

TUS

Technological University of the Shannon: Midlands Midwest
Ollscoil Teicneolaíochta na Sionainne: Lár Tíre Iarthar Láir

TUS Research

Functionality of Gold-Edge-Coated Triangular Silver Nanoparticles in Monitoring Extracellular Matrix Protein Conformations in C2C12/MC3T3-E1 Culture in the Presence of Biomimetic Bone Tissue Regeneration Scaffolds

Laura G. Rodriguez Barroso,^{*a} Farah Alwani Azaman^a, Robert Pogue^{a,b}, Declan Devine^a and Margaret Brennan Fournet^a

a. Technological University of the Shannon, Dublin Rd, N37 HD68, Athlone, Co. Westmeath, Ireland.

b. Universidade Católica de Brasília, Campus Asa Norte. SGAN módulo B 916 Avenida W5 - Asa Norte, 70790-160-DF, Brasília, Brazil.

Introduction

In the cellular environment high noise levels can both mediate and interfere with cellular functions.

The extracellular matrix (ECM) regulates protein dynamics and trajectories, which underpin critical biological processes involved in the development of human disorders and healing processes.

FRET and Raman Spectroscopy are conventional techniques for the study of proteins, however, they are elaborate, and their signals are hindered by the high noise levels of cellular environments.

Noble metal nanoparticles are known to have remarkable optical properties and have been researched for the development of highly sensitive nanobiosensors to study molecules and their interactions in the extracellular matrix.

