# BIOMINERALIZATION OF HYDROXYETHYL CELLULOSE/SODIUM ALGINATE IMPREGNATED WITH CELLULOSE NANOCRYSTALS BY USING SURFACE MODIFICATION TECHNIQUE

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MASTER OF SCIENCE

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**SUPERVISOR'S DECLARATION**

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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**STUDENT'S DECLARATION**

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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**ABSTRAK**

Kejuruteraan tisu tulang menghasilkan replikasi kerangka daripada pelbagai material biopolimerik untuk mendapatkan topographi tertentu dan pembenihan sel-sel tertentu sebelum dilaksanakan ke dalam badan yang cedera. Tujuan utama penyelidikan ini adalah untuk sintesis bahan biopolimer daripada hidroksil etil selulosa (HEC) (5% berat) yang dicampur dengan natrium alginat (SA) (10% berat) pada nisbah 1: 1, digabungkan dengan nanokristal selulosa (CNC) (11 w/v %) dan direka menggunakan teknik pengeringan beku. Tingkah laku peran cah seperti struktur kimia dan sifat terma diciikan dengan menggunakan FESEM, EDX, ATR-FTIR and UTM.. Pencirian adalah penting untuk memahami sifat fizikal, kimia dan mekanik kerangka. Biokompatibiliti in vitro kerangka telah disiasat dengan pengkulturan sel osteoblas janin manusia (hFOB) pada kerangka ini. Hasil imej SEM dipaparkan struktur berliang yang saling berkait dengan diameter antara 40 hingga 400 μm dengan peratusan keliangan dalam julat 75 ± 5% hingga 90.5 ± 5%. Pengembangan ratio yang ketara pada HEC/SA tidak dirawat denagn kerangka SBF disebabkan kekuatan ikatan hydrogen dan interaksi antara Van der Waals dengan rantai polimer. Kerangaka mula hancur selepas hari ketujuh yang mana berat menurun sehingga ~60%. Kemungkinan, ditu njukkan oleh ATR-FTIR disebabkan oleh interaksi diantara HEC, SA, dan CNC dalam campuran. Hasil TGA menunjukkan empat bahagian yang berlainan kehilangan jisim, mewakili suhu peralihan amorfus dan pelupusan air, pecahan ikatan rantaian sisi, pirolisis SA dan kelakuan dehidrosilasi kalsium fosfat. Interaksi sel menunjukkan sel hFOB menunjukkan perbezaan merebak sangat baik ke atas percambahan sel dan lebih menonjol terhadap HEC/SA/CNC yang dirawat dengan kerangka SBF. Oleh kerana biokompatibil dan terbiodegradasi ini menghasilkan keputusan yang baik, kerangka ini boleh digunakan untuk reka bentuk kejuruteraan tisu tulang yang akan dihasilkan oleh generasi akan datang..

**ABSTRACT**

Bone tissue engineering utilizes scaffolds fabricated from various biopolymeric materials to obtain a specific topography prior to seeding with specified cells and implantation into an injured body. The aim of this research is to synthesize biopolymeric materials from hydroxylethylcellulose (HEC) (5 wt%) blended with sodium alginate (SA)(10 wt%) at 1:1 ratio and incorporated with cellulose nanocrystals (CNC) (11 w/v%). The scaffolds was fabricated using the freeze-drying technique. For the mineralization process, these HEC/SA and HEC/SA/CNC scaffolds were treated with simulated body fluid (SBF) by immersion technique through the depositing of calcium phosphate on the scaffold's surfaces. The behavior of scaffolds such as chemical structures and thermal properties were characterized by using FESEM, EDX, ATR-FTIR, and UTM. In-vitro biocompatibility of the scaffolds was investigated by culturing human fetal osteoblast (hFOB) cells on these scaffolds. The SEM images displayed interconnected porous structures with diameters ranging from 40 to 400 μm and porosity percentages ranging from 75 ± 5% to 90.5 ± 5 %. The high swelling ratio of HEC/SA untreated with SBF scaffold was ascribed to the strong hydrogen bonding and Van der Waals interactions between polymer chains. After 7 days of incubation, the scaffolds began to disintegrate, which leads to the increase in weight loss (simultaneously up to ~60%). ATR-FTIR results exhibit possible interactions between hydroxyl groups of HEC, SA and CNC in the blends suggests there is chemical interaction between scaffolds. The TGA results showed four different regions of mass losses, represents the degradation temperature and water disposal, side- chain bond breaking, pyrolysis of SA and dehydroxylation behavior of calcium phosphate, respectively. The cell-scaffolds interaction demonstrated that hFOB cells differentiated and spread well on the scaffolds with better cell proliferation and attachment on HEC/SA/CNC treated with SBF porous scaffolds. Since these biocompatible and biodegradable scaffolds showed promising results, these scaffolds could be adopted for the design of next-generation tissue-engineered bone grafts.

