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3D Cell Culture in Periodontics

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Abstract: ***The complexity of periodontal tissues and the multifactorial nature of periodontal diseases necessitate advanced in vitro models that more accurately replicate in vivo conditions. This review critically evaluates the evolution, classification, and application of three-dimensional (3D) cell culture systems in periodontics. Compared to conventional two-dimensional (2D) cultures, 3D systems—comprising scaffold-based, scaffold-free, organ-on-a-chip, and microfluidic platforms—offer enhanced physiological relevance by simulating native extracellular matrix architecture, cellular heterogeneity, and microenvironmental cues. Scaffold-based models utilize biocompatible materials to support cell proliferation and differentiation, while scaffold-free techniques enable natural cell aggregation into spheroids and organoids. Organ-on-a-chip systems integrate dynamic flow conditions to mimic tissue interfaces and systemic interactions. These models facilitate improved understanding of periodontal pathogenesis, host–microbial interactions, inflammatory responses, and regenerative mechanisms. This review synthesizes evidences and emphasizing the utility of 3D cultures in drug screening, tissue engineering, and development of personalized therapies. Despite significant progress, challenges such as reproducibility, scalability, and cost must be addressed. Identification of research gaps and future directions underscores the transformative potential of 3D cell culture systems in advancing periodontal diagnostics and therapeutics. Further interdisciplinary efforts are warranted to integrate these platforms into clinical and translational research.***

Keywords: 3D Cell cultures, Periodontal Regeneration, Scaffold-Based Models, Host–Pathogen Interactions, Tissue Engineering

1. Introduction

Periodontal diseases are complex chronic inflammatory disorders that lead to the gradual destruction of the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. These diseases result from intricate interactions between dysbiotic microbial communities and the host immune response, influenced by genetic and environmental risk factors. While current treatment approaches—such as scaling and root planing, antimicrobial therapy, and regenerative techniques—can effectively manage disease progression, they do not fully address the underlying cellular and molecular mechanisms that drive tissue breakdown and repair. Conventional two-dimensional (2D) cell culture systems, commonly used for in vitro studies, lack the structural and functional complexity of in vivo periodontal tissues, limiting their translational relevance [1]-[3].

Three-dimensional (3D) cell culture systems offer a more physiologically relevant alternative, replicating native tissue architecture, cell–cell communication, and extracellular matrix (ECM) dynamics. These models facilitate deeper investigation into host–pathogen interactions, inflammatory responses, and regenerative pathways. In the field of periodontology, 3D cultures have become increasingly valuable for studying disease pathogenesis and evaluating therapeutic interventions under conditions that closely mimic those found in the human oral environment [2].

Types of 3D culture models include scaffold-based systems, scaffold-free spheroid models, organ-on-a-chip platforms, and microfluidic technologies. Each approach offers unique advantages for simulating specific aspects of periodontal biology. Despite these advances, barriers such as high cost, lack of standardization, and scalability issues remain challenges to broader application [2]-[3].

This review aims to provide a comprehensive evaluation of 3D cell culture methods in periodontics, examining their current applications, benefits, and limitations. It also highlights future research directions, emphasizing the transformative potential of 3D models in advancing personalized, regenerative, and translational strategies for periodontal therapy [3].

2. Traditional Cell Culture Technique In Periodontics

In the realm of biomedical research, cell culture techniques have been instrumental in elucidating cellular behavior, molecular mechanisms, and disease processes. The traditional 2D cell culture methods usually involve growing cells as monolayers on flat surfaces, such as plastic dishes or flasks, under controlled culture conditions [4]. While these techniques have provided invaluable insights into cell biology, they have notable drawbacks when applied to periodontal research. Periodontal tissues exhibit complex architecture, comprising various cell types organized within a dynamic extracellular matrix (ECM) rich in proteins, glycoproteins, and growth factors [4]-[5]. The spatial arrangement of cells, cell-cell interactions, cell-matrix interactions, and mechanical cues play crucial roles in regulating cellular functions, tissue homeostasis, and responses to external stimuli. Thus, one of the key limitations of traditional 2D cell culture is its inability to replicate the three- dimensional (3D) microenvironment which is usually found in vivo [5]. In addition, the traditional cell culture models frequently oversimplify the cellular heterogeneity and microenvironmental gradients present in the periodontal tissues. For instance, periodontal ligament fibroblasts cultured in 2D may exhibit different phenotypic characteristics compared to their counterparts in vivo, where they are surrounded by a complex ECM and neighboring cell types such as osteoblasts and endothelial cells [5]-[6]. This discrepancy limits the translatability of findings from 2D cultures to the complex in vivo scenarios encountered in

periodontal diseases. Despite their limitations, traditional cell culture techniques have played a crucial role in advancing our knowledge of fundamental cellular processes, cell signalling pathways, and the initial screening of therapeutic agents [6]-[8].

3. Emergence of 3D Culture Methods

With advancement in biomedical research, 3 D cell culture methods have emerged and they represent a paradigm shift in the field of research offering an enhanced physiologically relevant platform to study behavior cells, development of tissues, disease modeling and screening of drugs. The main aim of 3D cell culture method is to recreate the spatial organization, cellular interactions, ECM composition, and mechanical forces present in native tissues, thereby providing a closer approximation of in vivo conditions compared to traditional 2D cultures [9]-[11]

In the context of periodontics, the adoption of 3D cell culture methods holds immense promise for advancing our understanding of periodontal diseases and developing innovative therapeutic strategies. Periodontal tissues are composed of various cell types, including fibroblasts, osteoblasts, and immune cells, embedded within a dynamic extracellular matrix (ECM) that provides structural and biochemical cues essential for cell behaviour and function. 3D cultures recreate this intricate architecture, allowing cells to interact with each other and the ECM [11]. This realism is vital for studying the pathogenesis of periodontal diseases, which involve multifaceted interactions between bacterial biofilms, host immune responses, and tissue remodelling processes. Additionally, 3D cell cultures enable the evaluation of potential therapeutic agents and regenerative strategies in a setting that better reflects the true physiological environment, leading to more predictive and translatable results [12]-[14]

3.1 Types of 3D cell culture methods

3D cell culture models can be broadly classified into scaffold-based, scaffold-free, organ-ona-chip models and microfluidic systems. Each of these models offers unique advantages and is suited to different research applications [15].

