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DEVELOPMENT OF COMPOSITE GELATINE-BASED 3D MEMBRANE FOR PERIODONTITIS TREATMENT

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1 INTRODUCTION

Periodontal disease is currently a major public health challenge worldwide, with around 62% of the global population affected by moderate stages of the disease, and approximately 23.6% suffering from its most severe form [1]. This condition is characterised by chronic inflammation and progressive degradation of the periodontium, often associated with bacterial biofilms [1]. Developing effective strategies for managing this disease is essential, as untreated cases have been linked to serious oral and systemic health complications, including an increased risk of cardiovascular diseases, diabetes, and other chronic conditions [1]. This work proposes the development of a personalised hydrogel membrane using 3D printing technology, as the delivery for a nanoparticle-based therapy. The biomaterial chosen to incorporate in the hydrogels was zinc oxide nanoparticles (ZnO NPs), obtained by green synthesis from *Glycyrrhiza glabra* extract. This root has been used for centuries in traditional Chinese medicine because of its active compounds anti-inflammatory and antibacterial properties. [2]. As for the ZnO, they stand out as antibacterial biomaterials due to their remarkable oxidative stress activity. ZnO NPs have the downside of being potentially harmful to healthy cells when in higher concentrations in the organism, however, green synthesis has been proven to reduce the toxicity level [2]. 3D printing of hydrogels has gained increasing interest due to the membrane's unique flexibility, and its resemblance with the characteristics of various soft tissues. Additionally, its ability to personalisation allows for precise control over a wide range of physical and biological properties, while offering the ability to regulate porosity providing an ideal platform for promoting cell proliferation and survival, making 3D printing particularly valuable in biomedical applications [3].

2 METHODOLOGIES

2.1 GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

To prepare the *Glycyrrhiza glabra* extract, the root was stirred and heated at 60 °C in distilled water for 10 min. The pH of the plant extract was 5.8. The extract was filtered and centrifuged, and Zn $(NO_3)_2.6H_2O$ was added, followed by KOH, while the solution was stirred, to induce precipitation. This solution was centrifuged and the pellet dried and deagglomerated, with a yield of 44.8%.

2.2 COMPOSITE GELATINE-BASED HYDROGEL PRODUCTION

Two gelatine-based hydrogel formulations were developed. The control formulation, Gel, consisted of 40% gelatine, 10% glycerine, 10% sucrose, and 40% H_2O. The second formulation, Gel_ZnO, modified the H_2O content to 37.5% and added 2.5% ZnO NPs. Once all the components were combined, the

hydrogels were in the thermostatic bath for 1 hour, allowing gelation to occur, resulting in a semi-solid or gel-like consistency. The hydrogel was then transferred to a refrigerator for 24 hours at 4ºC to stabilise further the crosslinking between gelatin chains. To evaluate the degradation time of the hydrogels, two phosphate-buffered saline (PBS) solutions were prepared, one to mimic infection at pH 5.6, and the other to represent a healthy organism, at pH 7.4. FTIR analysis of the hydrogels was also obtained, to verify the composition of the chemical bonds created.

2.3 3-D BIOPRINTING OF A PERSONALISED MEMBRANE

The hydrogels were printed using the Allevi 2 bioprinter, which offers numerous advantages for tissue engineering applications. Its extrusion-based approach allows for precise placement of bioinks, enabling the creation of accurate 3D structures. By controlling the pneumatic force and temperature during the extrusion process, the printer ensures consistent material deposition, minimising errors and optimising the structural integrity of the final construct. To simulate the extracellular matrix of the target tissues, the 3D models used to test the hydrogel and composite hydrogel printing parameters were designed to have porosity. This was achieved by printing the layers at varying angles of 45º, 90º, and 135º. Visual inspection of the extruded filaments ensured the desired outcome of continuous and uniform strands, with the pressure set at 25 PSI for all tests, as this was previously determined to provide optimal extrusion performance in gelatine-based hydrogels.

3 RESULTS AND CONCLUSION

The synthesised ZnO NPs exhibited a two-dimensional amorphous structure with a tendency to cluster and fold, with an average size of 100 nm in the dimensional sides as observed through TEM and SAED analyses, being only nanometric in one dimension. Despite the challenges, both hydrogels were successfully printed into 3-layer structures with the desired porosity. The printed membranes demonstrated uniform filament extrusion, confirming that incorporating ZnO NPs did not compromise the hydrogel's printability. This is crucial for creating personalised, patient-specific membranes for periodontitis treatment. In addition, degradation tests were conducted at 25°C, room temperature, and 37°C, the temperature of the human body. Among the hydrogels, Gel_ZnO demonstrated the highest absorption capacity and the lowest degradation rate, being capable of resisting degradation at body temperatures for a few hours, it is crucial to address to improve this time in future investigations, and possible solutions may involve allowing the hydrogels to dry for several days to enhance molecular bonding or enhancing the formulation by incorporating components that confer temperature resistance. The FTIR analysis of the hydrogels showed the amide I, II, and III bands, characteristic of the structure of a gelatine-based hydrogel [4]. In conclusion, the successful 3D printing of personalised hydrogel membranes opens new avenues for patient-specific periodontal treatments, reducing reliance on invasive procedures and antibiotics, and addressing a significant global health challenge.

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