## TABLE OF CONTENT

# REFERENCES


Affatato, S., Ruggiero, A., & Merola, M. (2015). Advanced biomaterials in hip joint arthroplasty. A review on polymer and ceramics composites as alternative bearings. *Composites Part B: Engineering, 83*, 276-283.

Ahmed, S., & Ikram, S. (2016). Chitosan based scaffolds and their applications in wound healing. *Achievements in the life sciences*, *10*(1), 27-37.

Ali, M. E., & Lamprecht, A. (2017). Spray freeze drying as an alternative technique for lyophilization of polymeric and lipid-based nanoparticles. *International Journal of Pharmaceutics*, *516*(1-2), 170-177.

Altman, G. H., Diaz, F., Jakuba, C., Calabro, T., Horan, R. L., Chen, J., Lu, H., Richmond, J., & Kaplan, D. L. (2003). Silk-based biomaterials. Biomaterials, 24(3), 401–416. http://www.ncbi.nlm.nih.gov/pubmed/12423595

Agrawal, P., & Pramanik, K. (2019). Enhanced chondrogenic differentiation of human mesenchymal stem cells in silk fibroin/chitosan/glycosaminoglycan scaffolds under dynamic culture condition. *Differentiation*, *110*, 36-48.

Agarwal, S., Wendorff, J. H., & Greiner, A. (2009). Progress in the field of electrospinning for tissue engineering applications. *Advanced Materials, 21*(32-33), 3343-3351.

Barnes, C. (2007). sell sa, Boland eD, simpson DG, Bowlin Gl. nanofiber technology: designing the next generation of tissue engineering scaffolds. *Adv Drug Deliv Rev*, *59*(14), 1413-1433.

Bhardwaj, N., Kundu, S.C, (2011), Silk fibroin protein and chitosan polyelectrolyte complex porous scaffolds for tissue engineering applications**.** *Carbohydr. Polym.*, 85, pp. 325-333

Brien, F. (2011). Biomaterials & scaffolds Every day thousands of surgical procedures are performed to replace. *Mater Today*. *Elsevier Ltd*, *14*, 88-95.

Catauro, M., Mozzati, M. C., & Bollino, F. (2015). Sol–gel hybrid materials for aerospace applications: Chemical characterization and comparative investigation of the magnetic properties. *Acta Astronautica*, *117*, 153-162.

Cen, L., Liu, W. E. I., Cui, L. E. I., Zhang, W., & Cao, Y. (2008). Collagen tissue engineering: development of novel biomaterials and applications. *Pediatric research*, *63*(5), 492-496.

Chan, B., & Leong, K. (2008). Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *European spine journal*, *17*(4), 467-479.

Chen, L., Liu, Y., Lai, C., Berry, R., & Tam, K. (2015). Aqueous synthesis and biostabilization of CdS@ ZnS quantum dots for bioimaging applications. Materials Research Express, 2(10), 105401.

Chen, G., Ushida, T., & Tateishi, T. (2002). Scaffold design for tissue engineering. *Macromolecular Bioscience, 2*(2), 67-77.

Chemin, M., Heux, L., Guérin, D., Crowther-Alwyn, L., & Jean, B. (2019). Hybrid Gibbsite Nanoplatelet/Cellulose Nanocrystal Multilayered Coatings for Oxygen Barrier Improvement. *Frontiers in Chemistry, 7*(507). doi: 10.3389/fchem.2019.00507

Chiaoprakobkij,N.,Sanchavanakit,N.,Subbalekha,K.,Pavasant,P.,&Phisalaphong,M (2011).Characterization and biocompatibility of bacterial cellulose/alginate composite sponge with human keratinocytes and gingival fibroblasts. *Carbohydrate Polymers*, 85, 548–553.

Chinnappan, A., Lee, J. K. Y., Jayathilaka, W., & Ramakrishna, S. (2018). Fabrication of MWCNT/Cu nanofibers via electrospinning method and analysis of their electrical conductivity by four-probe method. *International Journal of Hydrogen Energy, 43*(2), 721-729.