Scaffold-Based Models: Scaffold-based models involve seeding cells onto or within a scaffold material that mimics the ECM. These scaffolds can be made from a variety of materials, including natural substances like collagen and alginate, or synthetic polymers such as polylactic acid (PLA) and polyglycolic acid (PGA). The scaffolds provide a 3D structure that supports cell attachment, proliferation, and differentiation, and can be engineered to release growth factors and other bioactive molecules. In periodontics, scaffold-based models are particularly useful for studying tissue regeneration and repair, as they can be designed to mimic the structural and biochemical properties of periodontal tissues [16]-[19]. Scaffold biomaterials used for periodontal regeneration:

1) **Natural scaffolds**: Natural scaffolds are derived from biological materials that inherently possess the properties of native ECM. Common natural materials used in periodontology include collagen, gelatin, hyaluronic acid, chitosan, and alginate. These materials provide excellent biocompatibility and bioactivity, promoting cell adhesion, proliferation, and differentiation [16].
2) **Synthetic scaffolds**: Synthetic scaffolds are created from engineered polymers such as polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL). These materials offer tunable mechanical properties and degradation rates, which can be customized to meet the specific requirements of periodontal tissue engineering. Synthetic scaffolds can be fabricated with controlled porosity and architecture to facilitate cell infiltration and nutrient diffusion.
3) **Hydrogel:** Hydrogel comprises of interconnected large-molecule polymers that are crosslinked, displaying absorbent qualities and a preference for water. The benefits of hydrogel formation encompass its high water content, biocompatibility, and the adaptability in structuring and shaping. A primary limitation of hydrogel in tissue engineering application is the weak mechanical stability [19]
4) **Bioceramics:** Bioceramic-based materials like hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and bioactive glass (BG) are extensively utilized to aid in the healing of alveolar bone (AB) in the periodontium [18]. These scaffolds typically offer robust mechanical stability and biodegradability, making them well-suited for periodontal regeneration. A significant advantage of bioceramic-based scaffolds compared to other natural and synthetic materials lies in their exceptional osteoconductive and osteoinductive properties. Furthermore, bioceramics can be administered into periodontal defects in various forms such as granules, paste, and injectable formats [18]-[20].

Scaffold based 3D cell culture models: Cells embedded in hydrogel: A frequently utilized hydrogel application in 3D cell culture is embedding. This process involves several steps to ensure an even distribution of cells. Initially, cells are mixed with hydrogel and added to a culture vessel, such as a 96-well plate. Subsequently, culture medium is dispensed on top of the gel [20]. The embedded cells form heterogeneous populations of spheroids. For high content screening (HCS) applications, the easy degradation and transparency of hydrogels are crucial properties [25]. Retrieving cells for RNA/DNA extraction can be challenging with some hydrogels. Synthetic hydrogels cannot be degraded, while the degradation of animal-derived matrices can affect the cell surface proteins of cultured cells [24].

Dome type 3D cell culture: Dome culture is a method suitable for cultivating organoid and spheroid 3D cell models typically utilizing animal-derived hydrogels. These hydrogels are temperature-sensitive and polymerize at 37°C. During preparation, the hydrogel material, pipet tips, and cell suspension must be maintained at 4°C to keep the matrix in a liquid state. This temperature requirement necessitates a swift workflow [21]. The culture vessel, where the cold matrix-cell suspension is added, is kept at 37°C to promote matrix polymerization and dome formation. Once the domes are formed, culture media is carefully added on top to cover them. To recover 3D cell models from the matrix, the temperature must be lowered to 4°C to liquefy the matrix. Enzymatic digestion, used during this process, can damage the cell surface of the cultured cells. Due to the temperature changes

required, this method is not ideal for high-throughput screening (HTS) applications [21]-[22].

Seeding cells on top of hydrogel: The use of hydrogel extends beyond embedding solutions, as it can also serve as an underlay material for cell suspension, allowing cells to grow on top of the gel. This method is particularly suitable for endothelial and epithelial cells, which typically are not surrounded by an extracellular matrix (ECM) under physiological conditions [22]. Depending on the type of hydrogel, cells may attach to the surface proteins, creating a more 2.5D than 3D culture. This is common with animal-derived matrices, but plant-based hydrogels can be modified to include binding proteins on their surface, better mimicking the natural ECM composition [22]. If the cells are not adherent and the hydrogel lacks surface proteins for binding, they tend to form spheroids. As cells grow on top of the hydrogel, they can produce their own ECM proteins and create a matrix. The hydrogel effectively divides the cell culture into distinct layers, allowing simultaneous growth of some cells within the hydrogel and others in the media layer [23]. However, if cells do not migrate into the hydrogel or attach to its surface, maintaining optical focus can be challenging, similar to other suspension cell models [23].

Cell culture inserts: Cell culture inserts are used in conjunction with multi-well plates, forming a setup that divides into two distinct chambers [24]. At the base of the insert is a microporous membrane that permits the passage of signaling molecules or cells. The membrane's permeability can be tailored by choosing an appropriate micropore size [23]-[24].

Scaffold-Free Models: Scaffold-free models rely on the self-assembly of cells to form 3D structures without the need for an external scaffold. In these systems, cells aggregate and interact with each other to form tissue-like constructs. Scaffold-free models are advantageous for studying cell-cell interactions, tissue organization, and the effects of various treatments in a more natural context. These models are useful in periodontics for examining the behavior of periodontal ligament fibroblasts, osteoblasts, and other cell types in a more physiologically relevant environment [24]-[25].

3.2 Techniques used in scaffold free models

3.2.1 *The hanging drop technique:* In the hanging drop method, approximately 10 µl drops of cell suspension are placed on a flat surface of the culture vessel. Once all the cell suspension droplets are in their designated positions, the surface is inverted. The surface tension of the cell suspension causes the droplets to hang from the attached surface, and gravity pulls the cells to the bottom of the droplet. At the bottom, the cells aggregate and form a spheroid. These spheroids are uniform, and their size can be adjusted by changing the cell seeding density. However, changing the cell culture media in the hanging drop method without disturbing or losing the spheroids is challenging. The technique can also become tedious when scaled up, limiting its use in high-throughput screening (HTS) applications. Additionally, the method's properties are not ideal for visualization. Droplets often fall when moving plates for imaging and the focal distance can be challenging for many microscopes since the droplets can be far from the plate's bottom [23]-[25].

