MICROTUBULAR STRUCTURED SCAFFOLDS FOR TESTICULAR TISSUE ENGINEERING

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF TECHNOLOGY

in

BIOTECHNOLOGY

by

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CERTIFICATE

Dated

This is to certify that the work in the thesis entitled "**MICROTUBULAR STRUCTURED SCAFFOLDS FOR TESTICULAR TISSUE ENGINEERING"** submitted by **Mr. Gokula Nathan K (213BM2025)**, in partial fulfilment of the requirements for the award of M.Tech (Biotechnology) at the National Institute of Technology-Rourkela, is an authentic work performed by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any University/Institute for the award of any Degree or Diploma.

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ACKNOWLEDGEMENT

I would really like to take this opportunity to thank my research guide, **Prof. Mukesh Kumar Gupta**, Department of Biotechnology and Medical Engineering, NIT Rourkela, for believing in me and allowing me to work on this research and motivating me throughout the time. I am sincerely thankful to **Prof. B. P. Nayak,** and **Prof. S. S. Ray**, Dept. of Biotechnology and Medical Engineering, NIT, Rourkela, for providing the necessary facilities for this work.

I give my special thanks to my labmates **Ms. Srishti Gupta, Mr. Iqbal Hussain. Mr. Praveen Kumar Guttula, and Ms. Tanushree Patra,** and I gladly accept that without their constant guidance, I would never be able to complete this research.

I extend my gratitude and wishes to **Mr.K.Senthilguru PhD Scholar.,** who helped me on this study whenever needed. I thank him for his support and hands for this work. I also extend my wishes to the juniors who worked all along with us for this study.

Finally, I would like to express my heartfelt thanks to my parents for their blessings, my good friends for their support and motivation that put me forward.

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Abstract

The study was aimed at synthesizing a macro-porous scaffolds through ionotropic gelation of sodium alginate and copper sulphate. The synthesized scaffolds were observed with regularly aligned pore channels of diameter about 25 to 40µm. Produced scaffolds were further analysed by SEM, EDS, FTIR, and protein adsorption, hemocompatbility for their biocompatibility. EDS results showed the removal of copper from gel scaffolds and hemocompatability proved the scaffolds are highly biocompatible.

Keywords: ionotropic gelation, macroporous, pore channels, sodium alginate

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6. References

[1] S.O. Rutstein, I.H. Shah, Infecundity infertility and childlessness in developing countries, (2004).

[2] R.A. Hess, L.R. de Franca, Spermatogenesis and cycle of the seminiferous epithelium, Molecular mechanisms in spermatogenesis, Springer2008, pp. 1-15.

[3] C. Leblond, Y. Clermont, Definition of the stages of the cycle of the seminiferous epithelium in the rat, Annals of the New York Academy of Sciences, 55 (1952) 548-573.

[4] K. Reuter, S. Schlatt, J. Ehmcke, J. Wistuba, Fact or fiction: in vitro spermatogenesis, Spermatogenesis, 2 (2012) 245-252.

[5] S. Seminara, W. Crowley Jr, Kisspeptin and GPR54: discovery of a novel pathway in reproduction, Journal of neuroendocrinology, 20 (2008) 727-731.

[6] R. McLachlan, N. Wreford, L. O'Donnell, D. De Kretser, D. Robertson, The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH, Journal of Endocrinology, 148 (1996) 1-9.

[7] N. Sofikitis, N. Giotitsas, P. Tsounapi, D. Baltogiannis, D. Giannakis, N. Pardalidis, Hormonal regulation of spermatogenesis and spermiogenesis, The Journal of steroid biochemistry and molecular biology, 109 (2008) 323-330.

[8] J.H. Lee, H.J. Kim, H. Kim, S.J. Lee, M.C. Gye, In vitro spermatogenesis by three-dimensional culture of rat testicular cells in collagen gel matrix, Biomaterials, 27 (2006) 2845-2853.

[9] A.G. Mikos, G. Sarakinos, S.M. Leite, J.P. Vacant, R. Langer, Laminated three-dimensional biodegradable foams for use in tissue engineering, Biomaterials, 14 (1993) 323-330.

[10] S. Wang, D. Nagrath, P.C. Chen, F. Berthiaume, M.L. Yarmush, Three-dimensional primary hepatocyte culture in synthetic self-assembling peptide hydrogel, Tissue Engineering Part A, 14 (2008) 227-236.

[11] E. Sachlos, J. Czernuszka, Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds, Eur Cell Mater, 5 (2003) 39-40.

