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Advancements in Three-Dimensional Stem Cell Culture Techniques: Implications for Regenerative Medicine and Therapeutic Applications

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Abstract

Background: Stem cell research is pivotal for advancements in regenerative medicine and therapeutic applications. Traditional two-dimensional (2D) cell culture techniques fail to adequately replicate the complex in vivo cellular microenvironment, leading to compromised cell functions and phenotypes. Recent developments in three-dimensional (3D) cell culture systems offer promising alternatives that more closely mimic the physiological conditions found in living organisms.

Methods: This review systematically explores emerging techniques in 3D stem cell culture and differentiation, categorizing them into scaffold-free and scaffold-based systems. It highlights innovative methodologies such as liquid overlay, hanging drop culture, magnetic suspension culture, and chemically defined media formulations, which facilitate the formation of multicellular spheroids and enhance cellular self-renewal and differentiation capabilities.

Results: The findings indicate that 3D culture systems significantly improve cell viability, proliferation, and functionality compared to conventional 2D cultures. Spheroid formation enhances intercellular communication and mimics tissue architecture, thereby optimizing the regenerative properties of stem cells. Moreover, the use of natural and synthetic hydrogels as scaffolds has been shown to support cellular activities and promote tissue-specific differentiation.

Conclusion: The adoption of 3D culture techniques represents a significant advancement in stem cell research, offering a more accurate model for studying cell behavior and drug responses. Future directions involve refining these technologies to enhance scalability and cost-effectiveness, ultimately bridging the gap between in vitro models and in vivo applications in regenerative medicine.

Keywords: Stem cells, three-dimensional culture, regenerative medicine, cell differentiation, scaffolds.

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1. Introduction

Cell culture is a crucial and foundational procedure in biology and medicine. This process entails isolating cells from biological tissues, replicating the in vivo survival environment to promote their growth and reproduction, and preserving their primary structures and functions under sterile settings with appropriate temperature, pH, and sufficient nutrition availability. Currently, in vitro cell culture techniques include two-dimensional (2D) adherent culture and three-dimensional (3D) spherical culture [1], with the former being the most often used. This approach utilizes a glass or polystyrene dish to provide mechanical support for the cells while maintaining consistent conditions for supplying exogenous nutrients and eliminating metabolites. The circumstances are meticulously regulated, and the cells are readily observable and collectible. Nevertheless, the 2D technique has limitations, since it cannot accurately replicate the intricate cellular milieu [1].

In vivo, most cells engage with adjacent cells and the extracellular matrix (ECM) to establish a sophisticated communication network of biochemical and mechanical signals, which underpins the maintenance of normal cellular activities [2]. Oxygen, hormones, and nutrients may be conveyed between cells, metabolic waste can be eliminated from cells, and cells can migrate in response to mechanical or chemical stimuli [3]. In 2D culture, cells proliferate in a confined environment, resulting in contact inhibition. Consequently, cell proliferation is diminished, and cell shape and function are altered [4]. For instance, stem cells are susceptible to losing their self-renewal capacity, entering senescence, or spontaneously differentiating into osteocytes or adipocytes [5]. Prolonged cultures will progressively result in the loss of tissue specificity, highlighting discrepancies between in vitro cell culture outcomes and in vivo animal investigations [6]. Consequently, comprehending how to enhance the simulation of the physiological milieu under in vitro circumstances is crucial for medical research [7].

Ongoing technological improvements have necessitated enhanced standards for cell culture models, resulting in the emergence of 3D cell culture. In contrast to 2D culture, 3D culture more closely resembles the organism in both structure and function, hence providing a more faithful simulation of the in vivo cellular microenvironment [1, 3, 8]. This 3D cell culture may influence cell growth and proliferation, enhance the self-renewal of stem cells, and suppress their differentiation. Moreover, analogous to in vivo cells, three-dimensional culture enhances the transfer of molecules across cells and between cells and the extracellular matrix, facilitates nutritional absorption, gas exchange, and the expulsion of metabolic waste in a balanced manner [9, 10]. Consequently, to preserve the intrinsic properties of cells and more accurately replicate their in vivo conditions, researchers have created several three-dimensional culture techniques. In drug research, 3D culture serves as an intermediary between 2D culture and animal experimentation [11, 12].

2. Three-Dimensional Stem Cell Cultivation Systems

3D cell culture technology involves the cocultivation of carriers with three-dimensional structures composed of diverse materials and various cell types in vitro, enabling cell migration and growth within the carrier's 3D architecture to create a 3D cell-carrier complex. This technology can be categorized into scaffold-free and scaffold-based culture systems, each with distinct applications in various studies [13].

