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# 3D Bioprinting – A Leap Into The Future

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***Abstract:***
*Numerous Approaches Have Been Introduced To Regenerate Artificial Dental Tissues. However, Conventional Approaches Are Limited When Producing A Construct With Three-Dimensional Patient-Specific Shapes And Compositions Of Heterogeneous Dental Tissue. 3d-Bioprinting Is An Emerging Technology In Regeneration Medicine/Tissue Engineering, Because Of Its High Accuracy And High Efficiency, Providing A New Strategy For Oral Tissue Regeneration. Bioprinting Technology Was Applied To Produce A Three-Dimensional Dentin–Pulp Complex With Patient-Specific Shapes By Inducing Localized Differentiation Of Human Dental Pulp Stem Cells Within A Single Structure. Recent Advances In The Field Of 3d Bio-Printing Have Given Rise To New Possibilities In The Manufacturing Of Customized Patient-Tailored (Bioactive Tissue) Constructs Which Show A Great Degree Of Resemblance To The Patient's Native Tissue For Periodontal Reconstruction. The Improved Quality And Cost Effectiveness Has Contributed To Their Increased Use On Patient. Tissue Engineering Recovers The Injured Tissue Through Seed Cells, Bio-Capable Scaffold And Bioactive Factors. Employing Custom-Designed 3d Printed Scaffolds That Securely And Effectively Reconstruct The Defects By Using Tissue Engineering And Regenerative Medicine Techniques Can Revolutionize Surgical Procedures. This Paper Explains The Upcoming Novel Field Of Bio Printing, Which Shows Promising Solutions For Treating Comorbidities Using Tissue Engineering And Regenerative Medicine.*

***Keywords:*** *Scaffold, Tissue Engineering, Periodontal Regeneration, Stem Cells, Regenerative Medicine*

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## I. INTRODUCTION

Periodontitisisahighlyprevalentdiseasecausedbyabacterialbiofilm.Reducedperiodontal support result in tooth loss and might need tissue augmentation orregenerative procedures to restore the form and function of the tooth supportingapparatus. Various procedures and techniques such as allografts, gene therapy,root surface conditioning and biomodifications were attempted in the field ofperiodontalregenerationbutmostofthemhadtheirownpartofclinicaldrawbacks.Thereforetheneedfortreatmentprocedureswithefficacyandefficiency stillpersist [1].

Recent developments in science and technology allow for alternative methods toface several difficulties in the treatment of periodontal disease. One of suchdevelopments includebioprinting which regenerate tissues withthe help ofscaffolds andbioactive factors [2].

3D bioprinting is one of the cutting-edge technologies which can regeneratemulticellular,biomimetictissueswithcomplexarchitecture.Thisrangeoftechniques,alsoreferredtoassolidfreeformfabricationoradditivebiomanufacturing, enables precise positioning of cells and biomaterials in a 3Dprinterwithfinelytunedinternalandexternalarchitectures,whilebeingcustomizable to patient-specific needs. It represents a powerful approach forengineeringbiomimetictissueconstructsinperiodontal regeneration.

## II. TISSUEENGINEERINGANDREGENERATIVEMEDICINE

Tissueengineeringevolvedfromthefieldofbiomaterialsdevelopmentandrefersto the practice of combining scaffolds, cells, and biologically active moleculesinto functional tissues. The goal of tissue engineering is to assemble functionalconstructsthatrestore,maintain,orimprovedamagedtissuesorwholeorgans [3].

Artificial skin and cartilage are examples of engineered tissues that have beenapproved bythe FDA; however,currently they have limited use in humanpatients.

Regenerative medicine is a broad field that includes tissue engineering but alsoincorporates research on self-healing – where the body uses its own systems,sometimes with help foreign biological material to recreate cells and rebuildtissues and organs.The terms “tissue engineering” and “regenerative medicine”havebecomelargelyinterchangeable,asthefieldhopestofocusoncuresinsteadoftreatmentsforcomplex,oftenchronic,diseases.

## III. TRANSITIONINGFROM2D TO3D

Eventhoughmanygreatdiscoveriesareattributedto2Dcellcultureandregenerationtechniques,therearecertainshortfallsthatexistinexecutingthe2Dcellculturetechniquesfortherapies.2Dcellculturetechniqueslackthecomplexity of actual tissues and organ systems. While animal models have beenemployed to test drugs and therapeutic measures in a preclinical setup, there hasbeen a desire to not use animal modeling due to high costs, ethical concerns, andpoor simulation to clinical applications [4]. To overcome these disadvantages, cellcultures were constructed in a three-dimensional environment. 3D cell culturetechniquesthatareunderpinnedbybioprintinginvolveintheprocessofgenerating scaffolds for tissue engineering and regeneration. Bioprinting willfacilitate the automated fabrication of multifaceted constructs to be used inresearchandsimulationoforiginalstructuresinconjunctionwith3Dcellculturetechniques. Bioprinting systems possess the precision necessary to incorporatethepatternsoftissue,cellandmatrixwithinthese3Dsystemswhichhelpsintheconstructionofdesired material and regeneration oftissuestructure [5].