3.2.2 *Ultra- low attachment plates:* Ultra-low attachment (ULA) plates were designed to enable large-scale, scaffold-free 3D cell cultures. These plates are fabricated using liquid overlay techniques, where the bottom of a cell culture dish is coated with a non-adhesive material to prevent cell adhesion and protein absorption. Typically, ULA plates are produced by covalently binding a hydrophilic and biologically inert material to the plate's surface. When cell suspension is added to a well of a ULA plate, the cells settle at the bottom without attaching to the surface, promoting their aggregation and spheroid formation [23]-[25].

3.2.3 *Organ-on-a-Chip:* Models Organ-on-a-chip models utilize microfluidic technology to recreate the microarchitecture and physiological functions of organs or tissues on a small, chip-like device. These models incorporate channels and chambers that simulate the flow of blood and other fluids, providing a dynamic environment for cell culture. Organ-on-a- chip technology allows for precise control over the mechanical and biochemical conditions, enabling the study of complex tissue responses and interactions. In periodontics, these models can be used to replicate the periodontal microenvironment, study disease progression, and evaluate the efficacy of new therapeutic interventions under conditions that closely mimic the in vivo setting [24]-[25]

4. Development and implementation of 3D cell culture models in periodontal research

Several studies have explored the development and implementation of 3D cell culture models in periodontal research, aiming to evaluate their effectiveness in replicating the in vivo environment of periodontal tissues and their potential applications in understanding disease mechanisms and advancing regenerative therapies. A study by Miryam Adelfio et al., [26] developed an in vitro humanized gingival based on a silk protein porous scaffold to support the growth and persistence of native gingival multicellular and microbial populations. On human microbiome exposure reduction in cell viability was observed within the pocket. In both samples, pro-inflammatory (granulocytemacrophage colony-stimulating factor [GM-CSF], IL-1RA, IL-6, IL-8, IL-12p40, IL-17A, and tumor Necrosis factor alpha and anti-inflammatory (IL- 2, IL-10, IL-3, and IL-4) cytokines were above the detection limits. Thus, the tissue model supported a functional response to human oral microbiome interactions in healthy conditions [26]. A similar study by M. Adelfio et al., [27] developed an in vitro gingival model which would be able to mimic the spectrum of periodontal disease presentation, for the identification of predictive biomarkers for earlystage diagnosis. In the model, optimized artificial saliva did not hinder the normal growth curve of gingival cells in comparison to co-culture media and supported the formation of a functional epithelial barrier expressing major components of the junctional network [27]. A study by Karanth et al., [28]

developed a poly(L-lactic acid) (PLLA) scaffold and evaluated critical characteristics essential for its biologic use as a craniofacial implant [28]. The crystallinity reduced from 27.5% to 13.9% during the 3D printing process. The hydrolytic degradation was minimal during a 12-week period. Osteoblast-like cells did not attach to the uncoated scaffold but attached well after coating the scaffold with fibrinogen and proved that 3D-printed PLLA scaffolds had promising properties akin to the natural bone. A similar 3D culture model was developed by Koskinen Holm and Qu, 2022 [29] using hTERTimmortalized gingival fibroblasts (hGFBs)-populated collagen gel directly crosslinked with genipin/cytochalasin D and seeding hTERT-immortalized gingival keratinocytes (TIGKs) on the upper surface for a 2-week air-liquid interface co-culture [29]. It was shown that genipin is a promising crosslinker with the ability to reduce collagen contraction while maintaining normal cell function in collagen-based oral tissue engineering. Another model developed by Vurat et al., [30] used three-dimensional bioprinting (3DBP) technology for developing a multi-cellular microtissue model resembling PD ligament-alveolar bone (PDL-AB) biointerface and showed that 3D-bioprinted multi-cellular periodontal/osteoblastic microtissue model has potential as an in vitro platform for studying processes of the human PDL [30].

5. The different techniques of 3D cell culture techniques in periodontics:

Amongst the various techniques used in 3D cell culture, scaffold based and non-scaffold based techniques seem to have garnered the ability to effectively harness periodontal tissues and prove its use in periodontal therapy. A study by Asad et al., [30] showed that culturing human gingival fibroblasts in a novel three-dimensional fibrin gel scaffold containing collagen-stimulating media can provide tissue-equivalent construct that mimics human gingival connective tissue. A study by Gauthier et al., [31] showed that polycaprolactone-based electro spun 3D fibrous scaffolds could be used instead of collagen to undergo human periodontal cell mechanobiological investigations. A study by Xuan et al., [31] studied the structurally ordered BRT (BRT-O) scaffolds fabricated by a three dimensional (3D) printing technique and compared it with clinically available β-tricalcium phosphate (β-TCP) scaffolds and found that BRT-O scaffolds released higher concentrations of ionic products than the β-TCP scaffolds thus promoting immunomodulatory roles in promoting critical-sized bone defects by enhancing the polarization of M2 macrophages. Another 3D cell culture technique is the spheroid based technique. A study by Yan et al., [32] developed a 3D cell culture method, which was based on spheroid formation of PDL cells on chitosan films. The viability of PDL cells in spheroids was assessed after 1, 3 and 6 days and it was found that the majority of cells in spheroids were living cells on day 1, 3, and 6. While on day 6, the number of dead cells increased in the central part [32].

6. Effectiveness of 3D cell culture in periodontics:

The effectiveness of 3D cell culture in periodontics lies in its ability to more accurately replicate the complex in vivo environment of periodontal tissues, thereby enhancing our understanding of disease mechanisms and improving the development and testing of regenerative therapies.

A study by Kollmuss et al., [33] has shown that all oral splint materials showed overall acceptable biocompatibility to the 3D cell culture model. It showed that oral splints fabricated with subtractive manufacturing techniques elicited the weakest cytotoxic response of hGF-1thus proving that 3D printing could be a viable alternative to milling for producing oral devices [33].

A study by Ivanov et al., [34] proved that the addition of exogenous components of the ECM (HA, Fn, and Lam) to the dECM most effectively induces the differentiation of PDLSCs into osteoblast-like and odontoblast-like cells under 3D culture conditions [34].

