Chapter-5

Conclusions and future scope of work

The primary objective of this thesis is to explore the potential applications of Luffa cylindrica (LC) and to investigate the physico-chemical and mechanical characteristics of scaffolds fabricated using advanced technologies for biomedical and environmental applications. LC exhibits remarkable attributes, including high mechanical strength, cost-effectiveness, eco-friendly, abundant availability, hydrophilicity and good oleophilic properties; rendering it suitable for various therapeutic applications. Nutritional investigations have revealed numerous medicinal properties associated with LC. The utilization of LC as a reinforced composite material in diverse fields such as packaging, automobiles, water treatment, in vitro studies and medicinal uses can be facilitated through surface modification. The performance analysis of LC encompasses a broad range of applications owing to its minimal environmental impact, biodegradability, biocompatibility, renewability and stability. Accordingly, to achieve a few aforementioned objectives, this thesis presents the development and fabrication of numerous three-dimensional scaffolds using the freeze-drying and hand lay-up approaches. In the first chapter, we explore the fundamental distribution patterns of luffa worldwide. By identifying these basic geographic locations, significant information into luffa's natural habitat and distribution patterns of luffa can be obtained. In addition, this section explores the advantages and limitations of employing natural and synthetic materials. We explore numerous applications of luffa and the benefits it brings to a wide range of industries. This chapter thoroughly investigates the potential benefits of luffa, encompassing its medical uses to its adaptability in the industrial sector. However, we also address the limitations and difficulties associated with using luffa. We expedite to comprehend the full scope of luffa and its potential effects on a diverse array of economic sectors and ecological systems. Additionally, we have explored the concept of 2D and 3D architecture, scaffold fabrication techniques, and their advantages and limitations. We anticipate that our efforts will contribute to advancing the research and application of luffa worldwide.

In chapter two of the thesis, porous composite scaffolds based on luffa were created by employing the freeze-drying method, incorporating LC, HA, PH and G in various combinations (w/v) i.e. 3% LC, 5% LC and control (C) (without luffa powder). The structural stability of the scaffolds was achieved through chemical crosslinking using glutaraldehyde (GTA). These scaffolds were subjected to comprehensive in vitro characterization. Morphological analysis of the freeze-dried scaffolds using scanning electron microscopy (SEM) revealed a pore size ranging from 20 to 240 μm. Degradation studies suggested that the degradation rate of the scaffolds could be controlled by adjusting the LC ratio based on specific application requirements. The scaffolds were found to be noticeably stable for 50 days at 37℃ in a lysozyme solution. Cellular biocompatibility of the microporous scaffolds was evaluated through the MTT assay, which demonstrated the favorable viability of mammalian cells. A qualitative cell attachment and growth assessment was conducted using SEM and fluorescence microscopy, revealing positive outcomes. Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The hydrophilic behavior of the samples was quantified by water contact angle measurements. As per the obtained data, the apparent and effective porosities were estimated as $57.08 \pm 4.38\%$, $50.58 \pm 4.09\%$, $59.45 \pm 1.60\%$ and $51.37 \pm 3.36\%$, $47.94 \pm 4.57\%$ and $53.09 \pm 5.45\%$ for 3% LC, 5% LC and control (C) scaffolds, respectively. Furthermore, all the luffa-based scaffolds exhibited excellent mechanical stability resembling native bone tissue, and no cytotoxicity was observed. Based on these exceptional characteristics, we anticipate that the microporous luffa-based scaffolds exhibit promising material for a wide range of tissue engineering applications.

The third chapter of the thesis highlights the biocompatibility efficacy of fabricated scaffolds in rat models. Chapter two provides a comprehensive procedure utilized for scaffold fabrication, encompassing the fabrication methods for the 3% LC, 5% LC and Control scaffolds. The results presented in chapter two reveal the compatibility of the scaffold and excellent cellular proliferation. Consequently, in this chapter, we have explored a comprehensive biocompatibility investigation of the scaffolds by implanting them in the subcutaneous tissue of rat models and performed histological studies and analysis of biochemical markers over a duration of 21 days. The histological analysis was investigated in all the groups, indicating that the animal models did not exhibit any signs of inflammation or toxicity, suggesting favorable tissue response to the implanted scaffolds. Initial observations revealed elevated levels of enzymes and biomarkers, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, creatine kinase-MB (CK-MB), and serum creatinine within the experimental groups after a 24-day interval. However, it was observed that these parameters became normalized after a period of 3 weeks following implantation, and there was no further significant increase in these parameters compared to the control groups. The outcomes thus suggested that the implanted luffa-based scaffolds did not induce any adverse effects on the heart, liver, and kidney. Our study demonstrates highly promising results; indicating the excellent in vivo biocompatibility of the luffa-based composite scaffolds. The successful integration of the scaffolds, absence of inflammatory responses, and overall tissue compatibility highlight the immense potential of these luffa-based scaffolds in various tissue engineering and regenerative medicine applications. In addition, the drug release behavior was evaluated by investigating the inhibitory impact of ampicillin incorporated into the scaffolds against the prevalent pathogen E. coli. All the scaffolds exhibited a distinct inhibition zone against E. coli bacterial strains. The 5% and 3% LC, exhibited a larger inhibition zone of 13 mm and 10 mm, respectively, in comparison to the control scaffold (without luffa) and the positive control (ampicillin-loaded Whatman filter paper) when combating the pathogens. This study implies that a controlled release of antibiotics can significantly increase their inhibitory efficacy against bacterial strains by ensuring consistent and localized delivery of therapeutic agents. The pores within the scaffold can function as reservoirs for the antimicrobial agents, facilitating a sustained release over time.

