Efficient bone substitutes and osteogenic biosensors: an integrated approach to regenerative medicine

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Abstract

Bone regeneration poses a significant clinical challenge, as conventional grafting techniques are limited by donor availability, risk of rejection, and poor integration. In this context, biomimetic scaffolds, which replicate the hierarchical structure and anisotropy of native extracellular matrix, are the focus of advanced tissue engineering, combining additive manufacturing techniques with natural polymers [1]. Additionally, recent research highlights the significance of graphene and nanofiber reinforcement for improved mechanical performance and osteogenic stimulation [2, 3, 4]. Conversely, the complementary electrochemical biosensing platforms allow for the sensitive detection of osteogenic biomarkers, linking bone substitutes to diagnostic monitoring [5]. These techniques serve as examples of how multicomponent biomaterials and bioelectronic tools hold the promise to convert bone tissue engineering (BTE) into a truly personalized regenerative solution.

In this regard, we offer a solution based on electrospun chitosan/polyethylene oxide nanofibers (NFs) reinforced 3D-printed scaffolds composed of gellan gum (GG) and cellulose nanofibrils (CNFs). After assessing the rheological characteristics and the printability of the base ink, as well as the scaffold morphology, we found the optimum GG/CNF weight ratio to be 3/1. The resulting formulations were then assessed once more for rheology and printability after different concentrations of NFs (0.5, 1, 2, and 4 wt%) were added to the base ink. Scanning electron microscopy (SEM) and micro-computed tomography analyses of the printed scaffolds revealed a well-organized internal architecture with interconnected pores and enhanced volumetric fidelity, particularly for 2% NFs addition (GCN-2.0). While nanomechanical indentation demonstrated an increase in storage modulus relative to control and mechanical anisotropy as a result of filament orientation and NFs alignment within the printed structures, structural characterization verified the successful integration of NFs within the polymeric matrix. *In vitro* swelling and degradation studies demonstrated an increase in water uptake capacity and long-term stability for GCN-2.0. Biological evaluation revealed good cytocompatibility with MG-63 cells for all samples and increased mineralization in the scaffolds with higher NFs content. In summary, the findings show that 2% NFs reinforcement represents an appropriate balance between printability, nanomechanical characteristics, and biological response, suggesting that this hybrid formulation has high potential for successful application in BTE.

Complementary to bone substitutes, we also propose an electrochemical approach for the detection of osteogenic processes associated with the activity of alkaline phosphatase (ALP) and Runt-related transcription factor 2 (RUNX2) biomarkers. This technique involved the modification of screen printed carbon electrodes (SPCEs) with electrochemically reduced graphene oxide (RGO), which were characterized by cyclic voltammetry and electrochemical impedance spectroscopy after each stage, revealing the proper electrochemical properties. Additionally, contact angle measurements, Raman spectroscopy, X-ray photoelectron spectroscopy, and SEM results confirmed the successful modification of the electrodes. Physical adsorption was used to immobilize the specific bioreceptors on the SPCEs, as demonstrated by contact angle measurements, structural characterization, and electrochemical techniques. Moreover, the detection method involved monitoring changes in the electrochemical signal following the hybridization of the target molecules with the bioreceptors. According to our findings, the pilot platform can detect both biomarkers up to 1 nM, with RUNX2 exhibiting higher sensitivity. This suggests that, with additional optimizations, the RGO-based platform holds considerable promise as an osteogenic biosensor in the future.

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References

[1] Dobrisan, M.R., Lungu, A., Ionita, M., “A review of the current state of the art in gelatin methacryloyl-based printing inks in bone tissue engineering”. Virtual and Physical Prototyping, 19.1 (2024), p.e2378003.

[2] Cojocaru, E., Oprea, M., Vlăsceanu, G.M., Nicolae, M.C., Popescu, R.C., Mereuţă, P.E., Toader, A.G., Ioniţă, M., “Dual nanofiber and graphene reinforcement of 3D printed biomimetic supports for bone tissue repair”. RSC advances, 14.44 (2024), pp.32517-32532.

[3] Dobrisan, M.R., Toader, A.G., Chiticaru, E.A., Vlasceanu, G.M., Popescu, R.C., Nicoara, A.I., Podaru, A.I., Toader, G., Rotariu, T., Ionita, M., “Nanostructured gelatin-gellan inks double reinforced with cellulose nanofibrils and graphene derivatives for 3D printing in bone regeneration applications”. Materials Today Advances, 28 (2025), p.100621.

[4] Chiticaru, E.A. and Ioniță, M., “Commercially available bioinks and state-of-the-art lab-made formulations for bone tissue engineering: a comprehensive review”. Materials Today Bio, 29 (2024), p.101341.

[5] Chiticaru, E.A. and Ioniță, M., “A Novel Approach Using Reduced Graphene Oxide for the Detection of ALP and RUNX2 Osteogenic Biomarkers”. Current Issues in Molecular Biology, 46.5 (2024), pp.4489-4505.

Figures

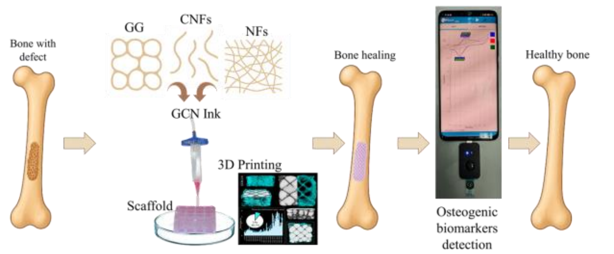

Figure 1. Integrated strategy for bone regeneration: 3D-printed substitutes coupled with point-of-care monitoring.