A study by Bhatt et al., [30] showed that Hydrogen water has antioxidative potential. In the study, the gingival fibroblasts which were obtained from patients with chronic periodontitis, after treatment with hydrogen water, showed the mean viability of 80% after 24 h and 73% after 48 h. The fibroblasts treated with distilled water showed condensation and shrinkage, indicating the cell death [30].

A study by Nowak-Terpiłowska et al., [35] has found that human gingival Fibroblasts irradiated with laser 1064 nm after 48 h and 72 h showed that best outcomes wherein the cell viability increase ranged from 0.6_ (3 J/cm2, 50 mW) to 1.3_ (64J/cm2,1000 mW). This indicated that the appropriate use of low-level laser irradiation could increase the proliferation rate of cultured cells and be of use in tissue engineering [35].

Another similar study by (Ferrà-Cañellas et al., 2023 [32] has proven that MIM-seq treatment restored collagen production levels in the culture models. The complete sequence of MIM-seq decreased PGE2 release and restored collagen deposition levels induced by IL-1_ treatment in hGFs exposed to IL-1 suggesting that MIM-seq can be used for treatment of periodontal diseases [32].

A study by Colangelo et al., [34] has further shown that polynucleotide, hyaluronic acid (PN, HA) compound has synergic effects on primary fibroblasts and promotes their viability, increases the spheroid size and perimeter and decreases spheroid circularity, thus proving its efficacy in periodontal therapy [34].

Another study by Hwa et al., [35] showed that adding an enamel matrix derivative to the culture of the 27 _g/mL group raised the level of RUNX2 mRNA expression proving that derivative of the enamel matrix may be used to promote osteogenic differentiation in stem cell spheroids [35].

A study by Kurosawa et al., [29] examined the effect of butyrate on gingival layers of a developed gingival 3D culture system and showed that DAMPs released following butyrate treatment can bind to receptors on surrounding cells and induce the production of proinflammatory cytokines [29].

In another study by Yuan et al., [31] has shown that adipose derived stromal or stem cells are potential seed cells for

pTDM-induced bio-root regeneration and thus helpful in periodontal therapy [31]

A study by Panduwawala et al., [28] showed that the 3D cell sheet-based approach may be potentially beneficial in regenerative periodontal therapy as histological evaluation revealed that after 2, 4 and 8 week of implantation, periodontal ligament-like tissue arrangements were observed around the implanted roots in experimental groups [28].

Similarly a study by Pandula et al., [35] showed that a higher ALP gene expression was observed at 3 days in 1 : 1 in the 3D model and thus the novel 3D cell sheet-based approach may be potentially beneficial for periodontal regenerative therapy [35].

A comparison of 2D and 3D cell culture models was done in a study by de Souza Castro et al., [27] wherein micro and titanium dioxide nanoparticles (TiO2 NPs) were added to the culture model and differentiation and mineralized matrix production of human osteoblasts SAOS-2 were compared. The results showed that mineralization increase was higher in the 3D model when compared with the 2D model, suggesting that 3D could present better mineralization evaluation [27].

7. Discussion

The development and implementation of three-dimensional (3D) cell culture models mark a significant advancement in the field of periodontal research. Unlike conventional two-dimensional (2D) cultures, which often fail to replicate the structural and functional complexity of periodontal tissues, 3D culture systems offer more physiologically relevant environments by mimicking the native extracellular matrix (ECM), spatial cell orientation, and dynamic cell-cell and cell-matrix interactions [20].

Periodontal tissues are composed of a unique triad of hard and soft tissue components, including gingival epithelium, connective tissue, periodontal ligament (PDL), cementum, and alveolar bone, each with distinct cellular and molecular functions [28]. The chronic inflammatory nature of periodontal disease leads to breakdown of these complex structures. To effectively study these interactions and explore novel regenerative strategies, models that closely resemble the in vivo microenvironment are critical. 3D cell culture systems have thus emerged as valuable tools, enabling a deeper understanding of the pathogenesis of periodontitis and the evaluation of therapeutic interventions in a biomimetic context [30].

Among various types of 3D systems, scaffold-based models using natural or synthetic polymers have shown immense utility in periodontal regeneration studies. Hydrogels such as collagen, gelatin, and hyaluronic acid offer excellent biocompatibility and support for cell adhesion, migration, and differentiation [22]. These scaffolds can be engineered to deliver growth factors or simulate specific ECM properties, making them highly effective in tissue engineering applications. For instance, hydrogel-embedded gingival fibroblasts or periodontal ligament stem cells have demonstrated increased collagen production, osteogenic potential, and ECM remodeling—features essential for periodontal repair [26].

Scaffold-free systems, including spheroids and organoids, are equally important, especially in studies where the aim is to examine natural cell aggregation, differentiation, and intercellular communication [33]. Hanging drop and ultra-low attachment plate methods have been successfully employed to culture periodontal ligament fibroblasts and gingival epithelial cells in spheroid formats. These models facilitate the study of cell responses to inflammatory cytokines, bacterial endotoxins like lipopolysaccharide (LPS), and regenerative agents such as enamel matrix derivatives or antioxidants [26]. Moreover, the 3D spheroid models have shown promise in simulating chronic inflammation and drug response scenarios more accurately than their 2D counterparts [21].

The emergence of organ-on-a-chip platforms and microfluidic models has further expanded the frontiers of periodontal research. These systems incorporate dynamic fluid flow, mechanical forces, and multi-cellular co-culture environments to mimic physiological conditions such as salivary shear stress, immune cell infiltration, and bacterial colonization. Gingival tissue-on-chip models have been used to replicate oxygen gradients and structural heterogeneity within the gingival sulcus, offering new avenues to investigate host–microbiome interactions and epithelial barrier integrity in the early stages of periodontal disease [28]-[31].

Despite these advancements, 3D culture models face notable limitations. Standardization of protocols, reproducibility of results, and cost-effectiveness remain significant challenges. Furthermore, many models lack the inclusion of vascular, neural, and immune components, limiting their ability to fully replicate the periodontal microenvironment. The integration of immune cells, such as macrophages and neutrophils, is particularly crucial for simulating the inflammatory responses observed in periodontitis [30]-[33].

Translational applicability is another area that requires attention. While in vitro 3D models offer compelling insights, their predictive value for in vivo human outcomes must be validated through preclinical and clinical studies. There is also a need to develop high-throughput screening platforms using 3D systems for evaluating biomaterials, pharmaceuticals, and biologics tailored to periodontal therapy [33].

