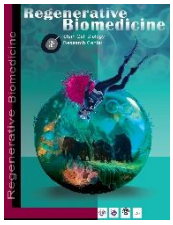

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Mini-Review Article

A Mini Review on Testicular Tissue Engineering: A Roadmap for Future Research

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Abstract

Testicular tissue engineering (TTE) is an emerging interdisciplinary field combining principles of regenerative medicine, biomaterials, and stem cell therapy to address male infertility caused by testicular dysfunction. The aim of this study is to investigate recent advancements, challenges, and future directions in TTE, focusing on scaffold-based approaches, stem cell therapies, and 3D culture systems, by integrating recent studies, up to December 2024 in databases including PubMed, Scopus, and Web of Science. Search terms included “testicular tissue engineering,” “male infertility,” “spermatogenesis,” “scaffolds,” and “stem cell therapy.” Present study highlights novel strategies, such as 3D bioprinting and decellularized testicular matrices, and justifies their originality in overcoming limitations of traditional fertility preservation methods. By integrating findings from diverse academic sources, and an overview of TTE’s potential to revolutionize male reproductive health, while identifying critical areas for current theoretical gaps and future research.

Keywords: Infertility, Regenerative medicine, Scaffolds, Testicular tissue engineering, 3D culture

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Introduction

Male infertility affects approximately 50% of infertility cases globally, with a reported 76.9% increase in prevalence between 1990 and 2019 (1, 2). Testicular dysfunction, resulting from congenital disorders, gonadotoxic treatments, or trauma, is a significant contributor. Traditional interventions, such as semen cryopreservation or testicular tissue preservation, are limited by their inability to restore fertility in prepubertal boys or men with non-obstructive azoospermia (NOA). Tissue engineering offers a promising alternative by creating bioartificial testicular constructs that replicate the native testicular microenvironment essential for spermatogenesis. The testicular microenvironment comprises sertoli cells, leydig cells, peritubular myoid cells, and a complex extracellular matrix (ECM) that supports germ cell development. Tissue engineering aims to recreate this niche using scaffolds, stem cells, and bioactive molecules. This study integrates insights from regenerative medicine, urology, and biomaterials, while addressing ethical and regulatory considerations and provides a comprehensive perspective justified by the increasing global burden of male infertility.

Present study was conducted by systematically searching peer-reviewed literature up to december 2024 in databases including PubMed, Scopus, and Web of Science. Search terms included “testicular tissue engineering,” “male infertility,” “spermatogenesis,” “scaffolds,” and “stem cell therapy.” Inclusion criteria encompassed original research, reviews, and clinical studies published in English, focusing on TTE applications. Exclusion criteria included non-peer-reviewed articles, conference abstracts, and studies unrelated to testicular function.

TTE is based on the principles of tissue engineering that combines cells, scaffolds, and signaling molecules to regenerate functional tissues (3, 4). The testicular microenvironment, consisting of sertoli, leydig, and germ cells within the matrix of the seminiferous tubules, is complex and dynamic. Theoretical models emphasize the need for biomimetic scaffolds that support cell-cell interactions, nutrient diffusion, and mechanical stability (5-10). Despite significant progress, theoretical gaps persist in understanding cell signaling dynamics, optimizing biomaterial properties, and ensuring vascularization of engineered tissues. This mini review addresses these gaps, emphasizing the novelty of TTE in providing functional solutions for infertility and justifying its originality through advancements in 3D culture systems and biomaterial design. Several theoretical gaps have been identified in studies, including:

Microenvironment replication:

Current models incompletely replicate the testicular niche, particularly the blood-testis barrier and hormonal gradients (11-13).

Cell differentiation pathways:

The mechanisms governing the differentiation of stem cells into functional germ cells are not well understood (14-17)

Long-term function:

Few studies address the durability of engineered tissues beyond short-term in vitro or animal models (18).

Current strategies in testicular tissue engineering

Biomaterials utilized in reproductive tissue engineering may be categorized as synthetic polymers, natural polymers (including scaffolds and biogels), and decellularized matrices. Some research attempted to produce efficient decellularized tissues for use as three-dimensional platforms for reconstructing an in vitro testicular microenvironment, potentially facilitating various uses in reproductive medicine. The main human experimental approaches are summarized in table 1.

Scaffold-based approaches using three - dimensional, biomimetic designs support testicular cell viability, differentiation and maintenance in vitro, but none has proven conclusively better for restoring testicular function in patients. Synthetic polymers (polycaprolactone, poly L lactic acid) and natural polymers (alginate, fibrin, collagen, Matrigel) were evaluated alongside decellularized matrices, nanofiber constructs, and microfluidic systems for their ability to mimic the native testicular microenvironment. Germ cells (including spermatogonial stem cells), Sertoli cells, Leydig cells, and testis derived cells have been maintained on these matrices (19).