## IV. HISTORYOF3DBIOPRINTING

The first commercial inkjet printer was manufactured by Siemens in the year 1951. Following which Gary Starkweather invented the laser printer in 1969 at the Xerox research lab in Webster, New York. In 1981, scientists found that chondrocyte differentiate in 3D environment and based on which in 1983, Charles Chuck Hull developed a prototype system referred to as stereolithography, in which layers are added by curing photopolymers with ultraviolet (UV) lasers. In 1989, Fused deposition modelling printing was developed by innovator Scott Crump. Early 2000s are considered to be an era of tissue engineering where inkjet printing of cells and organs were printed using 3D techniques

## V. PRINCIPLEOFBIOPRINTING

3D bioprinting is a method that is basically derived from additive manufacturingtechnology. In this method, objects are fabricated by adding materials layer bylayer, forming a 3d volumetric structure. The printed structures are designedusing a CAD-CAM software (or) CT (or) MRI (or) XRAY. Traditionally, 3dprinting has been primarily used to fabricate scaffolds constituted of syntheticinks such as polymer hydrogels, phosphate ceramics, inert metals etc which arethen seeded with living cells. However now-a-days the concept of bio-ink seemstobe emerging.

The principle of 3D printing is based on the precise placement of biologicalcomponents, biochemicals, and living cells in a layer-by-layer fashion with thespatial control of the placement of functional constituents onto the fabricated 3Dstructure [6].

## VI. APPROACHESOF3DBIOPRINTING

Theprocessof3Dbioprintingisbasedonthreedistinctapproaches:
Biomimicryorbiomimetics, Autonomousself-assembly, Mini-tissuebuildingblocks

Biomimicryistheprocessofidenticalreproductionofcellularandextracellular components of tissues and organs after an intricate examinationofnatureitself.Autonomous self-assembly is the method of replicating biological tissue byusingthemechanismofembryonictissueandorgandevelopmentasaguide. Mini-tissue building blocksapproachutilizesthemethod of bothof thepreviousstrategies [7].

**STEPSIN3D BIOPRINTING**

- ***Pre-bioprinting***: It is the first step in the process where the structure to beprinted is designed and modelled as a 3D structure using the ComputedTomography(CT)andMRIscans.Everyfinedetailisrecordedandtomographic reconstruction done on the images so that it can be printed. Thebioinks are prepared by isolation from living tissues and they are left tomultiply.

- ***Bioprinting*:**Itistheprintingprocesswherethedesignedstructuresareyettobe printed using the printers. Here the bio-inks are introduced to the printercartridgesandbasedonthedigitalmodelthecellsareaccumulatedinalayeredfashion.
- ***Post bioprinting*:** Post bioprinting process involves maintaining mechanicalintegrityandfunctionofthe3Dprintedstructure.Theycontroltheremodellingandthegrowthoftissuesb ysendingsignals.Evolutionofbioreactor technologies have caused rapid tissue maturation, vascularizationoftissuesand increasedthesurvivalrateofthetransplants.

**TYPESOF3DBIOPRINTING**

Various3Dprintingtechnologyhaveevolvedoverlasttwodecades.Eachoneofthemhavetheirapplicationsandl imitations.The different types of 3D bioprinting techniques are available such as inkjet bioprinting, laser assisted bioprinting, extrusion based bioprinting, pressure-assisted bioprinting and stereolithography[8].

***Inkjetbioprinting:***

ThefirstBio-printingtechnologydevelopedwasInkjetBio-printing.Itproduceddropletsofwell regulatedandcontrolledsizes,sourcedfromapreloadedcartridgecontainingthebioinkmaterial.Itfunctionsontheprinci pleofthermal/piezoelectric deformation of printer head. However, one of the majordrawbackisitsnon-compatibilitywithBio-inkdropletshavinghighviscosity.

