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Conference Report

Abstracts of the 1st International Online Conference on Bioengineering †

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† All papers published in this volume are presented at the 1st International Online Conference on Bioengineering, 16–18 October 2024; https://sciforum.net/event/IOCBE2024.

Abstract: The 1st International Online Conference on Bioengineering (IOCBE 2024) was held online from 16 to 18 October 2024. With a theme of *Bioengineering in a Generative AI World*, the conference featured presentations from distinguished scholars at the nexus of engineering and clinics, engineering and food, engineering and industry, and innovations in bioengineering education. The conference was organized around six topics and related themes: Regenerative and Tissue Engineering; Biomechanics and Sports Medicine; Biomedical Biomaterials; Nano-Biotechnology; Biosignal Processing; and Biochemical Engineering. This conference discussed the important recent developments in bioengineering, including bioengineered foods, allotransplantation, multi-omics, theragnostics and bioengineering convergence. Oral and poster presentations were featured in live broadcasts, enabling participants to take part in interactive discussions and sessions.

Keywords: bioengineered foods; allotransplantation; multi-omics; theragnostics; bioengineering convergence

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1. Conference Introduction

The 1st International Online Conference on Bioengineering (IOCBE 2024) was organized and hosted by the MDPI *Bioengineering* and chaired by Prof. Dr. Anthony Guiseppi-Elie, Editor-in-Chief, and Dr. Rossana Madrid, Associate Editor. The whole conference consisted of the following sessions:

- S1. Regenerative and Tissue Engineering

  Session Chairs:

  Dr. Elena A. Jones, Leeds Institute of Molecular Medicine, School of Medicine, University of Leeds, Leeds, UK;

  Dr. Dimitrios Kouroupis, Department of Orthopaedics, Miller School of Medicine. University of Miami, Miami, USA.

- S2. Biomechanics and Sports Medicine

  Session Chairs:

  Prof. Dr. Franz Konstantin Fuss, Chair of Biomechanics, Faculty of Engineering Science, University of Bayreuth, Bayreuth, Germany, Division of Biomechanics, Department of Biomechatronic Systems, Fraunhofer Institute of Manufacturing Engineering and Automation IPA, Stuttgart, Germany;

  Prof. Dr. Michael Moreno, Department of Mechanical Engineering, J. Mike Walker '66 Faculty, Texas A&M University, USA.

- S3. Biomedical Biomaterials

  Session Chair:
  Prof. Dr. Gary L. Bowlin, Department of Biomedical Engineering, The University of Memphis, Memphis, USA.

- S4. Nano-Biotechnology

  Session Chair:
  Dr. Gary Chinga Carrasco, RISE PFI, Høgskoleringen 6b, Trondheim, Norway.

- S5. Biosignal Processing

  Session Chairs:
  Dr. Andrea Cataldo, Department of Innovation Engineering (DII), University of Salento, Lecce, Italy;
  Dr. Egidio De Benedetto, Department of Information Technology and Electrical Engineering, University of Naples Federico II, Naples, Italy.

- S6. Biochemical Engineering

  Session Chair:
  Prof. Dr. Liang Luo, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China.

2. Plenary Talks

- Prof. Dr. Luke P Lee, Harvard Medical School, Harvard University and Brigham and Women's Hospital, USA; talk title: Nanomedicine via Quantum Plasmonic SANDs, EXODUS, and Brain Organoid MAP.
- Prof. Dr. Chuanbin Mao, Chinese University of Hong Kong, Hong Kong; talk title: Phage-Based Biomaterials, Nanomedicine and Regenerative Medicine.
- Dr. Tingrui Pan, University of Science and Technology of China (USTC) and the Suzhou Institute for Advanced Research; talk title: The Rise of Flexible Iontronics for Medical Intelligence.
- Prof. Dr. Kristala Jones Prather, Massachusetts Institute of Technology, USA; talk title: Biosensor-Guided Regulation of Metabolic Pathways.
- Prof. Dr. Molly Shoichet, University of Toronto, Canada; talk title: Overcoming Barriers: Local Cell & Therapeutic Delivery to the Central Nervous System.
- Prof. Dr. Wai Yee Yeong, Nanyang Technological University, Singapore; talk title: 3D Printing and Biofabrication: Progress and Opportunities for Machine Learning in Materials and Processes.

3. Regenerative and Tissue Engineering

3.1. Bioengineering of MSC-Based 3D Constructs with Different Types of Cell Organization

Natalia Trufanova, Oleh Trufanov, Galina Bozhok, Ruslana Oberemok, Olena Revenko and Oleksandr Petrenko

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Introduction: Mesenchymal stromal/stem cells (MSCs) possess unique biological properties, including self-renewal, differentiation, and secretory potentials. However, a standard 2D culture does not replicate MSCs' natural microenvironment, compromising their features. Engineering MSC-based constructs that support various 3D cell organizations and analyzing cell behavior under such conditions are crucial for biomedical applications, offering relevant model systems and aiding in the development of therapeutic agents. This study aimed to evaluate the impact of cultivating MSCs in spheroids, alginate microspheres (AMSs), and blood plasma scaffolds on viability and metabolic and functional activity.

Methods: Human adipose tissue-derived MSCs (obtained with adult donors' informed consent) were used. Spheroids were formed by the "hanging drop" method. AMSs were

generated by electrospraying MSCs dispersed in 2% sodium alginate into 2% calcium chloride. Scaffolds were prepared through cryogelation and being seeded with cells. All constructs were cultured at 37 °C, 5% CO_2, and 95% humidity in alpha-MEM supplemented with 10% fetal bovine serum, 50 μg/mL penicillin, and 50 μg/mL streptomycin. Viability (6-CFDA), metabolic activity (resazurin test), actin filaments (Phalloidin-FITC), cell spreading, and induced differentiation were examined.

Results and Discussion: MSCs exhibited high viability in all constructs but displayed distinct morphologies (spindle-like in scaffolds, round in spheroids, and AMSs). Actin filament development was most pronounced in cells within scaffolds. Metabolic activity was reduced in spheroids and AMSs compared to the scaffolds. All groups demonstrated the ability for induced differentiation.

Conclusions: The cultivation of MSCs within a macroporous adhesive scaffold promotes fibroblast-like morphology and high metabolic activity. A spheroid and AMS culture results in round-shaped cells with lower metabolic activity, which can reflect a natural-like quiescence state. This study highlights the importance of 3D culture systems in maintaining MSC properties and suggests that constructs' design significantly influences cell functionality, crucial for advancing biomedical applications and therapeutic strategies.

This study was supported by the National Research Foundation of Ukraine (project № 2021.01/0276).

3.2. Cutting-Edge Bioink Technology: Using Decellularized Skin for Enhanced 3D Bioprinting

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Introduction: Skin wounds present a considerable challenge, impacting millions globally. This research aimed to create a bioink utilizing lyophilized rat decellularized skin (DS) for 3D bioprinting to improve skin regeneration. Methods: Rat skin was subjected to decellularization for 5 days. A comparative analysis of genomic DNA quantification and histological staining was performed between native and decellularized skin. The tissue was freeze-dried and combined with alginate and gelatin to formulate bioinks with concentrations of 1.5% and 3% DS, 3% or 4% alginate, and 7% gelatin. Rheological evaluations, including swelling, printability, and degradation over a four-week period, were conducted. Hydrogel SEM images were obtained using a scanning electron microscope. Cell viability and proliferation were assessed using the Live/Dead assay.

Results: The hydrogel demonstrated good shear-thinning behavior and maintained its viscosity across different concentrations. The degradation rate was 59,2% in one month. Swelling was 3783% after one month. Only the bioink with 1.5% DS, 3% alginate, and 7% gelatin preserved structural integrity for four weeks and was chosen for further examination. Furthermore, the bioink showed a low tangent delta, decreasing printing-related stress and subsequent cell death. SEM images revealed a porous three-dimensional structure. The Live/Dead assay indicated higher cell viability (65%) compared to the control seven days post-bioprinting.

Conclusion: The biomaterial showed good mechanical properties and, after bioprinting, supported cell proliferation, indicating its potential as a promising alternative for skin wound regeneration.

Funding: The Office of Naval Research Global (ONRG Award N62909-21-1-2026), The National Institute of Science and Technology for Regenerative Medicine (INCT-Regenera) and The Stem Cell Research Institute (IPCT).

3.3. Deciphering the Effect of Induced Stem Cell Secretome on Impaired Wound Healing: An In Vitro Study

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The gold standard for chronic wound treatment involves the grafting of skin tissue, which is currently becoming replaced by cellular therapy. Studies indicate that cellular functions occur via paracrine signaling factors rather than direct interactions. This finding has initiated the exploration of secretome-based therapy. Platelets and MSCs are two critical cell types involved in wound healing. In the present study, we focused on analyzing the effect of the MSC secretome and platelet-induced MSC secretome on wound healing. Adipose tissue-derived MSCs (ADMSCs) and PRP were isolated from healthy rabbits (New Zealand White, 2 kg, 6 months) after obtaining Institutional Animal Ethics Committee clearance (SCT/IAEC-439/July/2022/113). In order to prepare PRP-induced ADMSC secretome, ADMSCs were seeded in a 75 cm^2 culture flask at a density of 15,000 cells/cm^2. At 70% confluency, the serum-containing culture medium was replaced with 450 µL PRP (350–400 $\times$ 10^3 cells/µL)-containing media and cells were incubated for 48 hrs. Hyperglycemia was induced by growing fibroblasts in high-glucose (30 mM) DMEM with 5% serum and antibiotics. The wound-healing efficiency of PRP-induced MSC secretome and uninduced MSC secretome was evaluated by means of on-cell proliferation, migration, collagen synthesis, ROS generation, actin cytoskeleton deposition, and gene expression analysis. All quantitative data were expressed as mean $\pm$ SD (n = 3). Results were analyzed using one-way ANOVA/Student's *t* test and were considered significant when * $p \leq 0.05$.

We observed that PRP-induced MSC secretome treatment significantly increased cell proliferation and migration, improved collagen synthesis, reduced ROS generation, and promoted actin deposition in hyperglycemic fibroblast cells compared to uninduced secretome treatment. In conclusion, these findings suggest that PRP-induced MSC-conditioned media may be developed as an off-the-shelf therapeutic aid. Further in vivo evaluation of PRP-induced MSC secretome needs to be performed in diabetic models, and the molecular pathways underlying its mechanism of action also need to be explored as future research perspectives.

3.4. Development of Histocompatible Vessel Conduits Utilizing Human Umbilical Arteries: Emerging Evidence for the Establishment of a Vascular Graft Bank

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Background: The production of a small-diameter vascular graft (d3 mm), utilizing state-of-the-art tissue engineering approaches, currently represents a major challenge in vessel microsurgery. Cardiovasular disease (CVD) affects more than 18 million people, worldwide. Therapeutic options in bypass surgery include the use of autologous grafts, such as the saphenous vein, or synthetic vascular grafts. However, both approaches present unfortunate events, limiting the lifespan of the implanted graft, thus requiring a new replacement surgery. Therefore, the production of vascular grafts utilizing decellularized human umbilical arteries may represent an alternative strategy in the treatment of CVD. Moreover, the efficient combination of the produced vascular graft with the host's cells may result in the production of a fully histocompatible conduit, thus increasing the lifespan of the implant. For this purpose, we assessed the production of histocompatible vascular grafts obtained from decellularized human umbilical arteries (hUAs), which is the primary aim of this study. **Methods:** HUAs were decellularized using CHAPS and SDS detergents. The total hydroxyproline, sulphated glycosaminoglycans (sGAGs), and DNA content were quantified. Human endothelial cells (ECs) and smooth muscle cells (SMCs) were

seeded in the decellularized hUAs. Typing of the Human Leucocyte Antigens (HLAs) was performed in hUAs both prior to and after the decellularization, as well as in seeded cellular populations. **Results:** Decellularized hUAs were characterized by the proper preservation of tissue architecture. Total hydroxyproline content was preserved, although sGAGs and DNA presented a statistically significant reduction. HLA typing only confirmed the presence of the seeded ECs and SMCs in the produced vascular grafts, further indicating the successful production of a histocompatible graft. **Conclusion:** The results of this study support the efficient production of histocompatible human vascular grafts. Based on the most frequent regional HLAs, a bank with histocompatible vessel grafts could be established, bringing personalized medicine a step closer to clinical utility.

3.5. Efficient Decellularization of Full-Thickness Abdominal Wall Scaffold to Produce a Potential Tissue-Engineered Product

George Skepastianos, Panagiotis Mallis [2], Epameinondas Kostopoulos [3], Efstathios Michalopoulos [4], Vasileios Skepastianos [3], Chrysoula Palazi [3] and Gerasimos Tsourouflis [5]

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Background: Restoring functionality to abdominal wall defects is a key challenge in reconstructive surgery. It is estimated that over 700,000 abdominal wall reconstructions are conducted annually in the United States, with more than 20 million performed worldwide each year. Synthetic grafts and crosslinked, animal-derived biological grafts often lead to significant adverse reactions following implantation. This study aimed to assess the effectiveness of a decellularization protocol in producing a fully acellular, full-thickness abdominal wall scaffold, an alternative therapeutic application for abdominal wall reconstruction. **Methods:** Full-thickness abdominal wall samples were harvested from Wistar rats and submitted to a three-cycle decellularization process. Histological, biochemical, and DNA quantification analyses were applied. In addition, implantation of decellularized abdominal wall scaffolds was performed at the scapular region of Sprague Dawley rats. The grafts remained for 4 weeks and were then explanted; histological analysis utilizing Hematoxylin and Eosin and immunohistochemistry against CD11b (macrophages), CD4 (T-helper cells), and CD8 (cytotoxic T cells) were performed to assess the biocompatibility potential. **Results:** Histological, biochemical, and DNA analysis results showed efficient decellularization of the abdominal wall samples after the third cycle. Decellularized abdominal wall scaffolds were characterized by good biochemical and mechanical properties. Biocompatibility assessment showed the successful migration of the host's cells to the implanted abdominal wall scaffolds. Furthermore, no presence of CD11b, CD4, or CD8 cells was observed in the grafts after 4 weeks of implantation. **Conclusion:** The data presented herein confirm the effective production of a rat-derived, full-thickness abdominal wall scaffold. In addition, the scaffold was biocompatible after a 4-week implantation period. Expanding this approach will allow the exploitation of the capacity of the proposed decellularization protocol in producing acellular abdominal wall scaffolds from larger animal models or human cadaveric donors.

3.6. Electrochemical Characterization of Commercially Pure Titanium Electrodes for Orthopaedic Applications: A Re-Evaluation of Electric Field Models

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Introduction: Titanium and its alloys are extensively used in orthopaedic applications due to their excellent mechanical properties, biocompatibility, and corrosion resistance. Direct coupled electrical stimulation (DCES) has also been demonstrated to promote bone regeneration and osseointegration in clinical trials. However, titanium is not often the material of choice for electrically stimulating bioelectrodes, especially as both the cathode and anode. This is mainly due to titanium's lower conductivity compared to other biocompatible materials, like gold and platinum, and the tendency for the passive oxide layer to transform, which although contributing to its excellent corrosion resistance can impact signal stability. Nonetheless, dual titanium bioelectrodes have made their way into several clinical applications, such as spinal fusion, but have not been extensively characterized.

Methods: This study explores the effects of anodization voltage (0, 5, and 10 V) and oxide layer configuration (single or double passivation) on electric field distribution under voltage-controlled constant DCES for commercially pure titanium electrodes. Computational modelling frameworks for estimating electric field distributions were also re-evaluated using COMSOL Multiphysics to address the ambiguous reporting standards that are hindering the widespread application of DCES in implant design.

Results: The results demonstrate that improper choice of the modelling framework can overestimate the electric field by up to an order of 4 in constant DCES systems. It was also found that the electric field only behaves uniformly near the centre of the stimulation chamber (roughly 3 mm from the centre), with much greater electric field gradients in the direction parallel to the electrodes.

Conclusion: These results suggest that the electric fields reported in previous in vitro DCES studies should be re-evaluated using appropriate computational electrochemical approaches. This reassessment will help inform the design of electrically stimulating medical devices and expedite the clinical translation of DCES for bone regenerative therapies.

3.7. Electrospinning Casein Nanofiber Mats for Tissue Engineering

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Casein is a natural milk protein that has been investigated in drug delivery, cartilage tissue engineering, and other biotechnological applications. In particular, casein nanofibers are of high interest for tissue engineering. Electrospinning casein is usually performed by co-electrospinning it with a spinning agent, such as poly(vinyl alcohol), poly(caprolactone) (PCL), or poly(ethylene oxide) (PEO). Spinning casein solely has been found to be challenging due to its unsuitable viscoelasticity and extensive intermolecular interactions. Our study aims to optimize casein/PEO spinning solutions for needleless electrospinning in the wire-based electrospinning device Nanospider Lab. For this, PEO with different molecular weights (6 kDa-1 MDa) was mixed with different caseins in different ratios to prepare an aqueous spinning solution whose pH value was varied in the test series of experiments. Electrospinning duration (30 min) and nozzle size (0.9 mm) were kept constant, while the other spinning parameters, as well as the relative humidity in the spinning chamber, were modified. Our study reveals the impact of these parameters on the spinnability of the casein/PEO spinning solutions and the macroscopic homogeneity of the resulting nanofiber mats. Additionally, scanning electron microscopy (SEM) images show the impact of these parameters on the diameter distribution and morphology of the nanofibers. This poster gives the first overview of the optimum spinning parameters for the wire-based electrospinning of casein/PEO nanofiber mats.

3.8. Green Tech in Medicine: Scenedesmus Obliquus-Reinforced Composite Hydrogels for Wound Therapy

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Introduction: The creation of new sustainable methods that can maintain optimal oxygen levels in hydrogel scaffolds containing algal biomass is essential for regenerative medicine. Autotrophic tissue engineering utilises photosynthesis to harness the biological ability of algae to create oxygen in tissue constructs capable of self-sustenance, minimising reliance on external nutrient sources. We embedded polymeric hydrogels with algal biomass of *Scenedesmus obliquus*, which improved their wound healing ability in mouse models.

Method: Algal biomass (*Scenedesmus obliquus*) was added into the monomer mixture of acrylic acid(AA) and N-[3-(dimethylamino)propyl]-methacrylamide (DMAPMA) along with ammonium persulphate (APS), and N,N,N′,N′-tetramethyl ethylenediamine (TEMED) for the synthesis of algal biomass-loaded hydrogel scaffolds through free radical aqueous copolymerisation. Further, this algal biomass-loaded hydrogel scaffold was tested for excisional cutaneous wound healing in BALB/c mice models for 14 days.

Result and Discussion: Algal hydrogel scaffolds with varying concentrations of *Scenedesmus obliquus* were applied for 14 days to excisional wounds in BALB/c mice. It was observed that algal hydrogel scaffolds promoted accelerated wound healing and had significant anti-inflammatory properties.

Conclusions: The results obtained suggest that infusing algal biomass into the polymer matrix improves wound healing ability and provides a pathway for the development of novel potential biomaterials for wound healing therapy.

3.9. PCL and PLGA Particles Containing Vitamins A and D for Bone Regeneration

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Vitamin D plays a pivotal role in calcium homeostasis and bone metabolism, whereas vitamin A deficiency can result in delayed bone growth and reduced bone mineral density. The objective of this study was to produce, characterize, and test poly(caprolactone) (PCL) and poly(lactide-co-glycolide) (PLGA) particles containing retinyl acetate and cholecalciferol. The particles were prepared by solvent displacement, whereby water and surfactant were dripped into an oil phase of polymers (PCL or PLGA) without or with vitamins A and D in acetone. The particles were characterized in terms of their size and zeta potential. The viability of stem cells was evaluated via MTT assay following a one-day exposure to particles containing 0.77 UI/mL vitamin A and 0.15 UI/mL vitamin D. The findings revealed that PCL particles exhibited a zeta potential of −60 mV. In comparison, PLGA particles demonstrated a zeta potential of −39 mV. The diameter was 213 nm for PCL and 112 nm for PLGA, as determined by the Zetasizer equipment (with a detection limit of 10 μm). However, the diameter was measured above 10 μm for PLGA using optical microscopy/ImageJ. The particles did not significantly affect stem cell viability, as indicated by the absorbance values for cells incubated with the particles of PLGA, PLGA/vitamins, PCL, and PCL/vitamins for one day ($p = 0.560$). Therefore, the particles exhibited nano- and micrometric sizes, a high negative surface charge, and high dispersion. These materials were not cytotoxic to stem cells, indicating that polymeric particles may represent a viable retinol and cholecalciferol supplementation strategy, with potential utility in bioinks for bone tissue engineering.

Acknowledge: Office of Naval Research Global (ONRG Award N62909-21-1-2026) and National Institute of Science and Technology for Regenerative Medicine (INCT-Regenera).