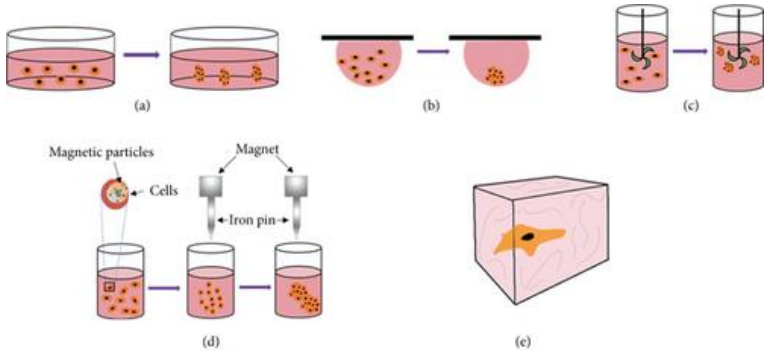

Figure 1. Diagram of three-dimensional cell culture systems.

3. Scaffold-Free Cultivation System

This culture method lacks a supporting framework for cell adhesion, growth, and diffusion, resulting in cells inside the culture medium aggregating into tissue-like formations known as spheroids. These spheroids generate their extracellular matrix, independent of foreign scaffolding or matrices. The extracellular matrix comprises glycosaminoglycans, proteoglycans, structural proteins, adhesion proteins, and other macromolecules, influencing activities like cell morphology, metabolism, function, migration, proliferation, and differentiation [14].

The liquid overlay approach is among the most straightforward and cost-effective ways for 3D cell culturing [15]. This technique utilizes substances that prevent cell adherence to the base of the cell culture container, such as agar, agarose, HEMA, or ultralow attachment plates [16]. The intercellular force exceeds that between cells and the material surface, and this force may spontaneously induce cell aggregation into spheres within 24–72 hours [17–20]. Corning ultralow attachment microplates are often used, distinguished by a covalently bonded hydrogel layer that reduces cell adhesion, protein adhesion, and cell activation, with spontaneous cell assembly dependent on self-secreted extracellular matrix (ECM) [21].

The hanging drop culture is a prevalent technique for 3D cell culture, using gravity to facilitate the aggregation of individual cells into 3D spheres [22, 23]. This approach involves depositing droplets of cell suspension onto the inner lid of a tissue culture plate, with each droplet having a volume of 10–20 μL and containing roughly 50–500 cells. Subsequently, the lid is inverted, and the droplets are secured by surface tension [24]. The microgravity conditions of each droplet consolidate the cells, creating a single sphere at the droplet's apex and facilitating proliferation [25]. The dimensions of the cellular sphere may be regulated by modifying the cell density of the suspension. The acquired cell spheres are tightly aggregated and homogenous in shape, resulting in spherical cells of the same size [26, 27]. This approach has many benefits, including cheap cost, ease of operation, high production efficiency, and the ability to coculture several cell types. Nonetheless, the drawbacks include that the volume of the cell solution must not exceed 30 μL; otherwise, the droplets may detach. Moreover, the task is demanding, making bulk manufacturing challenging [2]. Due to the tiny volume of the cell suspension and its propensity for evaporation, altering the cell culture medium poses significant challenges for sustaining long-term cell culture. The 384-hanging drop array enhances the hanging drop culture technique. The reservoir structure's design efficiently minimizes the evaporation of small-volume suspended droplets. The culture medium may be partially altered to extend the duration of cell spheroid growth. The mass manufacture of 3D spheres may facilitate fundamental biological research [26].

This approach involves placing a high-density cell suspension in the bioreactor, where it is maintained in motion by rotation and agitation, preventing the cells from settling and adhering to the substrate, hence optimizing cell interaction to facilitate the formation of 3D spheres [18]. The system comprises a container for cell culture and a constantly agitated impeller to maintain cell suspension and facilitate medium mixing. The movement of liquid not only inhibits cell attachment but also guarantees the equal distribution of nutrients and oxygen, facilitating the development and metabolism of 3D cell spheres. This technique is quite straightforward and can generate a substantial quantity of spheres rapidly. This approach facilitates straightforward cell culture and mass manufacturing; dynamic culture enhances nutrient delivery, and spheroids are readily obtainable. Nonetheless, the drawbacks of this technique are evident. The froth and shear stress of the fluid produced during churning may harm the cells; 3D cell spheres differ in size; therefore, specialized equipment is essential. Research indicates that rotating cell culture may promote osteogenic differentiation of human bone marrow mesenchymal stem cells [28]. The potential explanation is that a rotating culture, which closely resembles the in vivo cellular environment, facilitates bone formation and enhances the early production of osteocalcin and calcium deposition [29].