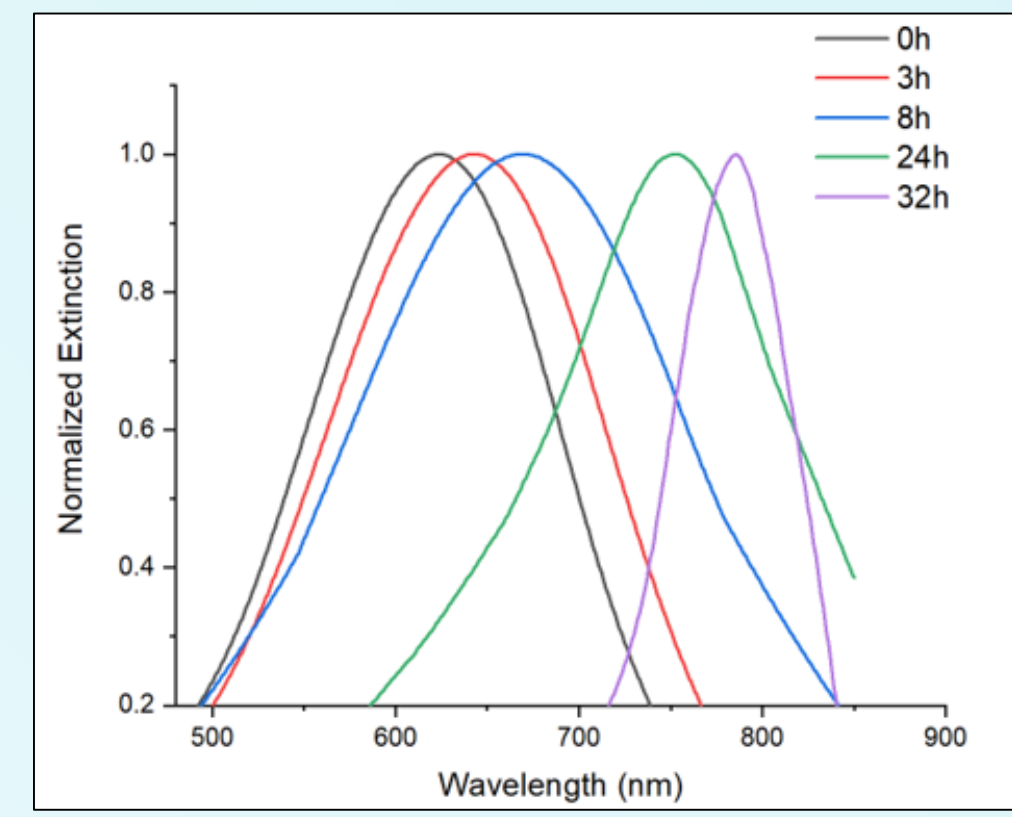
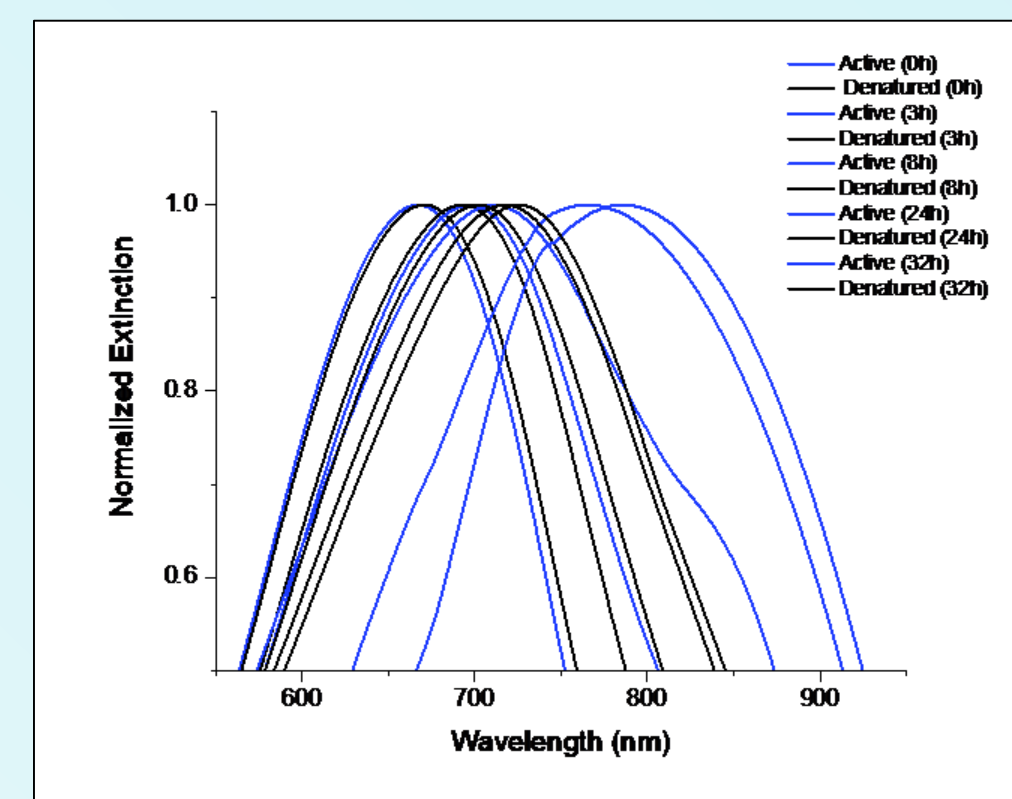
Gold edge-coated triangular silver nanoparticles (AuTSNP) were validated as a promising new tool to point protein conformational transitions in cultured cells, and to monitor protein activity in the presence of a biomimetic chitosan-based scaffold, since it mimics the ECM as a natural scaffold.

Scaffolds of different formulations were characterised to obtain the strongest construct, with regard to the strength of the linkage formation under photo-crosslinking procedures.