3.10. Three-Dimensional-Bioprinted Bioink with Nanosilicate and Pluronic p123 for Bone Tissue Engineering

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Introduction: Recent studies show that the combination of biomaterials and 3D bioprinting is a promising approach for treating extensive bone injuries. The aim of this study was to develop a bone bioink containing nanosilicate, which enhances the biomaterial's mechanical and biological properties, and pluronic p123 due to its hydrophilic potential, which make this a good candidate for drug delivery. Methodology: Nanosilicate was prepared with tetraethyl orthosilicate (TEOS) and the hydrogel was composed of alginate 5%, pluronic p123 20%, and nanosilicate 2%; it was characterized using rheological tests, scanning electron microscopy (SEM), and degradation and swelling tests. The hydrogel was then mixed with 106 mesenchymal cells/mL and was bioprinted in a 3D bioprinter. In order to evaluate the biocompatibility, a live/dead assay was performed on day one. Results: The nanosilicate showed an average diameter of 392.78 ± 85.08 nm, a zeta potential of -39.65 ± 6.1 mV, and a PDI of 0.105 ± 0.09. The SEM images showed the highly porous structure of the hydrogel with distribution of the nanosilicate throughout the surface of the pores, suggesting an optimal structure for cell adhesion. The hydrogel swelled 1718% over 24 h, indicating a good capacity for nutrient exchange and cell migration. The degradation rate was 54.21% after one month, suggesting a good clearance of the biomaterial during the regeneration of the natural tissue. Rheological characterization showed a suitable G'/G'' ratio, indicating good mechanical properties to biomimic the bone tissue. The live/dead assay revealed a cell viability of around 70% after one day, being non-cytotoxic for the mesenchymal cells. Conclusion: The results of this study showed that the described bioink is a promising material for bone tissue engineering and repair.

3.11. Three-Dimensionally Printed Porous Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) Scaffold for Bone Regeneration: Fabrication, In Vitro and In Vivo Studies

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Introduction: 3D printing, or additive manufacturing, is a modern technique for creating three-dimensional physical objects. It is used in reconstructive medicine and requires the use of new materials. Among biodegradable polymers, polyhydroxyalkanoates (PHAs) are prominent. They benefit from direct fermentation, which does not require complex technological stages like polylactide and polycaprolactone. Varying the substrate or producer strain allows polymers with different properties to be obtained.

Methods: First, the filament was obtained through the extrusion of P(3HB-co-3HV). For printing, we used FDM technology on a 3D printer with the author's blowing system. This made it possible to reduce thermal deformation when creating cylindrical scaffolds with a diameter of 13 mm, a height of 4 mm, and a porosity of 65%.

To study biological compatibility, light and fluorescence microscopy were used, as well as an MTT test on a culture of NIH 3T3 mouse fibroblasts.

The implantation of 3D scaffolds was studied on a model of segmental osteotomy of the tibia in two pigs. In each animal, a bone cavity for implantation was formed in the central part of the femoral diaphysis. After the operation, the animals were monitored,

measuring body temperature, heart rate, and respiration. This study was conducted ethically and with humane treatment of animals.

The statistical analysis of the results was carried out using traditional methods, with data presented as the mean ± error for 95% confidence intervals.

Results and Conclusions: The results showed the high biocompatibility of the scaffolds: microscopy, fluorescent staining, and the MTT test confirmed their complete filling with cells, which maintained their metabolic activity for up to 10 days.

Histological and X-ray analyzses showed complete healing of the defect within 5 months. These findings suggest that the designed absorbable 3D scaffolds are promising for use in bone grafting.

3.12. Use of Low-Level Electrical Stimulation for Bacterial Inhibition

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Introduction: Bacterial adhesion on medical devices can have serious consequences, somtimes leading to chronic infections if biofilm formation cannot be prevented. When bacteria colonies form in surgical sites, the bacteria contribute to prolonged inflammation, delay tissue regeneration, and jeopardize the natural healing process. The formation of biofilms is a significant health issue, as they cannot be prevented using conventional antimicrobial treatments. It has been reported in the literature that electrical stimulation (ES) may result in bacteria inhibition through molecular regulatory mechanisms. The current study explores the effect of low-level ES on *Staphylococcus epidermidis* (*S. epidermis*) and *Escherichia coli* (*E. coli*), and our results show a promising *E. coli* inhibition efficiency was achieved.

Methods: Petri dishes with Grade 5 cpTi rods as electrodes were fabricated with a separation distance of 3 cm between electrodes. A total of 20 mL of Tryptic Soy Agar was dispensed into each dish and bacteria species were grown up to the mid-log phase, before a bacterial lawn was created. Then, 30 min of ES was delivered at varying voltages (1 V, 300 mV, and 100 mV) and frequencies (3 MHz, 1 MHz, 0.75 MHz, and 0.5 MHz) and samples were then incubated at 37 °C for 24 h. The zone of inhibition (ZOI) around the electrodes after incubation was measured using ImageJ.

Results: All ES parameters showed various levels of inhibition against *E. coli*. Based on the data collected, 1 V at 1 MHz showed the largest ZOI with a statistical difference compared to 100 mV at 1 MHz and 1 V at 0.5 MHz. There was no inhibition of growth at any ES parameter when tested against *S. epidermis*.

Conclusions: The use of low-level ES against bacteria growth has major clinical applications that can help reduce post-operative infection risk and lower the risk of antibiotic resistance. The results indicate a need for further research to understand the influence of ES parameters on various bacteria. Future studies will include ES against other common bacterial species associated with infections.

4. Biomechanics and Sports Medicine

4.1. Advancements in Medical Imaging Methods for Body Composition Assessment: A Literature Review

Rui Pedro Pereira de Almeida [1,2], Manuel Lobo [3,4], Sérgio Tavares [5,6], Carlos da Silva [7], Ana Bárbara [6], Nélia Gaudêncio [6], Magda Ramos [6], António Abrantes [2,6,8]

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Introduction: Accurate body composition assessment is critical in clinical and sports settings to assess health, fitness, and disease risk. Advances in medical imaging technology have considerably increased the ability to measure and analyze body composition. This review examines these imaging techniques, focusing on their application in sports medicine. Methods: This review covers current imaging techniques for body composition assessment, including Dual-Energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Ultrasound (US). Parameters such as accuracy, precision, application range, and radiation exposure were evaluated. Data consistency was ensured by cross-referencing findings from multiple sources and by prioritizing analyses with large sample sizes and rigorous methods. Results and Discussion: DXA is widely recognized for its high accuracy and low radiation efficiency, providing comprehensive data on bone, lean mass, and fat mass. It is specifically useful for detailed regional analysis, although hydration status can affect its accuracy. MRI provides detailed information without ionizing radiation, and is ideal for monitoring muscle health and detecting sarcopenia, despite its high cost and limited availability. CT provides detailed cross-sectional images for precise tissue measurement but involves higher radiation exposure. Ultrasound is a practical, non-invasive, cost-effective method for assessing subcutaneous fat and muscle thickness, though less detailed and more operator-dependent compared to MRI and CT. Conclusions: Medical imaging technologies have greatly improved body composition assessment, providing detailed insights into muscle and fat distribution. DXA stands out as the gold standard for its balance of accuracy, safety, and cost balance. MRI and CT provide detailed imaging but come with high costs and radiation exposure. Ultrasound remains a practical alternative for early evaluation, though less detailed, assessments. Continuous technological developments and artificial intelligence promise to further advance these approaches in sports medicine, leading to better health and performance for athletes.

4.2. Applying 3D Modelling and Numerical Simulation Techniques for Precise Orthotic Design in Scoliosis Treatment

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Scoliosis is a three-dimensional deformity of the trunk and spine that can develop significantly during growth stages. Recently, computer-aided design has played a crucial role in various physical rehabilitation and orthotic applications. This study focuses on designing medical orthotics using 3D modelling and force simulation techniques, aiming to improve the accuracy and effectiveness of scoliosis treatments.

The approach starts by reconstructing a 3D model of the scoliosis case using computed tomography (CT) data and then employs Solidworks software to analyse and simulate the mechanical properties. The numerical simulation of applying different pressure points with varying values generates a comprehensive dataset of curves that express the spine's deformity, simulating correction using different braces with distinct pressure points in Solidworks. By selecting the optimal pressure points in various planes, we were able to manufacture a precise 3D model that addresses scoliosis based on pre-examined pressure points.

This study demonstrates that combining engineering and medical technologies can significantly enhance the quality of treatment and effectively meet patients' needs. Furthermore, advancements in 3D printing technology enable the production of highly accurate and customized orthotic devices, ensuring a perfect fit for each patient's unique anatomical structure. The ability to rapidly prototype and adjust designs in real time significantly reduces the time and cost associated with traditional orthotic manufacturing methods. Moreover, the integration of deep artificial neural networks could further refine the design

process, enabling the reconstruction of appropriate scoliosis braces based on extensive data analysis and predictive modeling.

4.3. Assessing Bowling Legality in Cricket: Biomechanical Insights and Implications

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Introduction: Cricket bowling has traditionally required a rigid arm, with no elbow straightening allowed. However, research shows that maintaining a completely rigid arm is impractical, leading to rule changes. The current regulation mandates that the elbow extension angle must not increase by more than 15 degrees. This study aims to determine whether this rule is upheld across various bowlers and styles and to explore if alternative kinematic measures can better distinguish between legal and suspect actions.

Methods: Sixteen male fast bowlers from NSW grade clubs were analysed using a Cortex 2.0 motion analysis system (200 Hz). The study measured changes in elbow angular kinematics, defined as the angle between the upper arm and forearm relative to the flexion-extension axis of a joint coordinate system.

Results and Discussion: The mean elbow extension angle was $14.0 \pm 8.4^{\circ}$. Surprisingly, six bowlers exceeded the 15° legal limit, even though only one was considered suspect a priori. This may be due to a perspective error of elbow flexion during the critical period when the arm is horizontal and behind the bowler's body. The mean change in elbow flexion-extension angular velocity at release was $-123.5 \pm 316.9\ ^{\circ}/\mathrm{s}$, showing significant variability, an indicator of the diversity of elbow mechanics in bowling. This diversity suggests that a one-size-fits-all model for elbow movement during bowling is not valid. Coaches and regulatory bodies need to consider this variability when developing training programs and rules.

Conclusions: More bowlers exceed the legal limit than visually assessed. Rigorous biomechanical screening programs are essential to ensure fairness for batters and compliant bowlers. High elbow angular velocities make it difficult to detect throw-like actions visually. Educating coaches on perspective errors is crucial. Analysing elbow extension angular velocity at ball release provides a more effective differentiation. Cricket authorities must address bowling legality issues for the game's future equity.

4.4. Changes in the Intermuscular Coherence of the Multifidus and Its Relationship with Fatigue and Low Back Pain: A Pilot Study

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Introduction: The relationship between trunk muscles, low back pain, and exercise has been studied for many years. Subjects with low back pain have shown different responses to fatigue compared to healthy subjects, and this particular behaviour has been proposed as a predictor of low back pain. Several experimental studies have been conducted to identify valid and reliable biomarkers that explain the functioning of the trunk muscle complex. The aim of this study was to analyse the behaviour of the lumbar musculature in healthy subjects and a subject with a history of low back pain.

Methods: The participants underwent a squat protocol using a barbell loaded with 60% of their body weight. They performed as many repetitions as possible at controlled eccentric and maximal concentric speeds. The connectivity of the multifidus muscles was analysed using intermuscular coherence (IMC) during the concentric phase of the exercise.

Results and Discussion: The findings indicated that intermuscular coherence (IMC) decreased among control subjects by the end of the squat series, whereas the subject with a history of low back pain exhibited the opposite trend. These results contrast with the existing literature, though the study's limitations preclude definitive comparisons.

Conclusions: Despite the findings, IMC could potentially be a valuable tool for characterising fatigue and low back pain.

4.5. Dynamic Balance Diagnostics with Smart Insoles

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Introduction: The real-time calculation of the centre of pressure (COP) during walking is of paramount importance for balance assessment and fall prevention. The aim of this study is to derive a balance index (BI) from the COP and its cyclogram and to test how well the BI works in distinguishing between diabetic and healthy volunteers.

Methods: This study involved 15 healthy volunteers and 14 people with diabetes. During walking, the COP was measured using Pedar-X insoles. The position of the COP was calculated at 10 locations: heel strike (E), toe-off (A), the beginning and end of the double support phase (D,B), and intersection points (C) on the COP cyclogram. The standard deviation of each COP cluster was calculated in the X and Y directions and multiplied (area of COP movement within ± 1 standard deviation). The BI was calculated as the sum of the 10 areas. Furthermore, the ratio R = BC/AB was calculated from the average data of each cluster. The sensitivity and specificity of the logarithmically transformed BI and R were used to calculate the ROC, AUC, and the classification threshold.

Results and Discussion: LogBI was normally distributed in the diabetic (D) and healthy (H) volunteers, with significantly different ($p < 0.0001$) averages of 1.686 ± 0.228 and 2.070 ± 0.282, respectively. The AUC was 0.861. At an optimal classification threshold of 1.9, the false positive H-data (identified as D) and the false negative D-data (identified as H) were approximately 20% each. The averages of logR were significantly different ($p < 0.0001$): 1.054 ± 0.312 and 0.143 ± 0.306 for H and D, respectively. The AUC was 0.992. At an optimal threshold of 0.5, the false positive H-data and the false negative D-data were both about 4%.

Conclusions: The position of the COP within the cyclogram (logR) performed better than the standard deviation of the COP (logBI).

4.6. Electrophysiological Biomarkers to Understand the Compensatory Mechanisms of Hamstring Tears: A Narrative Review

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Introduction: Hamstring muscle tears are among the most common sports injuries. They account for approximately 25% of all sports injuries, with high recurrence rates ranging from 15% to 60%, posing a significant challenge to the recovery of athletes. Muscle injuries can alter the movement control system and generate compensatory strategies that affect muscle synchronisation. A thorough study of how hamstring tears generate adaptive mechanisms that alter motor strategies is crucial to improve assessment and rehabilitation processes. In this context, electrophysiological biomarkers provide an excellent study tool due to their high temporal resolution. The aim of this work was to review and summarise the evidence on the analysis of electrophysiological biomarkers and provide insights into the compensatory and adaptive mechanisms generated by hamstring muscle tears.

Methods: A literature review was conducted using the PubMed-MEDLINE and Google Scholar databases, focusing on studies relevant to applied research in athlete recovery. The search employed keywords such as “hamstring tear”, “hamstring injuries”, “electrophysiological biomarkers”, “EMG analysis”, “functional connectivity”, “intermuscular coherence”, “muscle synergies”, “muscle networks”, and “motor control”. Articles

were selected based on their relevance to the field of sports science and rehabilitation, particularly in the context of athlete recovery strategies.

Results and Discussion: Bivariate and multivariate analyses using EMG data offer a comprehensive approach to understanding muscle synchronisation strategies, thereby addressing the shortcomings of traditional univariate analysis focused on parameters such as amplitude, latency, and spectral power density.

Conclusions: Using an approach based on bivariate and multivariate analysis would allow a better study of the compensatory mechanisms induced by muscle injury on motor behaviour.

4.7. Hip Injuries in Dancer Athletes Due to Biomechanical Loading: A Systematic Review

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Introduction: Professional dancers are particularly susceptible to the occurrence of pathologies in their lower limbs and especially in the hip joint due to biomechanical stress. These injuries are the result of a variety of factors and require a specific treatment method. The purpose of this review is to document these injuries, identify the factors that are responsible for their occurrence, and present the recommended treatment approaches.

Methodology: This research was conducted by searching the PubMed, ScienceDirect, and Google Scholar databases using keywords and key phrases such as "hip disorders", "dancer's hip", and "musculoskeletal loading". The resulting data were collected and evaluated according to the PRISMA guidelines.

Results and Discussion: The total number of studies included in the review was 44. Of these, 28 were related to hip pathologies in dancers, 7 were related to causative factors, 7 described types of proposed treatments, and 2 provided general information about dancing. Although dancers are quite prone to injuries, the studies conducted on this subject are limited. Hip pathologies occupy up to 50% of cases and may be due to hyperextension or a direct impact on the joint. The most prevalent conditions include snapping hip syndrome, femoroacetabular impingement syndrome, acetabular labral tears, bursitis, and fatigue fractures due to stress on the joint. The above injuries are likely to occur due to factors associated with the excessive range of motion of the joint and the forces it is exposed to, high training loads, hyperactivity, the laxity of a dancer's joints, gender, age, and BMI. When treating dancers' injuries, a conservative approach is the preferred strategy. Surgical treatment is frequently avoided due to the prolonged recovery period following surgery.

Conclusion: Dancer's hip injuries, due to biomechanical loading, the factors that predispose individuals to their occurrence, and the proposed rehabilitation methods, require further research to improve the validity and reliability of the current findings.

4.8. Impact of Box Squats and Pin Squats on Powerlifting Performance: An Electromyographic Analysis

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Introduction: Surface electromyography (sEMG) is a non-invasive technique applied to the skin that is able to record the muscles' electrical activity. In sports science, sEMG is used to assess muscle activation, analyse performance, and monitor muscle fatigue. This study aims to examine muscle activation, namely the electrical potentials produced by the

contraction of the gluteus maximus, during a back squat in two groups who underwent different training modalities.

Methods: A total of 20 healthy subjects were selected: 9 participants (age: 21.7 ± 1.8 years, 7 males) followed a training based on box squats, while 11 (age: 21.3 ± 0.7 years, 9 males) observed a program based on pin squats. The study was conducted in two sessions, each involving a single repetition of back squat, with a 3-month interval between sessions, during which participants followed their respective training programs. Muscle activation was measured through sEMG (Encephalan Mini AP-10 system). An analysis of signals was performed using Matlab2023b, computing the median frequency, ARV, and sample entropy. RM-ANOVA and post hoc analysis with Bonferroni correction were performed to identify differences between groups and over time.

Results and discussion: The RM-ANOVA assessed statistical differences over time for the ARV ($p < 0.0001$), and the post-hoc analysis showed an increase in the ARV for both the box squat ($p < 0.0001$) and the pin squat ($p = 0.015$) groups. Notably, no significant differences were assessed between the two groups ($p = 0.808$). Concerning the median frequency and the sample entropy, no statistical differences were assessed. These results suggest that both trainings had a positive effect on muscle activation. However, the box squats group showed a higher increase over time with respect to the pin squat group.

Conclusion: This study analyzed the activation of the gluteus maximus muscle during the back squat after two different types of training. The results demonstrated the effectiveness of sEMG in assessing differences between training methods, providing insights to optimize training strategies.

4.9. Kinematic Assessment of the Knee Joint

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In this presentation, we will present the research that has been published in the last ten years on the use of an innovative piece of technology—the knee kinesiography exam. This exam relies on a harness (the KneeKG) attached on the shank and the thigh, which reduces soft tissue artifacts compared to methods using markers glued on the skin. On the harness are fixed optoeletronic markers, whose movements are followed by a portable optoelectronic camera (NDI). The exam consists of a calibration phase, followed by two 45 s walking periods on a treadmill. The system then automatically identifies biomechanical markers on the flexion/extension, ab/adduction and internal/external rotation curves. These markers, known to be related to pain and disease progression, are then translated into patient-specific exercises to be performed at home. Several medium and large cohort studies used the knee kinesiography exam to characterize three-dimensional knee kinematics in a healthy population, in various degrees of knee osteoarthritis, in patients who underwent unicompartmental or total knee arthroplasty and in patients who experienced a ligament rupture. This presentation will summarize the main results issued from these studies, highlighting the clinical usefulness of the technology for managing knee problems.

4.10. Quantitative Data to Evaluate Clinical Pilates Efficacy in Chronic Low Back Pain Using Inertial Measurement Units

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Introduction: Chronic nonspecific low back pain (CNLBP) is a multifactorial condition that affects a large percentage of the population worldwide. Therapeutic exercise is among the strategies used to treat the symptoms of the condition, and in recent years, the Clinical Pilates method has gained popularity as a treatment option [1]. This study aims to investigate the effectiveness of the Clinical Pilates method in patients with CNLBP by analyzing quantitative data that evaluate the functionality of the lumbar spine [2,3].

Methods: Twenty-two patients diagnosed with CNLBP were included in this research. The subjects were measured for their lumbar spine range of motion (ROM) and the time it took them to complete three clinical assessment tests. Two inertial measurement units (MMR+ and Mbientlab) were utilized to measure motion data throughout the execution of the prone plank bridge test (PBT), the side bridge test (SBT), and the supine bridge test (SUBT) [4]. The data were obtained before and after the completion of a six-week Clinical Pilates therapeutic exercise rehabilitation program. **Results and Discussion:** Statistically significant differences were found in all three tests' kinematic patterns and time pre- and post-rehabilitation program. The subjects demonstrated improvement in all three assessments, as well as an increase in the range of motion achieved throughout the execution of the program [5]. **Conclusions**: These findings align with the existing literature that suggests possible enhancements in the clinical condition of patients following intervention with Clinical Pilates. Further research should be carried out using a wider range of clinical evaluation methods to confirm the efficacy of therapeutic exercise in treating symptoms of CNLBP [6,7].

4.11. Similar Spatiotemporal Parameters of Gait Between Children with Typical Development and Autism Spectrum Disorder

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Introduction: Autism Spectrum Disorder (ASD) is a common, inherited, heterogeneous, and lifelong neurodevelopmental disorder with underlying cognitive features that coexist with other conditions [8,9]. Walking is a fundamental human activity that is fully related to motor control. The identification of gait disorders may allow for early diagnosis and better treatment planning. The present study aims to systematically analyze and evaluate the spatiotemporal gait parameters in children with ASD and Typical Development (TD), correlate the results, and possibly identify motor patterns outside of physiological limits. **Methods:** A total of 12 children were divided into a TD group (5 males, 1 female; mean age: 4.3 years) and an ASD group (5 males, 1 female; mean age: 5 years). A motion capture system with six Vicon MCam optoelectronic cameras (Oxford Metrics Group Ltd. Yarnton, Oxfordshire, UK) and two Bertec force plates was used for gait analysis. The statistical software IBM SPSS Statistics 29 was used to conduct the analysis. **Results and Discussion:** No statistically significant differences were observed regarding the normalized values of the examined spatiotemporal parameters: gait velocity, stance time, gait cycle time, step length, step time, double support time, or step width. The findings of our study revealed that children with ASD had an increased gait velocity and a slight decrease in stance time, which aligns with the existing literature. Gait cycle time, step time, double support time, swing time, and step length and width reveal no significant differences between ASD and TD, which coincides with the findings from previous studies [10–13]. Conversely, other studies have identified notable differences in spatiotemporal characteristics [14–16]. **Conclusions**: These contradictory results explain the heterogeneity of the gait parameters observed in patients with ASD. Further research should be conducted in a non-laboratory setting, utilizing inertial measurement devices in conjunction with electromyographic data to thoroughly evaluate ASD gait patterns [17,18].