Magnetic suspension culture is a technique that uses magnetic nanoparticles, such as iron oxide or gold nanoparticles, and magnetism to aggregate individual cells into three-dimensional spheres. Cells are treated overnight with magnetic nanoparticles to render them magnetic; thereafter, the magnetic cells are removed and recultured [30]. During the cell culture process, a magnetic field is supplied, causing the cells

to aggregate into three-dimensional spheres at a height where magnetic force and gravity are in equilibrium [31]. Spheroids may be generated swiftly within five minutes, exhibit reproducibility and size stability, and can be removed and manipulated using magnetic instruments [24]. This strategy facilitates the coculturing of many cell types; nevertheless, the possible influence of nanoparticles on cellular signaling and function constitutes a restriction of this approach [32-34].

Chemical reagent culture is a methodology that employs certain chemical reagents to facilitate the self-assembly of cells into three-dimensional spheres. Chen et al. discovered that variations in batches of human or bovine serum albumin led to conflicting experimental outcomes before and after growing stem cells using TeSR media. The constituents of TeSR media were further examined. Following a systematic screening process, they successfully formulated a practical, defined, and albumin-free TeSR-E8 medium, including eight components: DMEM/F12, insulin, selenium, transferrin, L-ascorbic acid, FGF2, TGFβ, and NaHCO3, which is appropriate for stem cell cultivation [35]. Zhao et al. have confirmed that human mesenchymal stem cells cultured in a chemically specified serum-free TeSR-E8 medium may autonomously aggregate into three-dimensional spheres. The study indicated that, in comparison to 2D cells, the stemness of 3D cells is augmented, hence enhancing therapeutic efficacy in endotoxemic mice and decreasing death rates [6].

4. Scaffold-Based Culture System

Natural extracellular matrix (ECM) has suboptimal mechanical characteristics and heightened susceptibility to enzymatic degradation, hence limiting its use [36]. In recent years, advancements in biomaterial technology have led to the widespread usage of scaffolds made from artificial extracellular matrix, which can replicate the intricate three-dimensional structure and primary properties of real tissues. The scaffold serves to provide a spatial habitat for cells, promoting their adherence, proliferation, and cytokine production. Moreover, this scaffold may enhance connections among cells and between cells and the extracellular matrix, thereby influencing cell morphology, metabolism, functionality, migration, proliferation, and differentiation [37]. Furthermore, it functions as a conduit for the dissemination of soluble substances.

3D cell culture scaffolds may be categorized into two categories based on material origin: natural material scaffolds and synthetic material scaffolds [38], which include hyaluronic acid, collagen, polylactic acid, and polyethylene glycol. Hydrogel is one of the most extensively used materials for three-dimensional culturing [39]. Hydrogels may be categorized into natural polymer hydrogels and synthetic polymer hydrogels [40]. Hydrogel has a network structure abundant in hydrophilic groups, enabling it to retain substantial quantities of water. The hydrogel's network structure facilitates the unobstructed exchange of nutrients and oxygen, ensuring that the embedded cells get sufficient nourishment [41]. Simultaneously, it may cross-link bioactive molecules to modulate cell proliferation and differentiation, making it an exceptional alternative to ECM.

5. Natural Polymer Hydrogel

Natural polymer hydrogels mostly consist of natural substances augmented by additional biological elements or molecules [42-46]. Natural materials are derived from animal, plant, or human tissues or cells, including hyaluronic acid, collagen, fibrin, silk fibroin, alginate, chondroitin sulfate, gelatin, agarose, and chitosan sugar. They independently or collectively accumulate to create a three-dimensional network structure akin to the organism under certain circumstances.

Natural polymer materials have constrained mechanical capabilities, and the composition of the extracellular matrix (ECM) in humans or animals remains ambiguous. Consequently, there may be dangers of pathogens and discrepancies among batches [13, 47, 48]. Natural polymers often exhibit excellent biocompatibility, environmental responsiveness, minimal toxicity, and sites for cell attachment. Additionally, a broad range of sources and a cheap price are significant benefits. Moreover, these natural materials provide distinct benefits, and their amalgamation demonstrates exceptional performance [49-51]. Some researchers have amalgamated gelatin and polysaccharides to create a gel scaffold, using the medicinal and regenerative attributes of gelatin with the mechanical capabilities of polysaccharides.