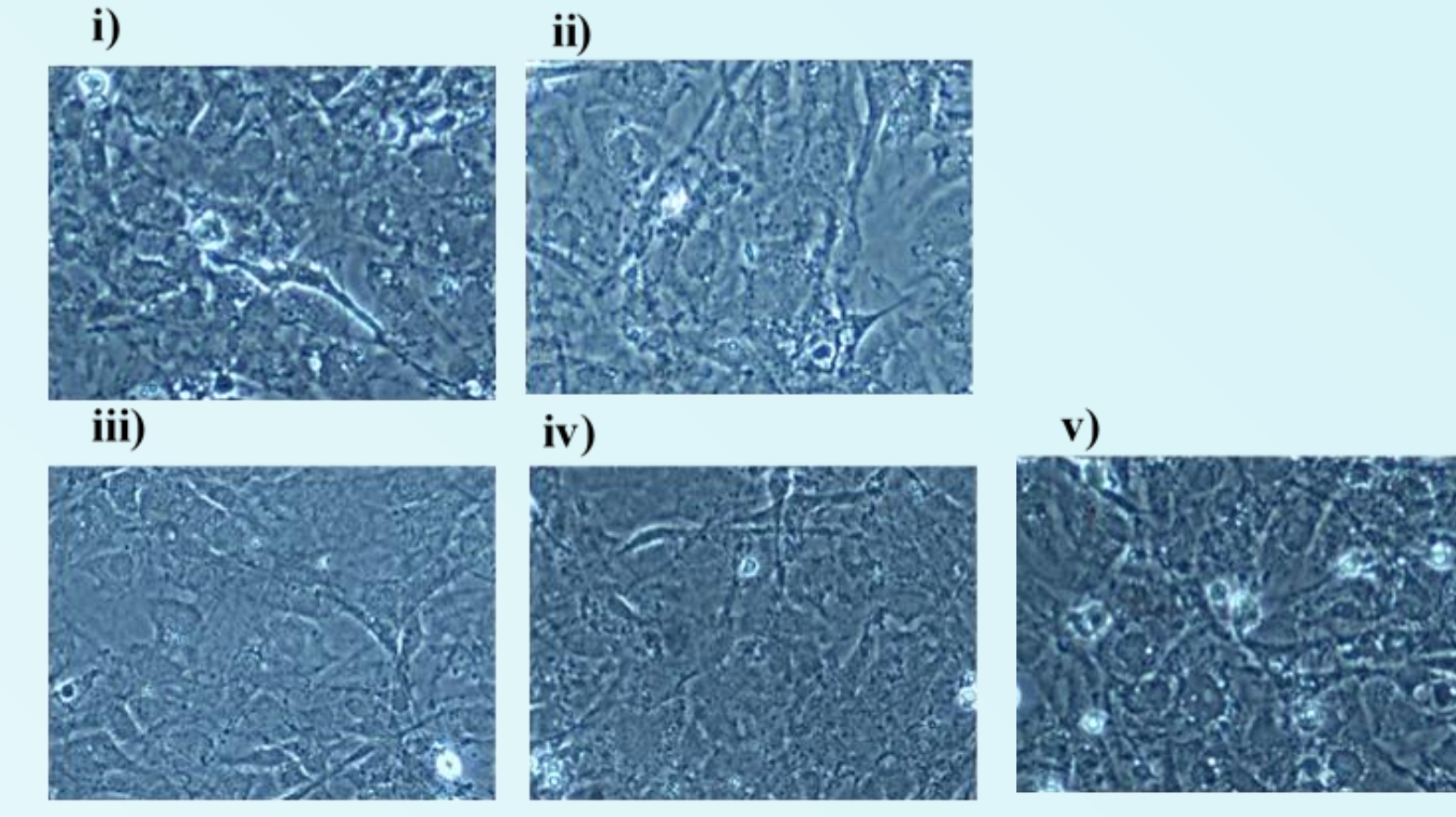
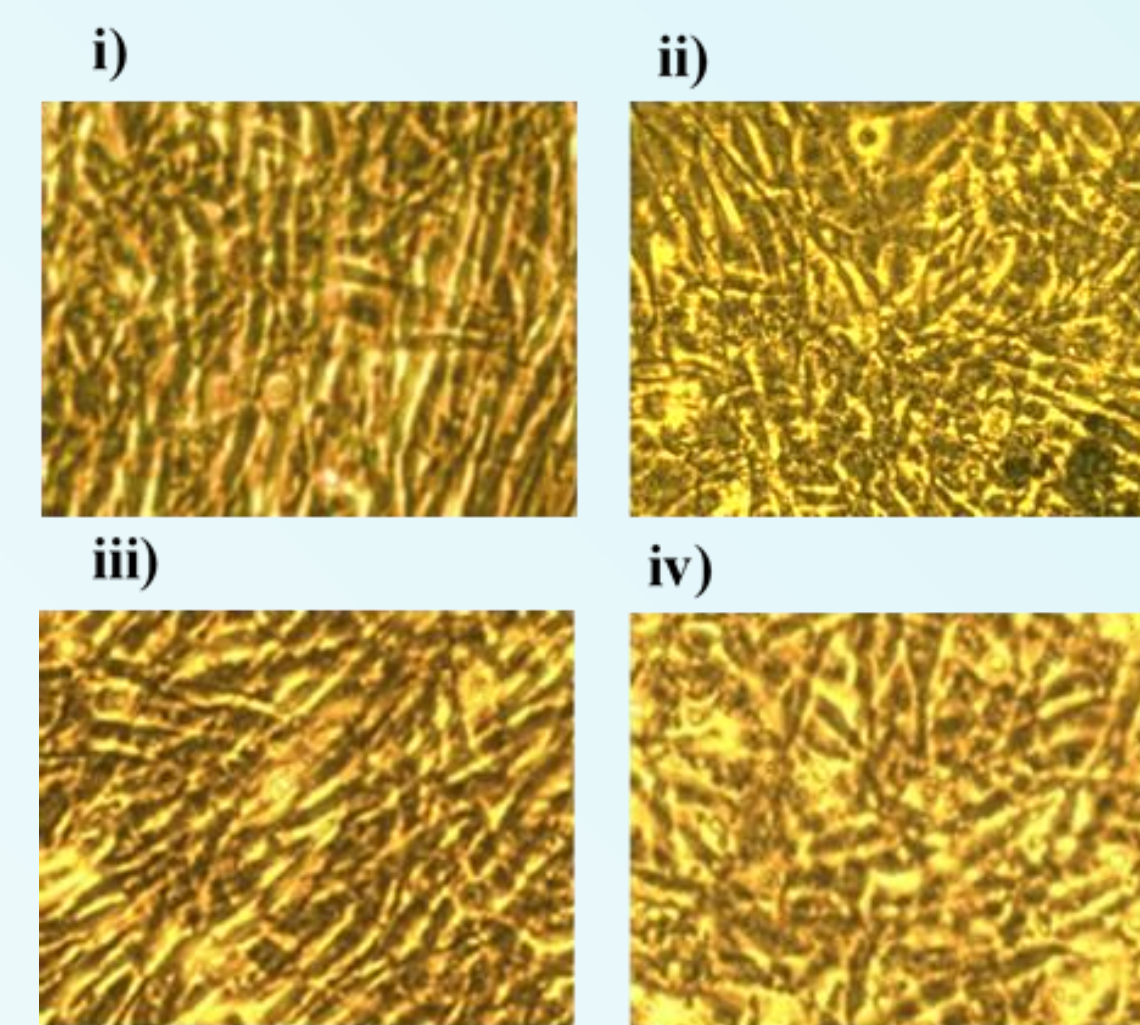
