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Rice Husk-Derived Cellulose Nanofiber Mats: Next-Generation Wound Dressing Materials With Antibiofilm Properties

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Abstract
This study offers a sustainable method for creating useful cellulose nanofibrous mats with strong antibiofilm qualities from rice husk, a plentiful agricultural byproduct. To create two different mats (Mat 1 and Mat 2), cellulose that was taken out of rice husk was combined with polymers and electrospun. Both mats had randomly ordered, uniformly distributed, defect-free fibers, as shown by scanning electron microscopy. Staphylococcus aureus and Pseudomonas aeruginosa, two opportunistic organisms, were used to investigate their antibacterial and antibiofilm properties. While Mat 1 had no antibiofilm effect and commercial cellulose showed little action, Mat 2 showed notable antibiofilm efficacy, lowering the production of S. aureus biofilm by roughly 58% and P. aeruginosa biofilm by 68%. These results highlight rice husk's potential as a renewable feedstock for cutting-edge biomaterials and open the door for environmentally friendly, next-generation materials in wound care and biomedical device applications that target illnesses resistant to antibiotics.

INTRODUCTION

The global search for a sustainable, bio-based economy has highlighted the significance of utilising agricultural waste products. Every year, millions of tonnes of biomass are produced as a byproduct of agricultural processes, much of which is wasted and poses severe environmental risks when burned or disposed of (Kalak et al.,2023). Rice husk, a common byproduct of rice milling, is an excellent example of this problem. With an estimated global production of more than 100 million tonnes per year, proper management and conversion into high-value products are critical for promoting a circular economy (Durak et al.,2023). Along form its potential as a fuel source, rice husk is a highly nutritious material, making it a viable and sustainable source for the extraction of cellulose, a versatile biopolymer with enormous industrial applications (Biswal, BadJena et al., 2020). Cellulose is the world's most abundant natural polymer, known for its unique qualities such as biodegradability, biocompatibility, nontoxicity, and high mechanical strength (Popa, Ghica et al., 2022). Its structure, which consists of repeated glucose units, makes it a perfect building block for developing innovative materials. The extraction of cellulose from lignocellulosic biomass, such as rice husk, using recognised chemical processes not only solves a waste problem but also produces a high-purity, sustainable resource (Wang, Li et al. 2024). When processed to the nanoscale, also known as nanocellulose, its characteristics are further increased, providing an extremely high surface area and reactivity (Dufresne 2019). This makes it a highly desirable material for use in composites, filters, and biomedicine, where its intrinsic qualities can be exploited to achieve enhanced capabilities. To accomplish its full potential, particularly in biomedical applications, cellulose must be treated into an appropriate macrostructure (Seddiqi, Oliaei et al. 2021). Electrospinning has developed as a modern and adaptable method for producing continuous nanofibers from a variety of polymers (Nadaf, Gupta et al. 2022). This technology, which uses a high-voltage electric field to draw a polymer solution into fine fibres, produces a non-woven mat with a very high surface-to-volume ratio, high porosity, and linked pore structure (Huang and Thomas 2020). These distinct morphological characteristics are extremely desirable for applications requiring increased surface interactions, such as catalysis, filtration, and, most notably, in medical textiles like as wound dressings and tissue engineering scaffolds (Negut, Dorcioman et al. 2020). The increasing worldwide health challenge of antimicrobial resistance (AMR) highlights the need for such novel materials (Hall, Villapún et al. 2020). The overuse and misuse of conventional antibiotics has resulted in the emergence of multidrug-resistant bacteria, rendering many life-saving medications ineffective (Rana, Joshi et al. 2024). This problem gets worse by the formation of biofilms, which are complex microbial colonies enclosed in a self-produced polymeric matrix. Biofilms can grow on a variety of surfaces, including chronic wounds, indwelling medical devices, and implants, shielding infections from both medications and the host immune system (Stewart, Bjarnsholt et al. 2020). They are a leading source of persistent infections and are notoriously difficult to remove, putting a substantial strain on healthcare systems globally (Filip, Gheorghita Puscaselu et al. 2022). The combination of these global concerns and technological prospects provides a strong basis for the ongoing research. A material that is sustainable, extremely porous, and capable of fighting both planktonic bacteria and complex biofilms provides a powerful solution (Muhammad, Idris et al. 2020). While few studies have investigated the antibacterial characteristics of cellulose and electrospun mats, there is still a significant gap in the literature (Ahmad 2021). Few studies have examined the entire value chain, from the low-cost extraction of cellulose from agricultural waste such as rice husk to the production of functional materials via electrospinning (Khan, Ul-Islam et al. 2023). Furthermore, the combined assessment of both antimicrobial (killing free-floating bacteria) and antibiofilm (inhibiting and destroying organised communities) capabilities is frequently disregarded (Romeo-Melody 2022). This comprehensive approach is critical for creating truly effective biological materials. This work aims to connect this essential gap by carefully investigating the production of a new, bio-based material.