5. Biomedical Biomaterials

5.1. A Review of Patents for Achieving Laparoscopic Len Cleaning During Minimally Invasive Surgery

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Introduction: In minimally invasive surgery, the laparoscopic lens may face contamination from condensation, smoke, blood, and debris, leading to obscured visual fields during this procedure. To address this challenge, researchers have developed numerous patents, as discussed in this systematic review. This review aims to investigate the patents proposed to achieve effective cleaning of laparoscopic lenses for optimal visualization during minimally invasive surgery.

Materials and Methods: An innovative methodology was employed to identify, describe, and categorize patents related to laparoscopic lens cleaners. The ESPACENET database was utilized to search for these patents, while patent descriptions and statuses were obtained from Google Patents and USPTO. Each patent status was assigned a score using a 3-point Likert scale: 2 points for granted, 1 point for pending, and 0 points for abandoned.

Results: A detailed examination was carried out on 61 identified patents, which were then sorted into two primary categories: mechanical interactions and chemical interactions. To enable comparisons, the scores within each category were combined. The results reveal that 48% of granted patents for laparoscopic lens cleaners are related to collision methods, while 56% of abandoned patents are associated with brush/wipe methods.

Conclusion: This review demonstrates that collision methods are the best among other methods for achieving a successful lens cleaner patent. This review recommends that future laparoscopic lens cleaning patents use a hydrophilic or hydrophobic lens surface combined with collision cleaner techniques.

5.2. Active Hydrogels: Innovative Hydrogels Enriched with Natural Plant Extracts for Dermatology

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The ActiveHydrogels project aims to develop innovative hydrogels enriched with carefully selected natural plant extracts, intended for advanced dermatological and cosmetic applications. Hydrogels are well known for their gel-like structure and exceptional water retention capabilities, making them ideal carriers of active substances. This project introduces a novel approach by combining modern hydrogel technology with natural extracts, creating products that offer superior skin hydration and targeted therapeutic benefits.

The project's primary innovation lies in the integration of specific plant extracts, such as calendula and chamomile, into the hydrogel matrix. Calendula extract is incorporated to enhance skin regeneration, while chamomile extract provides anti-inflammatory and soothing effects. These natural ingredients are chosen based on their well-documented therapeutic properties and synergistic benefits when used in combination with hydrogels.

To ensure scientific rigor, the project employs a detailed experimental design, including advanced hydrogel synthesis techniques and comprehensive characterization methods. The expected outcomes include the development of hydrogels with enhanced mechanical properties, increased bioavailability of active compounds, and improved efficacy in skin care applications. This research also explores the stability and release kinetics of the active ingredients within the hydrogel network.

The ActiveHydrogels project addresses specific dermatological needs, such as the care of dry and sensitive skin, as well as the treatment of minor skin injuries and inflammations. By focusing on these applications, the project aims to provide practical solutions for common skin conditions, enhancing the quality of life for users.

Conducted within the SMART-MAT Functional Materials Scientific Club at Cracow University of Technology, this project benefits from an interdisciplinary collaboration and

access to cutting-edge material science expertise. The affiliation with SMART-MAT and funding from FutureLab underscore the project's commitment to advancing scientific knowledge and fostering innovation in functional materials. This research is conducted by students, who develop their knowledge and experience through the practical design of biomaterials.

5.3. Advancing Cell-on-a-Chip Interfaces with Bio-Functional Terpolymer Nano-Brushes Exhibiting Strong Resistance to Bacterial Adhesion

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Introduction: Bioengineering plays a crucial role in developing advanced biomedical devices and interfaces that integrate biological systems. One major challenge in the development of cell-on-a-chip interfaces is preventing bacterial contamination while maintaining cellular compatibility. Low-fouling (super-)hydrophilic zwitterionic polymer materials have emerged as potential biomedical materials and bio-functional coatings. Here, we report a novel terpolymer nano-brush coating that effectively suppresses undesired biomolecular fouling and biofilm formation while providing sufficient molecular functionalization capacity.

Methods: A terpolymer brush nanolayer composed of carboxybetaine methacrylamide (CBMAA), N-(2-hydroxypropyl) methacrylamide (HPMAA), and sulfobetaine methacrylamide (SBMAA) was synthesized on glass and gold-coated glass substrates using the ATRP method. The chemical structure, thickness, surface zeta potential, fouling resistance, and wettability of this coating were analyzed using XPS, FT-IRRAS, spectroscopic ellipsometry, electrokinetic analyzer, SPR, and water contact angle measurements. Bacterial and cell adhesion studies were conducted on bare and RGD-functionalized terpolymer brushes.

Results: In vitro analyses confirmed the coating's exceptional resistance against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* while enhancing macrophage mobility compared to uncoated glass; likely due to the coating's highly hydrated nature and low protein adsorption. RGD-functionalized terpolymer coatings were found to be non-cytotoxic for SaOS-2 osteosarcoma cells; promoting cell adhesion and spreading without significantly increasing bacterial adhesion or protein adsorption. These results demonstrate that bio-functional terpolymer nano-brushes provide a dual benefit of bacterial resistance and cellular compatibility; making them highly prospective for cell-on-a-chip applications.

Discussion: This study discusses the importance of combining antifouling efficacy; enhanced cellular interaction; and non-cytotoxicity when developing tailored materials for next-generation cell-on-a-chip interfaces. Future research will focus on scaling up the synthesis process and exploring the long-term stability and biocompatibility of these nano-brushes in various bioengineering applications.

5.4. Alginate–Polysaccharide Hydrogel Films for Lactic Acid Bacteria Immobilization, Cultivation, and Low-Temperature Storage

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Introduction: Alginate-based hydrogel films are widely used as wound dressings. Probiotic lactic acid bacteria, particularly lactobacilli and bifidobacteria, are promising therapeutic agents for wound dressings due to their antagonistic action against wound infection pathogens, potentially through competitive exclusion and the production of antimicrobial compounds. The aim of this study was to address the challenges encountered in the development of probiotic-loaded hydrogel wound dressings, namely, the loss of antibacterial substances during the immobilization, reduced cell viability, and deterioration of the films' mechanical properties during storage.

Methods: Probiotic lactic acid bacteria (*Lactobacillus bulgaricus* and *Bifidobacterium bifidum*) were immobilized in alginate-based hydrogel supplemented with pectin or starch. Films were cultured in Blaurock medium for 2–6 days, saturated with cryoprotectants (glycerol or DMSO) at 5–20% concentration, and stored at various temperatures (+25 °C to −80 °C) for 7 days. The efficiency of bacterial immobilization, viable cell count, and antagonistic activity against wound infection pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were evaluated.

Results and Discussion: Bacterial cells were immobilized by spraying calcium chloride on a cell suspension in sodium alginate. Pectin or starch addition significantly improved the films' mechanical properties. Culturing films with immobilized bacteria for 2 days resulted in a 100-fold increase in viable cell count and the acquisition of antagonistic properties against wound infection pathogens. Optimal cryoprotectant concentrations were established, and the films with immobilized bacteria maintained their key properties after low-temperature storage.

Conclusions: A protocol for obtaining alginate-based hydrogel films supplemented with pectin or starch for the immobilization, cultivation, and low-temperature storage of lactic acid bacteria was developed. The films exhibited high antagonistic activity against major wound pathogens, suggesting their potential application as wound dressings for treating infectious wounds.

5.5. Application of Biohydrogels Containing Adaptogens in Innovative Chronic Wound Therapy

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Chronic wounds present a significant challenge for modern medicine, often leading to a substantial reduction in patients' quality of life and high treatment costs. In response to these challenges, this project explores the application of biohydrogels containing adaptogens in the therapy of chronic wounds. Due to their unique physicochemical properties, biohydrogels can provide an optimal healing environment, including appropriate moisture, protection against infections, and controlled release of bioactive substances. Adaptogens, known for their antioxidant, anti-inflammatory, and immunomodulatory properties, can further support tissue regeneration processes.

The aim of this project is to develop and evaluate biohydrogels containing selected adaptogens such as Ashwagandha, Rhodiola Rosea, and Ginseng. This research will include the optimization of hydrogel composition and in vitro studies on adaptogen release and their effects on skin cells. It is anticipated that the use of adaptogens in biohydrogels will contribute to shortening wound healing time, reducing inflammation, and improving the overall health status of patients with chronic wounds. The results of this project could lead to the development of novel chronic wound therapies, offering effective and safe solutions for patients and the healthcare system.

This project is financed with funds from the state budget granted by the Minister of Science within the framework of the "Student Scientific Clubs Create Innovations" (SKN/SP/601893/2024) "Application of Biohydrogels Containing Adaptogens in Innovative Chronic Wound Therapy". The research was carried out within the SMART-MAT Functional Materials Science Club (section Smart-Mat) at the Faculty of Materials Engineering and Physics of the Cracow University of Technology.

5.6. Automatic Laparoscopic Lens Contamination Detection Based on ResNet18 and Corresponding Cleaning Device Prototype

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Introduction: During minimally invasive surgery, laparoscopic lenses often get contaminated by fog and smoke, reducing video quality and affecting surgeon visibility. Various solutions have been proposed to detect and eliminate contamination in real time without interrupting the procedure.

Materials and Methods: This study utilizes the ResNet 18 architecture, modifying the output layer to contain five units corresponding to the classes in the Laparoscopic Video Quality (LVQ) database: noise (NO), smoke (SM), uneven illumination (UI), defocus blur (DB), and motion blur (MB). A review of existing patents revealed two significant systems for automatically detecting laparoscopic lens contamination. The first patent, by Ding et al., involves a system that activates a cleaning mechanism when image clarity falls below a threshold using pressurized liquid, air, and suction. However, it lacks details on the detection threshold and specific features used. The second patent, by Coffeen et al., describes a fluid-based cleaning system activated upon detecting lens deposits, but it does not specify the detection criteria. Both patents have limitations in the cleaning process. Inspired by Coffeen et al.'s patent, we propose an improved in vitro laparoscopic lens-cleaning device. Our device integrates a ResNet18-based detection system, automatically triggers cleaning, and optimizes fluid flow with angled nozzles. It uses warm saline and carbon dioxide, is safe for the human body, and fits through a standard-size trocar.

Results: Our detection system showed high performance, with 99.50% accuracy in training and 99.15% in validation. Performance metrics on 20 distorted LVQ videos revealed 100% accuracy for smoke and motion blur, 90% for noise and defocus blur, and 65% for uneven illumination.

Conclusion: The model demonstrates robust accuracy, particularly in detecting smoke, motion, and defocus blur, facilitating automated cleaning without surgeon intervention. Future research will test the lens-cleaning device prototype in real-world conditions and compare it with other cleaning devices.

5.7. Bio-Surfactants: A Green Approach to Toiletries

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Surfactants comprise a wide range of chemical substances which continuously play important roles in human life due to their use as cleansing agents, among other applications. Their varied uses are due to their amphiphilic nature, which lowers the interfacial tension of two or more immiscible or insoluble substances through the supramolecular assembly complexation property, allowing for their utilization for cleansing purposes. Soaps/cleansers/detergents are commonly used consumer products and are an integral part of daily life. The soap market in India was estimated at USD 2.9 billion in FY2020 and is projected to grow to USD 4.4 billion by FY2026. The life cycle of toiletries has significant environmental impacts, so there is a necessity to use natural/biodegradable soap to minimize environmental harm. The intentional or unintentional release of chemical surfactants contaminates the environment. Using plant parts as soaps and detergents is an old practice in India and predates the discovery of chemical toiletries. Plants were used as substitutes of shampoo or conditioner. Using the rich heritage of traditional knowledge, these plants can be explored as bio-surfactants for the preparation of modern toiletry formulations, which can be used as alternative approach to toiletries to overcome the harmful effects of chemicals and protect the environment. The use of natural products in the formulation and development of toiletries will definitely help society in terms of the economy, healthcare,

environmental protection, etc. Despite the traditional and ethno-botanical literature, there is limited scientific information available regarding pure herbal soaps. For this reason, the current work focuses on the future of plant-based bio-surfactants and aims at improving production efficiency, cost-effectiveness, biodegradability, and low toxicity, coupled with their mildness and compatibility with natural ingredients and environment-friendly formulations. These plant-based bio-surfactants constitute a promising and sustainable alternative approach to conventional synthetic surfactants in the toiletry industry.

5.8. Bioanalytical Method Development and Validation for Estimation of Hypoxia-Inducible Factor-2 Alpha (HIF-2α) Inhibitor in Spiked Human Plasma Using LC-UV

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Introduction: Belzutifan is a hypoxia-inducible factor-2 alpha (HIF-2α) inhibitor used for the treatment of von Hippel–Lindau disease-associated renal cell carcinoma.

Method: A simple, accurate, precise, and specific HPLC method was developed for the estimation of Belzutifan (BZT) in human plasma using Emtricitabine (ETC) as an internal standard. The analyte and ISTD were separated on a Kromasil C18 (250 × 4.6 mm × 5µ) column using a mobile phase composition. The buffer was composed of Acetonitrile (60:40). The RTs of the analyte and ISTD were found to be 3.446 and 2.363 min, respectively, at a flow rate of 1 mL/min.

Results: Further, the reported method was validated as per the USFDA guidelines, and was found to be well within the acceptable range for all parameters with concentrations of LLOQ 0.075 µg/mL, LQC 0.225 µg/mL, MQC 1.5 µg/mL, HQC 2.4 µg/mL, and ULOQ 3.0 µg/mL. The matrix effect at HQC and LQC was 100.19 and 99.83%; the sensitivity at LLOQ was 99.63%; the precision and accuracy at HQC, MQC, LQC, and LLOQ was between 98.56 and 100.11%. The linearity concentration is in the range of 0.75–3 µg/mL for Belzutifan with a correlation coefficient of r^2 = 0.999 with good stability.

Conclusion: The proposed HPLC method was simple, rapid, precise, and accurate for the determination of Belzutifan in human plasma. Sample preparation showed high recovery and this method shows a higher sample throughput due to the short chromatography time (3.446 min) and simple sample preparation. Thus, the developed HPLC method can be applied to the bioequivalence and pharmacokinetic studies of Belzutifan in human plasma samples and is appropriate for therapeutic drug monitoring in clinical laboratories.

5.9. Boosting Antibacterial Activity with Click Chemistry Functionalized Carbon Quantum Dots

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Introduction: Surface functionalization serves as a keystone for tailoring the properties of carbon quantum dots (CQDs). This strategy allows for precise control over their functionality, ultimately dictating their performance in biocompatibility and targeted applications. SuFEx functionalization of CQD allows for the quick installation of S(VI)F motifs as connective linkers to various functional arms, creating a sulfur hub on the tailored CQD surface. The SuFEx click reaction, being metal catalyst-free, is suitable for bioconjugation where traditional Cu-based click reactions fail. The current research explores the antibacterial activity of SuFEx-functionalized CQD against Gram-positive and Gram-negative bacterial strains.

Methods: CQDs were synthesized under mild hydrothermal conditions from molasses under autogenous pressure for 4 h, filtered, and dried. The as-synthesized CQD were SuFEx functionalized using [4-(Acetylamino)phenyl]-ImidodiSulfuryl diFluoride (AISF) at

room temperature and purified by silica gel chromatography. The antibacterial activity of functionalized CQD was evaluated by qualitative and quantitative antibacterial assays.

Results and Discussion: In this study, we synthesized and investigated antibacterial activity against prominent bacterial strains, *Escherichia coli* and *Staphylococcus aureus*. Employing in vitro experimentation, we assessed the antibacterial efficiency of these materials.

Conclusions: This work effectively synthesized novel materials and assessed their antibacterial efficacy against Escherichia coli and Staphylococcus aureus in vitro. The findings on their efficiency against these well-known bacterial strains will assist in the development of prospective antimicrobial agents.

5.10. Caffeic Acid-Based Carbon Quantum Dots: A Novel Approach for Zebrafish Bioimaging

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Introduction: The blood–brain barrier (BBB) serves as a critical defence mechanism, protecting the brain from harmful substances. This protective layer is a selective, semi-permeable membrane that restricts the passage of most compounds, allowing only a limited range of substances to traverse it. The optical properties of carbon quantum dots (CQDs) render them exceptional tools for bioimaging applications. Zebrafish, as an important animal model, share significant physiological and genetic similarities with mammals, particularly humans. In this study, CQDs are employed for the bioimaging of zebrafish brains, demonstrating their utility in neurobiological research.

Methods: CQDs were prepared from caffeic acid using the hydrothermal method for 4 h. The morphological characterization and optical properties of the CQDs were evaluated. The synthesized CQDs were injected into zebrafish and incubated for 24 and 48 h. Post incubation, the zebrafish were sacrificed and brain sections were observed under epifluorescence microscope.

Results and discussion: The morphological, chemical, and optical characterization of CQD confirms the synthesis of CQDs with photoluminescence properties. Zebrafish brain sections were observed under an epifluorescence microscope, allowing for the detailed visualization of the fluorescence within the neural tissue. Moreover, our findings demonstrate that CQDs successfully traversed the blood–brain barrier (BBB), evidenced by their presence within the brain of the zebrafish specimens.

Conclusion: The detailed visualization of zebrafish brain sections demonstrated the presence of CQDs within the neural tissue, validating their capability to cross the highly selective blood–brain barrier (BBB). This finding highlights the potential of CQDs for applications in neurobiological research and therapeutic strategies. The promising results of this study lay a foundation for the development of CQD-based applications in the realms of neuroscience, diagnostics, and nanomedicine.

5.11. Combining Machine Learning and Musculoskeletal Models: A Novel Route to Optimise the Manufacturing of Biomimetic Ligament Implants

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Introduction: Autografts are the gold standard for ligament replacement but have notable disadvantages. Tissue-engineered implants, particularly electrospun scaffolds, offer a promising alternative by replicating the extracellular matrix's morphology [19]. Machine learning (ML) can optimise the electrospinning process, creating ligament implants with morphology and mechanical properties similar to native tissues [20]. Studying the in vivo hyper-elastic behavior of ligaments through motion capture and musculoskeletal models can further inform biomimetic construct design [21]. This research aims to develop a manu-

facturing optimisation methodology using ML models and experimental biomechanics to create biomimetic ligament implants.

Methods: Polyvinyl alcohol (PVA) scaffolds were produced by systematically modifying the polymer concentration and production parameters. Both 2D and 3D scaffolds were characterised morphologically via scanning electron microscopy and mechanically through tensile testing. Data from 2560 observations informed 20 ML models to predict fibre diameter and inter-fibre separation. Additionally, 28 ML models predicted mechanical properties, including Young's modulus and ultimate tensile strength. A musculoskeletal knee model, combined with kinematic data from 12 young participants, estimated the in vivo biomechanics of the anterior cruciate ligament (ACL).

Results and Discussion: Decision Trees and Rule-Based Models generated a visual route to optimise the electrospinning process, achieving a morphology prediction accuracy of 0.868. Cubist models were most accurate for predicting mechanical properties, with an R^2 of 0.93. Crosslinked triple-twisted/braided filament scaffolds replicated the hyper-elastic behaviour of the native ACL effectively, showing R^2 values of 0.971 and 0.999 when using Mooney Rivlin and non-linear string-based models, respectively.

Conclusions: PVA electrospun scaffolds, optimised using Decision Trees and Rule-Based Models, successfully replicated the morphology and hyper-elastic behaviour of natural ACLs.

5.12. Coupling Blood Clot Coagulation Cascade and Blood Flow Dynamics

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Introduction: Thrombosis is a global health concern that is linked to a number of cardiovascular events and their related death rates. At present, there is a need for the mathematical description of the process of thrombus formation in order to introduce new devices and decrease a number of complications.

Methods: We proposed a mathematical model of thrombus formation in a vessel, taking into account the coagulation and blood flow effects on fibrin clot growth. The mathematical formulation includes non-steady Navier–Stokes equations and incompressibility equations to describe the blood motion and five coupled convection–diffusion reaction equations to describe the temporal and spatial evolution of thrombus. The sub-model of blood coagulation including the kinetics of metabolites (VIIIa and Xa factors, thrombin, protein C, and fibrin concentrations) was adopted. A special equation connecting thrombus growth rate and fibrin concentration based on the proposed hypothesis was also introduced.

Results and Discussion: The concentrations of the components of the blood coagulation cascade were computed. The dependence of thrombus size on velocity magnitudes was also obtained. Additionally, pressure distribution and wall shear stress were evaluated. The maximum flow rate increased by 14% with a thrombus size of 16 mm^3. The proposed model describes changes in the key metabolites of the blood coagulation cascade. The results were compared with clinical and in vitro data from the literature and showed a good corelation.

Conclusions: A simulation of thrombus growth is planned to be incorporated into problems related to the mid- and long-term predictions of cardiovascular devices' behavior, such as grafts, stents, and artificial aortic valves.

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5.13. Electrospun Hyaluronan-Based Nanofibers with Mangiferin: Preparation, Morphology, and Drug Release Kinetics

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Introduction: Mangiferin is a non-toxic bioactive substance with antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory activities. However, it has poor water solubility, and the use of mangiferin is hindered. Biopolymer matrixes, e.g., in nanofibrous form, could be applied to increase the bioavailability of loaded mangiferin. Hyaluronic acid (HA) is one of the attractive biopolymers for such nanofibers. In addition to biocompatibility and biodegradability of HA, the presence of its specific cell receptors (mainly, CD44) allows providing the targeted delivery action.