Chung, S., Ingle, N.P., Montero, G.A., Kim, S.H., King, M.W. (2010). Bioresorbable elastomeric vascular tissue engineering scaffolds via melt spinning and electrospinning. *Acta Biomater*, *6*, 1958–1967.

Cooper, L. N., & Maas, M. C. (2018). Bones and teeth, histology of *Encyclopedia of Marine Mammals* (pp. 114-118): Elsevier.

Corazzari, I., Nisticò, R., Turci, F., Faga, M. G., Franzoso, F., Tabasso, S., & Magnacca, G. (2015). Advanced physico-chemical characterization of chitosan by means of TGA coupled on-line with FTIR and GCMS: Thermal degradation and water adsorption capacity. *Polymer Degradation and Stability, 112*, 1-9.

Cox, S. C., Thornby, J. A., Gibbons, G. J., Williams, M. A., & Mallick, K. K. (2015). 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. *Materials Science and Engineering: C, 47*, 237-247.

Cyster, L. A., Grant, D. M., Howdle, S. M., Rose, F. R. A. J., Irvine, D. J., Freeman, D., ... & Shakesheff, K. M. (2005). The influence of dispersant concentration on the pore morphology of hydroxyapatite ceramics for bone tissue engineering. *Biomaterials, 26*(7), 697-702

De France, K.J. , Chan K.J.W. , Cranston E.D. Cranston, Hoare T. (2016), Enhanced mechanical properties in cellulose nanocrystal-poly(oligoethylene glycol methacrylate) injectable nanocomposite hydrogels through control of physical and chemical cross-linking *Biomacromolecules,* 17 (2) pp. 649-660

Deluzio, T. G., Seifu, D. G., & Mequanint, K. (2013). 3D scaffolds in tissue engineering and regenerative medicine: beyond structural templates? *Pharmaceutical Bioprocessing, 1*(3), 267-281.

Dogan, Ö., & Öner, M. (2008). The influence of polymer architecture on nanosized hydroxyapatite precipitation. *Journal of nanoscience and nanotechnology, 8*(2), 667-674.

Evangelopoulos, P., Kantarelis, E., & Yang, W. (2015). Investigation of the thermal decomposition of printed circuit boards (PCBs) via thermogravimetric analysis (TGA) and analytical pyrolysis (Py–GC/MS). *Journal of Analytical and Applied Pyrolysis*, *115*, 337-343.

Geng, F., Tan, L., Zhang, B., Wu, C., He, Y., Yang, J., & Yang, K. (2009). Study on β-TCP coated porous Mg as a bone tissue engineering scaffold material. *Journal of Materials Sciences and Technology*, *25*(01), 123-129.

George, J., & Sabapathi, S. N. (2015). Cellulose nanocrystals: Synthesis, functional properties, and applications. Nanotechnology, Science and Applications, 8. https://doi.org/10.2147/NSA.S64386

Ghaee, A., Nourmohammadi, J., & Danesh, P. (2017). Novel chitosan-sulfonated chitosan-polycaprolactone-calcium phosphate nanocomposite scaffold. *Carbohydrate polymers*, *157*, 695-703.

Gómez, S., Vlad, M., López, J., & Fernández, E. (2016). Design and properties of 3D scaffolds for bone tissue engineering. *Acta Biomaterialia*, *42*, 341-350.

Goonoo, N., Bhaw-Luximon, A., Bowlin, G. L., & Jhurry, D. (2013). An assessment of biopolymer-and synthetic polymer-based scaffolds for bone and vascular tissue engineering. *Polymer International*, *62*(4), 523-533.

Grishkewich, N., Mohammed, N., Tang, J., & Tam, K. C. (2017). Recent advances in the application of cellulose nanocrystals. Current Opinion in Colloid & Interface Science, 29, 32–45. https://doi.org/10.1016/J.COCIS.2017.01.005

Hakkou, K., Molina-Pinilla, I., Rangel-Núñez, C., Suárez-Cruz, A., Pajuelo, E., & Bueno-Martínez, M. (2019). Synthesis of novel (bio) degradable linear azo polymers conjugated with olsalazine. *Polymer Degradation and Stability*, *167*, 302-312.

Han, S., Lee, H., Hong, I., Kim, U., & Lee, S. (2017). Non-structural cancellous bone graft and headless compression screw fixation for treatment of scaphoid waist non-union. *Orthopaedics & Traumatology: Surgery & Research*, *103*(1), 89-93.