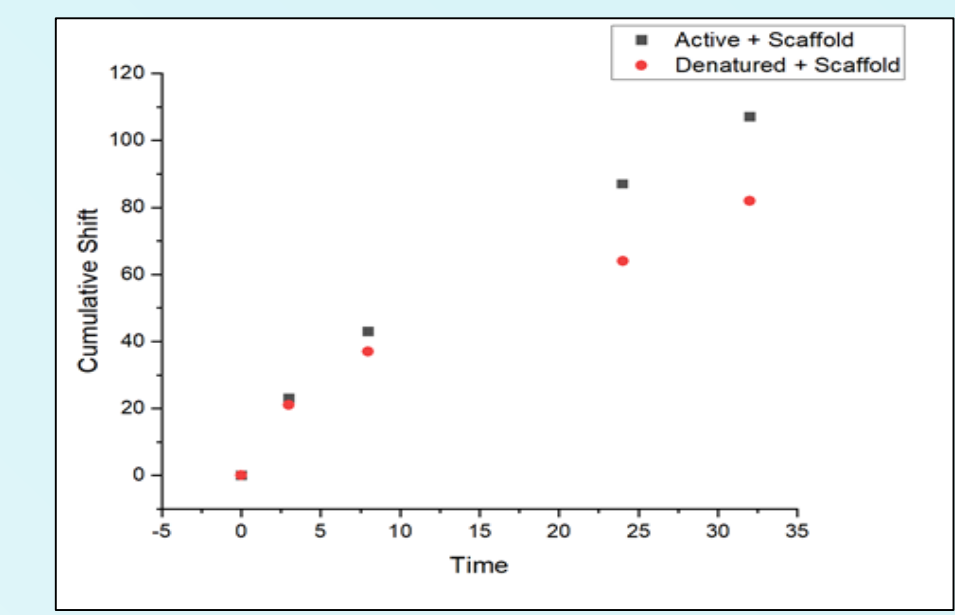
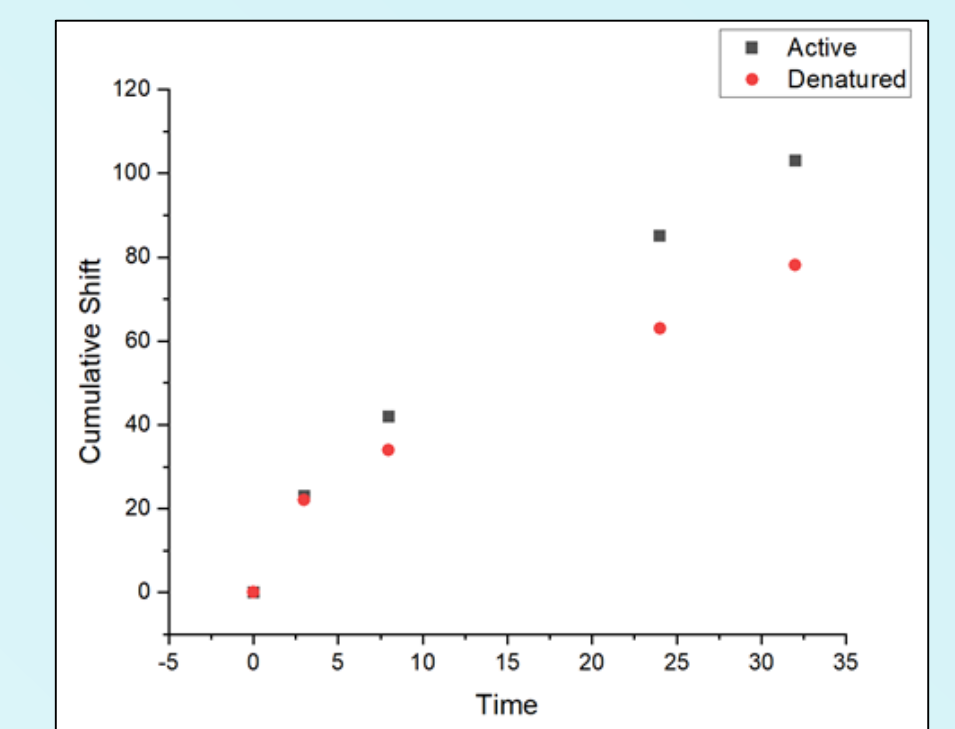
Results

