Abstract

This study presents an integrated approach of advanced biomedical applications through the exploration of single nucleotide polymorphisms (SNPs) within the GSK-3β protein and its effect on wound healing, the development of biopolymer-based materials for wound healing, and the utilization of winery waste-derived grape seed extract (GSE) for its anti-inflammatory and wound healing properties. By intertwining genetic analysis with material science and bioactive compound optimization, the research offers a cohesive methodology to tackle complex biomedical challenges.

The first objective focused on GSK-3β, a critical serine/threonine kinase with diverse cellular functions. However, there is limited understanding of the impact of non-synonymous single nucleotide polymorphisms (nsSNPs) on its structure and function. Through an exhaustive in-silico investigation 12 harmful nsSNPs were predicted from a pool of 172 acquired from the NCBI dbSNP database using 12 established tools that detects deleterious SNPs. Consistently, these nsSNPs were discovered in locations with high levels of conservation. Notably, the three harmful nsSNPs F67C, A83T, and T138I were situated in the active/binding site of GSK-3β, which may affect the protein's capacity to bind to substrates and other proteins. Molecular dynamics simulations revealed that the F67C and T138I mutants had stable structures, indicating rigidness, whereas the A83T mutant was unstable. Analysis of secondary structures revealed different modifications in all mutant forms, which may affect the stability, functioning, and interactions of the protein. These mutations appear to alter the structural dynamics of GSK-3β, which may have functional ramifications, such as the formation of novel secondary structures and variations in coil-to-helix transitions. In conclusion, this study illuminates the possible structural and functional ramifications of these GSK-3 nsSNPs, revealing how protein compactness, stiffness, and interactions may affect biological activities. Thus, various possibilities might cause a non-healing wound. In the following work, we will try to study how to achieve better and faster wound healing using various therapeutics. We also aim to create therapeutics agents which would control the inflammatory cytokines after the onset of the wound and also promote angiogenesis and neovascularization thus nullifying the effect of dysregulated GSK-3β.

The second objective was the preparation of starch-gelatin composite mats incorporating grape seed extract (GSE). The composite mats were created by heating potato starch and gelatin, followed by the addition of grape seed extract and ethanol to form a polymer lump, which was

then pressed into a film. Material characterization included FTIR, AFM, SEM, mechanical testing, swelling studies, in-vitro degradation, thermal analysis, and contact angle measurements. The biological evaluation involved culturing L929 mouse fibroblast cells on the mats, assessing cell viability with the MTT assay, and hemocompatibility using a hemolysis test. In-vivo biocompatibility was tested by implanting mats in Wistar rats and conducting histological examination after 14 days. Results indicated that a balanced ratio of starch and gelatin (1:1) produced mats with optimal properties, including enhanced mechanical strength, smooth surface morphology, high swelling capacity, rapid degradation, and excellent biocompatibility and hemocompatibility. These composite mats serve as a promising platform for integrating bioactive compounds such as GSE, leveraging their properties for enhanced wound healing applications. However the material developed was not mechanically very stable and was of brittle nature which is not mostly suitable for wound dressing material.

In the third objective, the study delved into optimizing the extraction of proanthocyanidins from grape seeds and evaluating their biological effects. Grape seeds were cleaned, dried, crushed, and extracted using ethanol gradients, followed by cold maceration and centrifugation. UV-VIS spectroscopy, FTIR, and LCMS/MS characterized the extract, revealing high proanthocyanidin and total phenolic content. In-vitro assays on L929 cells demonstrated high cell viability and significant cell migration at optimal concentrations of GSE. Anti-inflammatory and antioxidant properties were confirmed through reduced expression of NF-κB, IL-6, TNF-alpha, and ROS levels in LPS-stimulated RAW cells. A GSE-loaded starch-glycerite gel was formulated and characterized, showing significant wound healing potential in rabbit models, enhanced collagen deposition, and improved epidermal regeneration. This objective highlighted the therapeutic potential of GSE, derived from winery waste, as a potent bioactive compound for wound healing applications.

The fourth objective connected these findings by developing and characterizing PVA-starch composite films loaded with GSE for wound healing applications. The GSE not only acted as an active component but also participated as a crosslinking agent by inducing hydrogen bonds between polymers. Three composites with varying PVA and starch ratios were fabricated using solvent casting, followed by material characterization through FTIR, XRD, SEM, AFM, thermal analysis, mechanical testing, contact angle measurements, nano-indentation, swelling index, and degradation studies. Antibacterial efficacy against Staphylococcus aureus and Escherichia coli, hemocompatibility, cytocompatibility, and in-vivo wound healing in rabbits were assessed. The PS@2:1 composite exhibited superior properties, including antibacterial activity, biocompatibility, and enhanced wound healing, with significant collagen deposition

and minimal inflammation in treated wounds. This final objective underscored the successful integration of genetic insights, material science, and bioactive compound optimization, resulting in a holistic approach to developing advanced wound healing materials.

In brief, this comprehensive study successfully identified deleterious SNPs within the GSK-3β gene, developed effective starch-gelatin composite mats, optimized grape seed extract for anti-inflammatory and wound healing applications, and fabricated PVA-starch composites with promising wound healing properties and overcame the limitations of the starch-gelatin mats. The therapies hence developed controlled inflammation and also promoted angiogenesis which is a common problem during dysregulation of GSK-3β. These therapies and wound dressing thus have a high potential in nullifying the adverse effect of deleterious mutation of GSK-3β related to wound healing. These interconnected objectives provide valuable insights into SNP analysis, biopolymer-based wound healing materials, and the utilization of winery waste-derived extracts, offering potential avenues for innovative therapeutic approaches in biomedical applications.