8. Research Gaps Identified

With the rapid advancements in tissue engineering and regenerative medicine, there has been a proliferation of 3D culture techniques, including scaffold-based cultures, spheroid models, organotypic cultures, and bioprinting technologies.

However, the literature lacks a comprehensive synthesis of these techniques, their applications, strengths, and limitations in the context of periodontics.

9. Future Directions

As 3D cell culture systems continue to evolve, their integration into periodontal research and clinical translation

offers immense promise. However, several critical areas demand focused advancement to maximize their impact on periodontal diagnostics, therapeutics, and tissue engineering.

First, standardization and scalability of 3D culture models remain pressing challenges. There is an urgent need for reproducible protocols that allow consistent fabrication, seeding density, matrix composition, and endpoint analyses across laboratories. Development of commercially available, validated 3D kits specific to periodontal tissues could enhance accessibility and comparability across studies [30].

Second, the incorporation of immune and vascular components into 3D models will be pivotal. Periodontitis is an immunoinflammatory disease, yet most current models exclude key players such as neutrophils, macrophages, T-cells, and vasculature. Next-generation systems should strive for co-culture models or immune-responsive scaffolds that better mimic host responses, immune cell recruitment, and vascularized tissue dynamics [32]-[33].

Third, patient-specific (personalized) 3D models using autologous cells or stem cell-derived organoids hold potential for individualized therapeutic testing. These personalized models could revolutionize precision periodontics by allowing ex vivo evaluation of drug responses, biomaterial compatibility, and regenerative potential in a patient-tailored manner [16]-[17].

Fourth, microfluidics and organ-on-a-chip technologies should be further explored in periodontics. These dynamic platforms can simulate salivary flow, bacterial colonization, and mechanical stress in real-time, offering more physiologically relevant conditions for studying disease onset, progression, and treatment outcomes [10]-[12].

Fifth, integration of omics technologies—including transcriptomics, proteomics, and metabolomics—with 3D culture platforms could yield deeper insights into the molecular mechanisms governing periodontal regeneration, inflammation, and microbial interaction. High-throughput platforms combining 3D culture with single-cell RNA sequencing or spatial transcriptomics may reveal novel biomarkers and therapeutic targets [2]-[5].

Lastly, regulatory and translational pathways must be addressed. For 3D models to influence clinical practice, regulatory frameworks should be developed to validate their predictive value and safety [10]. Bridging the gap between laboratory-based innovation and clinical utility requires collaborative efforts among researchers, clinicians, material scientists, and regulatory authorities [6].

10. Conclusion

Three-dimensional (3D) cell culture systems represent a transformative advancement in the field of periodontal research and regenerative therapy. By more accurately replicating the cellular architecture, microenvironment, and biological behavior of periodontal tissues, these models have bridged critical gaps left by traditional two-dimensional cultures [2]. Scaffold-based and scaffold-free systems, along with emerging organ-on-a-chip technologies, have enabled researchers to explore host–microbe interactions, inflammatory responses, and tissue regeneration with greater fidelity and relevance to in vivo conditions [4].

Despite their potential, current 3D models face limitations in standardization, immune cell integration, and clinical translation [15]. Addressing these challenges through interdisciplinary collaboration, technological refinement, and personalized model development will be key to fully harnessing their capabilities [20]. As the field progresses, 3D cell culture systems are expected to play a pivotal role not only in understanding periodontal pathogenesis but also in advancing predictive diagnostics, drug screening, and patient-specific treatment planning [12].

In conclusion, 3D cell culture techniques offer a powerful platform for the future of periodontology—one that is more biologically accurate, translationally relevant, and capable of supporting next-generation therapeutic innovation [30]s.

