

Abstract

Uncontrolled haemorrhage remains a critical challenge in wartime and civilian trauma care emergencies, necessitating the development of effective haemostatic materials to reduce rebleeding during patient transportation. The unmet clinical need has led to exploring drug loaded haemostat dressings, providing mechanical haemostasis and drug delivery to the bleeding site to stabilize clots. It is desirable to employ sustainable biomaterials for fabrication of the patches. This thesis specifies that such biomaterials were successfully produced and evaluated in different forms: nanofiber sheets, freeze-dried sponges etc. Simultaneously, oyster shell waste presents an emerging ecological concern. Also, this research explores the development of novel haemostatic biomaterials by repurposing oyster shell waste as a functional component in bleeding control applications. Various formulations were investigated, including: calcium-enriched chitosan nanofibers derived from *Magallana cuttackensis* shells marine shell waste nanofiber (MSWNF); dual-drug loaded polyvinyl alcohol/chitosan nanofibers incorporating tranexamic acid and ethamsylate (PVA/CS/TXA-E); chitosan sponges reinforced with oyster shell powder (PVA/CS/MSW-O); and haemostatic films combining polyvinyl alcohol, chitosan, and oyster shell powder.

Comprehensive characterization using XRD, EDX, XPS, Raman spectroscopy, FTIR, and SEM confirmed the presence of calcium compounds (CaO , Ca(OH)_2 , CaCO_3) from the marine shell waste. Calcined MSW at 1200°C (MSW-T) showed a large zone of inhibition surrounded by it and had an excellent antibacterial activity against *Staphylococcus Aureus*. In vitro assays demonstrated significant improvements in haemostatic performance across all formulations, with reductions in clotting time (CT), prothrombin time (PT), activated partial thromboplastin time (aPTT), and plasma recalcification time (PRT). The in vitro clotting time (CT) under (PVA/CS/TXA-E) and MSWNF nanofibers were 230 ± 11 s and 168 ± 4 s, which, along with other parameters- prothrombin times (27 ± 0.4 s and 22 ± 0.6 s), and plasma recalcification time (76 ± 4 s and 47 ± 1 s), indicated enhancement in hemostasis performance by MSWNF over (PVA/CS/TXA-E). The PVA/CS/TXA-E nanofiber patches comprised fiber strands with a 347 ± 22 nm average diameter and showed a swelling ratio of 459%. The nanofiber possessed intermittent hydrophilicity (water contact angle 32). Drug release through the nanofiber followed a non-Fickian diffusion model. Dual-drug loaded nanofibers showed a decrease in the clotting time by 24%, while activated partial thromboplastin time (aPTT), Prothrombin time (PT), and Platelet recalcification time (PRT) decreased by 6%, 20% & 15% over the single-drug

loaded nanofibers. The PVA/CS/MSW-O sponge demonstrated superior performance in controlling both oozing and pressurized bleeding.

In vivo studies using rabbit models verified enhanced haemostatic efficacy, with MSWNF showing a 22% improvement in haemorrhage control. After evaluation in the rabbit incision model, the bleeding time of the control group (274 ± 6 s) was longer than (PVA/CS/TXA-E) (97 ± 8 s) or MSWNF (75 ± 5 s). Blood oozing until haemostasis was 0.380 g, 0.354 g, and 0.121 g under control gauge, (PVA/CS/TXA-E), and MSWNF, respectively, demonstrating enhancement in haemostasis performance by MSWNF. Histopathological examination confirmed biocompatibility and wound healing progression, evidenced by epithelialization, capillary formation, and granulation tissue development after 7 days of scaffold application. Gene expression analysis in RT-PCR revealed upregulation of key wound healing markers (VEGF-A, PDGF, IGF-1).

These findings demonstrate that natural biomaterials can be effectively employed to enhance haemostatic efficacy. Calcium-based compounds extracted from marine waste products substantially boost the ability of different biomaterial formulations to stop bleeding, offering promising, sustainable, and cost-effective alternatives for managing severe haemorrhage while addressing ecological concerns associated with oyster shell waste. The novel utilization of *Magallana Cuttackensis* shells, a specific marine ecological waste material, as a high-value functional biomaterial for biogenic calcium represents a significant and innovative contribution that effectively bridges the fields of biomaterials science, hemostasis management, and sustainable bioeconomy.