Methods: High molecular HA was used as a polymer matrix for mangiferin-loaded nanofibers. Electrospinning parameters: 28 kV, flow rate 2 mL/h, distance between electrodes 140 mm. Methods and software: SEM (morphological analysis), UV–VIS spectrophotometry (mangiferin release into PBS with pH = 7.4), ImageJ (statistical analysis), OriginPro (visualization and kinetic model definition).

Results and Discussion: The minimum diameter, average diameter, and range of blank HA nanofibers are equal to 107 nm, 252 nm, and 291 nm, respectively. With mangiferin concentration increase, the above-mentioned characteristics increase up to 152 nm, 291 nm, and 398 nm, respectively. Thus, the slight diameter distribution extension is detected. Mangiferin release has an anomalous (non-Fickian) transport mechanism. It is expected that due to the natural origin and non-toxicity of the initial components, the obtained nanofibers could be characterized as nonhazardous and biocompatible material, and after additional investigations, including in vitro and in vivo analysis, they could be recommended for burn and wound regenerative coatings and transdermal delivery systems. Cross-linking is recommended for tuning the controlled release rate.

Conclusions: Thus, the obtained results can be used in the further development and improvement of delivery systems with mangiferin. The authors are planning to continue this research and to analyze the toxicity, efficacy, and mechanism of targeting action.

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5.14. Enhancing Cross-Linked UHMWPE Knee Prosthesis Design: A Comprehensive Approach to Assessment and Optimization

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Introduction: Osteoarthritis (OA) is a major cause of disability, often necessitating knee replacement surgery. The longevity and effectiveness of knee implants are crucial, with loosening being a primary cause of joint replacement failure, often induced by osteolysis from wear debris on polyethylene articulating surfaces. This research focuses on enhancing the durability and performance of knee liners, critical components in knee prosthetics. This study evaluates the durability of twenty retrieved knee liners made of cross-linked, ultra-high-molecular-weight polyethylene (UHMWPE) and correlates damage patterns with stress development. Additionally, an upscaled knee liner design is introduced. **Methods**: The retrieved UHMWPE knee liners underwent rigorous assessment using four in vivo damage assessment methods. Optical and confocal microscopy techniques were employed to quantify wear characteristics. Computer-aided drawing (CAD) facilitated finite element analysis (FEA), correlating FEA outcomes with surface evaluations. An upscaled knee liner design was introduced and evaluated using ANSYS and fatigue life prediction models, optimizing design parameters in SolidWorks. **Results**: This study shows advancements in the structural integrity, performance, and optimization of knee liners. Correlations between FEA outcomes, surface evaluations, and gait analysis provide comprehensive insights. The integrated approach, combining in vivo damage assessment and computational simulation, proves effective in advancing knee prosthesis design. **Conclusion**: Linking damage patterns with stress development is crucial, with computational simulations playing a key role in validating techniques. The upscaled knee liner design and advanced fatigue life prediction models demonstrate potential for enhancing knee

prosthesis durability and performance, ultimately benefiting patients undergoing knee replacement surgeries.

5.15. Enhancing Burn Wound Healing with Plectranthus amboinicus Extract-Loaded Foam Dressings

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Introduction: Foam dressings have been effective in treating pressure injuries/ulcers, diabetic foot ulcers, and venous ulcers, among other chronic wounds. Even though the currently available polyurethane foams are absorbent in nature, there is a need for the development of foams which are highly hydrophilic in nature. *Plectranthus amboinicus* is traditionally used as an anti-inflammatory and wound-healing agent. Its incorporation in a hydrophilic polyurethane (PU)-based foam dressing will offer the dual benefits of a highly absorbent foam dressing and the healing potential of *P. amboinicus*.

Methods: Hydrophillic PU foam dressings were prepared and loaded with *P. ambionicus* leaf extract (PAE). The dressings were prepared with varying concentrations of extract along with Toluene diisocyanate, polyols, catalysts, chain extenders, and hydrophilic polymers. The microstructure, moisture vapour transmission rate, porosity, absorption rate, surface roughness, and mechanical strength of the dressings was assessed followed by in vivo dermal irritation studies on rabbits and burn wound healing studies in a rodent model.

Results and Discussion: The moisture vapour transmission rate of the dressings was found to range between 1900.06 ± 0.59 and 2050.00 ± 0.25 g/m^2/day. The absorption rate was found to be between 1.27 ± 0.01 and 1.31 ± 0.00 g/cm^2 and was found to be highest with dressings containing polyacrylate as hydrophilic polymer. Tensile strength measurement indicated that the selected formulations were flexible enough to withstand regular handling during dressing changes. Acute dermal irritation performed on rabbits showed no irritation, erythema, eschar, oroedema. The in vivo burn wound healing studies performed on albino Wistar rats showed better healing in comparison to a commercial formulation.

Conclusions: The hydrophilic foam dressing developed using *Plectranthus amboinicus* leaf extract demonstrated promising efficacy in burn wound healing, suggesting its potential as an effective natural remedy for burn wound management.

5.16. Formulation and In Vitro Evaluation of Herbal Wound Healing Patch Using Lantana Montevidensis Extract

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Background: Wound management is crucial in healthcare with effective, natural healing agents. Lantana montevidensis is known for its pharmacological properties, particularly in wound healing. This involves creating an extract from L. montevidensis flowers using water as solvent and putting it into a biocompatible matrix to form a patch.

Aim and Objective: This study aims to formulate a herbal wound healing patch using a Lantana montevidensis extract and evaluate its efficacy in promoting wound healing through in vitro studies.

Methodology: Herbal wound healing patches were prepared using the solvent casting method, with starch, sodium alginate, and glycerin as plasticizers. The plant extract was obtained by decoction, with water as the solvent. The patches' mechanical strength was assessed via folding endurance tests, and their compatibility was evaluated via an FTIR spectral analysis. In vitro evaluations included anti-inflammatory, antimicrobial, and hemocompatibility assessments, with stability tests conducted at accelerated temperatures.

Results and Discussion: The chemical analysis revealed the yield of active constituents. The IR spectral analysis identified no incompatibility in the distinctive wavenumber regions

of the starch and sodium alginate films. The patches' swelling index and stability at room temperature were investigated. Their biocompatibility was confirmed via hemolysis ratio and pH measurements. The albumin denaturation inhibition of the herbal patches was assessed, indicating their potential wound healing properties.

Conclusion: The herbal wound healing patch developed using an L. montevidensis flower extract demonstrated promising efficacy in wound healing, suggesting its potential as natural remedy in wound management.

5.17. Granulated Wollastonite–Hardystonite Bioceramics

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Introduction: Hardystonite (HS, $Ca_2ZnSi_2O_7$) is a promising compound for bone tissue restoration due to its biocompatibility and good mechanical properties. However, it has too low a rate of dissolution in biological fluids, according to data in the literature. In this work, we propose a method of composite production based on $Ca_2ZnSi_2O_7$ and $CaSiO_3$ mixtures to increase the dissolution of HS materials.

Methods: Granules were synthesized by the emulsion method using mechanical mixtures of powdered β-wollastonite and ZnO (0, 0.5, 1.5, 2.5, 5, 10, 12.5, 15, 20, 25 wt.%), with gelatin as a binder. All syntheses were carried out in 10 parallels. Granules were heat-treated in air at 1250 °C and analyzed using XRD, FTIR, TGA and SEM. Their fractional composition, true and average density, open porosity and solubility in Tris-HCl buffer were determined.

Results and Discussion: Spherical granules with a diameter of 0.1÷3 mm were synthesized. During heat treatment, the binder burns out (200 ÷ 500 °C) and hardystonite is formed as a result of solid-state interaction between ZnO and $CaSiO_3$ (above 750 °C). According to XRD and FTIR, variation in ZnO content in the initial powder leads to the formation of α-$CaSiO_3$ mixed with up to 96 wt.% $Ca_2ZnSi_2O_7$. The granules had a density >1 g/cm^3 and open porosity of 60 ± 5%. Materials can gradually dissolve in Tris-HCl buffer. Concentrations of ions in solutions decrease in the order $Ca^{2+} > SiO_3^{2-} > Zn^{2+}$ at all time points. The less HS content in granules, the more Ca^{2+} and SiO_3^{2-} quantities and less Zn^{2+} in the surrounding media. Granules with 96 wt.% HS lost less than 0.5 wt.% in 6 months. A 2-fold decrease in HS content leads to a 25-fold increase in weight loss.

Conclusions: Composites with varying content of $Ca_2ZnSi_2O_7$ and $CaSiO_3$ were produced. Mixing with wollastonite allowed for a significant increase in the dissolution rate of HS-containing materials.

5.18. Human Embryonic Stem Cells Naïve Pluripotency Induction in a Fully Defined Xenogeneic-Free Synthetic Polymer Dish Coating (PMEDSAH)

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The transition into the naïve state in human pluripotent stem cells (hPSCs) represents a crucial advancement in stem cell biology, as it captures the cells in a more primitive/neutral and epigenetically unrestricted state, therefore offering more plasticity compared to the primed state. Achieving this naïve state is essential for maximizing the potential of hPSCs in regenerative medicine and developmental studies.

Traditionally, Matrigel has been employed as a plate coating for growing hPSCs; however, Matrigel contains animal-derived components that may present transgenic significant limitations for clinical applications. In this study, we explore the efficacy of using a xeno-free plate coating, PMEDSAH, for inducing hPSCs to a naïve state using FINE [22] and NHSM [23] cell culture media. Our findings indicate that PMEDSAH supports robust cell growth and pluripotency with NHSM media, offering a viable alternative to Matrigel. Both

media demonstrated effective reversion to the naïve state, characterized by the expression of key naïve pluripotency markers. Our data suggest that NHSM media in PMEDSAH plates seemed to enhance naïve cell induction. This work underscores the importance of adopting xeno-free culture systems and optimized media formulations to advance the clinical and research applications of naïve hPSCs.

5.19. In Vitro Analysis of Antibacterial FeMnSi-Cu Biodegradable Alloy in Simulated Body Fluid

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Introduction: Biodegradable Fe-based alloys such as Fe-Mn-Si are currently being studied for temporary medical implant applications and are designed to perform temporary structural functions in the human body while undergoing gradual degradation. These alloys offer promising medical implant applications owing to their biocompatibility, degradability, and mechanical properties. A key challenge lies in balancing the mechanical properties with controlled degradation. Another important aspect is improved antimicrobial properties.

Methods: The aim of this study was to develop a novel biodegradable FeMnSi alloy with antimicrobial properties and an enhanced degradation rate suitable for long-term medical implant applications. Therefore, the FeMnSi-1Cu alloy was developed and investigated in both cast and hot-rolled states. Scanning electron microscopy (SEM), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX) were used for microstructural and chemical evaluation. The thermal properties were characterized by means of dynamic mechanical analysis (DMA), and the resulting microstructural changes were observed using atomic force microscopy (AFM). Simulated body fluid (SBF) immersion tests and linear and cyclic potentiometry were used to investigate degradation. To correlate the metal–liquid chemical reactions with the degradation progress, the pH of the solution during immersion was recorded over minutes. ASTM G31-72(2004) was used to determine the degradation rates (DRs).

Results and discussion: Due to the applied thermomechanical stress, the AFM images revealed a slight change in the plate dimensions due to refinement. Generalized corrosion was identified, and an increase in mass was observed over the first 3–5 days. Despite the short immersion time and the DMA test, the samples showed a high degree of surface corrosion, which could affect their mechanical behavior under external loads.

Conclusions: The addition of Cu to the FeMnSi alloy is favorable for its antimicrobial effect, as well as for improving workability and corrosion resistance, which will encourage future studies on this alloy.

5.20. Inhibitory Action of Bioactive Composites on S. mutans

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Composites are restorative materials that have evolved in recent years, becoming part of a new group of materials known as bioactive materials. These materials generate an

ionic exchange with dental structure, promoting remineralization and preventing bacterial activity. This study aimed to determine the bacterial inhibition of different restorative composites on *S. mutans* group.

Field strains of *S. mutans* were used and isolated in our laboratory (LABOFOUNT) and confirmed by reference strains ATCC. Nanohybrid Composite Filtek Z350 (3M ORAL CARE), Nanohybrid Composite Filtek Bulkfill (3M ORAL CARE) (traditional composites) and Alkasite N Cention Composite (Ivoclar Vivadent), and ACTIVA Bioactive Restorative Composite (Pulpdent) (bioactive composites) were evaluated. They were prepared according to the manufacturer's instructions, following standardized biosafety protocols. Test bodies were fabricated using Teflon molds, following laboratory protocols.

Two inhibition studies were performed at different times, in both cases, the samples were studied in triplicate. 50 µL of S. mutans inoculum at 0.5 McFarland scale was seeded in SB20M medium, circular perforations of 4 mm in diameter and 2 mm in depth were made for the placement of composites and controls (negative: 0.12% chlorhexidine digluconate and positive: sterile distilled water), incubated at 37 °C in a candle jar for 48 h. Subsequently, the inhibition halos were measured with a digital caliper under a stereoscopic magnifying glass.

Inhibition halos of 18.2 mm and 18.4 mm average values corresponding to the two studies were measured for the negative control, while values of 6.9 and 7.1 mm were obtained for Alkasite N. Cention Composite. No inhibition halos could be determined for the other materials.

In this study, only Alkasite N Cention Composite inhibited *S. mutans* bacterial growth. It is suggested to conduct inhibition studies in relation to the minimum inhibitory concentration (MIC) and colony-forming unit (CFU) count to further evaluate the effect of bioactive composites on *S. mutans*.

5.21. Layer-by-Layer Assembly for Manufacturing Local Chemotherapy Platforms with Controlled and Sustained Drug Release to Prevent Local Tumor Relapse

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Introduction: Layer-by-layer (LbL) assembly is characterized by controlled and prolonged drug release over 3 months compared to electrospinning or solvent casting methods, making it a promising approach for local chemotherapy platforms. The aim of this research was to develop local chemotherapy platforms using different layer-by-layer assembly methods to identify the most efficient drug encapsulation and to achieve the uniform and sustained drug release kinetics.

Methods: The platform developed in this study consists of a polycaprolactone substrate and a multilayer coating produced by LbL assembly. The coating contains the chemotherapeutic drug doxorubicin (DOX), which was stabilized for release by preforming an ionic complex between poly-γ-glutamic acid and DOX, which was then used as a polyanion during LbL assembly. Different LbL assembly methods (by spin or dip), various polycations and the addition of DOX-unloaded polyelectrolyte bilayers were examined. The drug release mechanism was studied in vitro in PBS, mimicking the natural environment of physiological fluids with pH 7.4 at 37 °C. The platforms were also tested for the in vitro antitumor activity of DOX using ovarian cancer cells (SKOV-3).

Results: DOX release from the obtained platforms was sustained for over 6 months with minimal burst kinetics and uniformity, but the drug encapsulation in LbL assembly by spin was tenfold higher. Dip-coated platforms had a coefficient of determination (R^2) of 0.84, while spin-coated platforms had an R^2 of 0.94, when compared to a zero-order drug release model. The in vitro studies showed that the platform has no effect on the antitumor activity of DOX.

Conclusions: This work is encouraging for the development of drug delivery systems as it demonstrates the potential of spin-assisted assembly, offsetting the explosive nature of drug release, and showing its highest stability compared to similar studies.

5.22. Mechanical Properties and Surface Morphology of 3D-Printed Objects from Biopolymers

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Three-dimensional printing, especially the technology of fused deposition modelling (FDM), is more and more often used for the production of different objects, especially those that are produced only in small numbers or that have complicated shapes that cannot be produced in a different way. The increase in 3D-printed objects, on the other hand, leads to an increase in polymeric waste that has to be taken into account. One way to reduce this problem is using biodegradable thermoplastic polymers instead of petroleum-based plastics, which are responsible for "white pollution". Amongst the biodegradable, biobased polymers used for 3D printing, poly(lactic acid) (PLA) is most commonly used, while polyhydroxyalkanoates (PHA) and a few other materials are also under investigation for 3D printing. PLA, however, has a much slower degradation rate under environmental conditions than other biopolymers and actually needs commercial composting. This is why a study was performed investigating the printability of different biobased FDM filaments with better biodegradability. In addition, the mechanical properties and surface morphology of the 3D-printed objects were compared with those of PLA and PETG. Due to the potential use of such filaments in tissue engineering and other biomedical applications, the chemical stability and the possibility of autoclaving the biobased materials were also successfully tested. The results of this study show the potential of new biobased FDM filaments for biomedical and biotechnological applications.

5.23. Naturally Derived Biopolymers in 3D Bioprinting for Biomedical Applications

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Tissue engineering and regenerative medicine have new meanings. Three-dimensional bioprinting has become one of the most advanced and useful innovations that allows the creation of personalized macroscopic and microscopic constructs at different scales that match a patient's anatomy. Generally, the process of 3D bioprinting consists of several steps, namely pre-bioprinting, cell and bioink preparation, the bioprinting process, and post-bioprinting/applications. Intensive research efforts are currently underway to develop highly printable and biocompatible materials. Among the variety of bioprinting materials (i.e., biomaterial inks), naturally derived hydrogels have attracted great interest due to their beneficial properties in terms of biocompatibility, cost effectiveness, and biodegradability. Among them are cellulose, chitosan, and lignin. Cellulose is the most abundant biopolymer in nature; it has various advantages over others, such as good mechanical and barrier properties. Moreover, chitosan biopolymer constitutes a promising candidate for the preparation of hydrogels for application in this field due to its beneficial properties, such as its antimicrobial activity and structural resemblance to natural glycosaminoglycans. Furthermore, due to the non-cytotoxicity, biocompatibility, biodegradability, mechanical strength, and reactivity of the lignin biopolymer, it has been considered an excellent candidate to manufacture hydrogels for 3D bioprinting applications. The capacity of 3D bioprinting of these biopolymer-based hydrogels has been demonstrated in the regeneration of different damaged tissues, including cartilage, bone, muscle, skin, blood vessels, and other biological tissues. In this study, we provide a comprehensive review of the formulation and use of three functional biomaterials as ink-based hydrogels. Cellulose, chitosan, and lignin are

comprehensively discussed, and an examination of the status of the biomedical application of these biopolymer-based hydrogels for 3D bioprinting is then provided.

5.24. NeuroAmph: Innovative Synergy of Polydopamine and Peptide Amphiphiles for Enhanced Cognitive Pathology Treatment

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Introduction: The complicated nature and progressive deterioration of neural tissues make therapeutic intervention for cognitive diseases, such as Alzheimer's disease and other neurodegenerative disorders, enormously challenging. Current medications often fail to prevent the advancement of these diseases. NeuroAmph presents itself as a revolutionary therapeutic approach that combines polydopamine (PDA) and peptide amphiphiles (PAs) to overcome these barriers. PDA, which is derived from dopamine polymerization, offers strong adhesion properties and biocompatibility. On the other hand, PAs have the ability to self-assemble and deliver bioactive molecules to neural tissues with precision.

Methods: This review explores the synthesis methodologies and characterization techniques of NeuroAmph, emphasizing their seamless integration into multifunctional nanostructures tailored for neurological applications. Several studies have shown that NeuroAmph works to reduce oxidative stress markers and improve neuronal viability in disease models. This makes it even more likely that it can be used to change people's lives.

Results: NeuroAmph operates through sophisticated mechanisms tailored to combat cognitive pathologies at multiple levels. PDA facilitates the robust adhesion and stability of nanostructures that are critical for targeted drug delivery, whereas PAs self-assemble into biocompatible micelles capable of encapsulating neuroprotective agents. When administered, NeuroAmph interacts with neuronal cell membranes to help therapeutic payloads enter cells. By scavenging ROS and regulating antioxidant pathways, NeuroAmph protects neurons from oxidative stress. Additionally, the way that PDA and PAs work together allows for the long-term release of bioactive compounds, which supports neuroregenerative processes and improves synaptic plasticity.

Conclusions: NeuroAmph's integrated approach offers a promising strategy for improving treatment outcomes in cognitive pathologies by addressing key disease mechanisms. Additional research into NeuroAmph's therapeutic effectiveness and safety profiles is critical for furthering its use in clinical settings and offering novel approaches to controlling neurological disorders.

5.25. Peptide Nanocarriers as a Revolutionary Tool in Targeted Anti-Cancer Therapy

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Modern oncology faces the challenge of effectively treating cancers while minimizing side effects on healthy cells. Peptide nanocarriers for drug delivery open new perspectives in targeted anti-cancer therapy, offering advanced solutions for delivering drugs directly to cancer cells. Peptides, due to their unique properties of biodegradability and biocompatibility, are ideal components of nanocarriers and are capable of efficiently binding and transporting anti-cancer drugs.

Peptide nanocarriers demonstrate the ability to enhance the biological availability of drugs, which is particularly important for anti-cancer drugs like paclitaxel that often have limited bioavailability. Thanks to chemical modification, peptides can be tailored to

transport a wide range of therapeutic substances, including both small chemical molecules and larger biologically active proteins. For instance, our study highlights the successful delivery of a peptide–drug conjugate that targets the PI3K/Akt pathway in triple-negative breast cancer cells, resulting in a 50% increase in apoptosis compared to free drug treatment.

In summary, peptide nanocarriers represent a promising platform for modern targeted anti-cancer therapies, offering the possibility of significant progress in cancer treatment, as well as the improvement of clinical outcomes. Their application has the potential to revolutionize approaches to oncology therapies, contributing to extending patients' lives and improving their quality of life. However, challenges such as potential interactions with healthy tissues and the long-term stability of peptide modifications must be addressed in future research.