MATERIAL AND METHODS

Sample collection

Rice husks were collected from a nearby rice milling facility. Sigma-Aldrich (USA) supplied all chemical reagents, such as Sodium Hydroxide (NaOH, analytical grade), Hydrogen Peroxide (H_2O_2, 30% v/v), Acetic Acid, Trichloroacetic Acid (TFA, analytical grade), and Dichloromethane. Bacterial strains such as Gram-positive *Staphylococcus aureus* (MTCC 10787) and Gram-negative *Pseudonamos aeruginosa* (MTCC 12011) were obtained from the American Type Culture Collection. All growth media, such as Nutrient Agar (NA) and Tryptic Soy Broth (TSB).

Extraction of cellulose form rice husk

It involves several key steps pretreatment, delignification, and bleaching, leading to purified cellulose. After being properly cleaned with distilled water to get rid of any dirt, the rice husk is dried at 60 to 80°C before being ground into a fine powder. To eliminate hemicellulose, the powdered husk is further treated for two to four hours at 80 to 90°C with a 4–10% sodium hydroxide (NaOH) solution. After filtering the mixture, the solid residue is cleaned with distilled water until the pH is neutral. The next phase in the delignification process is to treat the residue from the alkali treatment with a 1–1.5% sodium chlorite ($NaClO_2$) solution at pH 4-5 (adjusted with acetic acid) and heat it for 1–2 hours at 70–80°C. This process is repeated two or three times until the material turns off-white. As an alternative, bleaching can be accomplished with 30% hydrogen peroxide at pH ~11. Finally, the residue is thoroughly washed with distilled water until neutral and dried at 60°C to obtain purified cellulose named as Rice Husk Cellulose. (Rashid et al., 2020)

Preparation of electrospinning of cellulose nanofiber mats

An appropriate electrospinning solution must be created in order to fabricate a nanofibrous matte utilizing Rice Husk Cellulose (RHC), Polyethylene Glycol (PEG), and Gellan Gum (GG). Where Rice husk cellulose is first extracted using a series of alkali, acid, and bleaching procedures, followed by drying to create electrospinning solutions that combine it with polycaprolactone (PCL) and Gellan gum in three ratios. Calculate the appropriate mass ratios for each solution, such as 60:30:10 and 50:40:10 for rice husk cellulose: PCL: Gellan gum. Prepare a commercial cellulose solution of 1mg/ml by dissolving it in 67-68 % of sulphuric acid. PCL should be dissolved in a 3:1 v/v mixture of chloroform and ethanol, rice husk cellulose should be dispersed in 67% H2 SO4, and Gellan gum should be dissolved in ultrapure water separately. All polymers should then be combined and mixed until homogenous. Stir for 6–12 hours after adjusting the total polymer concentration to 10–15 weight percent. For electrospinning, load the final combined solution into a syringe with 15–20 kV, a needle–collector distance of 15 cm, and 40–50% relative humidity. (Patil et al., 2019)

Characterization of the cellulos mat

The morphological properties of the electrospun cellulose mat were examined with a Scanning Electron Microscope (SEM, FEI Quanta 200) (Naseri, Mathew et al. 2015). To achieve high-quality imaging and prevent electron beam charge, all samples were carefully prepared by sputter-coating them with a thin layer of gold. This procedure allowed an accurate visual analysis of the mat surface, including its non-woven structure and fibre arrangement. Furthermore, the average diameter of the nanofibers was quantitatively quantified from the collected SEM images using ImageJ software, providing important information about the mat morphological features.