Henmi, A., Okata, H., Anada, T., Yoshinari, M., Mikami, Y., Suzuki, O., & Sasano, Y. (2016). Bone matrix calcification during embryonic and postembryonic rat calvarial development assessed by sem–edx spectroscopy, xrd, and ftir spectroscopy. *Journal of bone and mineral metabolism*, *34*(1), 41-50.

Heydarkhan-Hagvall, S., Schenke-Layland, K., Dhanasopon, A. P., Rofail, F., Smith, H., Wu, B. M., ... & MacLellan, W. R. (2008). Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. *Biomaterials*, *29*(19), 2907-2914.

Hopkins, S., Toms, A., Brown, M., Welsman, J., Ukoumunne, O., & Knapp, K. (2016). A study investigating short-and medium-term effects on function, bone mineral density and lean tissue mass post-total knee replacement in a Caucasian female post-menopausal population: implications for hip fracture risk. *Osteoporosis International*, *27*(8), 2567-2576.

Hutmacher, D. W. (2000). Scaffolds in tissue engineering bone and cartilage. Biomaterials, 21(24), 2529–2543. http://www.ncbi.nlm.nih.gov/pubmed/11071603

Kanungo, B. P., Silva, E., Van Vliet, K., & Gibson, L. J. (2008). Characterization of mineralized collagen–glycosaminoglycan scaffolds for bone regeneration. *Acta biomaterialia*, *4*(3), 490-503.

Kokubo, T., Kushitani, H., Sakka, S., Kitsugi,T., & Yamamuro, T (1990).Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W, *J. Biomed. Mater. Res.*, 24, 721-734

Kong, L., Gao, Y., Lu, G., Gong, Y., Zhao, N., & Zhang, X. (2006). A study on the bioactivity of chitosan/nano-hydroxyapatite composite scaffolds for bone tissue engineering. *European Polymer Journal*, *42*(12), 3171-3179.

Kumar, A., Rao, K. M., & Han, S. S. (2017). Development of sodium alginate-xanthan gum based nanocomposite scaffolds reinforced with cellulose nanocrystals and halloysite nanotubes. *Polymer Testing*, *63*, 214-225.

Kumar, A., Lee, Y., Kim, D., Rao, K. M., Kim, J., Park, S., . . . Han, S. S. (2017). Effect of crosslinking functionality on microstructure, mechanical properties, and in vitro cytocompatibility of cellulose nanocrystals reinforced poly (vinyl alcohol)/sodium alginate hybrid scaffolds. *International journal of biological macromolecules*, *95*, 962-973.

Langer, R., Vacanti, J.P. (1993). Tissue engineering. *Science*, *260*, 920–926

Lee, S. H., & Shin, H. (2007). Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Advanced drug delivery reviews, 59(4-5), 339-359.

Lee, Y. B., Song, S.-J., Shin, Y. C., Jung, Y. J., Kim, B., Kang, M. S., . . . Jung, S.-H. (2019). Ternary nanofiber matrices composed of PCL/black phosphorus/collagen to enhance osteodifferentiation. *Journal of Industrial and Engineering Chemistry*.

Lei, Y., Xu, Z., Ke, Q., Yin, W., Chen, Y., Zhang, C., & Guo, Y. (2017). Strontium hydroxyapatite/chitosan nanohybrid scaffolds with enhanced osteoinductivity for bone tissue engineering. *Materials Science and Engineering: C*, *72*, 134-142.

Leukers, B., Gülkan, H., Irsen, S. H., Milz, S., Tille, C., Schieker, M., & Seitz, H. (2005). Hydroxyapatite scaffolds for bone tissue engineering made by 3D printing. *Journal of Materials Science: Materials in Medicine*, *16*(12), 1121-1124.

Liao, S., Murugan, R., Chan, C. K., & Ramakrishna, S. (2008). Processing nanoengineered scaffolds through electrospinning and mineralization suitable for biomimetic bone tissue engineering. *Journal of the mechanical behavior of biomedical materials*, *1*(3), 252-260.

Li, M., Yang, X., Wang, W., Zhang, Y., Wan, P., Yang, K., & Han, Y. (2017). Evaluation of the osteo-inductive potential of hollow three-dimensional magnesium-strontium substitutes for the bone grafting application. *Materials Science and Engineering: C*, *73*, 347-356.