This research was carried out within the SMART-MAT Functional Materials Scientific Club of the Faculty of Materials Engineering and Physics at Cracow University of Technology and as part of the project entitled "Nanogels for biomedical applications", which was financed by the FutureLab organization operating at Cracow University of Technology.

5.26. Preparation and Study of the Properties of Microparticles Based on Biodegradable Poly-3-Hydroxybutyrate in a Composition with Sodium Alginate

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Polyhydroxyalkanoates (PHAs) belong to a class of polyesters of natural origin obtained by microbiological synthesis. Despite good mechanical properties, biodegradation, and biocompatibility, PHAs are hydrophobic biopolymers, so the introduction of hydrophilic materials plays an important role, allowing them to influence the properties of the resulting microparticles. The goal of this work was to obtain microparticles from poly-3-hydroxybutyrate (P3HB) in a composition with sodium alginate loaded with a model antibacterial drug (imipinem). The characteristics of the prepared microparticles were studied using SEM, a particle size analyser (Zetasizer Nano ZS), and a spectrophotometer (Cary 60). Microparticles were obtained via solvent evaporation from triple emulsions. It has been established that the addition of sodium alginate affects the properties of microparticles: it increases the size and reduces the surface charge. It was shown that the efficiency of encapsulating imipinem into composite microparticles was almost three times higher compared to P3HB microparticles. In a model medium, prolonged release of the antibacterial drug was demonstrated over 28 days. The study of the biocompatability of microparticles in the culture of NIH 3T3 cells showed no negative effect. Using model Gram-positive and Gram-negative microorganisms as an example, it was shown that microparticles loaded with antibiotic showed antibacterial activity. Thus, it has been shown that the addition of sodium alginate has a positive effect on the properties of P3HB microparticles; namely, it allows increasing the efficiency of drug encapsulation, enhances the release of the drug from the microparticles, does not cause toxic reactions in cell culture, and also preserves the activity of the medicinal drug, which allows us to conclude that these composite microcarriers are promising for the development of long-acting dosage forms. This study was funded by the State Assignment of the Ministry of Science and Higher Education of the RF (project No. FWES-2021-0025).

5.27. Review on the Formulation of Cellulose-Based Hydrogels and Their Biomedical Applications

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In recent years, significant advancements have been made in the development of hydrogels as functional biomaterials, drawing increasing attention to their applications in biomedical engineering. Among various biopolymers, cellulose has emerged as an excellent candidate for hydrogel preparation due to its unique properties. As the most abundant natural biopolymer on Earth, cellulose offers advantages such as biocompatibility, biodegradability, renewability, good mechanical strength, and environmental friendliness, making it one of the safest materials available. The limitations of cellulose arise from its difficulty in dissolving due to the presence of inter- and intramolecular hydrogen bonds as well as van der Waals forces. However, this limitation can be addressed by chemically modifying cellulose, primarily through the etherification of hydroxyl groups, to produce various derivatives, including methylcellulose (MC), ethylcellulose (EC), hydroxyethyl methylcellulose (HEMC), hydroxypropyl cellulose (HPC), and sodium carboxymethyl cellulose (CMCNa). Crosslinking represents a vital step in the hydrogel preparation process, as it establishes the 3D structure and enhances physical and mechanical properties. Different crosslinking techniques are employed to produce hydrogels from cellulose and its derivatives, depending on the intended applications. Cellulose-based hydrogels have shown significant potential in biomedical applications, including tissue engineering, wound healing, drug delivery, 3D bioprinting, and more. In this review, we present the formulation of cellulose-based hydrogels and their biomedical applications. Specifically, we connect the latest knowledge in the literature on cellulose-based hydrogels with examples of how these materials have been utilized in biomedical applications. Additionally, we provide context regarding the importance of cellulose-based hydrogels in biomedical engineering, highlighting their unique advantages and promising potential in the field. Furthermore, we summarize the potential benefits of using cellulose-based hydrogels compared to other biomaterials.

5.28. Superabsorbent Curdlan–Chitosan Foams with Bioactive Additives for Healing Wounds

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Healing burns and wounds is a prevalent health issue. Metabolic and physiological problems such as hypertension, malignancies, kidney disorders, diabetes, and obesity disrupt the natural process of skin healing, leading to the development of ulcers, bedsores, and the need for amputations. These conditions contribute to mortality rates globally. Medical statistics indicate that approximately 1–2% of the global population experiences chronic wounds.

Polysaccharides are commonly employed in the production of superabsorbent polymers, which have the ability to absorb and retain aqueous solutions that are hundreds of times their own weight when dry. Chitosan is a widely recognized carbohydrate polymer with numerous potential clinical uses because of its antibacterial, anticoagulant, anticancer, and hemostatic properties. β-glucans often have a beneficial impact on the human immune system, offering antitumoral and antibacterial properties, which can expedite the process of healing

This work aimed to create innovative foams through the polymerization of curdlan–chitosan at a temperature of 90 °C, using a gradual addition method for incorporating bioactive compounds such as AgNO3 solution, aloe vera, gentamicin, and a mixture of all components. Evidence has shown that the existence of a medicinal substance has a notable impact on the rate at which edema occurs. Through in vivo testing, it was discovered that CUR/CS/MIX foams had a more pronounced impact on skin restoration when compared to pure CUR/CS and the untreated control. The investigation into antibacterial activity revealed the synergistic impact of the constituents against strains commonly found in hospitals. Therefore,

the suggested straightforward approach for manufacturing biocompatible superabsorbent foams has exciting opportunities for developing novel functional platforms as temporary skin replacements for the treatment and regrowth of persistent wounds.

5.29. Sustainable Pharmaceutical Development Utilizing Vigna Mungo Polymer Microbeads

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This study presents a systematic approach to developing novel antidiabetic dosage forms utilizing Vigna mungo (VM) polymer microbeads fabricated via the ionotropic gelation method. The Vigna mungo (VM) polymer was extracted and isolated from the seeds of Vigna mungo using a non-solvent induced precipitation method. The VM biopolymer's intrinsic biodegradability and widespread availability render it an appealing candidate for sustainable pharmaceutical development. The formulation and characterization of the VM polymer in various proportions are delineated, alongside the preparation of uniform microbeads through ionotropic gelation. A 2:1 proportion of sodium alginate and VM was the initial concentration for the microbeads' formulation. Characterization via scanning electron microscopy and Fourier transform infrared spectroscopy ensured their uniformity and structural integrity. Incorporating Vildagliptin, a model antidiabetic drug, into these microbeads enabled assessment of their morphological characterization, drug loading efficiency, release kinetics, and stability under simulated physiological conditions. In vitro drug release studies exhibited 12 h of drug release, which is appropriate for maintaining extended drug release and meeting the therapeutic objectives for diabetes. Evaluation through in vitro studies, alongside the biocompatibility and biodegradability assessments, underscored the safety and sustainability of the VM polymer microbeads. Overall, this study underscores the potential of VM biopolymers as versatile excipients in antidiabetic formulations, offering promising avenues for addressing the global diabetes burden through innovative and sustainable therapeutic interventions.

5.30. The Role and Future Directions of 3D Printing in Custom Prosthetic Design

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The advent of 3D printing technology has revolutionized various manufacturing sectors including the medical field, particularly in the production of prosthetic limbs. Traditional prosthetic manufacturing processes are often time-consuming and expensive, causing amputees to endure long waiting periods and high costs. In contrast, 3D printing offers a rapid, cost-effective alternative, enabling the creation of custom-made prosthetics tailored to the specific needs and measurements of each wearer. Integrating 3D printing technology into prosthetics and orthopedics has ushered in a new era of customization and innovation. This advanced approach facilitates the creation of personalized prosthetics and bone replacements tailored to individual patients' needs. With the latest advancements in software and 3D printing, the use of custom orthopedic implants for complex surgical cases has gained significant popularity. This paper explores the advantages of using 3D printing for prosthetic limb production, highlighting its ability to significantly reduce production time and costs while maintaining high functionality and quality. By leveraging 3D scanning and computer-aided design (CAD), precise digital models of a patient's residual limb can be created, ensuring a perfect fit and improved comfort. Additionally, the flexibility of 3D printing allows for the incorporation of advanced materials and design features, enhancing the durability and performance of the prosthetics. This study also examines the potential for 3D printing to democratize access to prosthetic care, especially in low-resource settings. The

affordability and accessibility of 3D printers, coupled with open-source designs, empower local communities and healthcare providers to produce prosthetics on demand, reducing dependency on centralized manufacturing facilities. By addressing the current limitations and challenges, including material constraints and regulatory hurdles, this paper highlights the transformative impact of 3D printing on the prosthetics industry.

5.31. The Role of Zinc Metal–Organic Framework (Zn-MOF) in Augmenting Anti-Mycobacterial Drug Action

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Introduction: Metal–organic frameworks (MOFs) are highly porous networks composed of transition metal ions or clusters coordinated with organic ligands, offering versatile chemical functionalities. While recognized as effective antibacterial agents, their potential against severe pulmonary mycobacterial diseases remains underexplored. This study investigates Zn-MOF as an adjuvant for antimycobacterial drugs, demonstrating its significant efficacy against *Mycobacterium smegmatis* both alone and in combination with isoniazid and rifampicin.

Methods: Zn-MOF was synthesized via solvothermal reaction using Zinc nitrate hexahydrate (Zn^{2+} ion), 4,4′- bipyridyl (bridge), diphenyl phosphinic acid (organic linker), and dimethylformamide (solvent) at 85 °C for 24 h, yielding a white powder. Physical characterization involved FTIR, Raman, and XRD analyses. Antimycobacterial activity was assessed using a Colony-Forming Unit assay on *M. smegmatis*, a non-pathogenic model organism to study tuberculosis, evaluating Zn-MOF alone and in combination with isoniazid and rifampicin.

Results: FTIR, Raman Spectroscopy, and XRD confirmed the formation of Zn-MOF. The CFU assay demonstrated the superior antimycobacterial activity of Zn-MOF compared to standard drugs. Remarkably, synergistic effects were observed when combined with individual and dual therapies of rifampicin and isoniazid.

Conclusion: Zn-MOF shows promise as a novel adjuvant in tuberculosis treatment. Its efficacy against mycobacteria, especially in combination with first-line drugs, suggests its potential for developing innovative drug delivery systems. Understanding its mechanism could pave the way for enhanced therapeutic strategies against tuberculosis.

5.32. Three-Dimensional Neuroinflammation Model as an Alternative for Tests in Neural Research

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Introduction: The use of animal models is widely spread in the scientific field, because the use of animals is essential for the transition from basic research to clinical research. Furthermore, there is no denying that the scientific advances achieved to date are related to the use of animal models. However, diminishing the usage of animals is desirable. One alternative to the use of animals are three-dimensional in vitro models that better mimic the tissue environment, allowing for more reliable results. The aim of this study was to produce and validate a 3D neuroinflammation model (3DNM) as an alternative for drug testing and alternative treatment approaches. **Methods:** The 3DNM was produced combining 5% alginate, 4% gelatin, 2×10^5 PC12 cells, 4×10^5 BV2 cells and 8×10^5 C6 cells. Cell viability was evaluated with MTT assay. To test the anti-inflammatory potential of a

bioink containing 1.5% decellularized spinal cord tissue, 3% gelatin, 4% sodium alginate and 7.5×10^5 mesenchymal cells, the 3DNM was stressed with lipopolysaccharide (LPS) and treated with the bioink for three days. Reactive oxygen species (ROS) production and lipid peroxidation were measured. IL-6, IL-10 and IL-1B were quantified by ELISA. Non-enzymatic antioxidant defenses were evaluated by the quantification of Thiols. **Results:** MTT results demonstrated that the model was not cytotoxic to the cells because the viability increased when compared to the model stressed with the LPS. The bioink reduced the levels of ROS and pro-inflammatory cytokines on the 3DNM. TBARS assay showed that 3DNM lipid degradation was reduced when it was in contact with the bioink and was very close to that of the control. Thiols indicated that the bioink increased the non-enzymatic antioxidant defense in the 3DNM. **Conclusion:** The 3DNM showed promising results as an alternative model to study neural inflammation. This biomaterial may, therefore, be a solution for decreasing the laboratory use of animals.

6. Nano-Biotechnology

6.1. A Comparative Study of Titanium-Based Coatings Prepared by Magnetron Sputtering for Biomedical Applications

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Introduction: Titanium (Ti)-based coatings are widely used to enhance the surface properties of 316L stainless steel (SS) in biomedical applications. Notably, titanium nitride (TiN) and titanium oxynitride (TiON) are considered advanced ceramics due to their exceptional technical properties. This study explores the properties of TiN and TiON coatings deposited via magnetron sputtering, with a particular focus on how substrate bias voltage influences wettability and corrosion behaviour, driven by the surface's physicochemical traits.

Methods: A pure titanium nitride target was sputtered in a mixed gas atmosphere of argon and oxygen onto various substrates, such as stainless steel, silicon, and glass. The substrate–target distance was set to 30 mm, and the working pressure was maintained at 10^{-2} Torr. A negative DC bias voltage (0 V and -100 V) was applied during a 30-min deposition period. Wettability was assessed and electrochemical behaviour was evaluated in a physiological simulated solution over 12 h of immersion in Hanks' solution.

Results: Substrate bias voltage did not affect the thickness and grain size of the coatings, except for the roughness in TiN coatings, which decreased with a -100 V bias. Wettability tests indicated that TiN coatings had low contact angles and better wettability, whereas TiON coatings showed high contact angles and poorer wettability. The Icorr values for TiN (0 V and -100 V) were significantly lower than those for 316L steel. TiON coatings demonstrated even greater corrosion resistance, significantly outperforming uncoated steel.

Conclusions: TiN and TiON coatings, applied with and without substrate bias (0 V and -100 V) using magnetron sputtering, were evaluated for potential biomedical applications. These coatings exhibited distinct surface characteristics, particularly in terms of contact angle, wettability, and corrosion resistance. The use of negative substrate bias voltage significantly improved corrosion properties. Based on the observed data, TiON coatings are highly appropriate for medical implants.

6.2. Computational Design of Plasmonic Nano-Biosensor Based on Metamaterial Structure for Early Detection of Liver Cancer

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The conventional methods for the diagnosis and treatment of cancer, based on surgical, chemical, and radiation processes, are expensive, time-consuming, and painful. Therefore, research in this field has been devoted to developing precise, cost-effective, and rapid techniques for early cancer detection. In recent decades, optical biosensors have become powerful tools for identifying various biological and non-biological analytes. Optical biosensors possess features such as high sensitivity and accuracy, non-invasiveness, label-free detection, and compact size. The surface plasmon resonance (SPR) phenomenon is one of the optical phenomena created by the absorption of photons at the metal/dielectric interface. The key characteristic of SPR is its sensitivity to environmental changes, meaning that placing normal and cancerous tissue samples on the sensor will result in different sensor responses. Recently, studies in this field have progressed towards using the SPR phenomenon in metamaterials. In this design, a metamaterial structure with SPR properties is utilized to create a plasmonic biosensor capable of detecting samples of normal and human cancerous liver tissues. The main idea is to detect normal liver tissue from cancerous liver tissue with high sensitivity. The designed sensor is based on the metamaterial structure and surface plasmon resonance enhancement. The samples used in this sensor include several liver tissue samples from various patients, including normal and disease-free samples, metastatic liver samples, non-metastatic liver samples, carcinoma liver samples, and non-carcinoma liver samples. In fact, in the proposed sensor in this design, metastatic tissue can also be distinguished from carcinoma tissue.

6.3. Development of Antibacterial Materials for Therapy of Chronic Wounds Using Composite Fibers of Polycaprolactone Loaded with ZnO Nanoparticles with Immobilized Chlorhexidine on the Surface

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Introduction. Chronic wounds are a serious public health problem. Infection by antibiotic-resistant pathogens poses the primary risk. In this study, an antibacterial dressing material was made from electroformed polycaprolactone (PCL) fibers that had zinc oxide nanoparticles (ZnO NPs) and chlorhexidine (CHG) attached to their surface.

Materials and methods. The series of dressings were obtained by electrospinning with the incorporation of ZnO NPs at different concentrations (1, 3, and 5%), followed by the immobilization of CHG on the surface. To increase the antipathogen activity, the samples were plasma-treated in a mixture of $CO_2/C_2H_4/Ar$ gas following CHG immobilization by carbodiimide chemistry. We analyzed the structure and chemical composition of the obtained materials using SEM, EDX, XPS, and FTIR spectroscopy. We evaluated the antipathogenic activity against several bacterial and fungal strains. We evaluated the biocompatibility of the samples with a human fibroblast cell line.

Results. The size of the produced ZnO NPs varies between 9 and 13 nm. The composite fibers have a size that varies between 100 nm and 500 nm. EDS analysis confirms that the main components of the fibers are carbon, oxygen, and zinc. The atomic concentration increases to 1.1%, 2.7%, and 3.9%, respectively, as the introduced wt% of ZnO increases. FTIR spectroscopy and XPS analysis confirmed the successful introduction of the ZnO NPs and the subsequent addition of CHG. Measurement of the wetting edge angles of the composite fibers reveals that the material's surface is hydrophilic. Further PCL modification with carboxyl groups and CHG leads to an improvement in the material's wettability. The results of the biological tests confirm that the developed dressings have promising bactericidal and proliferative activity.

Conclusions. We have created a series of new modified composite fiber materials with high potential for wound healing. This research was funded by the Russian Science Foundation (№ 24-79-10121).

6.4. Development of Antimicrobial PDMS Polymers Containing Silver-Copper Nanoparticles for Potential Applications in Biomedical Devices

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Introduction: Both urinary tract infections and primary bloodstream infections result from the use of PDMS catheters and are reported to be the most common nosocomial infections. Despite disinfection procedures used in hospitals, infections still occur, causing delays in discharge and potential mortalities. Bimetallic AgCu nanoparticles (AgCuNPs) are found to exhibit antimicrobial activity against pathogens. Hence, this work reports a manufacturing procedure for fabricating antimicrobial AgCu-PDMS films, which was shown to effectively reduce microbial growth of bacteria and fungi.

Methods: AgCuNP (0.1% wt/v) was dispersed in Sylgard™ silicone elastomer curing agent and then added to the silicone elastomer base. The mixture was heat-cured and cast, forming 0.1 mm thin films. The fabricated AgCu-PDMS films were treated with a UV_{254} or UV_{365} light source for 15 min. SEM was used to characterise the films, and the population of NP exposured was semi-quantified using ImageJ. Microbial broths ($\times 10^4$) were loaded onto the AgCu-PDMS films, and kinetic growth measurements were conducted to evaluate the antimicrobial properties against *E. coli*, *S. aureus*, and *C. albicans*.

Results and Discussion: Despite the higher frequency exhibited by the UV_{254}, more than double of the AgCuNPs were found exposed on the PDMS surface after etching the film using the UV_{365} source. This can be explained by the photothermal property owned by this bimetallic nanomaterial, which shows a board surface plasmonic resonance at λ_{max} (410 nm) and resulted in excessive polymer degradation due to the excitation of the nanoparticles. Therefore, as expected, the antimicrobial results obtained from the UV_{365}-treated AgCu-PDMS films achieved the optimal 72% reduction of microbial growth in relation to the control.

Conclusions: PDMS polymer with incorporated AgCuNPs exhibited antimicrobial properties after UV subjection to expose nanoparticles. Whilst it shows potential to challenge catheter infections, toxicity tests are required to research the suitability of these PDMS films for biomedical applications.

6.5. Effective Splicing Correction of SMN2 Gene in SMA Cells After Delivery of RNA Interpolyelectrolyte Complexes

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Introduction: Spinal muscular atrophy (SMA) is a genetic disorder caused by mutations in the *SMN1* gene, leading to a deficiency of survival motor neuron (SMN) protein. The human genome contains a paralog of the *SMN1* gene, *SMN2*, which, due to a splicing defect, produces insufficient levels of functional SMN protein. Therapeutic strategies aiming to correct this splicing defect in *SMN2* are supposed as treatments for SMA. The disadvantage of this therapy is intrathecal administration with associated side effects. Ternary oligonucleotide--peptide complexes coated by anionic polypeptide are designed to overcome the impossibility of systemic delivery antisense RNA oligonucleotide.

Methods: The study involved transfecting SMA fibroblast cell culture with the serum-stable oligonucleotide--peptide complexes, reverse transcription, semiquantitative PCR, and resazurin assay.

Results and Discussion: A significant increase was observed in the proportion of full-length SMN transcripts after therapeutic antisense RNA oligonucleotide delivery. Interpolyelectrolyte oligonucleotide--peptide complexes showed stability in the serum in contrast to cationic peptide complexes. The toxicity of the complexes remained within acceptable levels.

Conclusions: The delivery of antisense RNA oligonucleotides using interpolyelectrolyte complexes represents a promising strategy for the treatment of SMA. This strategy combines the specificity of antisense oligonucleotides with the protective and delivery-enhancing properties of interpolyelectrolyte complexes, potentially offering a more effective and sustained therapeutic option for SMA treatment.

6.6. Nitric Oxide-Generating Plasma-Deposited Coating for Improved Cell Proliferation and the Prevention of Biofilm Formation

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Introduction: Nitric oxide (NO) is known to alter coagulation by regulating platelet activity and plays a role in immunological response, infection prevention, and wound repair. Thus, NO-generating coatings have a high potential for wound healing applications. This study selected plasma polymerization to deposit a thin coating because of its high speed and low temperature, controlled film thickness, versatility on various surfaces, and environmental friendliness. Plasma modification offers the unique advantage of selectively enhancing surface properties without affecting the bulk attributes of the materials.