Composite applications provide a viable approach for the development of improved biomaterials [42]. The chitosan-alginate-gelatin composite hydrogel facilitates the chondrogenic differentiation of human mesenchymal stem cells and aids in cartilage repair in patients with associated cartilage disorders [52]. Certain researchers introduced hepatocyte-like cells, produced from human pluripotent stem cells, into the commonly used animal-derived hydrogel Matrigel, which is a plant-derived nanocellulose hydrogel inside agarose microporous 3D growth plates. These cells can all generate 3D spheres and enhance the liver maturation of hepatocyte-like cells, demonstrating that both plant-derived and animal-derived hydrogels possess identical activities; however, the former can circumvent drawbacks such as endotoxin presence and batch-to-batch variability [53-56].

Synthetic polymer scaffold materials including polylactic acid (PLA), polyethylene glycol (PEG), polycaprolactone (PCL), polylactic acid glycolic acid (PLGA), poly L-lactic acid (PLLA), and polyglycolic acid (PGA) [57-60]. These polymers are cross-linked to create a hydrogel, suitable for use as a three-dimensional cell culture substrate. This inert gel has a distinct chemical composition, elevated repeatability, substantial mechanical strength, straightforward processing and manufacture, as well as enhanced predictability of outcomes and increased adaptability [61, 62]. Consequently, the hydrogel has extensive potential for use in tissue engineering scaffold materials [51]. Regrettably, synthetic hydrogels often lack cell adhesion sites, integrin-binding peptides, and growth factor binding sites. ECM breakdown protease domains that facilitate cell-ECM cross-linking are essential [7], making the building process somewhat complex. The deficiencies of inadequate biocompatibility, insufficient toughness, and delayed water absorption restrict their direct employment in cell culture scaffolds, necessitating ongoing study and improvement.

Typically, a single material type struggles to fulfill the criteria for cell culture scaffold materials. Consequently, integrating many individual materials via an appropriate manner while thoroughly evaluating the merits and drawbacks of each material to create a composite material might provide favorable outcomes [63]. The exceptional water absorption capacity, robust biocompatibility, affordability, and abundance of natural materials, combined with cell adhesion sites and the tunable mechanical strength of synthetic materials, create an optimal formulation for the preparation of 3D cultured hydrogels. Researchers have created a collagen-bioceramic composite hydrogel that facilitates the osteogenic development of hADSCs, presenting a novel strategy for addressing bone deficiencies [64]. Certain researchers have used gelatin-methacryloyl hydrogel since the integration of biological and industrial techniques might expedite the practical application of tissue restoration. Nevertheless, the role of materials in modulating the activities and behaviors of cells in 3D culture requires more exploration.

6. Conclusions

In recent years, three-dimensional cell culture has become a significant cultivation method. Compared to 2D cell culture, 3D cells culture offers the advantage of creating a three-dimensional microenvironment that facilitates cellular proliferation, differentiation, motility, and apoptosis. It more accurately replicates the cellular conditions found in the human microenvironment, thereby exhibiting significant potential for development. Consequently, it has prospective uses in tissue engineering, regenerative medicine, pharmacological research, toxicity assessment, and the production of organoids and assembloids. Nonetheless, 3D cultures technology remains nascent; its expense is considerable, and because to suboptimal culture conditions, a disparity persists between in vitro cultures and physiological environments. The current research objective is to enhance technology to render the 3D culture system more analogous to the human body's actual environment, to establish an efficient and automated culture system while minimizing costs, and to optimize the utilization of diverse materials in design and the application of composite materials, among other considerations. We assert that as tissue engineering technology advances, issues such as the interdisciplinary integration of life sciences, engineering, and materials science, along with the persistent endeavors of scientific researchers, will be examined more thoroughly and will consequently be progressively addressed.