Chitosan-Hydroxyapatite biomimetic scaffold

C2C12 +/- scaffold



MC3T3 +/- scaffold



Methodology

AuTSNP optimization and preparation

Chitosan scaffold preparation and crosslinking characterisation

C2C12, MC3T3 cell culture

Monitoring of Fn in cell culture

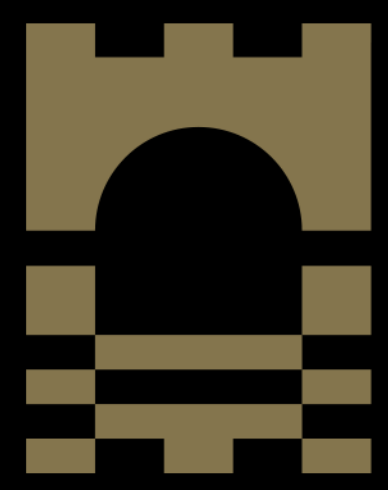
Monitoring of Fn in cells + chitosan-based scaffold

Conclusion

Functionalised AuTSNPs performance as Fibronectin (Fn) biosensors in the presence of ECM-mimicking bone regeneration scaffolds was demonstrated. The specificity of the Fn monitoring was confirmed through spectral monitoring denatured and active protein where it was successfully demonstrated that gold edge-coated triangular silver nanoparticles are powerful tools for non-labelling measurements for biomolecule dynamics in high background noise environments such as MC3T3 and C2C12 cell lines. The remarkable sensitivity of AuTSNPs enables their capability to interact with and sense tissue molecular signalling and hence can provide extraordinary possibilities for the development and progression of regenerative medicine.