Figures

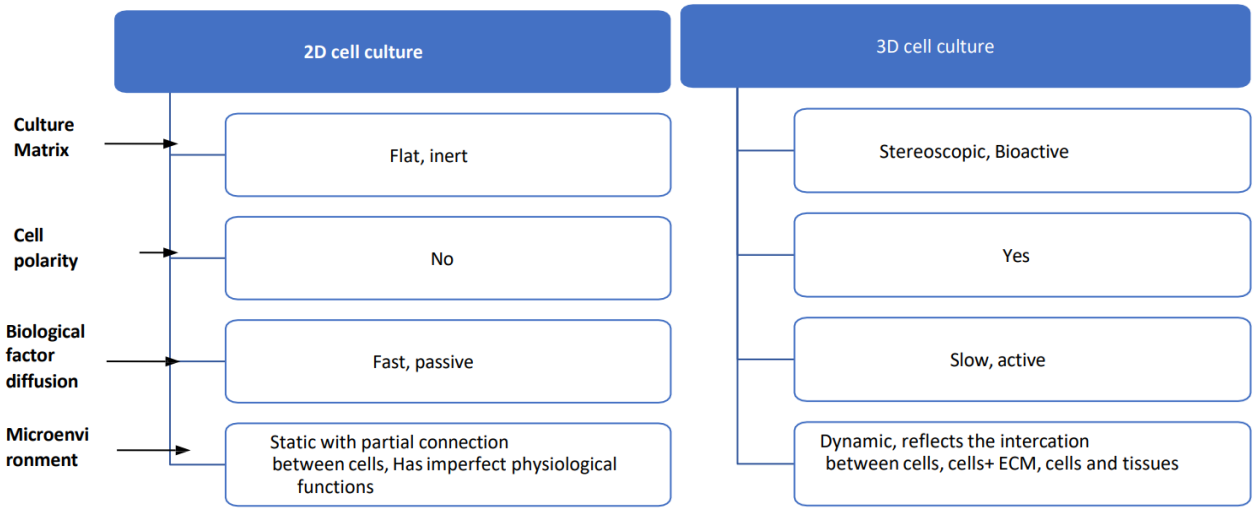

Figure 1: Differentiation between 2D and 3D cell culture methods

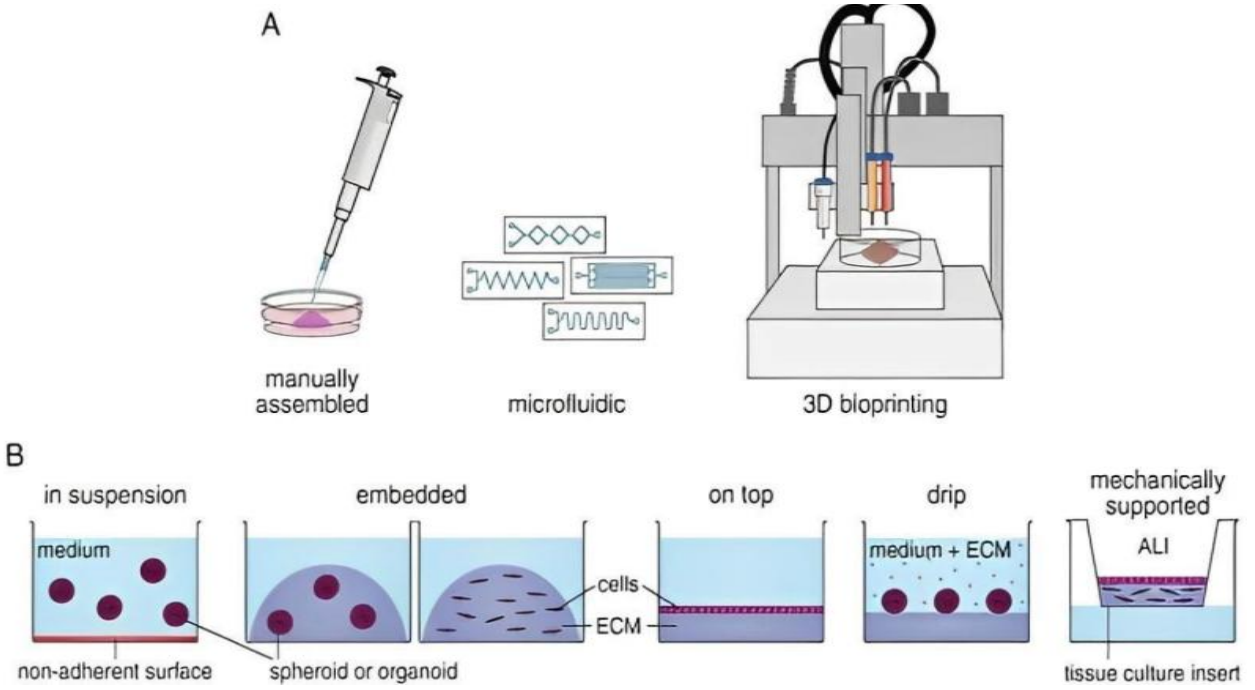

Figure 2: 3D cell culture technique

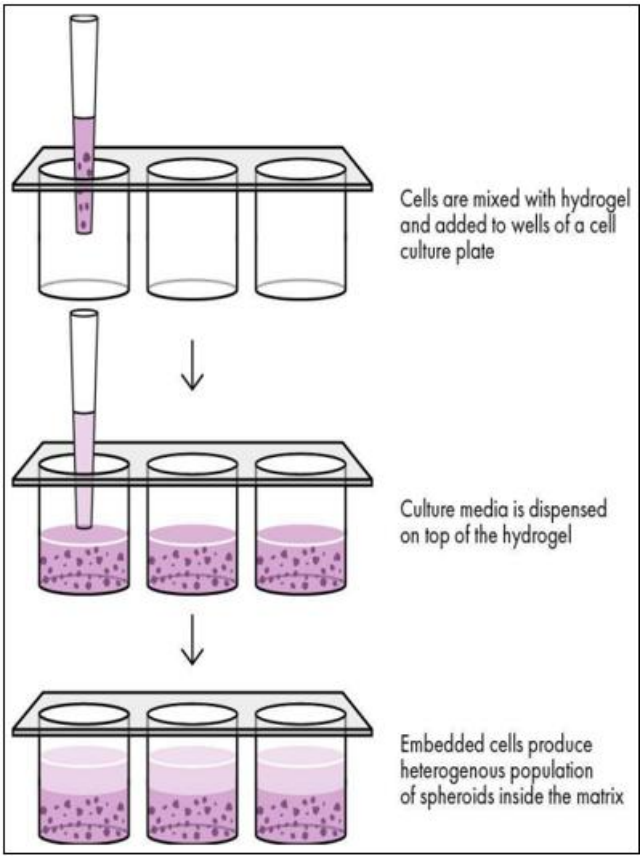

Figure 3: Hydrogel based 3D cell culture