Li, Y., Jia, H., Pan, F., Jiang, Z., & Cheng, Q. (2012). Enhanced anti-swelling property and dehumidification performance by sodium alginate–poly(vinyl alcohol)/polysulfone composite hollow fiber membranes. Journal of Membrane Science, 407–408, 211–220. https://doi.org/10.1016/j.memsci.2012.03.049

Lou, C. W., Huang, C. L., Chen, C. K., Liu, C. F., Wen, S. P., & Lin, J. H. (2015). Effect of different manufacturing methods on the conflict between porosity and mechanical properties of spiral and porous polyethylene terephthalate/sodium alginate bone scaffolds. *Materials*, *8*(12), 8768-8779

Lu, T., Li, Y., & Chen, T. (2013). Techniques for fabrication and construction of three-dimensional scaffolds for tissue engineering. International Journal of Nanomedicine, 8, 337–350. https://doi.org/10.2147/IJN.S38635

Liu, J., Ruan, J., Chang, L., Yang, H., & Ruan, W. (2017). Porous Nb-Ti-Ta alloy scaffolds for bone tissue engineering: Fabrication, mechanical properties and in vitro/vivo biocompatibility. *Materials Science and Engineering: C*, *78*, 503-512.

Limongi, T., Lizzul, L., Giugni, A., Tirinato, L., Pagliari, F., Tan, H., . . . Brusatin, G. (2017). Laboratory injection molder for the fabrication of polymeric porous poly-epsilon-caprolactone scaffolds for preliminary mesenchymal stem cells tissue engineering applications. *Microelectronic Engineering, 175*, 12-16.

Liu, W., Wang, D., Huang, J., Wei, Y., Xiong, J., Zhu, W., . . . Wang, D. (2017). Low-temperature deposition manufacturing: A novel and promising rapid prototyping technology for the fabrication of tissue-engineered scaffold. *Materials Science and Engineering: C*, *70*, 976-982.

Lu, J. W., Zhu, Y. L., Guo, Z. X., Hu, P., & Yu, J. (2006). Electrospinning of sodium alginate with poly (ethylene oxide). *Polymer*, *47*(23), 8026-8031.

Martin, T. J., & Sims, N. A. (2015). RANKL/OPG; Critical role in bone physiology. *Reviews in Endocrine and Metabolic Disorders*, *16*(2), 131-139.

Martin, V., & Bettencourt, A. (2018). Bone regeneration: Biomaterials as local delivery systems with improved osteoinductive properties. *Materials Science and Engineering: C*, *82*, 363-371.

Martin, V., & Bettencourt, A. (2018). Bone regeneration: Biomaterials as local delivery systems with improved osteoinductive properties. *Materials Science and Engineering: C*, *82*, 363-371.

Martins, A., Araújo, J. V., Reis, R. L., & Neves, N. M. (2007). Electrospun nanostructured scaffolds for tissue engineering applications.

Meryman, H. T. (1976). Historical recollections of freeze-drying. Developments in Biological Standardization, 36, 29–32. http://www.ncbi.nlm.nih.gov/pubmed/801137

Miranda-Nieves, D., & Chaikof, E. L. (2016). Collagen and elastin biomaterials for the fabrication of engineered living tissues. *ACS Biomaterials Science & Engineering, 3*(5), 694-711.

Nam, J.-Y., Kim, H.-W., Lim, K.-H., Shin, H.-S., & Logan, B. E. (2010). Variation of power generation at different buffer types and conductivities in single chamber microbial fuel cells. *Biosensors and Bioelectronics, 25*(5), 1155-1159.

Ngiam, M., Liao, S., Patil, A. J., Cheng, Z., Chan, C. K., & Ramakrishna, S. (2009). The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. *Bone, 45*(1), 4-16.

Novotna, L., Kucera, L., Hampl, A., Drdlik, D., Cihlar Jr, J., & Cihlar, J. (2019). Biphasic calcium phosphate scaffolds with controlled pore size distribution prepared by in-situ foaming. *Materials Science and Engineering: C, 95*, 363-370.

Pangon, A., Saesoo, S., Saengkrit, N., Ruktanonchai, U., & Intasanta, V. (2016). Hydroxyapatite-hybridized chitosan/chitin whisker bionanocomposite fibers for bone tissue engineering applications. Carbohydrate polymers, 144, 419-427.

Peresin, M. S., Habibi, Y., Zoppe, J. O., Pawlak, J. J., & Rojas, O. J. (2010). Nanofiber composites of polyvinyl alcohol and cellulose nanocrystals: manufacture and characterization. Biomacromolecules, 11(3), 674–681. https://doi.org/10.1021/bm901254n

Porter, J. R., Henson, A., & Popat, K. C. (2009). Biodegradable poly (ε-caprolactone) nanowires for bone tissue engineering applications. *Biomaterials, 30*(5), 780-788.