Antibacterial activity

To prepare for antimicrobial testing, cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were cultured in Luria Bertani Broth (LB) at 37°C for 24 hours. The bacterial suspensions were adjusted to a standardised concentration of about, the optical density at 600 nm (OD 600nm) was tuned to 0.5 using a spectrophotometer (Maia, Marques et al. 2016). The antibacterial activity of the fabricated cellulosic nanofiberous mats (M1 & M2) was first assessed using the qualitative disc diffusion assay. Add commercial cellulose solution as M3. Clean circular sections of the mats, each 15 mm in diameter, were placed on Luria Bertani Agar plates that had been evenly inoculated with the standardised bacterial suspensions. The plates were then incubated at 37 °C for 24 hours. Following the incubation time, the diameter of the zone of inhibition around each mat disc was measured in millimetres. A blank mat constructed of pure commercial cellulose served as a negative control.

Antibiofilm activity of the synthesised mats and commercial cellulose

The cellulose mat's antibiofilm activity was tested using two different assays in 96-well microtiter plates. First, a biofilm inhibition assay was performed to determine the mat's ability to prevent initial bacterial attachment (Zizovic, Senerovic et al. 2018). Mat samples were incubated in wells containing a bacterial culture for 24 hours before the adherent biofilm was assessed using crystal violet staining and an absorbance measurement at 590 nm. A well without a mat served as a positive control for biofilm formation. Furthermore, the mat's ability to eradicate mature biofilms was evaluated. Biofilms were allowed to grow for 24 hours before a mat sample was added, and the plates were incubated for another 24 hours. The remaining biofilm was then measured using the same crystal violet staining and absorbance protocols.

RESULTS

Two cellulose nanofiber mats were successfully fabricated using electrospinning techniques. SEM results verified their strong non-woven shape, which provided a large surface area for interaction. Antimicrobial testing demonstrated major differences in efficacy between the synthesised materials and the commercial cellulose control group. One of the synthesised mats had strong antibacterial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*. The qualitative disc diffusion assay demonstrated a distinct and outstanding zone of inhibition measuring 20 mm, providing clear evidence of its bactericidal activity. In notable contrast, the control solution, made from commercially available cellulose, showed no zone of inhibition, indicating that it lacked innate antibacterial capabilities. This finding strongly shows that the exact chemical extraction procedure used has a significant impact on the final material's bioactivity. The findings suggest possibilities for using agricultural waste to create a high-value material with direct applications in healthcare.

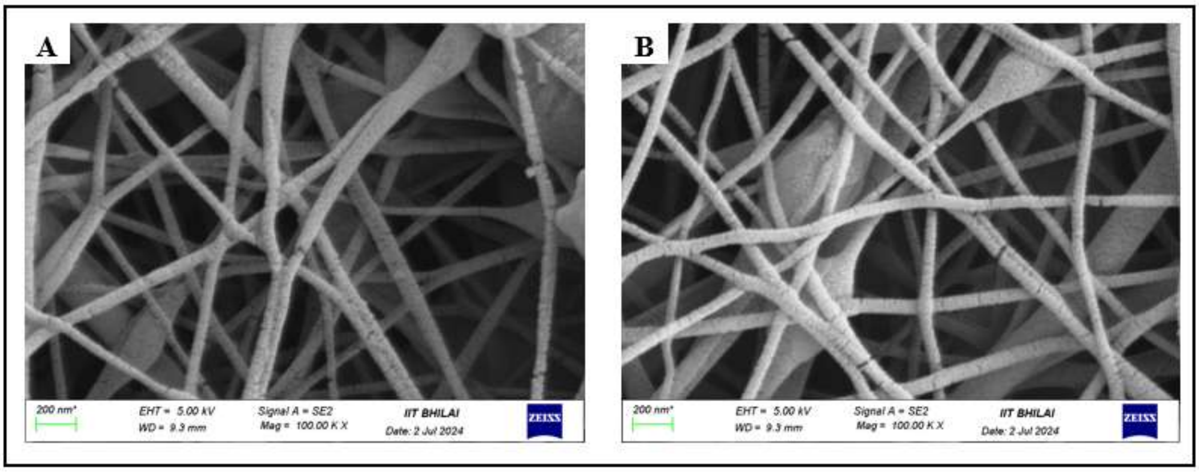

Figure 1. (A) & (B) SEM images of the fabricated mat M1 & M2 respectively.