A study measured 50% cell viability at 7 days and 66.66% at 14 days on a porous human serum albumin/tri-calcium phosphate scaffold, with no significant difference compared to monolayer culture (6). In qualitative comparisons, an alginate matrix improved cell survival over fibrin gel, and nanofiber scaffolds enhanced spermatogonial stem cell maintenance. In sum, the studies describe scaffold geometries and material properties that foster testicular cell function, yet no single approach demonstrates a clear advantage for clinically restoring testicular function(19).

Scaffolds provide structural support and mimic the ECM of the testis. They must be biocompatible, biodegradable, and mechanically robust (Table 1).

Natural scaffolds

Natural scaffolds, such as decellularized testicular ECM, retain native architecture and bioactive molecules. Studies demonstrate that decellularized scaffolds support Sertoli and germ cell attachment, promoting spermatogenesis in vitro (20). However, variability in decellularization protocols limits reproducibility.

Synthetic scaffolds

Synthetic scaffolds, including polylactic acid (21) and polyglycolic acid (PGA), offer customizable properties. Studies reported enhanced cell alignment on nanofibrous PLA scaffolds, improving spermatogenic efficiency (22).

Hybrid scaffolds

Hybrid scaffolds combine natural and synthetic materials to balance bioactivity and mechanical strength. A previous study demonstrated that collagen-PLA scaffolds support Leydig cell testosterone production, highlighting their potential for endocrine restoration (23).

Cell selection is critical for TTE success, common sources include:

Stem Cells, Embryonic Stem Cells (ESCs); ESCs differentiate into germ-like cells but raise ethical concerns (14, 24). Induced Pluripotent Stem Cells (iPSCs): iPSCs offer patient-specific solutions, with studies

Table 1. Human studies using synthetic and natural biomaterials in TTE approaches

Biomaterial	Types of cells used	Main findings	Ref.
Collagen-based hydrogels	Spermatogonial Stem Cell	In 3D culture in collagen gel matrix, spermatocytes were induced to differentiate into spermatids in vitro.	(25)
Fibrin	Endometrial stem cells (hEnSCs)	Scaffolds containing human serum albumin (HSA)/tri calcium phosphate nanoparticles are easily produced and do not show cytotoxicity to spermatogonial stem cells.	(26)
Chitosan-based hydrogel	Testicular tissue human (25 and 31 years of age)	The complete process of spermatogenesis was achieved both in vitro and in vivo. The culture system was defined using a bioreactor made of a hollow cylinder of a chitosan hydrogel that simulates the seminiferous tubules.	(27)
Agarose gel	Testis fragments(12 to 19 week fetuses)	Using agarose hydrogel, haploid spermatids recombined during meiosis, showing an increase in genetic diversity. Additionally, haploid spermatids performed the fertilization of oocytes, resulting in blastocyst formation.	(28)
Agarose gel	Testis fragments (4week-old)	In three-dimensional testicular tissue culture, freezing SSCs slowly can induce the production of haploid cells.	(29)
PCL/Gelatin nanofibrous scaffolds	Spermatogonial Stem cells	The planned scaffold provided a suitable self-renewal microenvironment for the permatogonial stem cells. The scaffolds produced have potential application in research and reconstructive medicine related to the field of male infertility	(30)
Decellularized scaffolds	Neonatal testicular cells	The scaffolds obtained after decellularization are not cytotoxic, providing adequate conditions that support the fixation and infiltration of testicular cells.	(31)
Decellularized scaffolds	Induced Pluripotent Stem cells (iPS)	A 3D cell culture model was developed to generate human male germ cells from iPSCs and this model was compared to conventional 2D culture. Considering the effect of the 3D scaffold in the induction of specific markers	(32)

showing differentiation into spermatogonial stem cells (33). Mesenchymal Stem Cells (MSCs): MSCs support testicular repair by secreting growth factors, though their differentiation potential is limited (34). Primary Cells; Sertoli and Leydig cells isolated from testicular biopsies maintain native functionality but are limited by donor availability (13).

3D culture systems

3D culture systems mimic the testicular architecture, enabling cell-cell interactions. Bioreactors and 3D bioprinting enhance nutrient delivery and spatial organization.

Vascularization; adequate vascularization is essential for nutrient and oxygen delivery. Current strategies, such as angiogenic growth factor incorporation, show promise but struggle with long-term stability (21). Immune Compatibility; immune rejection remains a barrier, particularly for allogeneic cell sources. Immunomodulatory scaffolds and autologous iPSCs are being explored to mitigate this issue (16). Clinical Translation; Translating TTE to clinical settings requires standardized protocols, regulatory approval, and long-term safety data. Preclinical studies in large animal models are limited, hindering progress (18).