References

[1] A. Bianco and G. Bianco, "Proteins," in *Medical Biochemistry*, Elsevier Inc., 2017, pp. 21-71. [2] V. Pollock, "Proteins," *J. Pharm. Comp. Pharmacol. Ref.*, no. 1992, pp. 1-11, 2007. [3] S. Nachimuthu and R. Ponnusamy, "Introduction to proteomics," in *Concepts and Techniques in Genomics and Proteomics*, vol. 367, Woodhead Publishing Limited, 2007, pp. 147-158. [4] B. Alberts, J. Lewis, M. Raff, K. Roberts, and P. Walter, "Protein Function," in *Molecular Biology of the Cell*, 4th ed., Garland Science, 2002, pp. 1-19. [5] B. Moore, K. Connell, R. B. Mortensen, C. T. Liu, S. J. Benkovic, and J. Salafsky, "Protein Conformational Changes Are Detected and Resolved Site Specifically by Second-Harmonic Generation," *Biophys. J.*, vol. 109, no. 4, pp. 806-815, 2015. [6] D. E. Charles et al., "Silver Nanoparticles for Highly Sensitive Plasmon Resonance Sensing," *ACS Nano*, vol. 4, no. 1, pp. 55-64, 2010. [7] N. Ostrowska, M. Feig, and J. Trylska, "Modeling Crowded Environment in Molecular Simulations," *Front. Mol. Biosci.*, vol. 6, no. September, pp. 1-6, 2019. [8] M. Feig, J. Yu, P. H. Wang, G. Nawroski, and Y. Sagita, "Crowding in Cellular Environments at an Atomic Level from Computer Simulations," *J. Phys. Chem. B*, vol. 121, no. 24, pp. 8009-8025, 2017. [9] H. Lu, W. Wu, J. An, D. Li, and B. Zhao, "A simple method to synthesize triangular silver nanoparticles by light irradiation," *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 64, no. 4, pp. 956-960, 2006. [10] Y. Zhang et al., "Wash-free highly sensitive detection of reactive protein using gold decorated triangular silver nanoparticles," *ACS Adv.*, vol. 4, no. 35, pp. 29022-29031, 2014. [11] K. A. Willets and R. P. Van Duyne, "Localized surface plasmon resonance spectroscopy and sensing," *Annu. Rev. Phys. Chem.*, vol. 58, pp. 267-297, 2007. [12] J. L. Lohmeijer, V. Aulin, G. Botel-Audien, M. Lam, M. Salama, and S. Boudry, "Silver-based plasmonic nanoparticles for and their use in biosensing," *Biosensors*, vol. 9, no. 2, 2019. [13] B. Petyayeva and U. J. Kroll, "Localized surface plasmon resonance: Nanostructures, biosensors and biosensing a review," *Anal. Chim. Acta*, vol. 706, no. 1, pp. 8-24, 2011. [14] D. A. Svyatoyukh, H. E. M. Mewhorst, and P. W. M. Fedak, "Using Acellular Bioactive Extracellular Matrix Scaffolds to Enhance Endogenous Cardiac Repair," *Front. Cardiovasc. Med.*, vol. 5, no. April, pp. 1-8, 2018. [15] T. G. Kim, H. Shin, and D. W. Lim, "Biomimetic scaffolds for tissue engineering," *Adv. Funct. Mater.*, vol. 22, no. 12, pp. 2446-2468, 2012. [16] R. Dorri et al., "Biodegradable scaffolds for bone regeneration combined with drug-delivery systems in osteomyelitis therapy," *Pharmaceutics*, vol. 10, no. 4, 2017. [17] V. Amaral, V. Kokol, K. Boltes, P. Leticia, and R. Rossi, "Incorporation of antimicrobial peptides on electrospun nanofibers for biomedical applications," *ACS Adv.*, vol. 8, no. 49, pp. 28013-28023, 2018. [18] F. Zhang and M. W. King, "Biodegradable Polymers as the Pivotal Player in the Design of Tissue Engineering Scaffolds," *Adv. Healthc. Mater.*, vol. 9, no. 13, pp. 1-22, 2020. [19] M. E. Brennan-Fournet, M. Huerta, Y. Zhang, G. Malliaras, and R. M. Owens, "Detection of fibronectin conformational changes in the extracellular matrix of live cells using plasmonic nanoparticles," *J. Mater. Chem. B*, vol. 3, p. 1, 2015. [20] M. Devine, E. Hector, J. S. Hayes, S. Sheehan, and C. H. Evans, "Extended release of proteins following encapsulation in hydroxyapatite/chitosan composite scaffolds for bone tissue engineering applications," *Mater. Sci. Eng. C*, no. November, pp. 1-9, 2017. [21] E. M. Ahmed, "Hydrogel: Preparation, characterization and applications: A review," *Adv. Res.