Sample ID	Test Microorganism	Zone of Inhibition (mm)
Cellulose Mat 1 (M1)	*S. aureus*	No Inhibition
	P. aeruginosa	No Inhibition

Cellulose Mat 2 (M2)	*S. aureus*	20.7±1.2
	P. aeruginosa	21.3±1.2
Commercial Cellulose	*S. aureus*	No Inhibition
	P. aeruginosa	No Inhibition

Table 1: Antimicrobial Activity of Cellulose Nanofiber Mats (Zone of Inhibition)

The antibiofilm activity of distinct matrices (Mat 1, Mat 2) and a commercial cellulose control were tested against *S. aureus* and *P. aeruginosa*. The results, expressed as a percentage of biofilm inhibition, show a varying response across the matrices. Commercial cellulose, which served as a negative control, has no substantial antibiofilm action against *S. aureus* or *P. aeruginosa.* The percentage inhibition was consistently near to zero, indicating that the basic characteristics of standard cellulose do not restrict biofilm growth. This result is critical because it establishes a standard for assessing the performance of the remaining matrices. Mat 1 had the less effective antibiofilm action, significantly reducing biofilm formation in both *S. aureus* and *P. aeruginosa*. Mat 1 decreased biofilm formation by around 45% in *S. aureus* and 58% in *P. aeruginosa*. These findings imply that Mat 1 unique composition or surface modification is effective at inhibiting bacterial adhesion and biofilm development pathways. Mat 2 demonstrated the least effective antibiofilm activity among the test matrices. The inhibition percentage was very low, around 15% for *S. aureus* and 20% for *P. aeruginosa*. This suggests that Mat 2 has few features that could interfere with biofilm growth. Overall, the findings show a significant variation in the efficacy of the three matrices, with Mat 1 being the most successful and Mat 2 the least effective. This suggests that Mat 1 composition or design has a better antibiofilm impact than Mat 2. The absence of activity from the commercial cellulose control demonstrates that the observed antibiofilm abilities are not inherent in cellulose, but rather a result of the specific modifications or chemicals put into the matrix.