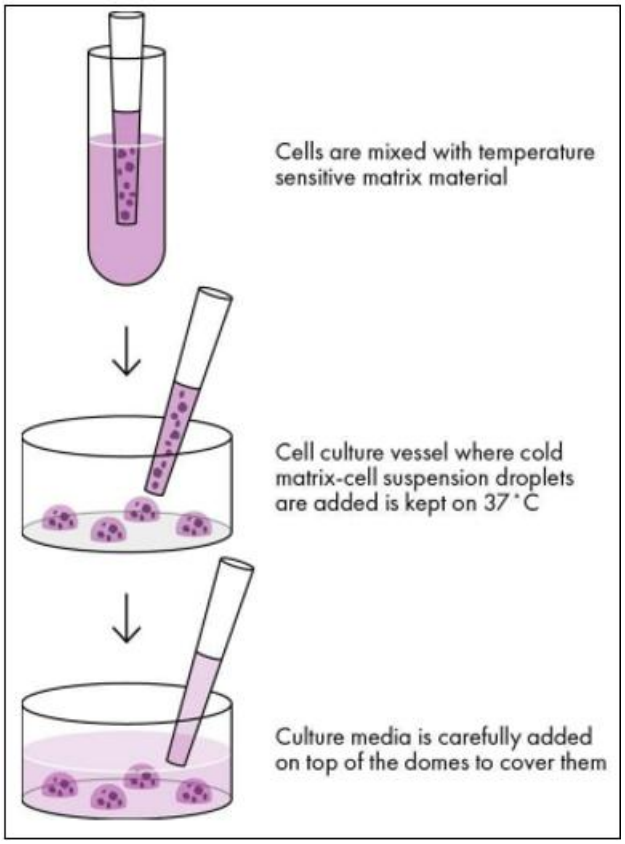

Figure 4: Dome type 3D cell culture

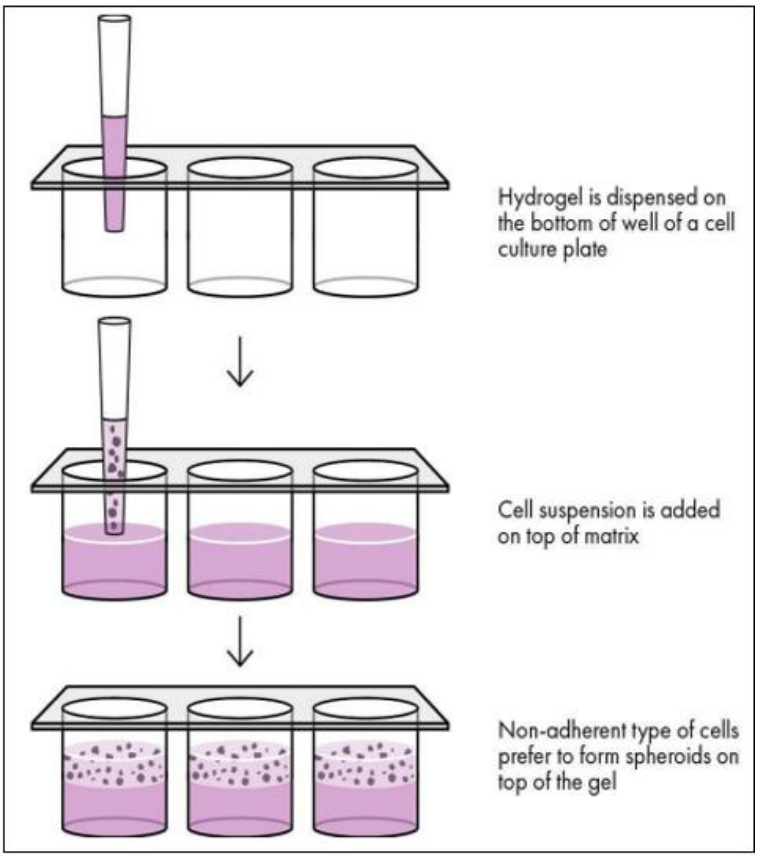

Figure 5: Seeding cells on top of hydrogel

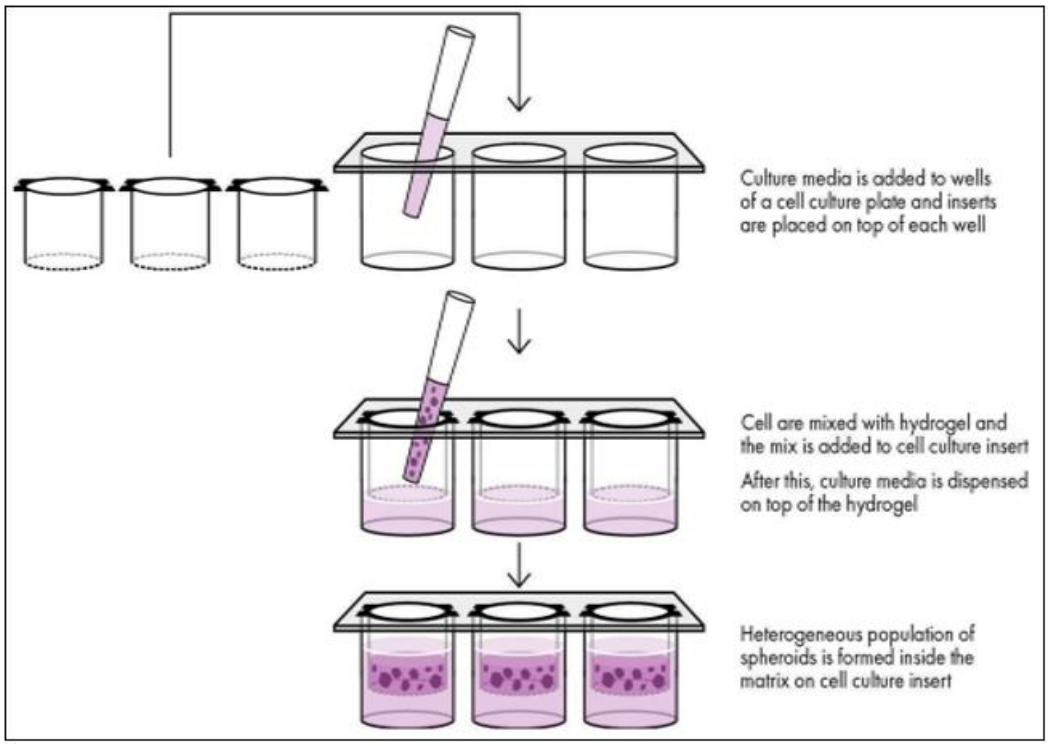

Figure 6: Cell culture inserts

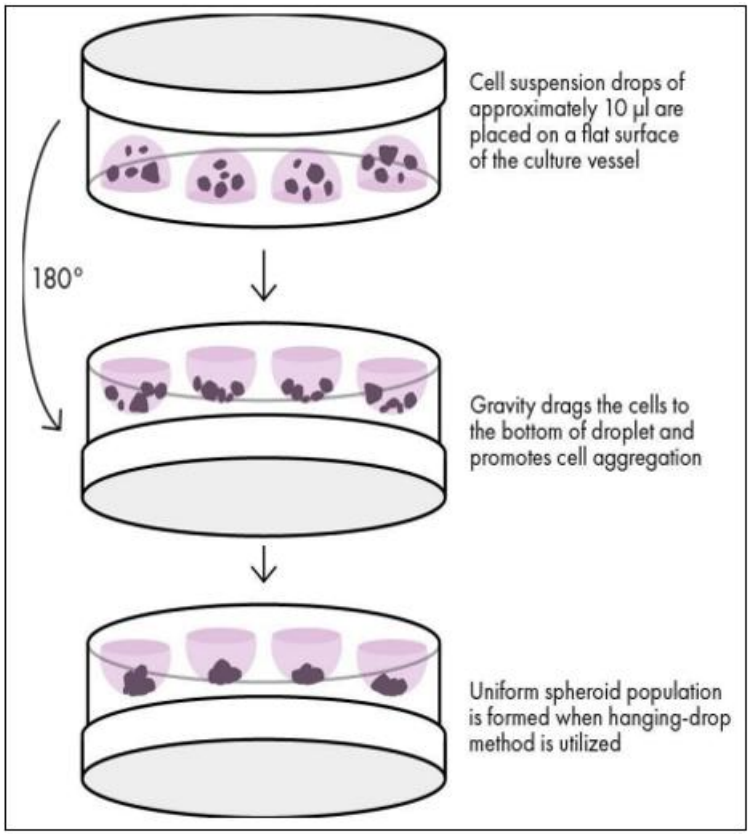