Future directions

Advanced Biomaterials; Next-generation biomaterials, such as self-assembling hydrogels, could enhance scaffold adaptability and cell signaling (35).Gene

Editing; CRISPR/Cas9-mediated gene editing could correct genetic defects in iPSCs, improving differentiation efficiency (36). Artificial Intelligence; AI-driven modeling could optimize scaffold design and predict cell behavior, accelerating TTE development (37).

Discussion and conclusion

TTE holds transformative potential for male infertility treatment, addressing limitations of current therapies. The integration of scaffolds, stem cells, and 3D culture systems has advanced our understanding of testicular biology, yet significant gaps remain. Not being able to completely recreate the testicular environment affects how well it works, and issues with scaling up and matching the immune system create real difficulties (3, 10). The theoretical gap in microenvironment replication underscores the need for advanced imaging and single-cell sequencing to map testicular dynamics. Practically, standardized protocols for scaffold fabrication and cell sourcing are essential for reproducibility. The proposed future directions, including AI and gene editing, offer innovative pathways to overcome these challenges, positioning TTE as a frontier in regenerative medicine. Consequently TTE represents a paradigm shift in treating male infertility and testicular dysfunction. By combining biomaterials, stem cells, and 3D culture systems, TTE aims to restore spermatogenesis and endocrine function. This review highlights key advancements, identifies critical gaps, and proposes a roadmap for future research. Addressing challenges in vascularization, immune compatibility, and clinical translation will be pivotal for realizing TTE's therapeutic potential. Collaborative efforts across disciplines, supported by ethical and regulatory frameworks, will drive the field toward clinical success.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reproductive biology and endocrinology. 2015;13:1–9.

2. Huang B, Wang Z, Kong Y, Jin M, Ma L. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of global burden of disease study. BMC Public Health. 2023;23(1):2195.

3. Langer RS, Vacanti JP. Tissue engineering: the challenges ahead. Scientific American. 1999;280(4):86–9.

4. Langer R, Vacanti JP, Vacanti CA, Atala A, Freed LE, Vunjak-Novakovic G. Tissue engineering: biomedical applications. Tissue engineering. 1995;1(2):151–61.

5.Gargus ES, Rogers HB, McKinnon KE, Edmonds ME, Woodruff TK. Engineered reproductive tissues. Nature biomedical engineering. 2020;4(4):381–93.

6. Borzouie Z, Hekmatimoghaddam S, Jebali A, Aflatoonian B. The viability of human testis-derived cells on human serum albumin-based scaffold as an artificial male germ cell niche. International journal of fertility & sterility. 2020;14(2):150.

7. Borzouie Z, Naghibzadeh M, Talebi AR, Pourrajab F, Jebali A, Nikukar H, et al. Development of an artificial male germ cell niche using electrospun poly vinyl alcohol/human serum albumin/gelatin fibers. Cell Journal (Yakhteh). 2019;21(3):300.

8. Anvari A, Movahedin M. Releasing Scaffold Can Improve Spermatogenesis. Regenerative Biomedicine. 2025;1(2):73–7.

9. Hamzeh M, Movahedin M. Appropriate Biomaterials for Scaffold Fabrication to Induce In Vitro Spermatogenesis. Regenerative Biomedicine. 2025;1(2):78–84.

10. Javidpou M, Golzadeh J, Tafti FH, Aflatoonian B. Tissue engineering for reconstitution of human testicular niche; one step closer to fertility preservation and male infertility treatment. Science. 2019;282(5391):1145–57.

11. Oliver E, Stukenborg JB. Rebuilding the human testis in vitro. Andrology. 2020;8(4):825–34.

12. Wanjari UR, Gopalakrishnan AV. Blood-testis barrier: A review on regulators in maintaining cell junction integrity between Sertoli cells. Cell and Tissue Research. 2024;396(2):157–75.

13. Reuter K, Ehmcke J, Stukenborg J-B, Simoni M, Damm OS, Redmann K, et al. Reassembly of somatic

cells and testicular organogenesis in vitro. Tissue and Cell. 2014;46(1):86–96.

14. Tahajjodi SS, Yazd EF, Agharahimi A, Akyash F, Hajizadeh-Tafti F, Golzadeh J, et al. Cumulus cells conditioned medium facilitates germ cell differentiation from human embryonic stem cells: An experimental study. International Journal of Reproductive Biomedicine. 2025;23(1):33.

15. Akyash F, Aflatoonian R, Farashahi-Yazd E, Hajizadeh-Tafti F, Golzadeh J, Tahajjodi SS, et al. Testicular cells derived conditioned medium supports germ cell differentiation of human embryonic stem cells. Cell Journal (Yakhteh). 2024;26(6):370–9.

16. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem cell research & therapy. 2019;10:1–22.

