

3d Printing - A New Vista for Periodontal Regeneration

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Abstract: Tissue Regeneration In The Oral Cavity Is Regulated By Signalling Molecules, Cells And By Matrix Formation. A Balance Between Bone Resorption And Bone Formation Is Key For Maintenance And Regeneration Of Alveolar Bone And Supporting Structures Around Implant And Tooth. Periodontal Tissue Engineering Focuses On Regenerating Form And Function Of Hard And Soft Tissues Using Signalling Molecules Scaffolds And Cells. Bony Defects May Vary In Size From Small Intrabony Defects To Large Horizontal And Vertical Bone Defects In Periodontal Diseases That Prove Critical For Implant Rehabilitation. Over Decades, Efforts Are Being Made To Achieve Predictable And Reliable Bone Regeneration Using Various Methods. This Review Focuses On Various Materials And Methods That Are Being Used And Which Are In Research Stage For 3 D Printing Of Patient-Specific Custom-Made Scaffolds For Periodontal Regeneration.

Keywords: Periodontal Regeneration, 3 D Scaffolds , Bony Defects.

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I. Introduction

Tissue engineering/regenerative medicine aims “to stimulate regeneration of tissues and organs by either implanting biomaterials for in vivo regeneration or by constructing substitutes in vitro”.¹ Tissue engineering is a translational research area including a broad range of disciplines, such as stem cell biology, material sciences, medicine, chemistry, and manufacturing.² Recently, nanotechnology was introduced as a new area in Tissue engineering and in periodontal tissue engineering, with emerging studies demonstrating significant influence of nano scaled topography and geometry on cell differentiation, behaviour, and enhanced 3-dimensional (3D) regeneration.^{3,4} A recent study showed that nano-pro resolving medicines designed specifically for treatment of inflammation-induced bone loss resulted in the increased bone formation in a large animal model.⁵ Ongoing research into the importance of nanoscale features for regeneration of periodontal complex tissues will further elucidate the required scaffold design parameters and therapeutic capabilities of nanotechnology-based applications

There is a need for effective strategies for implementation of tissue engineering into day-to-day practice. These strategies may be a hurdle with the regulatory agencies as these represent tissues, biological products, drugs requiring evaluation to be done in all of the applicable pathways.² In 2014, 28 tissue engineering products were approved for clinical use and made commercially available for various applications by the FDA.⁶

II. 3 D Printing

The term 3D printing is generally used to describe a manufacturing approach that builds objects one layer at a time, adding multiple layers to form an object. This process is more correctly described as additive manufacturing and is also referred to as rapid prototyping.⁷

The 3D printer uses a powder or liquid resin that is slowly built from an image on a layer-by-layer basis. All 3D printers also use 3D CAD software that measures thousands of cross-sections of each product to determine exactly how each layer is to be constructed. The 3D machine dispenses a thin layer of liquid resin and uses a computer-controlled ultraviolet laser to harden each layer in the specified cross-section pattern. At the end of the process, excess soft resin is cleaned away through use of a chemical bath.⁸

3D printing uses raw materials such as plastics, resins, super alloys, nickel-based chromium and cobalt chromium, stainless steel, titanium, polymers, ceramics composite materials and polycaprolactone.

III. Direct 3 D Printing

This technique can simultaneously arrange multiple cell types, deposit extra cellular matrix, and provide fine-tuned control over bioactive molecules deposition. To date, peptides, proteins, DNA plasmids, and living cells have been printed.⁹ 3D printing has also been used to produce a 3D cell culture model for generating ECM on scaffolds.¹⁰

IV. Indirect 3 D Printing

The 3D printing method can further be used for indirect printing, which refers to the printing of a mold that is then cast with the final polymer. With this technique, a computed tomography scan of the patient's defect can act as a template for making a 3D mold. This mold can then be used for making a scaffold for gene therapy and a growth factor delivery system. Park and co-workers^{11, 12} designed a 3D wax mold to produce a fiber-guiding scaffold to improve integration of PDL fibers into mineralized tissues. In a randomized controlled clinical trial, the use of prefabricated 3D polycaprolactone (PCL) scaffolds in post extraction sockets resulted in normal bone healing and better maintenance of the alveolar ridge as compared with extraction sockets without scaffolds.¹³

V. Fused Deposition Modeling

The fused deposition modelling technique for 3D printing of thermoplastic material, such as PCL and poly lactic-co-glycolic acid (PLGA) can create scaffolds with mechanical strength, high porosity, and controlled morphology. However, it does not allow for incorporation of living cells or temperature-sensitive biological molecules.¹⁴

VI. Hydrogel Scaffolds

Additionally, 3D plotting is a technique to make soft tissue scaffolds, such as hydrogels, with direct incorporation of cells while retaining their normal activity.¹⁴ A potential limitation of the hydrogel as a scaffold includes inhibition of cell-to-cell interactions, which may influence cell signalling.