[12] M.M. Matzuk, D.J. Lamb, The biology of infertility: research advances and clinical challenges, Nature medicine, 14 (2008) 1197-1213.

[13] M.G. Teles, S.D. Bianco, V.N. Brito, E.B. Trarbach, W. Kuohung, S. Xu, S.B. Seminara, B.B. Mendonca, U.B. Kaiser, A.C. Latronico, A GPR54-activating mutation in a patient with central precocious puberty, New England Journal of Medicine, 358 (2008) 709-715.

[14] I. Georgiou, M. Syrrou, N. Pardalidis, K. Karakitsios, T. Mantzavinos, N. Giotitsas, D. Loutradis, F. Dimitriadis, M. Saito, I. Miyagawa, Genetic and epigenetic risks of intracytoplasmic sperm injection method, Asian journal of andrology, 8 (2006) 643-673.

[15] I. Mateizel, G. Verheyen, E. Van Assche, H. Tournaye, I. Liebaers, A. Van Steirteghem, FISH analysis of chromosome X, Y and 18 abnormalities in testicular sperm from azoospermic patients, Human Reproduction, 17 (2002) 2249-2257.

[16] N. Schultz, F.K. Hamra, D.L. Garbers, A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets, Proceedings of the National Academy of Sciences, 100 (2003) 12201-12206.

[17] K.A. Groth, A. Skakkebæk, C. Høst, C.H. Gravholt, A. Bojesen, Klinefelter syndrome—a clinical update, The Journal of Clinical Endocrinology & Metabolism, 98 (2012) 20-30.

[18] D.T. Carrell, Contributions of spermatozoa to embryogenesis: assays to evaluate their genetic and epigenetic fitness, Reproductive biomedicine online, 16 (2008) 474-484.

[19] A.C. CHANDLEY, P. EDMOND, S. CHRISTIE, L. GOWANS, J. FLETCHER, A. FRACKIEWICZ, M. NEWTON, Cytogenetics and infertility in man*, Annals of human genetics, 39 (1975) 231-254.

[20] D.J. Elliott, H.J. Cooke, The molecular genetics of male infertility, Bioessays, 19 (1997) 801-809.

[21] J.W. de Vries, S. Repping, R. Oates, R. Carson, N.J. Leschot, F. van der Veen, Absence of deleted in azoospermia (DAZ) genes in spermatozoa of infertile men with somatic DAZ deletions, Fertility and sterility, 75 (2001) 476-479.

[22] C. Foresta, E. Moro, A. Ferlin, Y chromosome microdeletions and alterations of spermatogenesis 1, Endocrine reviews, 22 (2001) 226-239.

[23] A. Ferlin, B. Arredi, E. Speltra, C. Cazzadore, R. Selice, A. Garolla, A. Lenzi, C. Foresta, Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy, The Journal of Clinical Endocrinology & Metabolism, 92 (2007) 762-770.

[24] A. Sathananthan, W. Ratnasooriya, P. de Silva, J. Menezes, Characterization of human gamete centrosomes for assisted reproduction, Italian journal of anatomy and embryology= Archivio italiano di anatomia ed embriologia, 106 (2000) 61-73.

[25] V. Rawe, Y. Terada, S. Nakamura, C. Chillik, S.B. Olmedo, H. Chemes, A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage, Human Reproduction, 17 (2002) 2344-2349.

[26] B.R. Emery, D.T. Carrell, The effect of epigenetic sperm abnormalities on early embryo-genesis, Asian journal of andrology, 8 (2006) 131-142.

[27] M.T. Hemann, K.L. Rudolph, M.A. Strong, R.A. DePinho, L. Chin, C.W. Greider, Telomere dysfunction triggers developmentally regulated germ cell apoptosis, Molecular biology of the cell, 12 (2001) 2023-2030.

[28] M. Piasecka, J. Kawiak, Sperm mitochondria of patients with normal sperm motility and with asthenozoospermia: morphological and functional study, Folia histochemica et cytobiologica/Polish Academy of Sciences, Polish Histochemical and Cytochemical Society, 41 (2003) 125.

[29] J. Tesarik, M. Bahceci, C. Özcan, E. Greco, C. Mendoza, Restoration of fertility by in-vitro spermatogenesis, The Lancet, 353 (1999) 555-556.

[30] C. Staub, A century of research on mammalian male germ cell meiotic differentiation in vitro, Journal of andrology, 22 (2001) 911-926.

[31] S. Aizawa, Y. Nishimune, In-vitro differentiation of type A spermatogonia in mouse cryptorchid testis, Journal of reproduction and fertility, 56 (1979) 99-104.