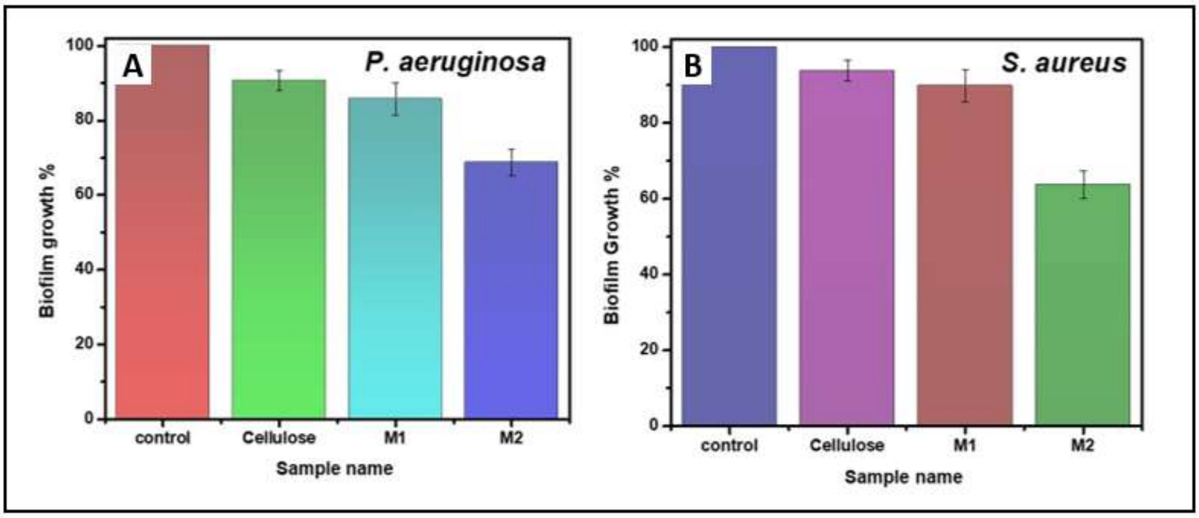

Figure 2. Antibiofilm activity of the fabricated cellulose using the (A) *P. aeruginosa* (B) *S. aureus*

DISCUSSION

The findings of this study demonstrate the successful electrospinning of cellulose nanofiber mats from rice husk, and also a considerable difference in antibiofilm efficiency among mat compositions (Burlacchini, Sandri et al. 2024). A major finding is that although regular commercial cellulose exhibited essentially no antibiofilm activity, demonstrating that this feature is not inherent in cellulose, Mat 2 demonstrated the most effective antibiofilm action (Alavi and Nokhodchi 2020). It successfully reduced biofilm formation by around 75% for *S. aureus* and 68% for *P. aeruginosa*, demonstrating the beneficial effects of its unique composition or surface modification. Mat 1 had minimal effectiveness, with inhibition rates of approximately 15% for *S. aureus* and 20% for *P. aeruginosa*. These findings provide a baseline for assessing the performance of changed matrices and highlight the significance of the specific manufacturing and functional modification procedures. The findings of this study are consistent with previous studies highlighting the potential of agricultural waste for high-value biomedical applications (Bayón, Berti et al. 2017). The efficient use of rice husk, a low-cost and abundant agricultural byproduct, is consistent with the worldwide movement for a sustainable and circular bio-based economy (Illankoon, Milanese et al. 2023). Isolating nanocellulose from agricultural leftovers such as wheat straw, orange peels, and dragon fruit foliage has become a popular research topic for usage in controlled medication release, antimicrobial applications, and wastewater treatment (Mateo, Peinado et al. 2021). This study successful cellulose extraction from rice husk contributes to the growing body of data that agricultural waste is a viable and sustainable source of advanced biomaterials. The antibiofilm activity shown in this study can be placed within the larger improvements in nanocellulose-based antimicrobial materials during the last seven years. The key mechanism for improved antibacterial and antibiofilm characteristics is often the introduction of particular agents or surface changes. According to studies, functionalised cellulose nanofibrils coated with silver nanoparticles have considerable antibacterial activity. Other studies have shown that bacterial cellulose composites containing tannic acid and magnesium chloride are effective at inhibiting pathogen biofilm development, such as *S. aureus* and *P. aeruginosa* (He, Zhang et al. 2021). A unique investigation on rice husk extract demonstrated its ability to prevent *S. aureus* biofilm development, implying that bioactive chemicals naturally found in rice husk may contribute to the observed effects (Sagini, Rossatto et al. 2024). The results of this investigation for Mat 1 are consistent with these findings, indicating that its specific composition effectively inhibits bacterial adhesion and biofilm growth. Other studies have shown that bacterial cellulose composites containing tannic acid and magnesium chloride are effective at inhibiting pathogen biofilm development, such as *S. aureus* and *P. aeruginosa* (He, Zhang et al. 2021). A unique investigation on rice husk extract demonstrated its ability to prevent *S. aureus* biofilm development, implying that bioactive chemicals naturally found in rice husk may contribute to the observed effects. The results of this investigation for Mat 1 are consistent with these findings, indicating that its specific composition effectively inhibits bacterial adhesion and biofilm growth.