Methods: Plasma surface modification was carried out by using the high-frequency plasma system ZP-COVANCE-RFPE-3MP operating at 13.56 MHz at 15 W. Isopentyl nitrite (99.995%), C_2H_4 (99.95%), and Ar (99.998%) were used as precursors to deposit thin films on silicon wafers and polycaprolactone nanofibers at a pressure below 30 Pa. The obtained plasma-deposited polymer films were studied using SEM, EDX analysis, XPS, FTIR spectroscopy, and WCA. The kinetics of the release of NO were investigated by means of spectrophotometry. The adhesion and proliferation of human fibroblast cells on the surface of plasma-treated samples were studied. The samples were tested against different pathogens in terms of biofilm formation.

Results: Plasma deposition resulted in homogeneous and well-bonded layers. SEM micrographs showed no pinholes, cracks, or other damage in the deposited layers. According to FTIR and XPS, the obtained spectra indicated the presence of nitroxyl compounds on the surface of the samples. The difference in wettability of the samples was determined to be $\approx 90^0$. The deposited polymer coatings were shown to promote better proliferation of human fibroblast cells. The plasma-treated samples completely prevented biofilm formation.

Conclusions: An approach was developed for the deposition of nitroxyl-containing films from a mixture of isopentyl nitrite/C_2H_4 with improved proliferation of human cells and high antipathogenic activity.

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6.7. Plasma Chemical Modification of Hydroxyapatite Surface to Immobilise Bioreceptors for Biosensor Applications

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Introduction: Biosensors are instruments capable of providing quantitative or semi-quantitative analytical information from an identifier in the form of a bioreceptor linked to signal transduction. Nanosized hydroxyapatite (nHA) is often used as an immobilisation matrix component due to its excellent biocompatibility and high adsorption capacity. Successful immobilisation of bioreceptor molecules on the surface of the working electrode

is the key to achieving high sensitivity of the biosensor. To achieve better binding of biomolecules to the nHA matrix, plasma chemical surface modification is used in the present work.

Methods: The synthesis of nHA was carried out by the chemical precipitation method. The obtained powder was characterised by XRD, FTIR, TEM, and BET, and the zeta potential was measured. To study the adsorption properties and surface modification, the nHA powder was pressed into 10 mm diameter tablets. To obtain carboxyl functional groups on the surface of the material, a perfected mode of plasma deposition from $CO_2/C_2H_4/Ar$ gas mixture on a ZP-COVANCE-RFPE-3MP unit was used. The treated materials were investigated by SEM, EDX analysis, XPS, and WCA. The adsorption capacity was investigated using glucose oxidase enzyme as an example. Cross-linking by carbodiimide chemistry was carried out to immobilise glucose oxidase.

Results: Plasma modification resulted in successful immobilisation of glucose oxidase on the nHA surface. According to XPS data, the spectra obtained indicated the presence of carboxyl compounds on the surface of the modified samples. Plasma chemical treatment was shown to increase the bond strength between the nHA substrate and immobilised glucose oxidase molecules.

Conclusions: An approach to immobilise biomolecules on hydroxyapatite matrix has been developed, which is promising for applications in biosensors.

Funding: This work was supported by the Russian Science Foundation (grant № 20-19-00120-P).

6.8. Rational Design of Single-Atom Nanozymes for Biomedical Applications

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Introduction: Natural enzymes play crucial roles in various biomedical fields, particularly in biosensing. Nevertheless, their use is often limited by high costs and limited stability. Therefore, there is an urgent need to develop artificial alternatives with enzyme-like properties and enhanced stability for low-cost biomedical applications. Nanozymes have emerged as a promising alternative to natural enzymes, offering advantages such as mimicking enzymatic functions, high catalytic stability, easy modification, and low fabrication costs. These attributes make nanozymes highly suitable for use in biosensors to amplify signals and improve detection performance. Precisely designing single-atom nanozymes (SANs) at the atomic level to achieve isolated metal active sites can significantly enhance their enzyme-like catalytic activity, thereby boosting their performance in biomedical applications.

Methods: We designed various SANs to mimic the structure of heme-based enzymes' active centres, achieving enzyme-like properties comparable to natural ones. Specifically, Fe-N-C-based SANs are reasonably designed via different strategies, including a secondary-atom-assisted method, MnO_x nanoconfinement, an ion-imprinted approach, and P atom adjustment strategies, etc.

Results: The SANs demonstrated superior robustness compared to natural enzymes, maintaining excellent stability under varying pH levels and temperatures. Their exceptional catalytic specificity for hydrogen peroxide suggests they could effectively replace natural peroxidases in high-sensitivity biosensing. To explore practical applications, we developed electrochemical sensors, immunosorbent assays, intercellular nanoprobes, and lateral-flow immunoassays based on SANs for biosensing and bioimaging.

Conclusions: The developed SANs exhibit excellent enzyme-like activity, selectivity, and stability due to their unique electronic and geometrical properties. These features offer substantial potential for substituting natural enzymes in various biomedical applications. To continuously monitor human health, we further integrated SANs into wearable microgrids. This development aims to meet the demands for autonomous, self-powered,

self-regulated, and flexible wearable energy management and sensors, thereby enabling comprehensive healthcare monitoring and advanced human--machine interfacing.

6.9. The Encapsulation of Oregano Oil in Natural Nanogels and Preliminary Studies on Its Antiviral Activity

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Introduction: Nowadays, the therapeutic administration of essential oils is gaining high interest. For instance, oregano oil possesses remarkable effects, such as antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral activity. However, it is characterized by low aqueous solubility, which makes its application difficult and compromises its effects. Loading the oil into nanoparticles is an appropriate strategy to improve its use. Nanogels prepared from natural polymers are considered alternative nanosized drug delivery systems capable of improving the characteristics of hydrophobic substances.

Methods: Oregano oil was loaded into nanogels prepared from chitosan and albumin by emulsification. The nanogel system was physicochemically characterized via DLS, TEM, IR and XRD analysis. A dialysis method was applied to study its in vitro release. The antiviral activity of the nanoparticles loaded with oregano oil was tested on Betacoronavirus 1 (S379 Riems) using a direct treatment approach. The MTT dye assay was applied for measurement of the cell viability. The cytopathic effect was evaluated microscopically.

Results and Discussion: The encapsulation efficiency of the oil in the nanogel particles was approx. 40%. The nanogels possessed a small mean diameter of 26 nm, a narrow size distribution and a positive zeta-potential (+20.6 mV). TEM confirmed their small size. The in vitro dissolution test showed that approx. 100% of the oil was released for 24 h. The IR and XRD analyses confirmed the successful entrapment of the oil. The nanoparticles showed more than 40% protection of the treated cells at concentrations between 15 and 30 μg/mL as compared to the non-treated virus-infected control.

Conclusions: Nanogels prepared from the natural polymers chitosan and albumin could be considered appropriate carriers for the delivery of oregano oil. Their moderate activity against Betacoronavirus 1 could be a platform for further investigations of the pharmacodynamics and possible combinations with clinically applied chemotherapeutics.

6.10. The Influence of Polymer Shell Composition on the Release Rate and Stability of Doxorubicin-Loaded Fe_3O_4 Nanoparticles

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Introduction: The development of theranostic systems could improve the quality of cancer treatment. Biocompatible Fe_3O_4 nanoparticles can be used in contrast agents and hyperthermia preparations. Doxorubicin (DOX) is the antitumor agent in the system. However, the surface modification of the developed systems is necessary for safe and prolonged circulation in the bloodstream. This study compared the influence of polymer shell composition on DOX release and particle stability in a saline solution. We analyzed polymers such as chitosan, polyethylene glycol (steric hindrance), and lysozyme.

Methods: We prepared Fe_3O_4 nanoparticles using the hydrothermal method. The synthesized particles were examined using SEM, EDX, FTIR, and BET analysis systems. The magnetic characteristics and surface charge of the nanoparticles were also studied. DOX loading and release were investigated using spectrophotometric methods at different

pH values (5.5, 7.4, and 8.5). The coating techniques of the developed systems with polymer shells were optimized. The DOX release kinetics of coated particles at different pHs were investigated. Polymer-coated particles provide pH-dependent drug release due to the swelling–shrinking behavior of polymers. The cytotoxicity of the obtained systems was evaluated through in vitro tests using the cancer Emt6 cell line and the NIH 3T3 healthy cell line.

Results: The obtained nanoparticles (20 nm) were superparamagnetic. We determined the DOX loading capacity of the particles to be 15%. The particles demonstrated a high percentage of drug release (80%) at pH = 5.5, whereas drug release was nearly absent in neutral and alkaline solutions. All types of polymer coatings provided particle stability and prolonged drug release. The chitosan shell demonstrated the highest colloidal stability, while the largest release rate (40%) was shown by lysozyme. Fe_3O_4 particles loaded with doxorubicin exhibited cytotoxicity to both cell lines, but the coating application reduced the cytotoxic effect on healthy cells.

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7. Biosignal Processing

7.1. A Machine Learning Approach to Classifying Electromyographic Signals of Cranial Nerves During Neurosurgical Procedures

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Introduction: Monitoring electromyograms (EMGs) during skull base surgeries is crucial to prevent cranial nerve injuries, which are common complications of skull base surgery. In order to enhance the safety and efficacy of surgical procedures, using machine learning (ML) algorithms to classify EMG signals can improve the recognition of muscle activation patterns.

Methods: This research utilized a public dataset [24] to monitor the EMG obtained from five cranial nerves of 11 patients during cerebellopontine angle tumour surgery. Specifically, the EMG data were collected using the Neuromaster G1 MEE-2000 (Nihon Kohden, Inc., Tokyo, Japan) from the V, VII, XI, X, and XII cranial nerves. An ML model was developed using MATLAB 2023b, based on an ensemble of decision trees, to classify EMG signals into 'Injury', 'Artifact', and 'Healthy' categories. The features used include the amplitude of the rectified value, root mean square value, median frequency, total power, and mean normalized frequency. The data were split using holdout with 80% for training and 20% for testing. Synthetic minority oversampling was applied to the training data to balance the classes; 800 maximum splits per tree were configured with limits of 5 observations per leaf and 10 per parent node. The model was trained through 250 learning cycles with pruning enabled to improve generalization. Subsequently, the model was validated using 5-fold cross-validation, ensuring a robust evaluation of its performance.

Results and discussion: The model achieved on the test set an overall accuracy of 81.12%, with 32.49% precision and 81.01% recall for Injury, 70.00% precision and 75.68% recall for Artifact, and 97.54% precision and 82.12% recall for Healthy, with F1-scores of 46.38%, 72.73%, and 89.17%, respectively.

Conclusion: This study demonstrated the potential of ML in EMG for intraoperative monitoring of cranial nerves, suggesting future optimizations and the integration of advanced algorithms to further improve diagnostic accuracy and clinical utility.

7.2. A Preliminary Investigation into the Feasibility of Probabilistic Blood Pressure Estimation from ECGs Using Compositional Bayesian Neural Networks (Auto-BNN)

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Introduction: The rising prevalence of cardiovascular disease, especially hypertension, necessitates continuous and non-invasive blood pressure (BP) monitoring. While previous studies have investigated the potential for estimating BP from electrocardiograms (ECGs), these claims require further verification. This study presents a preliminary investigation into the feasibility of estimating BP from ECG pulse morphology using a novel deep compositional Bayesian neural network (auto-BNN).

Methods: Our model leverages a deep learning architecture to capture the variations in ECG morphology associated with BP. It incorporates convolutional neural network (CNN) layers for ECG waveform feature extraction, a long short-term memory (LSTM) unit to capture temporal dependencies in ECG sequences, and variational layers based on auto-BNN to enable uncertainty estimation.

Results and Discussion: An initial evaluation of data from 130 individuals sourced from Physionet yielded mean errors of 3.38 mmHg (systolic) and 2.40 mmHg (diastolic) with standard deviations of 13.20 mmHg and 11.88 mmHg, respectively. These results suggest that our model could potentially capture correlations between BP variations and ECG signals, such as changes in R wave amplitude, ST-segment depression, T-wave inversions, and widened P waves associated with high BP, as well as sinus tachycardia and ST-segment/T-wave changes associated with low BP. However, it is important to note that these correlations may have captured the relationship between heart rate (HR) and BP. Further research should explore methods to exclude HR information from ECGs to ensure the validity of BP estimation findings.

Conclusions: This preliminary study demonstrates the potential feasibility of using ECG pulse morphology for BP estimation. However, further validation on larger and more diverse datasets is crucial to assess the generalizability of our approach. While our initial results are encouraging, it is important to note that achieving very high accuracy in BP estimation solely from ECGs may be inherently challenging due to the complex and multifaceted factors influencing BP.

7.3. A Robust Approach for Emotional Assessment: The Employment of Power-Normalized Cepstral Coefficient and Stacked Classifiers

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Introduction: Emotional assessment has become a primary focus in multiple fields, thanks to its power to encompass the real-time status of individuals. One of the traits most affected by emotional status is the voice, recognized as a signal carrying a great deal of information. Effective computing through voice recordings holds significant importance in various fields, ranging from healthcare to human–computer interaction. In fact, by analyzing vocal cues, effective computing systems can detect emotional states, providing critical insights into a person's mental health and well-being. This study aims to develop a machine learning (ML) approach for emotion recognition from vocal recordings.

Methods: Emotion classification was performed using audio recordings from the EMOVO dataset (EMOVO Corpus: an Italian Emotional Speech Database), comprising syntactically neutral phrases spoken by six actors across seven emotions: neutral, disgust, anger, surprise, fear, sadness, and joy. The approach began with audio preprocessing, where a set of 20 power normalized cepstral coefficients was extracted. Crucially, the training and testing sets were divided in a manner to ensure an equal representation of each emotion class, maintaining balanced compositions, bolstering the reliability of the model proposed. Subsequently, a stacked ML model was employed, comprising kNN and SVM as base models, augmented with Extreme Gradient Boosting.

Results: This model achieved remarkable accuracies of 87% during training and 81% during testing, showcasing robustness and premises for novel and diverse applications.

The methodology emphasized maintaining balanced distributions in predictions, ensuring unbiased and non-overfitted results.

Conclusion: This comprehensive approach integrated advanced features and a systematic classification strategy, contributing significantly to the advancement of emotion analysis in audio data, and fostering the development of more intuitive, responsive, and human-centered technology solutions.

7.4. A New Methodological Approach Based on the Stationarity and Permutation Entropy of EMG Bursts for Assessing Muscle Function Alterations in a Parkinson's Disease Animal Model

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Introduction: The EMG signal is the electrical manifestation of motor unit (MU) recruitment processes underlying the contractile dynamics of muscle fibers. The analysis methodology frequently carried out includes a preprocessing stage based on artifact removal and stationarity testing, as well as a feature extraction and interpretation stage. Generally, stationarity criteria are difficult to meet when EMG signals are evoked by momentary activations (bursting activity). Thus, the study and/or characterization of contractile patterns evoked in free-moving protocols require particular treatments.

Methods: Here, we propose a new approach for quantitatively measuring stationarity using the mean, variance, and autocovariance test (MVA test) and Permutation Entropy for measuring the uncertainty degree. This methodology was applied to EMG signals obtained from a Parkinson's disease (PD) lesion model to longitudinally study the muscle function alterations.

Results and Discussion: The MVA test was compared with the classic Reverse Arrangement test (RA-test). The RA test indicated that EMG signals become more stationary over post-injury time. However, the MVA test revealed that the temporal structure of EMG around the maximum recruitment zone of motor units presents incremental non-stationary characteristics (in variance and autocovariance) over post-injury time. Likewise, it was observed that the initial phase of motor recruitment in the biceps femoris (BF) muscle (around the onset) presents a high non-stationary component, which increases over post-injury time. Permutation entropy measures throughout the contractile dynamics of the BF muscle revealed that the uncertainty degree decreases in the initial phase of contraction as the animal's post-injury time increases.

Conclusions: The analysis proposed allowed for a longitudinal characterization of muscle function alterations in an animal model of PD in terms of the stationarity properties of EMG signals. Furthermore, it was observed that permutation entropy could serve as a robust biomarker for quantifying neuromuscular remodeling caused by PD progression.

7.5. Assessing the Relationship Between Gesture Intuitiveness and Muscle Network Efficiency: A Comparison of Non-Negative Matrix Factorization and Intermuscular Coherence Analysis Methods

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Introduction: Human–Machine Interaction is a research area that has been gaining increasing attention due to the search for new, more natural, and intuitive interfaces based on the use of new technologies which facilitate the interaction of users with smart devices. In this context, there have been attempts to develop gesture-based interfaces. However, a fundamental question still needs to be addressed centred around whether the gestures used are indeed intuitive. To this end, questionnaires answered by volunteers are usually used, but this type of response is quite subjective. The use of neurophysiological indicators may be an alternative to finding an objective and efficient metric of intuitiveness. This study aimed to test the hypothesis that the improved coordination of muscle synergies serves as a reliable indicator of gesture intuitiveness.

Methods: EMG signals from 16 muscles were monitored, and muscle networks were constructed from the extraction of muscle synergies obtained using Non-Negative Matrix Factorization (NMF) and also from Intermuscular Coherence (IMC). The muscle networks obtained using both approaches in four frequency bands were analyzed in their spatial structure and also using metrics (such as Weighted Global Efficiency (WGE) and Effective Average Strength (EAS)). The correlation of these metrics with the Intuitiveness Level (IL) associated with each gesture was then calculated.

Results and Discussion: The networks from muscle synergies show denser connectivity levels than IMC. Notably, WGE values of synergy muscle networks in the Beta and Gamma2 bands, as well as EAS values of IMC muscle networks in the Gamma1 band, positively correlate with IL values.

Conclusions: The results provide substantial evidence supporting a significant correlation between the intuitiveness level and muscle synergies analyzed using both NMF and IMC approaches.

7.6. Cardiovascular Health Analysis and Decision Making Using Artificial Intelligence

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Cardiovascular disease represents a significant global health challenge and the necessity for advanced techniques for early detection, diagnosis and management. This study explores Artificial Intelligence (AI) techniques in cardiovascular health analysis and decision-making processes. For instance, for patients experiencing Ventricular Ectopic Beats (VEBs), AI can recommend stress reduction and regular exercise. Using artificial neural networks, ElectroCardioGram (ECG) signals can be analyzed to detect abnormalities in various cardiovascular diseases. The proposed AI system includes a soft voting ensemble transfer learning method to process ECG data to classify different types of abnormalities in the heart, providing accurate and timely diagnostic support. Additionally, the system incorporates patient data to offer personalized treatment recommendations. Through extensive training and testing on a publicly available diverse dataset, the AI model demonstrates high accuracy and robustness in identifying critical cardiovascular conditions and decision making. This research underscores the potential for AI to revolutionize cardiovascular healthcare by enhancing diagnostic precision, facilitating early intervention, and ultimately improving patient outcomes. The implementation of such AI-driven solutions can significantly reduce the burden on healthcare systems and pave the way for more efficient and effective cardiovascular disease management. However, there are a number of issues with the medical application of AI techniques and applications and their findings and interpretations, such as confidential patient data, noisy data and biased data, which may lead to wrong conclusions. Still, AI is a next-generation technology that has significant potential in the medical field.

7.7. Cell Sorting Enhances the Training Efficiency of AI-Driven Automated Cell Morphology Analysers

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In the realm of haematology, the transition from manual microscopic examination to automated cell morphology analysers as the gold standard remains an unachieved milestone. A pivotal impediment to this advancement is the inadequacy in the volume and fidelity of labelled cellular samples essential for the training of artificial intelligence (AI)-driven cell classification models. The process of deriving labeled cells from digital microscopic imagery necessitates meticulous human curation, which, given the extensive quantity of cells required, becomes onerous and fraught with quality control challenges.

This manuscript introduces an innovative methodology designed to significantly enhance the efficiency of the labelling process. Initially, we employed some techniques to mitigate the confounding effects of erythrocyte populations on leukocyte identification, thereby expediting subsequent cellular sorting procedures. Subsequently, leveraging state-of-the-art cell sorting technology, we executed label-free segregation of a targeted leukocyte subset, exemplified by monocytes, culminating in the acquisition of a highly purified monocyte suspension. We adhered to established slide preparation and staining protocols, such as the Wright--Giemsa staining method, to fabricate blood smears that preserve cellular morphology with minimal alteration. In the final phase, human annotators perform batch labelling of monocytes through the mediation of digital microscopic imagery of the smears. Consequently, the resultant digital microscopic images predominantly feature monocytes, with a negligible presence of other leukocyte classes.

By delineating non-monocytic cells with the annotation "not monocytes," annotators can efficiently designate the remaining cells as monocytes, thereby achieving batch labelling of the specified leukocyte class.

This approach not only augments the efficacy of manual labelling but also diminishes the laboriousness associated with the task, with the anticipation of concurrently elevating the calibre of labelling accuracy.

7.8. Characterization of Cortical Patterns Using High-Density EEG in Motor Tasks Related to Musical Note Execution

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Introduction: The execution of complex motor actions involving processes such as planning, decision-making, and execution entails a certain cognitive workload (CWL), engaging the involvement of multiple brain areas and processes related to the coordinated activation of muscles. Factors such as context, previous experience, stimulus complexity, and required precision, among others, can establish basal cortical patterns from which those related to the specific motor task are generated. Here, we propose a study to characterize cortical patterns before and after the execution of musical motor tasks.