***Laserassistedbio-printing(lab)****:*

This technique involves the use of a laser to selectively solidify a bioink in a precise location. The laser is controlled by a computer to create a 3D structure, which is then filled with living cells. Theupperlayerisadonorlayercomprisingofanenergyabsorbingtopzoneandbio-ink suspended at the bottom layer. A pulsed laser beam is focused on theenergy absorbing zone. It absorbs the laser energy and creates increased gaspressure, causing the propulsion of the cell droplets towards the collector slide.Individualtissueparticlessubsequentlyintegratetoformafullyfunctionalorganincorporatedwithrequiredspatial construct [9].

***Micro-extrusion basedbioprinting***:

One of the most popular types of 3D bioprinting is extrusion-based bioprinting. This technique involves the use of a nozzle to extrude a bioink, which is a mixture of living cells and a supportive material such as a hydrogel. This method of bio-printing is based on fused deposition and solution depositionmodelling technology. It uses a fluid dispenser along with an automated roboticsystem for extrusion printing. Fluid dispenser is based on either an air-driven orPistonpressureassistedsystemthatdepositstheBio-Inkintheformofcylindricalfilamentsaccordingtotherequireddesign.Itproducesmechanicallyandstructurallystrong supportivepolymericconstructs and3Dscaffolds [10].

***Pressure-assistedbioprinting(pab):***

Pressure-assisted bioprinting is based on the extrusion of biomaterials out of theprinter nozzle in order to fabricate a 3D biological structure. The commonbiomaterials used in this technique include hydrogels, cells and proteins, andceramicmaterialsolutions,collagenandchitosanetc.Thismethodprovidesabout40-80%cellviability.Theuseofpressure-assistedbioprintinginvolvesroom temperature processing and direct incorporation of homogenous cells onto thesubstrate. It is based on the principle in which the pressure is induced by acoordinatedmotionofpneumaticpressureorplungerorviascrew-basedpressureinthe form ofthecontinuousfilament [9].

***Stereolithography:***

Stereolithography is a nozzle free technique used to produce the 3D structure ofbiological and non-biological materials. The stereolithography technique has thehighest fabrication accuracy, and a large number of materials can be used in theprocess.The technique involves light-sensitive hydrogels that are deposited in alayer-bylayerfashiontoforma3Dstructure.Thecellviabilityismorethan90%.Thistechnologyisbasedontheprincipleofsolidi ficationoftheliquidphotosensitivepolymer upon illumination.

**BIO-INK**

Theidealbioinkformulationshouldmeetspecificmaterialandbiologicalrequirements,includingprintability, mechanics,degradation,function,biocompatibility, cytocompatibilty and bioactivity. The most commonly usedbio-inksfortissueandorganprintingarecell-ladenhydrogelsanddecellularizedextracellularmatrix(decm)-

basedsolutions.

Cellladenhydrogelsareparticularlyattractiveduetotheirbiocompatiblepropertiesandtheirabilitytorecapitulatethecellularmicroenvironment.dECM-based bio-ink formulations or decellularized tissue inks are of great significanceduetotheirinherentpropertyofbioactivityandsimplificationoftheformulationintoa printable bio-ink[11].

Bio-inkscanbeclassifiedbroadlyas**scaffoldbased**and**scaffoldfreematerials**.

***Scaffoldbasedconstructs:***

a. Hydrogels are the most widely used bio-ink material used in conjunction withInkjet, laser assisted and extrusion based bioprinters. It is a combination of anExtra-cellular matrix and living cells in the form of a pre-polymer solution thatundergoes physical or chemical cross linking to form self-supported structures.The encapsulated cells in the Bio-ink are cultured in suitable media in thelaboratory and aredeposited atsub-humanbodytemperatures.

b. Decellularized matrix-based bio-ink is produced by the lysis and extraction ofthecellularcomponentsofthenativetissuewiththeconservationoftheextracellularmatrix.Itisemployedintheextrusion-basedbio-printingandoffersgood bio-mimicry. In this process, tissue specific-customized constructs can befabricatedbutitisexpensiveandlacksadequatemechanicalstrengthrequiredforfabrication ofloadbearinglarge constructs.

c. Microcarriers are porous constructs of natural or synthetic materials used inExtrusion based bio-printing that facilitate cellular attachments, growth andmaturation with improved mechanical properties. Clogging of the nozzle head,expenseandsubsequentdecreasedaccuracywhileprintingaresomeoftheissuesreportedpreviously.

***Scaffoldfreebio-inks**:*

Scaffold free bio-inks are used for printing highly dense cellular constructs withthe absence of any supporting hydrogel or matrix. It is used in extrusion-basedbio-printingmethod.Itconsistsofcellsuspensionsinsuitablegrowthmediathatfacilitate cellular interactions and deposition of extra cellular matrix. The tissuespheroidsproducedexhibitsenhancedtissuebio-mimicryandcellularinteractions. Tissue spheroids, are spherical aggregates of cells with favorablestructural integrity used for tissue engineering and drug testing. However, thewholeprocessisverylaborintensivewithdifficultyinextractionofprematurelyfusedcellularaggregates.Cellpelletsandtissuestrandsareviewedasalternativesinscaffoldfree Bio-inks.