References

1. Duval K., Grover H., Han L. H., Mou Y., Pegoraro A. F., Friedberg J., and Chen Z., Modeling physiological events in 2D vs. 3D cell culture, Physiology (Bethesda, Md.). (2017) 32, no. 4, 266–277
2. Seo J., Lee J. S., Lee K., Kim D., Yang K., Shin S., Mahata C., Jung H. B., Lee W., Cho S. W., and Lee T., Switchable water-adhesive, superhydrophobic palladium-layered silicon nanowires potentiate the angiogenic efficacy of human stem cell spheroids, Advanced materials (Deerfield Beach, Fla.). (2014) 26, no. 41, 7043–7050
3. Linand R. Z. and Chang H. Y., Recent advances in three-dimensional multicellular spheroid culture for biomedical research, Biotechnology Journal. (2008) 3, no. 9-10, 1172–1184.
4. Chaicharoenaudomrung N., Kunhorm P., and Noisa P., Three-dimensional cell culture systems as an in vitro platform for cancer and stem cell modeling, Journal of Stem Cells. (2019) 11, no. 12, 1065–1083
5. Kim J., Kang J. W., Park J. H., Choi Y., Choi K. S., Park K. D., Baek D. H., Seong S. K., Min H. K., and Kim H. S., Biological characterization of long-term cultured human mesenchymal stem cells, Archives of Pharmacal Research. (2009) 32, no. 1, 117–126
6. Zhao Y., Xiao E., Lv W., Dong X., He L., Wang Y., and Zhang Y., A chemically defined serum-free culture system for spontaneous human mesenchymal stem cell spheroid formation, Stem Cells International. (2020) 2020, 1031985.
7. Lutolfand M. P. and Hubbell J. A., Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering, Nature Biotechnology. (2005) 23, no. 1, 47–55
8. Su G., Zhao Y., Wei J., Han J., Chen L., Xiao Z., Chen B., and Dai J., The effect of forced growth of cells into 3D spheres using low attachment surfaces on the acquisition of stemness properties, Biomaterials. (2013) 34, no. 13, 3215–3222
9. Ylostalo J. H., 3D stem cell culture, Cell. (2020) 9, no. 10
10. Ryu N. E., Lee S. H., and Park H., Spheroid culture system methods and applications for mesenchymal stem cells, Cell. (2019) 8, no. 12
11. Koledova Z., 3D cell culture: an introduction, Methods in Molecular Biology. (2017) 1612, 1–11
12. Yamada K. M. and Cukierman E., Modeling tissue morphogenesis and cancer in 3D, Cell. (2007) 130, no. 4, 601–610
13. Knight E. and Przyborski S., Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro, Journal of Anatomy. (2015) 227, no. 6, 746–756
14. Lou J., Stowers R., Nam S., Xia Y., and Chaudhuri O., Stress relaxing hyaluronic acid-collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture, Biomaterials. (2018) 154, 213–222
15. Costa E. C., Gaspar V. M., Coutinho P., and Correia I. J., Optimization of liquid overlay technique to formulate heterogenic 3D co-cultures models, Biotechnology and Bioengineering. (2014) 111, no. 8, 1672–1685
16. Hickman J. A., Graeser R., de Hoogt R., Vidic S., Brito C., Gutekunst M., van der Kuip H., and IMI PREDICT consortium, Three-dimensional models of cancer for pharmacology and cancer cell biology: capturing tumor complexity in vitro/ex vivo, Biotechnology Journal. (2014) 9, no. 9, 1115–1128
17. Carvalho M. P., Costa E. C., Miguel S. P., and Correia I. J., Tumor spheroid assembly on hyaluronic acid-based structures: a review, Carbohydrate Polymers. (2016) 150, 139–148
18. Cesarz Z. and Tamama K., Spheroid culture of mesenchymal stem cells, Stem Cells International. (2016) 2016, 9176357.
19. Achilli T. M., Meyer J., and Morgan J. R., Advances in the formation, use, and understanding of multi-cellular spheroids, Expert Opinion on Biological Therapy. (2012) 12, no. 10, 1347–1360
20. do Amaral J. B., Rezende-Teixeira P., Freitas V. M., and Machado-Santelli G. M., MCF-7 cells as a three-dimensional model for the study of human breast Cancer, Tissue Engineering. Part C, Methods. (2011) 17, no. 11, 1097–1107
21. Chen H., Fu H., Wu X., Duan Y., Zhang S., Hu H., Liao Y., Wang T., Yang Y., Chen G., and Li Z., Regeneration of pulp-dentinal-like complex by a group of unique multipotent CD24a(+) stem cells, Science Advances. (2020) 6, no. 15.