Pourasghar, M., Koenneke, A., Meiers, P., & Schneider, M. (2019). Development of a fast and precise method for simultaneous quantification of the PLGA monomers lactic and glycolic acid by HPLC. *Journal of pharmaceutical analysis, 9*(2), 100-107.

Qi, H., Ye, Z., Ren, H., Chen, N., Zeng, Q., Wu, X., & Lu, T. (2016). Bioactivity assessment of PLLA/PCL/HAP electrospun nanofibrous scaffolds for bone tissue engineering. *Life Sciences, 148*, 139-144. doi: https://doi.org/10.1016/j.lfs.2016.02.040

Ramakrishna, S., Ramalingam, M., Kumar, T. S., & Soboyejo, W. O. (2016). *Biomaterials: a nano approach*: CRC press.

Ren, H., Yu, Y., & An, T. (2020). Bioaccessibilities of metal (loid) s and organic contaminants in particulates measured in simulated human lung fluids: A critical review. *Environmental Pollution*, 115070.

Rezaei, A., & Mohammadi, M. (2013). In vitro study of hydroxyapatite/polycaprolactone (HA/PCL) nanocomposite synthesized by an in situ sol–gel process. *Materials Science and Engineering: C, 33*(1), 390-396.

Ribeiro, C., Sencadas, V., Correia, D. M., & Lanceros-Méndez, S. (2015). Piezoelectric polymers as biomaterials for tissue engineering applications. *Colloids and Surfaces B: Biointerfaces, 136*, 46-55.

Rodrigues, M. T., Gomes, M. E., & Reis, R. L. (2011). Current strategies for osteochondral regeneration: from stem cells to pre-clinical approaches. *Current opinion in biotechnology, 22*(5), 726-733.

Roohani-Esfahani, S.-I., Newman, P., & Zreiqat, H. (2016). Design and fabrication of 3D printed scaffolds with a mechanical strength comparable to cortical bone to repair large bone defects. *Scientific reports, 6*, 19468.

Sanchez, C., Mazzucchelli, G., Lambert, C., Comblain, F., DePauw, E., & Henrotin, Y. (2018). Proteomic analysis of osteoblasts secretome provides new insights in mechanisms underlying osteoarthritis subchondral bone sclerosis. *Osteoarthritis and Cartilage, 26*, S89.

Saha, N., Shah, R., Gupta, P., Mandal, B. B., Alexandrova, R., Sikiric, M. D., & Saha, P. (2019). PVP-CMC hydrogel: An excellent bioinspired and biocompatible scaffold for osseointegration. *Materials Science and Engineering: C, 95*, 440-449.

Scaffaro, R., Lopresti, F., Botta, L., Rigogliuso, S., & Ghersi, G. (2016). Preparation of three-layered porous PLA/PEG scaffold: relationship between morphology, mechanical behavior and cell permeability. *Journal of the mechanical behavior of biomedical materials, 54*, 8-20.

Schoof, H., Apel, J., Heschel, I., & Rau, G. (2001). Control of pore structure and size in freeze-dried collagen sponges. Journal of Biomedical Materials Research, 58(4), 352–357. http://www.ncbi.nlm.nih.gov/pubmed/11410892

Shaabani, A., Hezarkhani, Z., & Badali, E. (2016). Natural silk supported manganese dioxide nanostructures: synthesis and catalytic activity in aerobic oxidation and one-pot tandem oxidative synthesis of organic compounds. *Polyhedron, 107*, 176-182.

Sheehy, E. J., Vinardell, T., Toner, M. E., Buckley, C. T., & Kelly, D. J. (2014). Altering the architecture of tissue engineered hypertrophic cartilaginous grafts facilitates vascularisation and accelerates mineralisation. *PloS one, 9*(3), e90716.

Shin, K., Acri, T., Geary, S., & Salem, A. K. (2017). Biomimetic mineralization of biomaterials using simulated body fluids for bone tissue Engineering and regenerative medicine. *Tissue Engineering Part A, 23*(19-20), 1169-1180.

Siqueira, P., Siqueira, É., De Lima, A. E., Siqueira, G., Pinzón-Garcia, A. D., Lopes, A. P., . . . Botaro, V. R. (2019). Three-Dimensional Stable Alginate-Nanocellulose Gels for Biomedical Applications: Towards Tunable Mechanical Properties and Cell Growing. *Nanomaterials, 9*(1), 78.