**APPLICATIONSOFBIO-PRINTINGINPERIODONTOLOGY**

Theperiodontaltissueshaveacomplexorganizationwhichrequiresmultilayeredbiomaterial constructs to restore the structural and functional integrity at thebone-ligament interface. Studies on modifying periodontal scaffolds are carriedout onthree aspects [12]

(1) findtheproperseedcells,withregardtoethicalandbio-safetyconcerns;
(2) fabricatebetterscaffolds,withtheinnovationofnewbiocompatiblematerialsand their future clinical application;
(3) identifymoreeffectivebioactivefactorstoachievethewholeregenerationofperiodontium.

The successful regeneration of the periodontium involves a coordinated multi-response from the periodontal fibers, gingiva, alveolar bone and cementum.Guidedtissueregenerationistheconventionalprocedurethatinvolvestheplacementof abarriermembrane in the periodontaldefect site to promoteselectiverepopulationoftheperiodontalcells.However,theclinicaloutcomesofthis method have been unpredictable. It has been well documented that wound stability and closure are essential for primary intention healing and the regeneration of periodontal defects. Moreover, 3D-printing techniques have attracted a great deal of attention in periodontal tissue regeneration therapies due to the sophisticated and challenging nature of this kind of reconstruction, which requires the regeneration of three different tissues including bone, cementum, and periodontal ligaments. Recently, use of multiphasic scaffoldswhich consists of a complex construct with varying microarchitecture such asporosity, pore organization as well as the chemical composition has shownguaranteeingclinical outcomes, asthese scaffoldscloselymimicthenativeperiodontal architecture.

## VII. STEMCELLSIN PERIODONTIUM

***PeriodontalLigamentStemCells:***

Periodontal ligament is a vital structure connecting teeth and alveolar bone, andperiodontalligamentstemcells(PDLSCs)arepluripotentstemcellsextracted fromperiodontalligament.PDLSCsareconsideredtobeimportantstemcellsforperiodontal regeneration and mandibular defects repair. They can differentiateintoalveolarboneandperiodontalligamentundertheinteractionwithextracellular membranes, and play an important role in regeneration of oral hardtissue. In vivo, PDLSCs can both differentiate into bonelike mineral tissue andligament-like fibric tissue with a certain arrangement depending on differentgrowth factors.

***GingivalMesenchymalStemCells:***

Gingivalmesenchymalstemcells(GMSCs)derivefromgingiva,andhaveshowntheir potential in periodontitis treatments. Studies showed the proliferation andosteogenic ability of the cells were promoted when cultured with the properscaffold.Asystemicreviewshowedthat,comparedwithotherstemcells,GMSCsareeasytoobtain,highinproliferationratesandcolony-formingefficiency, and can induce a stable periodontal tissue regeneration. Therefore,GMSCs can beasubstituteforPDLSCs inperiodontal recovery [13].

## VIII. LIMITATIONS OF3DBIOPRINTING

Periodontitis is a chronic inflammation disease caused by bacteria. The loss ofsurrounding tissue caused by periodontitis is unrecoverable. Periodontal surgeryis an effective restoration method, including GTR. The oral and maxillofacialregion has been considered as a rich source of adult stem cells. Several studiesand animal trials have proven that stem cells can perform a promising role inperiodontalregeneration.Thedevelopmentof3Dbioprintingtechnologyprovides a new solution for periodontal tissue regeneration, scaffolds usingbiocompatible materials and bioactive particles building a regeneration micro-environment. The current challenge for fabricating the scaffolds is focused onhow to regenerate the comparted tissue in the periodontium, respectively. Thedesignofscaffolds,includingmicropatternandmultiphasicstructure,etc.provides the possible solutions. Besides, using 3D bioprinting techniques toproduce scaffolds is both time-consuming and costly, because of individualizeddesign andthehighexpenseofrelative equipment.

## IX. CONCLUSION

Many clinical researches and case studies should be done in 3D-bioprinting ofthe periodontium using the available biomaterials and latest bioprinting methodstoregeneratetheperiodontium.Bioprintingandbioinkdesigningcouldbeestablishedasafieldofscientificspecialtyinthefutureorientedtowardsperiodontal regeneration. This gives us hope of a future where autologous graftsandalloplasticmaterialswill bereplacedby bio-printed products.

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