VII. 3d Printing With Live Cells

3D printing of living cells, either in cell aggregates or seeded onto 3D printed scaffolds may enhance cell signalling and promote tissue formation. Scaffold free approach is defined as layer-by-layer additive biomanufacturing and is a technique with the potential to remove the need for a scaffold.¹⁵ mini-tissue-based approach is a technique in which tissue spheroids are used as building blocks that fuse to form a tissue. Self-assembled vascular spheroids can form a branched vascular system within a 3D construct, thereby providing blood supply to all parts of newly forming tissue.¹⁶ interestingly, recent studies report on the use of 3DP to build complex tissues, such as constructing periodontium-like tissue.¹⁷ A 3DP bioresorbable scaffold has been used for periodontal repair.¹⁸ 3DP also has the potential to make complex, patient-specific constructs such as temporomandibular joints¹⁴

VIII. Emerging Concepts Of Tissue Engineering In Periodontology

New approaches are becoming available for periodontal regeneration. These include the use of advanced biomedical imaging such as cone beam computed tomography used for pathology visualization, Implant placement and to visualize topography of bone. Cone beam computed tomography technology offers high resolution and three-dimensional imaging of bony topologies that allow the development of image-based scaffolds that can be 'personalized' to fit precise defect morphologies around teeth.¹⁸ The image-based scaffolds can be made via several rapid prototyping techniques to manufacture polymeric or ceramic scaffolds. For example, three-dimensional printing has been utilized to make surgical guides and some first-generation regenerative scaffolds for clinical use. These scaffolding technologies can be used in combination with either biologics or cell therapies to create 'bioactive scaffolding systems' for tissue repair.¹⁸

IX. Additive Biomanufacturing Applied To Periodontal Scaffold Design And Fabrication

The design of scaffolds, which mimic the complex periodontal shape and organization, represents a significant challenge in regenerative Periodontology. Although additive biomanufacturing may help to surmount this hurdle, adoption and long-term success of these strategies greatly rely on the biomaterials being used. Regarding periodontal regeneration, the most commonly used materials for restoring and/or replacing lost oral tissues are ceramics and polymers.¹⁹

Ceramic biomaterials such as CaP, calcium sulfate (CS), and bioactive glass (BG) are ideal candidates for hard-tissue engineering and restoring the lost function due to their similar composition to bone mineral, the stimulating effects on cell proliferation and differentiation, and their relatively low degradation rate, the latter specifically facilitating prolonged guided tissue remodelling and structural support. Despite these advantages, the brittleness and low ductility need to be considered when using these materials.²⁰

Synthetic polymers on the other hand, such as polylactic acid (PLA), polyglycolic acid (PGA), the copolymer poly(lactic-co-glycolic acid) (PLGA), and PCL have highly adjustable characteristics, excellent production repeatability, and can potentially be mass produced. However, the process of printing synthetic polymers involves using parameters detrimental to cell viability (e.g. high temperature), making the incorporation of cells and growth factors into the polymer mixture complicated if not impossible.²⁰

Review of literature describes a wide range of different additive biomanufacturing technologies. These are classified into laser-assisted printing, inkjet printing, and extrusion-based printing. Common to all these technologies is the use of CAD software or digital images for the design.²¹ Extrusion-based printing is the most widely applied technique for potential application in periodontal regeneration. While a great variety of extrusion-based printers are described in the literature, several general characteristics can be identified. These include the temperature controlled material handling, dispensing system and stage, and an optional light source and piezoelectric humidifier.²²

As the name indicates, extrusion printing involves the controlled extrusion of a material through a printer head onto a collector. In most of these systems, mechanical movement (piston or screw) or a pneumatic system enables the extrusion of the polymer melt or ink leading to the deposition of a filament or strut, which dimensions can be adjusted by modifying the printing conditions (e.g. temperature, feed-rate and the velocity of the collector). An example of an extrusion-based printing technique evaluated for periodontal applications is fused deposition modeling (FDM). In FDM systems; a thermoplastic material is fed from a filament coil and inserted into a heated nozzle head that enables the deposition of semi-molten state polymer struts onto a substrate.²³

Electrospinning is a broadly used micro-nano fiber fabrication technique is another scaffold fabrication method explored for periodontal applications. A typical electrospinning setup consists of a syringe that contains the desired polymer, a syringe pump, a high voltage supply, and a collector plate. Electrospinning can be performed with either polymer solutions and polymer melts, also referred to as solution electrospinning and melt electrospinning respectively. In solution electrospinning, a polymer solution is extruded through a spinneret and electrified leading to the formation of a jet. Under the influence of the electrical field applied between the spinneret and the collector, the jet undergoes several physical instabilities inducing a whipping and oscillating motion as it travels towards the collector. The high frequency of the oscillation results in a drastic reduction of the jet diameter, which enables the deposition of micro- to nanofibers. On the other hand, the higher viscosity of polymer melts diminishes the electrical instabilities and allows for controlled fiber placement. Since melt electrospinning allows direct writing of the polymer melt, this method can be considered as an additive (bio) manufacturing technique.²⁴