Methods: Ten subjects participated in an experimental involving the unilateral execution of musical chord on an electronic piano. Nine musical chord (C, D, E_7, F, $F_{\#m}$, G, A, A_m, and B) were presented alternately and sequentially to the subjects on a 21-inch monitor. Throughout the experiment, EEG signals were recorded from 256 channels. Cortical activity was analyzed based on the spectral energy in different bands and patterns of connectivity evoked before and after the motor executions.

Results and Discussion: Event-related desynchronizations and connectivity patterns have shown differential characteristics before and after musical tasks. Moreover, the presentation of musical chords triggered cortical patterns different from those evoked by the instruction of a simple motor action (pressing a piano key).

Conclusions: With this study, we characterized the cortical dynamics evoked by the performance of musical chords. Likewise, we concluded that it would be possible to

determine the cognitive loads demanded on subjects by musical instructions measuring EEG signals.

7.9. Characterization of Contractile Dynamics in Postural Control During Demanding Balance Tests

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Introduction: Postural stability results from an effective interaction between sensory feedback integration and muscle modulation. However, the propensity for falls increases with age and has been extensively described for neuromuscular disorders. The comprehension of the muscle modulation mechanisms involved in postural control could help for handle balance deficits and fall prevention. In this study, we investigate the muscle contractile dynamics generated by challenging postures and how the balance is affected by the feedback visual privation.

Methods: This exploratory study examined muscle activation during different postural stability conditions. Postural conditions included three items of the Berg Balance Scale and were administrated in increasing difficulty order with both open and close eyes. Electromyographic (EMG) signals were obtained bilaterally from lower and upper leg muscles (tibialis anterior, gastrocnemius medialis, vastus medialis, rectus femoris, biceps femoris, and tensor fasciae latae) and from one trunk muscle. EMG recordings were synchronized with plantar sensors (F-Scan64 system) to extract Center of Force and pressure data. EMG amplitude, spectral analysis and intramuscular coherence were examined in relation to body movement during destabilizing postures.

Results and Discussion: This experimental approach allowed us to characterize muscle dynamic contraction under different postural conditions with and without visual information. We have found that different strategies and specific modulations were required for controlling balance in highly demanding postures. The amplification of muscle activity and coactivation of lower leg muscles were observed during the most challenging posture and without visual information. Also, specific behaviors consisting of intermittent muscle activity along the postural tasks were observed, especially in the muscles of the dominant leg.

Conclusions: In this study, we describe characteristic oscillatory modulations and synergistic activations as motor strategies for maintaining the balance after a demanding postural condition. These results are discussed in relation to the possibility of accurately assess the efficiency of postural motor strategies.

7.10. Code Development for Heartbeat Detection with Inexpensive Optical Pulse Sensors

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Smart textiles with embedded electronics can be used to monitor body functions, e.g., during sports or for medical reasons. One of the main problems of such embedded electronics is their washability. A recent study therefore investigated the washability of different inexpensive optical pulse sensors, as they could be integrated into clothing to enable pulse measurements. To evaluate the measured data, microcontrollers can also

be integrated into the garment, which is why an ATtiny85 microcontroller additionally underwent washing tests. The main part of this study concentrated on code development to improve heartbeat detection using inexpensive optical pulse sensors. While all sensors and microcontrollers remained unaltered by 10 washing cycles, significant differences in the quality of the inexpensive optical pulse sensors were found, depending on the producer. As the optimum hardware, a combination was defined of an ESP8266 D1 mini as the textile-integrated transmitter and an ESP32 with a 1.8″ display to receive and show the measured data outside the garment. Pulse detection criteria were defined, which could be used to calculate the heart beats per minute (BPM) with high reliability. The tests and developments can be used as the base for a complete textile-integrated optical pulse measurement with mobile data storage and depiction.

7.11. Comparative Analysis of Time Series Techniques for COVID-19 Forecasting: LSTM, Transformer, and ARIMA

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Introduction: The COVID-19 pandemic highlighted the critical need for accurate forecasting models to inform public health decision-making. This study compares the performance of three time series techniques—Long Short-Term Memory (LSTM) networks, Transformer models, and Autoregressive Integrated Moving Average (ARIMA)—in predicting the spread of COVID-19.

Methods: We trained and evaluated LSTM, Transformer (Temporal Fusion Transformer), and ARIMA models using the publicly available Johns Hopkins University Center for Systems Science and Engineering (JHU CSSE) COVID-19 Data Repository, encompassing confirmed cases, deaths, vaccination rates, and relevant socio-economic factors. Model performance was assessed using mean absolute error (MAE) and root mean squared error (RMSE) for 7-day and 14-day forecasting horizons.

Results and Discussion: The Transformer-based model consistently outperformed both the LSTM and ARIMA models in terms of forecasting accuracy. For 7-day forecasts, the Transformer achieved an MAE of 85 cases per 100,000 population and an RMSE of 120, while LSTM had an MAE of 90 and RMSE of 125 and ARIMA had an MAE of 105 and RMSE of 155. For 14-day forecasts, the Transformer maintained its superior performance with an MAE of 110 and RMSE of 150, compared to LSTM (MAE of 115 and RMSE of 155) and ARIMA (MAE of 138 and RMSE of 178). The Transformer's ability to capture long-range dependencies and incorporate diverse data sources contributed to its improved performance. Notably, all models were able to capture sudden shifts in the spread of the virus, enabling timely alerts for potential outbreaks.

Conclusion: This study demonstrates the superior performance of Transformer-based models in forecasting the COVID-19 pandemic compared to LSTM and ARIMA models. The findings underscore the potential of Transformers in epidemiological modelling and highlight the importance of leveraging advanced deep learning techniques for accurate and timely predictions in public health crises. Further research will explore the integration of additional data sources and model refinements to enhance forecasting capabilities for future outbreaks.

7.12. Cortical Dynamics of Phosphene Perception: A Study Using EEG Signals

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Introduction: Scientific evidence has shown that the electrical stimulation of the primary occipital cortex can evoke luminous visual perceptions known as phosphenes. This finding has provided the basis for the development of cortical visual prostheses for blind individuals. In this context, understanding and unveiling the cortical perceptual dynamics evoked during the process of perceiving phosphenes is crucial in order to improve the interaction between this technology and the user.

Methods: In this study, we investigated the cortical perceptual dynamics of three blind subjects who were implanted with a 10 × 10 Utah microelectrode array in their visual cortices. Cortical responses during the perception and non-perception of evoked phosphenes were monitored using electroencephalographic (EEG) techniques. Processing methods included the quantification of event-related synchronization/desynchronization (ERS/ERD) and directed transfer function (DTF)-based connectivity analysis.

Results and Discussion: The analysis of EEG signals revealed significant differences in ERS/ERD within the 1-to-45 Hz range (specifically between 4 to 7.5 Hz), predominantly in frontal and prefrontal regions, when subjects perceive phosphenes. These differences were observed between 250 and 750 ms following stimulus application. Connectivity analysis based on DTF determination showed that phosphene perception evokes directional connections from temporal regions to central and frontal regions.

Conclusions: In this study, we demonstrated that EEG signals allow the characterization of cortical dynamics during phosphene perception. It was observed that, in terms of evoked oscillation energy, frontal areas exhibit higher synchronization during phosphene perception, while in terms of directional connectivity, cortical directional information shows cross-modulation when the phosphene is perceived.

7.13. Detection of Cardiovascular Disorders in Patients Supported with Continuous-Flow Left Ventricular Assist Devices by Monitoring Electrical Current Signals

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Continuous-flow left ventricular assist devices (CF-LVADs) are miniaturised devices implanted in end-stage heart failure patients to support the failing left ventricle. CF-LVADs alter the blood flow in the cardiovascular system, causing further complications. Additional health disorders during CF-LVAD support may increase morbidity and mortality in patients. Therefore, continuously monitoring blood flow through CF-LVADs may help to detect the complications early, allowing for timely interventions and reducing mortality in patients implanted with CF-LVADs. The lack of long-term reliable and implantable sensors in CF-LVADs does not allow for the real-time continuous monitoring of haemodynamic signals in the cardiovascular system.

In this study, intrinsic CF-LVAD electrical current signals were continuously monitored and analysed to evaluate cardiac function and detect cardiovascular disorders. CF-LVAD electrical current signal waveforms over a 600 s period in patient-specific RR intervals were analysed to detect normal sinus rhythm, Atrial Fibrillation (AF) with unimodal and bimodal RR interval distributions, and right ventricular failure during CF-LVAD support.

The average RR interval duration in sinus rhythm was 0.828 ± 0.051 s and the coefficient of variation was 0.006. The average RR interval duration in AF with unimodal distribution was 0.512 ± 0.106 s and the coefficient of variation was 0.207, whereas the mean of the RR intervals in AF with a bimodal distribution was 0.884 ± 0.260 s and the coefficient of variation was 0.294. The CF-LVAD electrical current signal waveform was altered because of right ventricular failure and AF with both unimodal and bimodal cardiac RR interval distributions. The amplitude of the CF-LVAD electrical current signal was relatively small because of right ventricular failure and AF with bimodal cardiac RR interval distribution, which caused the amplitude to vary over each cardiac cycle.

Cardiovascular disorders can be detected by monitoring and analysing the features of CF-LVAD electrical current signals in patients supported with CF-LVADs.

7.14. Detection of Developmental Language Disorder Using Machine Learning and Mel-Frequency Cepstral Coefficients from Voice Recordings

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Introduction: Language disorder is the most frequent developmental disorder in childhood, impacting various aspects of language processing. Approximately 11–18% of children aged 18–36 months exhibit language delays, often improving by age 3, but some persist into developmental language disorder (DLD). The early detection of DLD is crucial, as it allows for timely intervention, improving long-term outcomes. This study aims to assess DLD though machine learning (ML) techniques applied to Mel-Frequency Cepstral Coefficients (MFCCs), which are features commonly used in voice analysis.

Materials and Methods: This study utilized a freely available dataset (DOI: https://doi.org/10.6084/m9.figshare.2360626.v2 (accessed on 20 March 2025)) that comprises voice recordings from 54 children (35 boys, 19 girls) aged 6 to 12 years diagnosed with DLD, recorded with MD SONY MZ-N710 (fs = 44.1 kHz, 16-bit resolution), and 44 typically developing children (15 boys, 29 girls) aged 4 to 12 years, recorded with a SONY digital Dictaphone (fs = 16 kHz, 16-bit resolution). Specifically, the dataset includes recordings of each letter of the alphabet. For the purposes of this study, MFCCs and their first and second derivatives were extracted from the recordings of the letter "A" to perform the classification task. Moreover, the mean and median frequencies of the power spectrum were computed, aiming to investigate eventual spectral distinctions between the two groups.

Results: The best performance was achieved by employing a set of 15 features selected through the MRMR procedure, which resulted in a test accuracy of 96.7% and an AUC of 0.98, utilizing a Cubic SVM. Moreover, a t-test assessed differences between the two groups concerning the mean frequency (p = 0.024) and the median frequency (p = 0.022).

Conclusion: This study demonstrated the feasibility of employing ML algorithms in diagnosing DLD through the analysis of MFCCs extracted from voice recordings. This approach could significantly enhance long-term outcomes for individuals affected by this disorder.

7.15. Effect of Data Collection and Environment on Machine Learning Performance in Screening Dysphonia

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Objectives/Introduction: Machine learning (ML) is a promising tool for assessing voice quality and dysphonia. Several public datasets containing recordings of both normal and pathological voices are available online. Since ML benefits from larger datasets, combining these available datasets could enhance ML performance. However, the varying environmental conditions under which these recordings were collected may impact ML accuracy, and the extent of this impact is unclear. This work aims to investigate how different data collection procedures affect ML efficacy in screening dysphonia.

Methods: Two datasets were considered. The first dataset included voice samples from 198 participants: 148 individuals with voice disorders and 50 vocally normal subjects. The second dataset, the publicly available PVQD database, included 276 subjects: 187 patients with voice problems and 89 without vocal issues. Various acoustic measurements (including perturbation, noise, cepstral, and spectral analyses) were estimated from the recordings using MATLAB scripts and Praat software. These measurements were derived from multiple types of speech productions: a sustained vowel /a:/ and running speech. Different ML models were trained on the extracted acoustic features from each recording and evaluated

for accuracy, sensitivity, specificity, and Receiver Operating Characteristic (ROC) curves to compare the impact of each dataset, collected under different procedures, on dysphonic voice classification.

Results and Conclusions: Accurate acoustic metrics were generated from the two datasets. Using these measurements, ML models were successfully trained and evaluated to classify dysphonic versus non-dysphonic speakers. The comparative analysis revealed discrepancies in classification accuracy among the models between the two datasets and when the datasets were combined. Identifying which ML models are robust or sensitive to changes in data collection environments helps in selecting appropriate models for tasks involving different datasets with varying data collection procedures. The outcome is an important step towards more reliable/effective ML tools in screening voice disorders.

7.16. Effect of Individual Abilities for Mental 3D Rotation in Learning EEG-Controlled BCIs

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Introduction: Brain–computer interface (BCI) systems aim to transmit control commands generated through brain activity to computer applications. Mastery of BCIs generally requires special training, in which some of the users (the so-called BCI-illiterates) might be lacking. Our research paper examines the relation between the users' individual mental rotation abilities and the effort associated with mastering motor-imagery-based BCI systems.

Methods: The mental rotation abilities in the test subjects were assessed with a dedicated test that complies with the paradigm developed by Vandenberg and Kuse. The number of correct answers on 3D figure rotations provided during the 90 s allocated for the test was recorded. Subsequently, the subjects were asked to control a robotic claw arm to transfer cubes from one place to another, within the motor imagination BCI paradigm. To reflect the BCI learning effort and effectiveness, the number of cubes transferred and the average time to transfer one cube were recorded.

Results and Discussion: We report on the positive relation between the individual mental 3D rotation abilities and the mastery of motor imagery BCI system. Then we put forward and discuss the ensuing recommendations for organizing effective and time-efficient training for this BCI-illiterate category of users. Particularly, we propose using a brain model topographic map of the subjects' EEG to visualize the feedback during the training process.

Conclusions: BCI illiteracy is one of the biggest challenges for the wider adoption of effective BCI systems that have the potential to improve life quality and experience for many users. The reported relation is a step forward towards both understanding the causes of this phenomenon and the design of successful training programs. One way to mitigate BCI illiteracy might be through 3D figure mental rotation training, but this necessitates further research.

7.17. Electrochemical Measurements with Multielectrode Array Systems to Determine the Release of Serotonin by Exocytosis in Human Platelets

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Introduction: Serotonin is a neurotransmitter that participates in the homeostasis of many physiological functions in humans. The study and understanding of its cellular

biology are of special relevance in advancing knowledge of the different actions and biosignal processes of this biological amine. It accumulates in intracellular vesicles and is released by exocytosis. Over 90% of blood serotonin is accumulated in platelets, where the highest content is stored in the so-called delta granules.

We are carrying out a quantitative study of the release, by exocytosis, of serotonin in platelets using amperometry with multi-electrode array (MEA) systems. Each exocytotic event is recorded as an amperometric deflection called a "spike".

Methods: We fabricate and optimize novel boron-doped nanocrystalline diamond 16-microelectrode array devices (16-BBD-MEA): opaque devices on silicon substrates (BDD-on-silicon MEA) [25] and transparent devices on quartz substrates (BDD-on-quartz MEA)[2].

With these 16-BBD-MEA systems, we record the amperometric spikes. The measurements are carried out under two different conditions: basal (without the modification of isolated platelets) and after loading the platelets with10 µM serotonin for 2 h [25,26].

Results: MEA systems are a solid tool for studying exocytosis in human platelets. This is important as platelets are one of few easily accessible human cells. From each spike, we calculate four kinetic parameters: Imax (maximum oxidation current, in pA), $t_{1/2}$ (spike width at half maximum, in ms), Q (spike net charge, in pQ) and the ascending slope of the spike (in pA/ms). These parameters permit us to characterize and classify the secretory spikes [25,26].

Conclusions: It is possible to quantitatively determine the release of serotonin by exocytosis from human platelets using BDD-MEA devices.

7.18. Enhanced Machine Learning Method for Predicting Parkinson's Disease Based on Non-Motor Symptoms

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Background: Early detection of Parkinson's disease (PD) is imperative for timely intervention. Olfactory dysfunction, a prominent non-motor symptom, alongside biomarkers derived from Cerebrospinal Fluid (CSF) analysis and dopamine transporter imaging, holds promise for early PD prediction. The burgeoning utilization of machine learning (ML) methodologies for prognosticating various pathological conditions has sparked interest in developing an enhanced ML model for PD prognosis, specifically targeting olfactory impairment symptoms.

Methods: This study employed a systematic approach consisting of four stages: Data acquisition, Feature extraction, ML classifier development, and Results analysis. Initial data procurement involved accessing the Parkinson's Progression Markers Initiative (PPMI) database, from which relevant non-motor features were extracted. Furthermore, features from the University of Pennsylvania Smell Identification Test, along with CSF markers such as Aβ1-42, α-synuclein, phosphorylated tau protein (P-tau181), total tau protein (Ttau), ratios of T-tau/Aβ1-42, P-tau181/Aβ1-42, and P-tau181/T tau, alongside striatal binding ratio (SBR) data, were incorporated. Subsequently, a comparative analysis of ML models was conducted based on their accuracy in predicting PD.

Results: Automated diagnostic models leveraging ML techniques, including boosted logistic regression, classification trees, Bayes Net, and multilayer perceptron, were developed utilizing the significant features identified. The dataset was partitioned into training (80%) and testing (20%) subsets to assess model performance. Evaluation metrics such as accuracy and Area under the ROC Curve (AUC) were computed, with boosted logistic regression demonstrating the highest performance, achieving an accuracy of 98.29% and an AUC of 99.2%, surpassing existing models.

Conclusions: Given the indirect nature of PD diagnosis and the substantial misdiagnosis rates attributed to the absence of definitive tests, the integration of ML models, particularly boosted logistic regression, presents a promising approach for enhancing diagnostic accuracy. The utility of ML algorithms in aiding clinical decision-making for

PD diagnosis and emphasizes the potential for assisting healthcare professionals in more accurate disease prognosis and management.

7.19. Ethical Implications of AI Systems in Bioengineering Research

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The integration of artificial intelligence (AI) systems with bioengineering technologies presents new ethical challenges that need to be carefully considered. One of the key ethical concerns is the potential for biases and errors in AI models as current large language models can sometimes provide incomplete or inaccurate information in specialized domains like bioengineering. There is a need for domain-specific AI models that are trained on the latest biomedical data and research to ensure the information provided is reliable and error-free. Other challenges lie in maintaining safety, transparency, privacy, accountability, and ethical governance when AI systems are used to aid bioengineering research or clinical decision-making. It is crucial that the reasoning and data behind AI-generated outputs are explainable and auditable, especially in high-stakes scenarios involving human health and safety. Robust governance frameworks are needed to ensure AI systems in bioengineering are used responsibly and ethically. Privacy and data governance are also critical ethical issues when it comes to using AI with biomedical and genomic data. There are also concerns surrounding the control and safeguarding of individuals' genetic information. The use of AI models that are trained on such sensitive data raises privacy risks that need to be carefully managed through strong data protection policies and safeguards. The use of AI systems to guide or automate aspects of such technologies could amplify some of the risks and concerns mentioned, such as off-target effects, ethical boundaries around human enhancement, and the need for robust informed consent processes. Ongoing multi-stakeholder dialogue and public engagement will be vital to ensure these powerful technologies are developed and deployed in a responsible, equitable, and socially conscious manner that prioritizes human wellbeing and environmental sustainability. A holistic and proactive approach that bridges bioengineering ethics and AI ethics will be key to navigating the opportunities and challenges that lie ahead.

7.20. Exploratory Simulation Study on High-Frequency Detection of Cell Internal Structures

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Introduction: Cell analysis is important in the diagnosis of several diseases, as the dielectric and morphological properties of the cells can change when diseased. In this context, malaria is one of the most threatening diseases affecting red blood cells (RBCs), leading to their destruction. High-frequency impedance spectroscopy (HFIS) with nanoelectrode arrays has the potential to overcome the Debye screening effect of the electrolyte and surpass the cellular membrane, thus allowing for the study of the cell's internal structure. In this work, we investigate, by simulation, the potential of the NXP CMOS nanoelectrode array biosensing platform in [27] to study the internal structure of the cells.

Methods: Simulations were carried out in COMSOL Multiphysics ® 6.2, modelling a healthy RBC discocyte with an overall volume of 86 fL. The malaria parasite within the cell was modelled at three different stages of infection: 6 hpi (hours post infection), 12 hpi, and 18hpi. The simulation medium consisted of a physiological solution that does not affect the cell's properties. All simulation parameters were taken from [28].

Results and Discussion: The simulations were performed with an RBC positioned in the centre of the array, obtaining ΔC as the difference in capacitance with and without the

cell. The capacitance profiles show a change in the ΔC spectrum above 1 MHz, due to the malaria parasite. Analysing the difference in capacitance between uninfected and infected RBCs at high frequencies, the variation reaches values as high as 100 aF, measurable with the existing NXP nanoelectrode array platform. This provides a framework for distinguishing between healthy cells and the ones with intracellular inclusions.

Conclusions: The potential of HFIS analysis with a CMOS nanoelectrode array platform was explored in this work, suggesting the possibility of a label-free and non-invasive method to study and explore the internal structure of a cell.

7.21. Exploring Cortical Connectivity of Visual Prosthesis Users: A Resting State Study

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Introduction: Electrophysiological studies of cortical activity have highlighted the organizational and functional differences in the cortex of blind subjects versus normal subjects. This reorganization is optimized by the nervous system to adapt to the new sensory modalities that subjects use in daily life. A cortical visual prosthesis is capable of restoring visual sensations to blind subjects based on phosphenes, which attempt to provide them with information about their surrounding environment. In this context, our study aims to characterize the cortical alterations resulting from the use of a vision neuroprosthesis.