Figure 7: The hanging drop technique

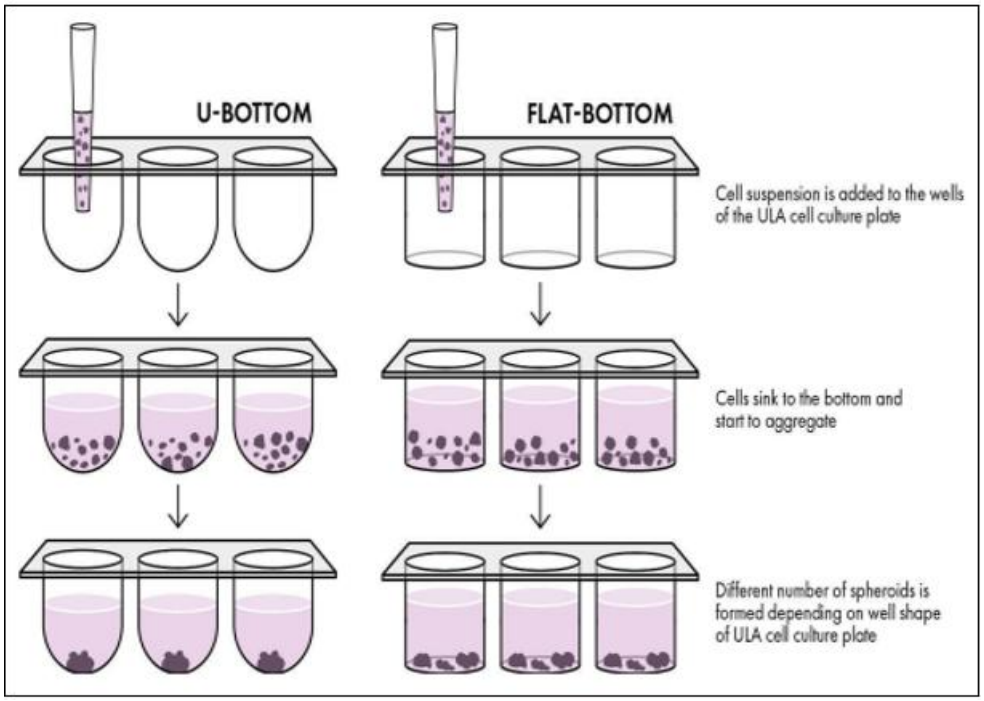

Figure 8: Ultra- low attachment plate technique

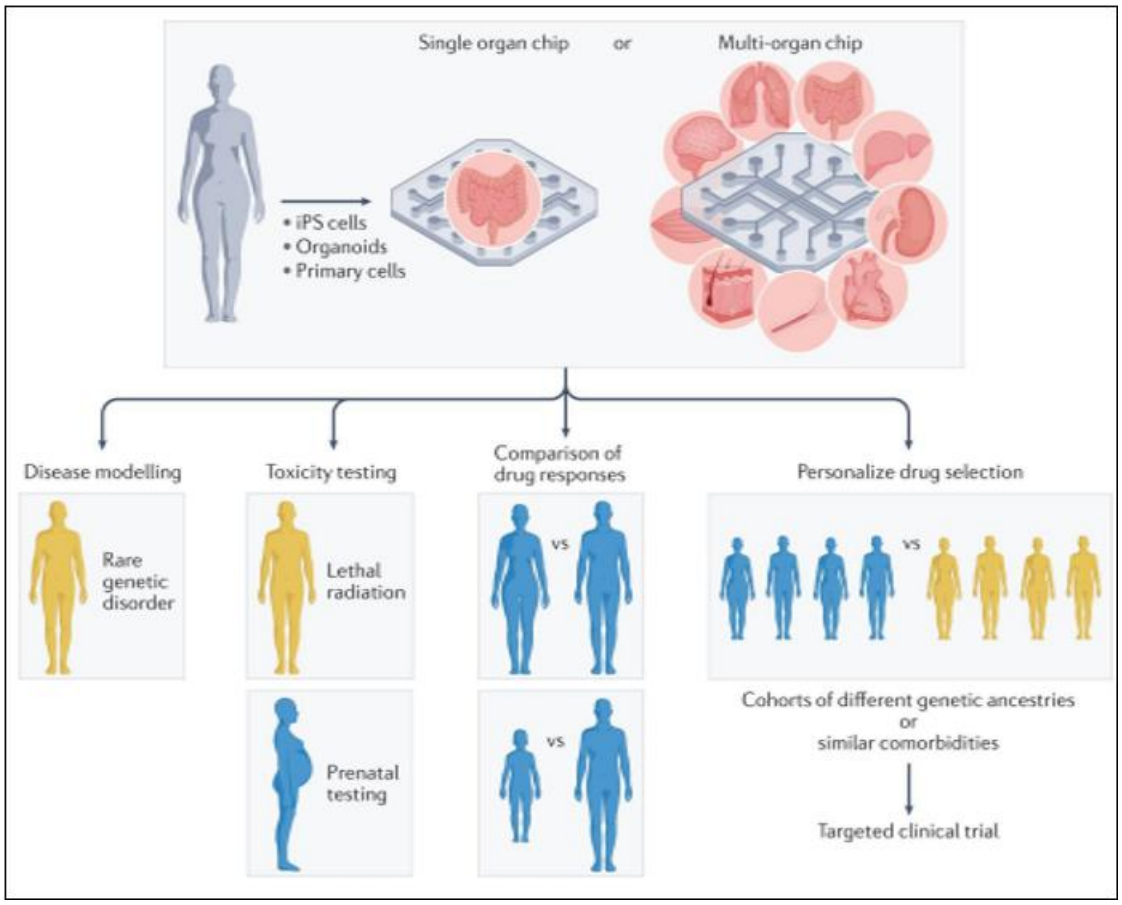

Figure 9: Overall use of Organ-on-a-Chip Model

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