17. Sadeghian-Nodoushan F, Aflatoonian R, Borzouie Z, Akyash F, Fesahat F, Soleimani M, et al. Pluripotency and differentiation of cells from human testicular sperm extraction: An investigation of cell stemness. Molecular reproduction and development. 2016;83(4):312–23.

18. Vermeulen M, Poels J, De Michele F, Des Rieux A, Wyns C. Restoring fertility with cryopreserved prepubertal testicular tissue: perspectives with hydrogel encapsulation, nanotechnology, and bioengineered scaffolds. Annals of biomedical engineering. 2017;45:1770–81.

19. Horvath-Pereira BdO, Almeida GHDR, Silva Júnior LNd, do Nascimento PG, Horvath Pereira BdO, Fireman JVBT, et al. Biomaterials for Testicular Bioengineering: How far have we come and where do we have to go? Frontiers in Endocrinology. 2023;14:1085872.

20. Baert Y, De Kock J, Alves-Lopes JP, Söder O, Stukenborg J-B, Goossens E. Primary human testicular cells self-organize into organoids with testicular properties. Stem Cell Reports. 2017;8(1):30–8.

21. Lovett M, Lee K, Edwards A, Kaplan DL. Vascularization strategies for tissue engineering. Tissue Engineering Part B: Reviews. 2009;15(3):353–70.

22. Eslahi N, Hadjighassem MR, Joghataei MT, Mirzapour T, Bakhtiyari M, Shakeri M, et al. The effects of poly L-lactic acid nanofiber scaffold on mouse spermatogonial stem cell culture. International journal of nanomedicine. 2013:4563–76.

23. Shams A, Eslahi N, Movahedin M, Izadyar F, Asgari H, Koruji M. Future of spermatogonial stem cell culture: application of nanofiber scaffolds. Current stem cell research & therapy. 2017;12(7):544–53.

24. Clark AT, Bodnar MS, Fox M, Rodriquez RT, Abeyta MJ, Firpo MT, et al. Spontaneous differentiation of germ cells from human embryonic stem cells in vitro. Human molecular genetics. 2004;13(7):727–39.

25. Lee J-H, Gye MC, Choi KW, Hong JY, Lee YB, Park D-W, et al. In vitro differentiation of germ cells from nonobstructive azoospermic patients using three-dimensional culture in a collagen gel matrix. Fertility and sterility. 2007;87(4):824–33.

26. Yadegar M, Hekmatimoghaddam SH, Saridar SN, Jebali A. The viability of mouse spermatogonial germ cells on a novel scaffold, containing human serum albumin and calcium phosphate nanoparticles. Iranian journal of reproductive medicine. 2015;13(3):141.

27. Perrard M-H, Sereni N, Schluth-Bolard C, Blondet A, d′ Estaing SG, Plotton I, et al. Complete human and rat ex vivo spermatogenesis from fresh or frozen testicular tissue. Biology of reproduction. 2016;95(4):89, 1–10.

28. Yuan Y, Li L, Cheng Q, Diao F, Zeng Q, Yang X, et al. In vitro testicular organogenesis from human fetal gonads produces fertilization-competent spermatids. Cell Research. 2020;30(3):244–55.

29. Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N. In vitro transplantation of spermatogonial stem cells isolated from human frozen–thawed testis tissue can induce spermatogenesis under 3-dimensional tissue culture conditions. Biological Research. 2019;52:1–9.

30. Bashiri Z, Zahiri M, Allahyari H, Esmaeilzade B. Proliferation of human spermatogonial stem cells on optimized PCL/Gelatin nanofibrous scaffolds. Andrologia. 2022;54(5):e14380.

31. Baert Y, Stukenborg JB, Landreh M, De Kock J, Jörnvall H, Söder O, et al. Derivation and characterization of a cytocompatible scaffold from human testis. Hum Reprod. 2015;30(2):256–67.

32. Ganjibakhsh M, Mehraein F, Koruji M, Aflatoonian R, Farzaneh P. Three-dimensional decellularized amnion membrane scaffold promotes the efficiency of male germ cells generation from human induced pluripotent stem cells. Experimental Cell Research. 2019;384(1):111544.

33. Eguizabal C, Montserrat N, Vassena R, Barragan M, Garreta E, Garcia-Quevedo L, et al. Complete meiosis from human induced pluripotent stem cells. Stem cells. 2011;29(8):1186–95.

34. Hsiao C-H, Ji AT-Q, Chang C-C, Cheng C-J, Lee L-M, Ho JH-C. Local injection of mesenchymal stem cells protects testicular torsion-induced germ cell injury. Stem cell research & therapy. 2015;6:1–12.

35. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnology and bioengineering. 2009;103(4):655–63.

36. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–78.

37. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nature medicine. 2019;25(1):44–56.