In the next part of the thesis, various forms of luffa-PDMS composite scaffolds were fabricated using a hand lay-up approach. Numerous characterizations were performed, including surface roughness, porosity, surface wettability, oil and PBS absorption, degradation, thermal stability, oil spillage and mechanical properties. The morphological analysis of SEM images showed the internal architecture of luffa; depicting microcapillaries and microporous structures. The P-L flakes scaffold had a high density of luffa microparticles resulting in the highest level of oil absorption. In contrast, the P-L powder appeared as an agglomerated material due to the majority of compromised capillaries. AFM images depicted that the P-L flakes scaffold had the highest surface roughness, a higher surface area-to-volume ratio, higher peak frequencies, and more crests. The surface of P-L powder scaffolds exhibited fewer peaks with fewer peak numbers due to its more tightly packed structure; reducing the microporous architecture. The existence of luffa within scaffolds exhibited exceptional oleophilic properties and PDMS displayed outstanding swelling in organic solvents. The P-L flakes have demonstrated a noticeably higher capacity for absorbing oil, PBS, and a few organic solvents, probably because of the high microcapillary properties of luffa flakes. The oxygen permeability of PDMS can enable continuous gaseous exchange between the water body and the atmosphere. In addition, the hydrophobic characteristics of PDMS enable buoyant forces to overcome the weight of the luffa-PDMS composite membrane and prevent it from sinking. Cell cytotoxicity testing demonstrates the cellular biocompatibility of the luffa-PDMS composite, indicating that it poses no threat to aquatic environments. Luffa fibers are found excellent for absorbing oil and adding PDMS renders them suitable for absorbing crude oil toxic materials. The fabricated scaffolds can be reutilized 10-12 times proficiently. Moreover, luffa has lower production costs than synthetic fibers and cotton; resulting in lower end-product prices. This easy and green synthesis method can produce vast quantities of a multifunctional biomass-based adsorbent material for anti-fouling, self-cleaning, and customized oil-water separation. Thus, the outcomes suggest that luffa-PDMS-based scaffolds might be a desired material for various environmental remediation applications. The utilization of LC in tissue engineering and environmental applications remains relatively unexplored, providing an avenue for further investigation. The findings of this study, along with the knowledge acquired in the development of luffa-based biomaterials, can serve as an underpinning for broader implementation in the realms of hard tissue engineering and environmental applications, based on the unique properties of LC. The results of this thesis demonstrate the compatibility of LC-based scaffolds in both in vitro and in vivo studies, as well as their potential in oil-water separation applications. These findings highlight the versatility and effectiveness of LC-based scaffolds and suggest that they could serve as a viable alternative to synthetic scaffolds commonly employed in diverse applications.

Since membrane wettability to oil and water affects oil removal effectiveness, membranes with higher oleophobicity are needed. In this field, nanoparticles, particularly clay nanoparticles, can regulate the wettability of oil/water separation surfaces. Another obstacle to oil/water separation is cleaning the materials used for oil removal, which must be economically viable to reuse the oil and materials. Recent discoveries and the importance of water/oil separation to the environment suggest that oleophobic materials will become increasingly popular. New natural materials with improved oil-water separation are promising research areas. Researchers are investigating using cellulose, chitosan, and lignin to create new oil-absorbing materials. These materials could make better, more sustainable oil-water separation filters and membranes. In-situ oil spill repair using natural materials is another potential field of research. In-situ remediation cleans up oil spills without removing soil or water. Microbes, enzymes, and plant-derived biosorbents could be employed to generate more effective and environmentally benign in-situ remediation approaches. New technologies to remove heavy metals, herbicides, and medications from the environment could also be developed using natural materials. It could improve water quality and protect ecosystems.

The future prospects in this research domain involve the fabrication of scaffolds using advanced techniques, enabling the development of 3D constructs using LC-based bioink. This approach holds immense potential for fabricating tissue-engineered structures. In addition, exploring the drug delivery potential of LC entails investigating its ability to encapsulate and release bioactive molecules to enhance tissue regeneration and promote specific cell behaviours. Long-term studies are considered to assess LC-based scaffolds' stability, biocompatibility and tissue regeneration capabilities. These studies should evaluate the scaffold's performance over an extended period to ensure its suitability for clinical applications. Furthermore, conducting animal studies by implanting LC-based scaffolds in appropriate animal models, such as rats or rabbits, is essential for the evaluation of tissue integration, host response, tissue formation, vascularization, biocompatibility and scaffold degradation over a specified period.