22. Jørgensen A., Young J., Nielsen J. E., Joensen U. N., Toft B. G., Rajpert-de Meyts E., and Loveland K. L., Hanging drop cultures of human testis and testis cancer samples: a model used to investigate activin treatment effects in a preserved niche, British Journal of Cancer. (2014) 110, no. 10, 2604–2614
23. Foty R., A simple hanging drop cell culture protocol for generation of 3D spheroids, Journal of visualized experiments: JoVE. (2011) 51, no. 51
24. Lv D., Hu Z., Lu L., Lu H., and Xu X., Three-dimensional cell culture: a powerful tool in tumor research and drug discovery, Oncology Letters. (2017) 14, no. 6, 6999–7010
25. Breslinand S. and O'driscoll L., Three-dimensional cell culture: the missing link in drug discovery, Drug Discovery Today. (2013) 18, no. 5-6, 240–249
26. Tung Y. C., Hsiao A. Y., Allen S. G., Torisawa Y. S., Ho M., and Takayama S., High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array, The Analyst. (2011) 136, no. 3, 473–478.
27. Huang S. W., Tzeng S. C., Chen J. K., Sun J. S., and Lin F. H., A dynamic hanging-drop system for mesenchymal stem cell culture, International Journal of Molecular Sciences. (2020) 21, no. 12
28. Suenaga H., Furukawa K. S., Suzuki Y., Takato T., and Ushida T., Bone regeneration in calvarial defects in a rat model by implantation of human bone marrow-derived mesenchymal stromal cell spheroids, Materials in medicine. (2015) 26, no. 11
29. Desai P. K., Tseng H., and Souza G. R., Assembly of hepatocyte spheroids using magnetic 3D cell culture for CYP450 inhibition/induction, International Journal of Molecular Sciences. (2017) 18, no. 5
30. Adine C., Ng K. K., Rungarunlert S., Souza G. R., and Ferreira J. N., Engineering innervated secretory epithelial organoids by magnetic three- dimensional bioprinting for stimulating epithelial growth in salivary glands, Biomaterials. (2018) 180, 52–66
31. Türker E., Demirçak N., and Arslan-Yildiz A., Scaffold-free three-dimensional cell culturing using magnetic levitation, Biomaterials Science. (2018) 6, no. 7, 1745–1753
32. Kim J. A., Choi J. H., Kim M., Rhee W. J., Son B., Jung H. K., and Park T. H., High-throughput generation of spheroids using magnetic nanoparticles for three-dimensional cell culture, Biomaterials. (2013) 34, no. 34, 8555–8563
33. Lewis N. S., Lewis E. E., Mullin M., Wheadon H., Dalby M. J., and Berry C. C., Magnetically levitated mesenchymal stem cell spheroids cultured with a collagen gel maintain phenotype and quiescence, Journal of tissue engineering. (2017) 8.
34. Yaman S., Anil-Inevi M., Ozcivici E., and Tekin H. C., Magnetic force-based microfluidic techniques for cellular and tissue bioengineering, Frontiers in Bioengineering and Biotechnology. (2018) 6
35. Chen G., Gulbranson D. R., Hou Z., Bolin J. M., Ruotti V., Probasco M. D., Smuga-Otto K., Howden S. E., Diol N. R., Propson N. E., and Wagner R., Chemically defined conditions for human iPSC derivation and culture, Nature Methods. (2011) 8, no. 5, 424–429
36. Huang Q., Zou Y., Arno M. C., Chen S., Wang T., Gao J., Dove A. P., and du J., Hydrogel scaffolds for differentiation of adipose-derived stem cells, Chemical Society Reviews. (2017) 46, no. 20, 6255–6275
37. Ivanovska J., Zehnder T., Lennert P., Sarker B., Boccaccini A. R., Hartmann A., Schneider-Stock R., and Detsch R., Biofabrication of 3D alginate-based hydrogel for cancer research: comparison of cell spreading, viability, and adhesion characteristics of colorectal HCT116 tumor Cells, Tissue Engineering. Part C, Methods. (2016) 22, no. 7, 708–715
38. Liu Z., Tang M., Zhao J., Chai R., and Kang J., Looking into the future: toward advanced 3D biomaterials for stem-cell-based regenerative medicine, Advanced materials (Deerfield Beach, Fla.). (2018) 30, no. 17, article e1705388
39. Nam S., Stowers R., Lou J., Xia Y., and Chaudhuri O., Varying PEG density to control stress relaxation in alginate-PEG hydrogels for 3D cell culture studies, Biomaterials. (2019) 200, 15–24
40. Chaudhuri O., Viscoelastic hydrogels for 3D cell culture, Biomaterials Science. (2017) 5, no. 8, 1480–1490
41. 1 Lu L., Yuan S., Wang J., Shen Y., Deng S., Xie L., and Yang Q., The formation mechanism of hydrogels, Current Stem Cell Research & Therapy. (2018) 13, no. 7, 490–496