[32] T. Sato, T. Yokonishi, M. Komeya, K. Katagiri, Y. Kubota, S. Matoba, N. Ogonuki, A. Ogura, S. Yoshida, T. Ogawa, Testis tissue explantation cures spermatogenic failure in c-Kit ligand mutant mice, Proceedings of the National Academy of Sciences, 109 (2012) 16934-16938.

[33] J.A. Rowley, G. Madlambayan, D.J. Mooney, Alginate hydrogels as synthetic extracellular matrix materials, Biomaterials, 20 (1999) 45-53.

[34] F. Despang, M. Gelinsky, R. Dittrich, Novel biomaterials with parallel aligned pore channels by directed ionotropic gelation of alginate: mimicking the anisotropic structure of bone tissue, INTECH Open Access Publisher2011.

[35] R. Dittrich, F. Despang, A. Bernhardt, A. Mannschatz, T. Hanke, G. Tomandl, W. Pompe, M. Gelinsky, Mineralized scaffolds for hard tissue engineering by ionotropic gelation of alginate, Advances in Science and Technology, 49 (2006) 159-164.

[36] J. Thumbs, H.-H. Kohler, Capillaries in alginate gel as an example of dissipative structure formation, Chemical physics, 208 (1996) 9-24.

[37] P. Prang, R. Müller, A. Eljaouhari, K. Heckmann, W. Kunz, T. Weber, C. Faber, M. Vroemen, U. Bogdahn, N. Weidner, The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels, Biomaterials, 27 (2006) 3560-3569.

[38] B. Wei, J. Wang, Z. Chen, G. Chen, Carbon-Nanotube–Alginate Composite Modified Electrode Fabricated by In Situ Gelation for Capillary Electrophoresis, Chemistry-A European Journal, 14 (2008) 9779-9785.

[39] S.T. Moe, G. Skjaak-Braek, A. Elgsaeter, O. Smidsroed, Swelling of covalently crosslinked alginate gels: influence of ionic solutes and nonpolar solvents, Macromolecules, 26 (1993) 3589-3597.

[40] B. Sarmento, D. Ferreira, F. Veiga, A. Ribeiro, Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies, Carbohydrate Polymers, 66 (2006) 1-7.

[41] D. Gan, L.A. Lyon, Synthesis and protein adsorption resistance of PEG-modified poly (N-isopropylacrylamide) core/shell microgels, Macromolecules, 35 (2002) 9634-9639.

[42] K. Pal, S. Pal, Development of porous hydroxyapatite scaffolds, Materials and manufacturing processes, 21 (2006) 325-328.

[43] H.J. Kong, D. Kaigler, K. Kim, D.J. Mooney, Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution, Biomacromolecules, 5 (2004) 1720-1727.

[44] C.H. Goh, P.W.S. Heng, L.W. Chan, Alginates as a useful natural polymer for microencapsulation and therapeutic applications, Carbohydrate Polymers, 88 (2012) 1-12.

[45] C.K. Siew, P.A. Williams, N.W. Young, New insights into the mechanism of gelation of alginate and pectin: charge annihilation and reversal mechanism, Biomacromolecules, 6 (2005) 963-969.

[46] B.J. Willenberg, T. Hamazaki, F.W. Meng, N. Terada, C. Batich, Self-assembled copper-capillary alginate gel scaffolds with oligochitosan support embryonic stem cell growth, Journal of Biomedical Materials Research Part A, 79 (2006) 440-450.

[47] C.G. Anderson, Treatment of copper ores and concentrates with industrial nitrogen species catalyzed pressure leaching and non-cyanide precious metals recovery, JOM, 55 (2003) 32-36.

[48] F. Ganji, S. Vasheghani-Farahani, E. Vasheghani-Farahani, Theoretical description of hydrogel swelling: a review, Iran Polym J, 19 (2010) 375-398.

[49] S.K. Papageorgiou, E.P. Kouvelos, E.P. Favvas, A.A. Sapalidis, G.E. Romanos, F.K. Katsaros, Metal–carboxylate interactions in metal–alginate complexes studied with FTIR spectroscopy, Carbohydrate research, 345 (2010) 469-473.

[50] C.K. Kuo, P.X. Ma, Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties, Biomaterials, 22 (2001) 511-521.

[51] K.Y. Lee, K.H. Bouhadir, D.J. Mooney, Controlled degradation of hydrogels using multi-functional cross-linking molecules, Biomaterials, 25 (2004) 2461-2466.