Stratton, S., Shelke, N. B., Hoshino, K., Rudraiah, S., & Kumbar, S. G. (2016). Bioactive polymeric scaffolds for tissue engineering. *Bioactive Materials, 1*(2), 93-108.

Stockert, J. C., Blázquez-Castro, A., Cañete, M., Horobin, R. W., & Villanueva, Á. (2012). MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta histochemica, 114*(8), 785-796

Subia, B., Kundu, J., & Kundu, S. C. (2010). Biomaterial scaffold fabrication techniques for potential tissue engineering applications. In D. Eberli (Ed.), Tissue engineering (Issue 3, pp. 141–159). InTech.

Sundberg, J., Götherström, C., & Gatenholm, P. (2015). Biosynthesis and in vitro evaluation of macroporous mineralized bacterial nanocellulose scaffolds for bone tissue engineering. *Bio-medical materials and engineering, 25*(1), 39-52.

Tamburaci, S., & Tihminlioglu, F. (2018). Biosilica incorporated 3D porous scaffolds for bone tissue engineering applications. *Materials Science and Engineering: C*, *91*, 274-291.

Tang, T., Ebacher, V., Cripton, P., Guy, P., McKay, H., & Wang, R. (2015). Shear deformation and fracture of human cortical bone. *Bone, 71*, 25-35.

Tang, J., Sisler, J., Grishkewich, N., & Tam, K. C. (2017). Functionalization of cellulose nanocrystals for advanced applications. Journal of Colloid and Interface Science, 494, 397–409. https://doi.org/10.1016/j.jcis.2017.01.077

Tanan, W., Panichpakdee, J., & Saengsuwan, S. (2019). Novel biodegradable hydrogel based on natural polymers: Synthesis, characterization, swelling/reswelling and biodegradability. *European Polymer Journal*, *112*, 678-687.

Tohamy, K. M., Mabrouk, M., Soliman, I. E., Beherei, H. H., & Aboelnasr, M. A. (2018). Novel alginate/hydroxyethyl cellulose/hydroxyapatite composite scaffold for bone regeneration: In vitro cell viability and proliferation of human mesenchymal stem cells. *International journal of biological macromolecules, 112*, 448-460.

Tomlinson, D., Erskine, R., Morse, C., Winwood, K., & Onambélé-Pearson, G. (2016). The impact of obesity on skeletal muscle strength and structure through adolescence to old age. *Biogerontology, 17*(3), 467-483.

Tuzlakoglu, K., Bolgen, N., Salgado, A. J., Gomes, M. E., Piskin, E., & Reis, R. L. (2005). Nano-and micro-fiber combined scaffolds: a new architecture for bone tissue engineering. *journal of materials science: materials in medicine*, *16*(12), 1099-1104.

Tzur-Balter, A., Gilert, A., Massad-Ivanir, N., & Segal, E. (2013). Engineering porous silicon nanostructures as tunable carriers for mitoxantrone dihydrochloride. *Acta Biomaterialia, 9*(4), 6208-6217

Venugopal, J., Low, S., Choon, A. T., Kumar, T. S., & Ramakrishna, S. (2008). Mineralization of osteoblasts with electrospun collagen/hydroxyapatite nanofibers. *Journal of Materials Science: Materials in Medicine*, *19*(5), 2039-2046.

Vepari, C., & Kaplan, D. L. (2007). Silk as a Biomaterial. Progress in Polymer Science, 32(8–9), 991–1007. https://doi.org/10.1016/j.progpolymsci.2007.05.013

Vig, K., Chaudhari, A., Tripathi, S., Dixit, S., Sahu, R., Pillai, S., . . . Singh, S. R. (2017). Advances in skin regeneration using tissue engineering. *International journal of molecular sciences, 18*(4), 789.

Vrana, N. E. (2016). Immunomodulatory biomaterials and regenerative immunology: Future Science.

Wang, W., & Yeung, K. W. (2017). Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioactive Materials, 2*(4), 224-247.

Wang, Y., Qian, J., Zhao, N., Liu, T., Xu, W., & Suo, A. (2017). Novel hydroxyethyl chitosan/cellulose scaffolds with bubble-like porous structure for bone tissue engineering. *Carbohydrate Polymers, 167,* 44-51.

Wang, F., Wu, H., Venkataraman, V., & Hu, X. (2019). Silk fibroin-poly (lactic acid) biocomposites: Effect of protein-synthetic polymer interactions and miscibility on material properties and biological responses. *Materials Science and Engineering: C*, *104*, 109890.