42. Afewerki S., Sheikhi A., Kannan S., Ahadian S., and Khademhosseini A., Gelatin-polysaccharide composite scaffolds for 3D cell culture and tissue engineering: towards natural therapeutics, Bioengineering & translational medicine. (2019) 4, no. 1, 96–115
43. Xu X., Gurski L. A., Zhang C., Harrington D. A., Farach-Carson M. C., and Jia X., Recreating the tumor microenvironment in a bilayer, hyaluronic acid hydrogel construct for the growth of prostate cancer spheroids, Biomaterials. (2012) 33, no. 35, 9049–9060
44. Elenjord R., Allen J. B., Johansen H. T., Kildalsen H., Svineng G., Maelandsmo G. M., Loennechen T., and Winberg J. O., Collagen I regulates matrix metalloproteinase-2 activation in osteosarcoma cells independent of S100A4, The FEBS Journal. (2009) 276, no. 18, 5275–5286
45. Noori A., Ashrafi S. J., Vaez-Ghaemi R., Hatamian-Zaremi A., and Webster T. J., A review of fibrin and fibrin composites for bone tissue engineering, International Journal of Nanomedicine. (2017) Volume 12, 4937–49611
46. Mao Z., Bi X., Ye F., du P., Shu X., Sun L., Guan J., Li X., and Wu S., The relationship between crosslinking structure and silk fibroin scaffold performance for soft tissue engineering, International Journal of Biological Macromolecules. (2021) 182, 1268–1277
47. Akeda K., Nishimura A., Satonaka H., Shintani K., Kusuzaki K., Matsumine A., Kasai Y., Masuda K., and Uchida A., Three-dimensional alginate spheroid culture system of murine osteosarcoma, Oncology Reports. (2009) 22, no. 5, 997–1003
48. Suriand S. and Schmidt C. E., Ccell-laden hydrogel constructs of hyaluronic acid, collagen, and laminin for neural tissue engineering, Tissue Engineering. Part A. (2010) 16, no. 5, 1703–1716.
49. Tytgat L., van Damme L., van Hoorick J., Declercq H., Thienpont H., Ottevaere H., Blondeel P., Dubruel P., and van Vlierberghe S., Additive manufacturing of photo-crosslinked gelatin scaffolds for adipose tissue engineering, Acta Biomaterialia. (2019) 94, 340–350
50. Saeedi Garakani S., Khanmohammadi M., Atoufi Z., Kamrava S. K., Setayeshmehr M., Alizadeh R., Faghihi F., Bagher Z., Davachi S. M., and Abbaspourrad A., Fabrication of chitosan/agarose scaffolds containing extracellular matrix for tissue engineering applications, International Journal of Biological Macromolecules. (2020) 143, 533–545
51. Carletti E., Motta A., and Migliaresi C., Scaffolds for tissue engineering and 3D cell culture, Methods in Molecular Biology. (2011) 695, 17–39
52. Sahai N., Gogoi M., and Tewari R. P., 3D printed chitosan composite scaffold for chondrocytes differentiation, 2021, Current medical imaging.
53. Toivonen S., Malinen M. M., Küblbeck J., Petsalo A., Urtti A., Honkakoski P., and Otonkoski T., Regulation of human pluripotent stem cell-derived hepatic cell phenotype by three-dimensional hydrogel models, Tissue Engineering. Part A. (2016) 22, no. 13-14, 971–984
54. Polonio-Alcalá E., Rabionet M., Guerra A. J., Yeste M., Ciurana J., and Puig T., Screening of additive manufactured scaffolds designs for triple negative breast cancer 3D cell culture and stem-like expansion, International Journal of Molecular Sciences. (2018) 19, no. 10
55. Jabbari E., Sarvestani S. K., Daneshian L., and Moeinzadeh S., Optimum 3D matrix stiffness for maintenance of cancer stem cells is dependent on tissue origin of cancer cells, Plo S one. (2015) 10, no. 7, article e0132377
56. Fu Y., Xu K., Zheng X., Giacomin A. J., Mix A. W., and Kao W. J., 3D cell entrapment in crosslinked thiolated gelatin-poly(ethylene glycol) diacrylate hydrogels, Biomaterials. (2012) 33, no. 1, 48–58
57. Mendez U., Zhou H., and Shikanov A., Synthetic PEG hydrogel for engineering the environment of ovarian follicles, Methods in Molecular Biology. (2018) 1758, 115–128
58. Jung J. W., Lee H., Hong J. M., Park J. H., Shim J. H., Choi T. H., and Cho D. W., A new method of fabricating a blend scaffold using an indirect three-dimensional printing technique, Biofabrication. (2015) 7, no. 4, article 045003
59. Zhao X., Sun X., Yildirimer L., Lang Q., Lin Z. Y. W., Zheng R., Zhang Y., Cui W., Annabi N., and Khademhosseini A., Cell infiltrative hydrogel fibrous scaffolds for accelerated wound healing, Acta Biomaterialia. (2017) 49, 66–77