X. Multiphasic Scaffolds For Periodontal Regeneration

For predictable periodontal regeneration to occur, hierarchical tissue formation with the appropriate interfacial connection is required. Equally important is establishing sufficient strength and mechanical integrity, which is mainly determined by adequate periodontal ligament fiber orientation and their incorporation into the newly formed tissue. To address these challenges, Park et al.²⁵ developed a biphasic PCL–PGA scaffold fabricated via computer-aided manufacturing. The scaffold consisted of both periodontal ligament-specific and bone-specific compartments facilitating the formation of human tooth dentin-ligament-bone complexes. In this approach, 3D wax-printing systems facilitated the manufacturing of molds, which were used in the fabrication of the hybrid scaffold. Mold characteristics were judiciously chosen in terms of pore size, channel orientation, and tissue specific compartments. After fabrication, the molds were cast with a PCL or PGA polymer solution. To form a single scaffold structure, both compartments were fused with a thin PCL layer.

In vivo evaluations of the scaffold design in subcutaneous pockets in mice have shown bone and periodontal ligament regeneration capacity and generation of parallel and obliquely oriented fibers. To further resemble the periodontium, adjustments in the design were made.²⁵ This approach has demonstrated control over fiber orientation and facilitates morphogenesis of periodontal tissue. Following on from this strategy, Park et al. examined the effect of controlled channel architecture in the scaffold design on the tissue interface.¹¹ In vivo evaluations of this scaffold in periodontal fenestration defects in athymic rats have shown controlled and predictable periodontal fiber orientation, controlled tissue infiltration, and a better organization of the ligament interface compared with random scaffold architectures. With this image-based, fiber-guiding scaffolding system, the authors intend to predictably facilitate regeneration and integration of dental supporting tissues.¹² It is noteworthy that although the main focus was put on periodontal applications, the general design with the controlled pore architecture is expected to suit diverse scenarios involving the regeneration of tissue interfaces.

Additively Manufactured Scaffolds in Combination with Cell Sheet Technology

As described above, another approach aiming at periodontal tissue regeneration involves cell sheet engineering. In addition to utilization of the cell sheets unaided, several groups have investigated the

combination of additively manufactured scaffolds with cell sheet technology. Vaquette et al.²⁶ described a biphasic scaffold in combination with cell sheet technology to regenerate alveolar bone and periodontal ligament simultaneously. The scaffold design was based on a fused deposition modeled component for the bone compartment and a more flexible solution electrospun component for the periodontal ligament compartments an extension of the biphasic scaffolds, Lee et al. developed a triphasic scaffold.¹⁷

This approach was based on previous work by Lee et al.²⁷ and involved printing of a seamless scaffold with region specific pore sizes for the purpose of facilitating integrated regeneration of various tissues. The scaffold was fabricated by using fused deposition modeling and consisted of a compartment for the cementum/dentin interface, a compartment for the periodontal ligament, and a compartment for alveolar bone. By combining biophysical properties with spatially released bioactive cues, regeneration of periodontal tissue was envisioned. Although promising, a possible limitation of this approach might be the stiffness of the PCL scaffold, which impedes adaptability to the complex 3D anatomy of different periodontal defects and, hence, limits from a translational research point of view.

Rasperini et al.¹⁸ fabricated the first reported personalized additively manufactured bioscaffold for periodontal osseous defect regeneration in humans. In this study, a customized scaffold was printed by using a computed tomography scan of the patient's defect. The scaffold, made from PCL powder containing HA, was manufactured by using selective laser sintering technology, which allowed for precise scaffold features, supporting tissue regeneration. Despite the fact that Rasperini et al. have demonstrated the potential of this additively manufactured scaffold for the treatment of large periodontal osseous defects, the scaffold became exposed after 12 months, which ultimately led to failure from a clinical point of view. A more rapidly resorbing biomaterial with a less bulky design would probably have better suited this application. Additionally, the observed limited bone regeneration indicated the need for incorporating scaffold design imperatives, such as larger pores and pore interconnections.

XI. Conclusion

3D imaging and modeling can create a huge impact in regenerative periodontics. Regenerative medicine and three-dimensional imaging, allows more predictability in management of complex interdisciplinary clinical scenarios. These 3 D scaffolding technologies can be used in combination with either biologics or cell therapies to create 'bioactive scaffolding systems' for tissue repair. A major hurdle in the usage of cell scaffolds in day-to-day practice is a difficult task of getting clearances from regulatory agencies as it involves cells and tissues. Further well-controlled clinical trials are required to prove the efficacy of this 3 D printed scaffolding systems.

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