*, vol. 6, no. 2, pp. 105-121, 2015. [22] K. Maji, S. Dasgupta, K. Pramanik, and A. Bissoy, "Preparation and Evaluation of Gelatin-Chitosan-Nanobioglass 3D Porous Scaffold for Bone Tissue Engineering," *Int. J. Biomed.*, vol. 2015, 2016. [23] S. Grabow-Zelinska, A. Sankowska, K. Kuczyńska, and E. Pamiul, "Physico-chemical characterization and biological tests of collagen/fibronectin/chitosan scaffolds cross-linked by dialdehyde starch," *Polymers (Basel)*, vol. 12, no. 2, 2020. [24] L. Tao et al., "In vitro and in vivo studies of a gelatin/carboxymethyl chitosan/LAPONITE composite scaffold for bone tissue engineering," *ACS Adv.*, vol. 7, no. 85, pp. 54100-54110, 2017. [25] A. Killion, L. M. Geever, D. M. Devine, J. E. Kennedy, and C. L. Higginbotham, "Mechanical properties and thermal behaviour of PEGDMA hydrogels for potential bone regeneration application," *J. Mech. Behav. Biomed. Mater.*, vol. 4, no. 7, pp. 1219-1227, 2011. [26] H. Maachou, K. E. Bai, Y. Bai, A. Chappes, G. Gote, and D. Allouche, "Characterization and in vitro bioactivity of chitosan/hydroxyapatite composite membrane prepared by freeze-gelation method," *Trends Biomed. Artif. Organs*, vol. 22, no. 1, pp. 15-24, 2008. [27] B. Cirina-Cimrina and N. Borodajenko, "Research of Calcium Phosphates Using Fourier Transform Infrared Spectroscopy," *Infrared Spectrosc.* - *Mater. Sci. Eng. Technol.*, [28] A. Killion, L. M. Geever, D. M. Devine, H. Farrell, and C. L. Higginbotham, "Compressive strength and bioactivity properties of photopolymerizable hybrid composite hydrogels for bone tissue engineering," *Int. J. Polym. Mater. Polym. Biomater.*, vol. 63, no. 13, pp. 641-650, 2014. [29] S. Kim et al., "Growth and osteogenic differentiation of alveolar human bone marrow-derived mesenchymal stem cells on chitosan/hydroxyapatite composite fabric," *J. Biomed. Mater. Res - Part A*, vol. 103, no. 6, pp. 1550-1558, 2013. [30] S. Kargazi, M. Mozafari, S. Hamzeshou, and P. B. Milan, "Bone Tissue Engineering Using Human Cells: A Comprehensive Review on Recent Trends, Current Prospects, and Regeneration Applications," *Appl. Sci.*, vol. 9, no. 174, pp. 1-49, 2019. [31] M. Anta, G. Baneyk, E. Kubow, and V. V. Abstrakt, "Fibronectin in aging extracellular matrix fibrils is progressively unfolded by cells and elicits an enhanced rigidity response," *Nih Public Access*, vol. 139, no. 1, pp. 229-220, 2008. [32] P. Singh, C. Carragher, and J. S. Schwarzbauer, "Assembly of fibronectin extracellular matrix," *Annu. Rev. Cell Dev. Biol.*, vol. 26, pp. 393-410, 2010. [33] B. Bhatia, A. Mittal, and D. E. Kiskal, "Antimicrobial potential and in vitro cytotoxicity study of poly(vinyl pyrrolidone)-stabilised silver nanoparticles synthesised from *Spirinibacillus boroniensis*," *ET Nanobiotechnology*, vol. 15, no. 4, pp. 427-440, 2021. [34] M. Hashimoto et al., "Microtopographical cellular responses of MC3T3-E1 and RAW264.7 after exposure to water-dispersible silver nanoparticles stabilized by metal-carbon bonds," *Dent. Mater. J.*, vol. 32, no. 5, pp. 725-733, 2013. [35] H. Y. Zhang, T. Ding, J. Liu, and H. Zhao, "Multifunctional Gold Nanoparticles: A Novel Nanomaterial for Various Medical Applications and Biological Activities," *Front. Bioeng. Biotechnol.*, vol. 8, no. August, pp. 1-17, 2020.



TUS

Technological University of the Shannon: Midlands Midwest
Ollscoil Teicneolaíochta na Sionainne: Lár Tíre Iarthar Láir

TUS Research