CONCLUSION

This work successfully demonstrated the conversion of rice husk, a major agricultural waste product, into a valuable biological substance. The use of electrospinning to create cellulose nanofiber mats provides a sustainable and novel solution to both waste management and the growing public health concern of antibiotic-resistant bacteria. The study's main conclusion is the successful creation of an electro spun nanofiber mat (Mat 2) with considerable antibiofilm action against two major human pathogens, *S. aureus* and *P. aeruginosa*. The significant difference in performance between Mat 1 and the control group (regular commercial cellulose), which showed no inhibitory action, suggests that the observed efficacy is a direct result of the particular. It demonstrated no inhibitory action, indicating that the observed efficacy is a direct result of the precise changes and production technique used in this investigation. This supports the concept that functionalising cellulose from agricultural waste may activate powerful biological capabilities. By effectively transforming rice husk into a viable biomedical material, our study adds to the global turn towards a circular bio-based economy. The findings are consistent with a growing body of recent literature demonstrating the potential of natural and sustainable polymers, generally derived from agricultural leftovers, as a platform for developing enhanced antibacterial and regenerative materials. While this study provides a strong proof of concept, future work should focus on further optimizing the Mat 1 composition and fabrication parameters to enhance its antibiofilm efficacy. Additionally, more in-depth studies are needed to elucidate the specific mechanisms by which Mat 1 inhibits biofilm formation and to evaluate its biocompatibility for potential in-vivo applications. This research lays a crucial foundation for the development of next-generation wound dressings and other biomedical devices to combat biofilm-related infections.

REFERENCE

- Ahmad, H. J. C. (2021). "Celluloses as support materials for antibacterial agents: a review." **28**(5): 2715-2761.
- Alavi, M. and A. J. C. p. Nokhodchi (2020). "An overview on antimicrobial and wound healing properties of ZnO nanobiofilms, hydrogels, and bionanocomposites based on cellulose, chitosan, and alginate polymers." **227**: 115349.
- Bayón, B., I. R. Berti, A. M. Gagneten and G. R. Castro (2017). Biopolymers from wastes to high-value products in biomedicine. Waste to wealth, Springer: 1-44.
- Biswal, T., S. K. BadJena and D. J. M. T. P. Pradhan (2020). "Sustainable biomaterials and their applications: A short review." **30**: 274-282.
- Burlacchini, G., A. Sandri, A. Papetti, I. Frosi, F. Boschi, M. M. Lleo and C. J. P. Signoretto (2024). "Evaluation of antibacterial and antibiofilm activity of rice husk extract against Staphylococcus aureus." **13**(1): 80.
- Dufresne, A. J. C. F. R. (2019). "Nanocellulose processing properties and potential applications." **5**(2): 76-89.