White, T. D., & Folkens, P. A. (2005). *The human bone manual*: Elsevier.

Woodard, J. R., Hilldore, A. J., Lan, S. K., Park, C., Morgan, A. W., Eurell, J. A. C., . . . Johnson, A. J. W. (2007). The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity. *Biomaterials, 28*(1), 45-54.

Wu, S., Liu, X., Yeung, K. W., Liu, C., & Yang, X. (2014). Biomimetic porous scaffolds for bone tissue engineering. *Materials Science and Engineering: R: Reports, 80,* 1-36.

Yang, S., Leong, K.-F., Du, Z., & Chua, C.-K. (2001). The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue engineering*, *7*(6), 679-689.

Yang, S., Leong, K. F., Du, Z., & Chua, C. K. (2001). The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Engineering, 7(6), 679–689. https://doi.org/10.1089/107632701753337645

Yamamoto, S., Matsushima, Y., Kanayama, Y., Seki, A., Honda, H., Unuma, H., & Sakai, Y. (2017). Effect of the up-front heat treatment of gelatin particles dispersed in calcium phosphate cements on the in vivo material resorption and concomitant bone formation. *Journal of Materials Science: Materials in Medicine, 28*(3), 48.

Yang, F., Wolke, J. G. C., & Jansen, J. A. (2008). Biomimetic calcium phosphate coating on electrospun poly (ε-caprolactone) scaffolds for bone tissue engineering. *Chemical Engineering Journal*, *137*(1), 154-161

Yin, H.-M., Huang, Y.-F., Ren, Y., Wang, P., Zhao, B., Li, J.-H., . . . Li, Z.-M. (2018). Toward biomimetic porous poly (ε-caprolactone) scaffolds: Structural evolution and morphological control during solid phase extrusion. *Composites Science and Technology, 156*, 192-202.

Yildirim, N., & Shaler, S. (2017). A study on thermal and nanomechanical performance of cellulose nanomaterials (CNs). *Materials, 10*(7), 718.

Zare-Harofteh, A., Saber-Samandari, S., & Saber-Samandari, S. (2016). The effective role of akermanite on the apatite-forming ability of gelatin scaffold as a bone graft substitute. Ceramics International, 42(15), 17781-17791.

Zhao, X., Sun, X., Yildirimer, L., Lang, Q., Lin, Z. Y. W., Zheng, R., ... & Khademhosseini, A. (2017). Cell infiltrative hydrogel fibrous scaffolds for accelerated wound healing. Acta biomaterialia, 49, 66-77.

Zhang, C., Yuan, X., Wu, L., Han, Y., & Sheng, J. (2005). Study on morphology of electrospun poly (vinyl alcohol) mats. European polymer journal, 41(3), 423-432.

Zhang, C., Salick, M. R., Cordie, T. M., Ellingham, T., Dan, Y., & Turng, L. S. (2015). Incorporation of poly (ethylene glycol) grafted cellulose nanocrystals in poly (lactic acid) electrospun nanocomposite fibers as potential scaffolds for bone tissue engineering. Materials Science and Engineering: C, 49, 463-471.

Zhang, Z., & Michniak-Kohn, B. B. (2012). Tissue engineered human skin equivalents. *Pharmaceutics, 4*(1), 26-41.

Zhao, Y., He, M., Jin, H., Zhao, L., Du, Q., Deng, H., . . . Chen, Y. (2018). Construction of highly biocompatible hydroxyethyl cellulose/soy protein isolate composite sponges for tissue engineering. *Chemical Engineering Journal, 341*, 402-413.

Zhao, C., Tan, A., Pastorin, G., & Ho, H. K. (2013). Nanomaterial scaffolds for stem cell proliferation and differentiation in tissue engineering. *Biotechnology advances, 31*(5), 654-668.

Zheng, X., Hui, J., Li, H., Zhu, C., Hua, X., Ma, H., & Fan, D. (2017). Fabrication of novel biodegradable porous bone scaffolds based on amphiphilic hydroxyapatite nanorods. *Materials Science and Engineering: C, 75*, 699-705.

Zhou, C., Yang, K., Wang, K., Pei, X., Dong, Z., Hong, Y., & Zhang, X. (2016). Combination of fused deposition modeling and gas foaming technique to fabricated hierarchical macro/microporous polymer scaffolds. *Materials & Design, 109*, 415-424.

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