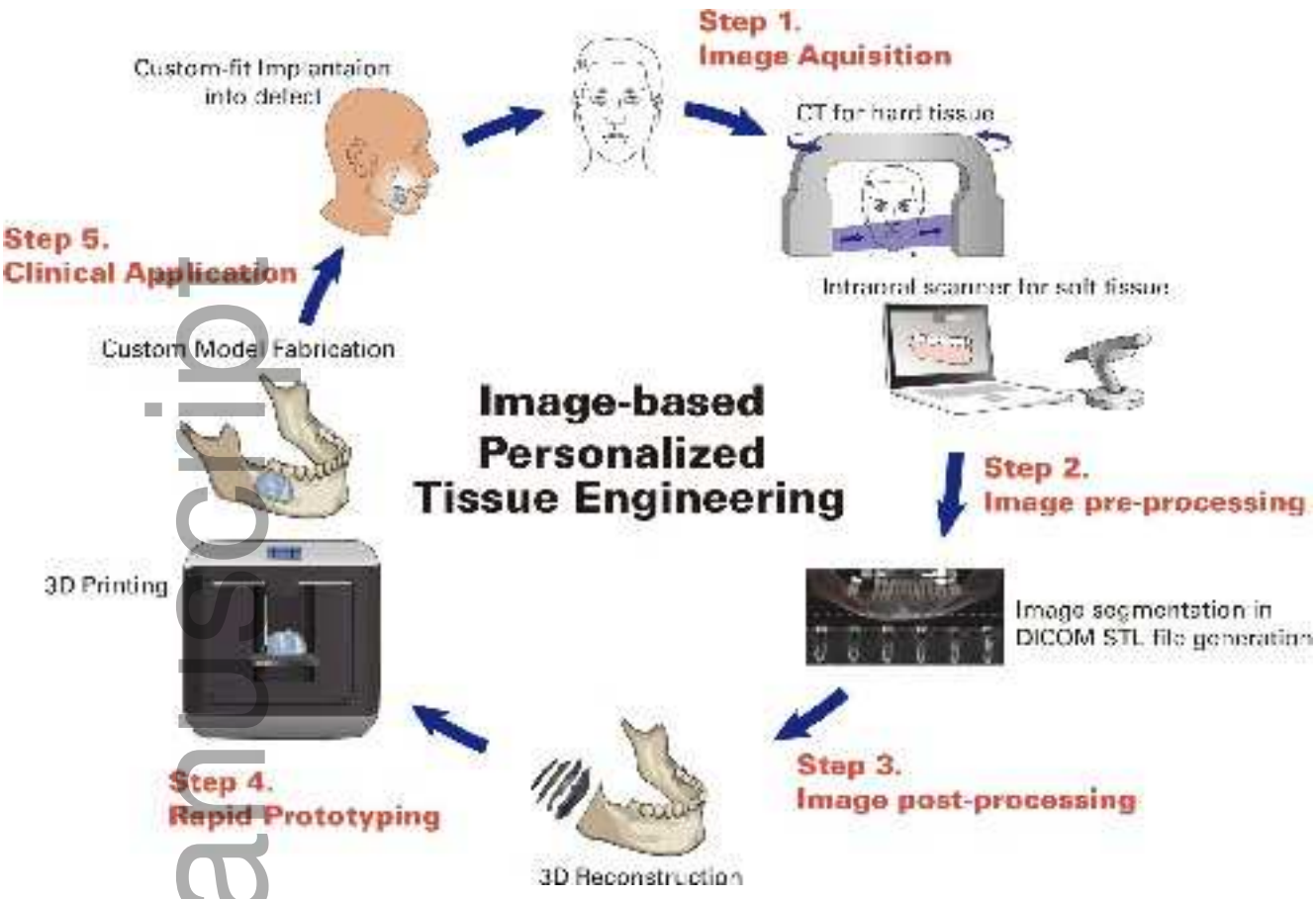

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