60. Song K., Ji L., Zhang J., Wang H., Jiao Z., Mayasari L., Fu X., and Liu T., Fabrication and cell responsive behavior of macroporous PLLA/gelatin composite scaffold with hierarchical micro-nano pore structure, Nanomaterials (Basel, Switzerland). (2015) 5, no. 2, 415–424
61. Kemmochi A., Tamura T., Shimizu Y., Owada Y., Ozawa Y., Hisakura K., Oda T., Kawano Y., Hanawa T., and Ohkohchi N., A novel hydrogel sheet prevents postoperative pancreatic fistula in a rat model, Journal of Hepato-Biliary-Pancreatic Sciences. (2021) 28, no. 2, 192–201
62. Li G., Zhang T., Li M., Fu N., Fu Y., Ba K., Deng S., Jiang Y., Hu J., Peng Q., and Lin Y., Electrospun fibers for dental and craniofacial applications, Current Stem Cell Research & Therapy. (2014) 9, no. 3, 187–195
63. Zhu Y., Zhang Q., Shi X., and Han D., Hierarchical hydrogel composite interfaces with robust mechanical properties for biomedical applications, Advanced materials (Deerfield Beach, Fla.). (2019) 31, no. 45, article e1804950
64. Kimand W. and Kim G., Collagen/bioceramic-based composite bioink to fabricate a porous 3D hASCs-laden structure for bone tissue regeneration, Biofabrication. (2019) 12, no. 1, article 015007.

التطورات في تقنيات زراعة الخلايا الجذعية ثلاثية الأبعاد: الآثار المترتبة على الطب التجديدي والتطبيقات العلاجية

الملخص

الخلفية :تعتبر أبحاث الخلايا الجذعية محورية لتحقيق تقدم في الطب التجديدي والتطبيقات العلاجية. تفشل تقنيات زراعة الخلايا التقليدية ثنائية الأبعاد (2D) في تكرار بيئة الخلايا المعقدة في الجسم الحي بشكل كافٍ، مما يؤدي إلى ضعف وظائف الخلايا والأنماط الظاهرية. توفر التطورات الأخيرة في أنظمة زراعة الخلايا ثلاثية الأبعاد (3D) بدائل واعدة تُحاكي الظروف الفسيولوجية الموجودة في الكائنات الحية بشكل أكثر قربًا.

الطرق :تستعرض هذه المراجعة بشكل منهجي التقنيات الناشئة في زراعة الخلايا الجذعية ثلاثية الأبعاد والتمايز، مصنفة إياها إلى أنظمة بدون دعامات وأنظمة مع دعامات. وتسلط الضوء على منهجيات مبتكرة مثل الطفو السائل، وزراعة القطرات المعلقة، وزراعة التعليق المغناطيسي، وصياغات الوسائط الكيمائية المعرفة، التي تسهل تشكيل كرات خلوية متعددة وتحسن من قدرات التجديد الذاتي والتمايز للخلايا.

النتائج :تشير النتائج إلى أن أنظمة الزراعة ثلاثية الأبعاد تحسن بشكل كبير من بقاء الخلايا وتكاثرها ووظائفها مقارنةً بزراعات 2 Dالتقليدية. يعمل تشكيل الكرات الخلوية على تعزيز التواصل بين الخلايا ويُحاكي بنية الأنسجة، مما يعزز الخصائص التجديدية للخلايا الجذعية. علاوة على ذلك، أظهرت استخدامات الهيدروجيلات الطبيعية والاصطناعية كدعامات دعم الأنشطة الخلوية وتعزيز التمايز الخاص بالأنسجة.

الخاتمة :تمثل اعتماد تقنيات الزراعة ثلاثية الأبعاد تقدمًا كبيرًا في أبحاث الخلايا الجذعية، حيث توفر نموذجًا أكثر دقة لدراسة سلوك الخلايا واستجابات الأدوية. تشمل الاتجاهات المستقبلية تحسين هذه التقنيات لتعزيز القدرة على التوسع وفاعلية التكلفة، مما يعزز في النهاية الفجوة بين النماذج المخبرية والتطبيقات في الجسم الحي في الطب التجديدي.

الكلمات المفتاحية :الخلايا الجذعية، الزراعة ثلاثية الأبعاد، الطب التجديدي، تمايز الخلايا، الدعامات.