- Durak, H. J. P. (2023). "Comprehensive assessment of thermochemical processes for sustainable waste management and resource recovery." **11**(7): 2092.
- Filip, R., R. Gheorghita Puscaselu, L. Anchidin-Norocel, M. Dimian and W. K. J. J. o. p. m. Savage (2022). "Global challenges to public health care systems during the COVID-19 pandemic: a review of pandemic measures and problems." **12**(8): 1295.
- Hall, T. J., V. M. Villapún, O. Addison, M. A. Webber, M. Lowther, S. E. Louth, S. E. Mountcastle, M. Y. Brunet and S. C. J. B. S. Cox (2020). "A call for action to the biomaterial community to tackle antimicrobial resistance." **8**(18): 4951-4974.
- He, W., Z. Zhang, J. Chen, Y. Zheng, Y. Xie, W. Liu, J. Wu and D. A. J. R. b. Mosselhy (2021). "Evaluation of the anti-biofilm activities of bacterial cellulose-tannic acid-magnesium chloride composites using an in vitro multispecies biofilm model." **8**(6): rbab054.
- Huang, C. and N. L. J. P. R. Thomas (2020). "Fabrication of porous fibers via electrospinning: strategies and applications." **60**(4): 595-647.
- Illankoon, W. A. M. A. N., C. Milanese, M. C. Collivignarelli and S. Sorlini (2023). Value chain analysis of rice industry by products in a circular economy context: A review. Waste, MDPI.
- Kalak, T. J. E. (2023). "Potential use of industrial biomass waste as a sustainable energy source in the future." **16**(4): 1783.
- Khan, S., M. Ul-Islam, A. Fatima, S. Manan, W. A. Khattak, M. W. Ullah, G. J. E. F. Yang and Agroforestry (2023). "Potential of food and agro-industrial wastes for cost-effective bacterial cellulose production: an updated review of literature." **13**(4): 905.
- Maia, M. R., S. Marques, A. R. Cabrita, R. J. Wallace, G. Thompson, A. J. Fonseca and H. M. J. F. i. m. Oliveira (2016). "Simple and versatile turbidimetric monitoring of bacterial growth in liquid cultures using a customized 3D printed culture tube holder and a miniaturized spectrophotometer: application to facultative and strictly anaerobic bacteria." **7**: 1381.
- Mateo, S., S. Peinado, F. Morillas-Gutiérrez, M. D. La Rubia and A. J. J. P. Moya (2021). "Nanocellulose from agricultural wastes: Products and applications—A review." **9**(9): 1594.
- Muhammad, M. H., A. L. Idris, X. Fan, Y. Guo, Y. Yu, X. Jin, J. Qiu, X. Guan and T. J. F. i. m. Huang (2020). "Beyond risk: bacterial biofilms and their regulating approaches." **11**: 928.
- Nadaf, A., A. Gupta, N. Hasan, S. Ahmad, P. Kesharwani and F. J. J. R. a. Ahmad (2022). "Recent update on electrospinning and electrospun nanofibers: current trends and their applications." **12**(37): 23808-23828.
- Naseri, N., A. P. Mathew, L. Girandon, M. Fröhlich and K. J. C. Oksman (2015). "Porous electrospun nanocomposite mats based on chitosan–cellulose nanocrystals for wound dressing: effect of surface characteristics of nanocrystals." **22**(1): 521-534.
- Negut, I., G. Dorcioman and V. J. P. Grumezescu (2020). "Scaffolds for wound healing applications." **12**(9): 2010.
- Popa, L., M. V. Ghica, E.-E. Tudoroiu, D.-G. Ionescu and C.-E. J. M. Dinu-Pîrvu (2022). "Bacterial cellulose—a remarkable polymer as a source for biomaterials tailoring." **15**(3): 1054.
- Rana, S., V. Joshi, G. C. Sahoo, M. Kumar and K. Pandey (2024). Upsurge in Antibiotic-Resistant Infections. Emerging Paradigms for Antibiotic-Resistant Infections: Beyond the Pill, Springer**:** 25-42.
- Romeo-Melody, I. L. (2022). Antimicrobial efficacy of XF-73 and photo-activated XF-73 against clinically relevant microorganisms in planktonic and biofilm mode of growth, Aston University.
- Sagini, J. P. N., F. C. P. Rossatto, F. Souza, E. Pilau, C. B. Quines, D. S. Ávila, R. Ligabue-Braun, A. R. Zimmer, R. I. Pereira and K. R. J. M. P. Zimmer (2024). "Inhibition of Staphylococcus epidermidis and Pseudomonas aeruginosa biofilms by grape and rice agroindustrial residues." **197**: 107019.
- Seddiqi, H., E. Oliaei, H. Honarkar, J. Jin, L. C. Geonzon, R. G. Bacabac and J. J. C. Klein-Nulend (2021). "Cellulose and its derivatives: towards biomedical applications." **28**(4): 1893-1931.
- Stewart, P., T. J. C. M. Bjarnsholt and Infection (2020). "Risk factors for chronic biofilm-related infection associated with implanted medical devices." **26**(8): 1034-1038.
- Wang, L., G. Li, X. Chen, Y. Yang, R. K. Liew, H. M. Abo-Dief, S. S. Lam, R. Sellami, W. Peng, H. J. A. C. Li and H. Materials (2024). "Extraction strategies for lignin, cellulose, and hemicellulose to obtain valuable products from biomass." **7**(6): 219.
- Zizovic, I., L. Senerovic, I. Moric, T. Adamovic, M. Jovanovic, M. K. Krusic, D. Misic, D. Stojanovic and S. J. T. J. o. S. F. Milovanovic (2018). "Utilization of supercritical carbon dioxide in fabrication of cellulose acetate films with anti-biofilm effects against Pseudomonas aeruginosa and Staphylococcus aureus." **140**: 11-20.