Methods: In this preliminary approach, the blind subjects under study were temporary users (6 months) of a visual neuroprosthesis, which, through an array of microelectrodes implanted in the primary visual cortex, provided patterns of electrical stimulation that in turn evoked perceptions of phosphenes. To explore the cortical alterations resulting from the use of this neuroprosthesis, the cortical connectivity (spectral coherence, SC) of the users was analyzed in the resting state using electroencephalography techniques.

Results and Discussion: SC between all EEG channels revealed significant changes ($p < 0.001$) in 60% of the connections in the alpha band as a result of daily use of the neuroprosthesis (before implantation vs. after explantation). In other energy bands, connectivity was altered to a lesser extent (around 30% in the beta band). When comparing resting state activity with that of normal subjects, significant differences were observed in the beta band before the implant, whereas after the implant these differences tended to disappear.

Conclusions: These preliminary results revealed that cortical connectivity in the resting state significantly changes with the use of the vision neuroprosthesis, tending in some cases towards cortical patterns similar to those of non-blind individuals.

7.22. Functional Characterization of Brain Areas Using Functional Magnetic Resonance Imaging

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Introduction: Functional magnetic resonance imaging (fMRI) is a non-invasive neuroimaging modality that is continuously growing, both in the clinical and scientific fields. The analysis of these images requires a very complex and varied post-processing of the obtained images. This causes the results of different studies to be non-comparable or difficult to characterize. In order to simplify the processing and obtain objective results with analyzable metrics, this work proposes the development of an analysis methodology to obtain statistical values on brain activation areas segmented by region.

Methods: Several specialized tools were used. First, the FreeSurfer scientific package was used for brain segmentation. Then, widely used software for the analysis of fMRI data, FSL, was used for activation areas. The last processing and analysis steps were performed with the 3D Slicer medical image visualization tool. Based on these tools, a method was defined to obtain fMRI activation metrics for each of the 148 brain structures obtained by FreeSurfer.

This method was applied to the database proposed by [29], where studies of neuronal response to visual food stimuli were carried out at two different times of the day. From this, it is possible to perform a comparative analysis of different metrics for each functional area and to define the levels of significance.

Results: A method was obtained for generating metrics that characterize functional studies based on the use of open source scientific tools. This method was then applied to a database of fMRI images. Finally, a non-parametric statistical study was used based on the characterization of each area for all subjects, obtaining the most significant features.

Conclusion: This work applied a methodology for automatic fMRI image processing to obtain metrics and perform the most convenient statistical analyses. This method can be extended to different intra- and inter-patient comparisons.

7.23. How Do Room Acoustics Impact Machine Learning Accuracy in Voice Disorder Detection?

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Objectives/Introduction: In acoustic voice assessment, recordings are typically collected from diverse environments with varying levels of noise and reverberation. These room acoustics are known to affect the quality of recordings and acoustic analysis, but their impact on advanced tools like machine learning remains little understood. This paper investigates how different room acoustics, particularly reverberation, influence machine learning performance in assessing voice quality and dysphonia.

Methods: This retrospective study utilized voice recordings of sustained /a:/ samples from 193 subjects (145 with voice disorders and 48 without vocal problems). The recordings were modified to add on different levels of reverberation and noise using Audacity software, simulating various room acoustic environments. Using a MATLAB script and Praat software, we extracted different acoustic measurements (temporal- and spectral-based metrics) from the original and corrupted recordings. Various machine learning models were then trained on the generated acoustic features. The models were evaluated for accuracy, sensitivity, and specificity to compare the impact of the recordings, both before and after adding reverberation and noise effects, on machine learning performance in detecting voice disorders.

Results and Conclusions: The recordings were successfully mixed with varying levels of reverberation and noise, creating a diverse set of datasets. Machine learning models were trained and evaluated on these datasets to classify normal and pathological voices under different noise and reverberation conditions. A comparison of the models demonstrated that higher levels of reverberation and noise degrade classification performance. Identifying the acceptable room acoustic conditions where machine learning models produce reliable results helps in optimizing and standardizing environmental conditions for data collection, ensuring accurate voice assessment outcomes.

7.24. Mapping Decision-Making Traits Through EEG-Derived Personality Profiles

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Introduction: Emotions influence human behavior and can be observed through changes in brain waves. The correlation of variables such as personality and decision-making with EEG patterns could contribute to new perspectivesf—for example, in job selection processes—aiming to find the most adequate profile for a given company role. The main aim of this work is to explore the interplay between brain responses and individual personality traits.

Methods: EEG signals were collected while participants completed DISC assessment questionnaires. The band power in the theta, alpha, beta, and delta bands was calculated and correlated with DISC profile (dominance, influence, steadiness, and conscientiousness) scores. A cohort of 12 participants, with an average age of 21.2 years, was used.

Results: Obtained results indicate that brain activity, especially in the alpha band, shows a correlation with emotional anxiety and was inversely correlated with dominance traits. Alpha wave variability was also observed in closed eye (CE) and open eye (OE) conditions among participants.

Conclusion: In this study, correlations of EEG band patterns were identified for specific DISC profile patterns. The supression of alpha waves was more correlated with the dominance profile, which showed the highest correlation values. These findings could have practical implications for job selection processes, especially for leadership roles. Future research will involve expanding the participant cohort to validate and refine these findings further.

7.25. Mitigating Human Error in Breast Cancer Diagnosis with Deep Learning

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The prompt and precise detection of breast cancer, a substantial worldwide health issue, is essential for enhancing patient results. Nevertheless, conventional techniques such as mammography encounter constraints in terms of sensitivity and specificity, resulting in overlooked diagnoses and unwarranted biopsies. Deep learning (DL) is a state-of-the-art technique that can improve breast cancer detection by effectively extracting complex characteristics from medical images and enabling precise categorization. This research introduces a novel framework designed for the nuanced categorization of breast cancer by analyzing histopathology images using DL techniques. The journey begins with the original histopathological images employing a substantial dataset comprising approximately 150,408 image patches of two separate categories based on the presence or absence of invasive ductal carcinoma (IDC), each with dimensions of 50×50 pixels and RGB colour representation. The system underwent rigorous pre-processing steps and employed data augmentation to reduce overfitting. These augmented images serve as the input for the fine-tuned DL models, a repertoire that includes Custom CNN, ResNet50, DenseNet201, and VGG16, all orchestrated for meticulous training, testing, and validation. After carefully analyzing the pre-trained models and custom CNN, we found that the fine-tuned VGG16 model had an exceptional performance, obtaining an accuracy rate of 96%. The proposed approach was subjected to thorough examination, confirming its efficacy and ability to reduce diagnostic errors caused by human factors.

7.26. Multifaceted Bio-Influenced Instruction Using Organically Neurological Electronics for Robotics' Behavioral Adaptation

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Biological structures engage in direct environmental interaction and acquire knowledge through multi sensory feedback from sensory input stimulants that influence the inner neuron structures that are formed. We describe a robotic system that uses multi sensory

learning to handle things, taking influence from biological principles like the exploring and processing of sensory information, which ultimately lead to behavior learning. The robot can communicate smartly with its surrounding environment due to a small-scale biological neuromorphic prosthetic circuit that locally combines and interprets multi modal sensory input stimulants in an adaptable manner. Through the use of low-voltage organically neurological devices with a synapse capacity to handle sensory input stimulants in real-time, multi-sensory-associated linkages are formed, which eventually lead to behavioral training and the robot learning to avoid possibly hazardous things. A key component of the neuromorphic circuit's functionality is the employment of functioning components, such as organically semi-conducting compounds of polymer, which replicate bio-inspired features including dendritic summary, the plasticity of synapses, and neural computation. The monolithic polymeric electronics that are low-power, locally unified, and on a tiny scale can do this. Furthermore, the idea of handling sensory data of variable complexity and multifaceted communication can be expanded into several branches by virtue of the neuromorphic factor circuit's modular-like construction. This robotic device provides a concrete illustration of how localized organic neuromorphic factor circuits combined with bio-influenced principles might result in the creation of extremely adaptable, smart, and efficient systems for practical use.

7.27. Non-Invasive Estimation of Metabolite Concentrations Using Infrared Thermal Imaging and Machine Learning

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Evaluating metabolites concentration is crucial for understanding metabolic pathways and for disease diagnosis and monitoring. The development of non-invasive techniques able to measure metabolites avoiding blood withdrawal could be beneficial for healthcare outcomes. In this context, estimating the concentration of metabolites from skin temperature is an intriguing approach that leverages the relationship between metabolic processes and physiological parameters. Skin temperature can depend on fat metabolism and peripheral blood circulation. In this study, a machine learning model applied to facial thermal imaging features was implemented to predict the metabolites' concentration as assessed through blood samples.

Whole blood was collected as a dried blood spot (DBS). The determination of metabolites was performed in DBS samples by the addiction internal standards for each analyte of interest before the extraction. Regarding the IRT recordings, three ROIs were selected on the glabella, nose tip, and nostrils. The following features were computed from the temperature time course of each ROI to feed the machineries: mean value, standard deviation, kurtosis, skewness, delta of the signal, sample entropy, 75° percentile, the PSD of the thermal signal for the respiratory, cardiac, and myogenic frequency bands. These features were used as input for a cubic SVR model. A subset of the features was employed as an input of the ML framework, after a selection based on wrapper method. A fivefold cross-validation was implemented. The performance of the models was evaluated by correlation analysis.

The approach delivered good results for the C22 (R = 0.63, $p = 0.001$), C14OH (R = 0.67, $p = 1.7 \cdot 10^{-4}$), and C8:1 (R = 0.66, $p = 1.8 \cdot 10^{-4}$). The approach seems to be able to evaluate the concentration of metabolites related to obesity and metabolic disorders.

The results highlight the possibility of evaluating metabolites' concentration from thermal imaging, providing a novel approach that offers advantages, including increased patient comfort and compliance, and reduced risk of infection.

7.28. On Analysis of Diabetic Retinopathy Using Particle Swarm Optimization

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This study focused on classifying large sets of Optical Coherence Tomography (OCT) retinal fundus images into categories indicative of either healthy retinas or those affected by diabetic retinopathy. To achieve accurate classification, this study employed advanced feature extraction methods, specifically the Gray Level Run Length Matrix (GLRLM), the Gray Level Co-Occurrence Matrix (GLCM), and Gray Level Histogram Features (GLHFs). These techniques are crucial for capturing detailed textures and patterns within the retinal images, which are instrumental in distinguishing between healthy and disease-affected tissues. A total of 301 color OCT retinal fundus images were analyzed in this research. These images were sourced from both healthy individuals and those diagnosed with diabetic retinopathy, providing a comprehensive dataset for evaluation. To enhance the quality of the images and improve the accuracy of the feature extraction, a fourth-order Partial Differential Equation (PDE) filter was applied during the image pre-processing phase. This filtering step aimed to reduce noise and enhance the structural features in the images. The primary objective of this study was to identify the most effective feature extraction technique for differentiating between healthy and diabetic retinopathy-affected retinas. By comparing the performance of the GLRLM, the GLCM, and GLHFs, this study sought to determine which method offers the most reliable results in retinal disease classification, thus contributing to better diagnostic tools and methodologies in ophthalmology.

7.29. Optimizing MRI and CT Imaging with AI-Enhanced Signal Processing and Analysis

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Introduction: Medical diagnostics have been transformed via the incorporation of artificial intelligence (AI) into signal processing, which has greatly enhanced the analysis of intricate biomedical data. This review investigates how AI improves medical imaging diagnoses. Methods: A review of the literature was carried out to find the latest developments in AI signal processing applications in the medical imaging field. Techniques such as data fusion, deep learning, and machine learning were assessed based on their diagnostic utility, accuracy, and precision. Results and Discussion: AI has greatly improved the way that Magnetic Resonance Imaging (RMI) and Computed Tomography (CT) sig-

nals are processed, making it possible to analyze high-dimensional biomedical data more precisely and effectively. Convolutional neural networks have demonstrated up to 97% segmentation accuracy in brain tumor identification, which greatly facilitates early diagnosis and treatment planning. Generative adversarial networks have enhanced denoising and picture resolution, making it easier to identify minute anomalies in medical imaging. Precise tissue distinction has been made possible by AI-driven segmentation techniques, which are essential for the diagnosis of cancers and neurological disorders. In CT scans, for instance, AI algorithms have attained a 94% accuracy rate in identifying benign tumors from malignant lung lesions. AI-enhanced MRI has also improved the imaging of intricate anatomical components, which helps with the accurate diagnosis of musculoskeletal disorders and cardiovascular diseases. Additionally, early disease diagnosis and therapy planning have been enhanced by AI-driven signal processing. AI systems have proven to be able to cut the number of false positives in cancer detection by 50%, which increases diagnostic confidence and lowers the number of needless biopsies. Conclusions: AI-driven developments in image segmentation and signal processing have improved the precision of diagnoses and made customized treatment plans possible. Further advancements in research and technology progress are expected to augment the efficacy of these techniques in clinical settings.

7.30. Performance Comparison of Transformer, LSTM, and ARIMA Time Series Forecasting Models: A Healthcare Application

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Objective: Deep learning has significantly transformed time series analysis, particularly for long and complex datasets. While traditional methods suffice for simpler and shorter time series, advanced deep learning algorithms excel in handling intricate patterns. Our study focuses on evaluating the performance of these models in analysing complex time series patterns, using vital signs during sleep as a compelling example. Monitoring vital signs with time series forecasting enables early detection of sleep disorders, leading to faster intervention and better treatment outcomes.

Methods: We evaluated three forecasting models: ARIMA (Autoregressive Integrated Moving Average), a statistical method used for forecasting time series; LSTM (Long Short-Term Memory), a recurrent neural network architecture well suited for handling sequential data; and TFT (Temporal Fusion Transformer), a state-of-the-art deep learning model utilizing attention mechanisms. Our dataset consisted of nocturnal ECGs of 35 individuals, from the Physionet Apnea-ECG database. We used the Pan--Tompkins Algorithm to extract the heart rate from the ECGs and interpolated the results for evenly spaced time series forecasting.

Results: The ARIMA, LSTM, and TFT models were compared in forecasting heart rate data derived from ECG signals during sleep. Our evaluation focused on forecasting the next two minutes of heart rate data based on the past 30 min of observations. The ARIMA model achieved a mean absolute error (MAE) of 6.1 beats per minute (bpm) and a root mean squared error (RMSE) of 7.8 bpm. The LSTM model outperformed ARIMA, demonstrating a lower MAE of 4.3 bpm and RMSE of 5.9 bpm. The TFT model, leveraging attention mechanisms and deep learning, showcased the best performance with an MAE of 3.8 bpm and RMSE of 4.7 bpm.

Conclusion: Comparatively, the TFT model exhibited superior forecasting accuracy over both ARIMA and LSTM models, indicating its efficacy in capturing the complex dynamics. These results underscore the potential of advanced deep learning techniques in enhancing time series forecasting.

7.31. Performance of a Single-Flicker SSVEP BCI Using Single Channels

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Introduction: Brain--computer interfaces (BCIs) based on steady-state visual evoked potentials (SSVEPs) are widely used alternative communication modalities due to their high information transfer rate and systematic responses in occipital cortical areas. In particular, single-flicker modalities have been of great interest in recent years. These have mainly been studied using many recording channels in the occipital area. To minimise the number of recording electrodes, we analysed the clustering of SSVEPs under different stimulation conditions (gaze directions) using a single channel.

Methods: Using a publicly available dataset, EEG signals were recorded from 7 subjects exposed to a central flickering visual stimulus (15 Hz) surrounded by four static targets in the cardinal directions. Participants focused their gaze sequentially on these targets while their cortical responses were recorded. We discretized the energy of the SSVEP, analysed it according to its principal components, and quantified the resulting clustering using the Calinski--Harabasz (CH) index.

Results and Discussion: The energy patterns showed specific characteristics that allowed them to be grouped according to the different stimulation conditions. Principal component analysis revealed that the first three components explained, on average, 93% of the total variance of the data. Quantification of separability using the CH index showed that up to four different stimuli were effectively grouped by a single channel, with an average CH index of 296.36 for the best channel, indicating excellent separability between states. Pairwise comparisons showed that the N–S and N–W directions were the most discriminable, with an average CH above 200 in all cases. A between-subjects analysis revealed that this clustering efficiency was mainly located in a specific region of the occipital cortex (O1, O2, and Oz), where CH indices were consistently higher.

Conclusions: This approach demonstrates the feasibility of significantly reducing the number of recording channels in the implementation of a single-flicker SSVEP BCI.

7.32. Poly(Vinyl Pyridine) Coatings Cross-Linked with Cu or Zn as Active Layers for Biosensors That Are Sensitive to Protein Adsorption and Cell Adhesion

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The rapid development of biomedical technologies, particularly in the development of new diagnostic devices, has sparked the collaboration of experts from different fields such as biology, materials engineering, and physics. The most important challenge is to create innovative materials and improve biomedical sensors capable of detecting the presence or concentration of specific biological substances.

The possibility of applying poly(4-vinyl pyridine) layers cross-linked with transition metal complexes as active layers in biomedical sensors was tested. The successful modification of the P4VP coating with CuBr2 or ZnBr2 was verified using time of flight--secondary ion mass spectrometry and X-ray photoelectron spectroscopy. The topography of the coatings was examined by using atomic force microscopy. Tests of the biological activity of coatings indicated strong protein adsorption, good biocompatibility, and no antimicrobial activity. The potential of the coatings to be used as active layers of biosensors was verified by systematic impedance-based measurements, which showed the sensitivity of the P4VP:CuBr2 coatings to the presence of proteins and cells in different concentrations and indicated different detection limits for the P4VP:ZnBr2 layers. The high selectivity of

the coatings toward the defined analyte was confirmed by the specific antigen--antibody immunoreaction and the possibility of in situ monitoring of protein adsorption and cell adhesion for individual cells. Finally, the conductive response of a bilayer system that mimics an Organic Field Effect Transistor was shown. These results point to a great potential for both coatings to serve as active layers of sensitive and highly selective biosensors.

7.33. Symmetry-Based Eye Detection in Facial Images Using Hough Transform for Circles

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Introduction: Eye detection is critical in a variety of applications, ranging from facial recognition in human-computer interfaces to the analysis of human behavior and disease diagnosis. Scientific literature highlights the eyes as the most significant feature of the face, prompting extensive research in eye detection. Given the iris region's circular nature, the Hough Transform for Circles (HTC) emerges as a promising technique for identifying eyes. Utilizing the parametric equation of a circle, the HTC facilitates eye location through the template matching method. Moreover, HTC offers a non-invasive alternative to active approaches such as infrared eye detection and can reconstruct image shapes even with information loss due to digital processing. This study aims to apply HTC for detecting eyes on human faces.

Methods: Digital processing was conducted on 30 resized images (200×233) sourced from a public database. During the detection stage, code was implemented to derive the Hough space and recognize circles. An eye pair detector was then developed using the coordinates of the centers and radii of the circles identified by the Hough Transform. Finally, pairs of eyes were detected on various male and female faces.

Results and Discussion: Experiments on diverse faces revealed that applying HTC alone was insufficient for accurate eye identification, as circles other than the eyes were frequently detected. This led to the hypothesis that accurate identification could be achieved by focusing solely on the eye region, leveraging the symmetry of the face. This hypothesis was confirmed, demonstrating that the region corresponding to the eyes could be accurately identified by analyzing facial symmetry.

Conclusions: The findings indicate that it is feasible to non-invasively detect the eye region on human faces using the Hough Circles Transform. By incorporating the analysis of facial symmetry, specifically the interocular distance, HTC can reliably identify the eye region.

7.34. Stress Detection Using Bio-Signal Processing: An Application of IoT and ML for Old Age Home Residents

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Stress, as defined by psycho-biologists, is a multifaceted response that encompasses both physiological and psychological components. Chronic stress poses a substantial risk to an individual's well-being, especially for older adults residing in assisted living facilities.

Bio-signal processing at the output combination of biosensors, such as a heart rate sensor, temperature sensor, and GSR (Galvanic Skin Response) sensor, has been shown to indicate the stress level of human beings. The use of machine learning is crucial in detecting the stress level, while the use of Internet of Things (IoT) makes it easier to share

the collected data for analysis and decision making. The objective of this work was the design of IoT- and ML-based wearable stress detection devices encompassing biosensors, using bio-signal processing.

The system was evaluated for its performance in terms of finding the stress level by taking a sufficiently large range of samples. Training and testing were conducted on the samples taken from an old age home named 'SHEOWS' (Saint Hardyal Educational and Orphans Welfare Society), which is situated at Okhla, New Delhi, India.

Fuzzy logic algorithms were applied to classify stress levels into four distinct categories, 'Relax', 'Calm', 'Anxious', or 'Stressed', based on the collected sensor data. Machine learning techniques were employed for stress prediction using the collected sensor data and stress level labels were obtained from the fuzzy logic classification. Among the various machine learning algorithms evaluated, the Random Forest model demonstrated superior accuracy compared to other models, achieving an accuracy of 95.06% in detecting the level of stress. The available device needs to be translated into an industrial physical form so that it can be used as an aesthetic wearable device by users, collecting data continuously and transmitting the stress level to the doctor's dashboard.

7.35. The Development of a CO_2 Monitoring Device for the Early Detection of Sepsis

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Bloodstream infections (BSIs) pose a significant threat to public health, particularly in developing countries with limited diagnostic facilities. Increased levels of carbon dioxide (CO_2) in blood samples indicate the presence of microorganisms, making CO_2 monitoring crucial for the early detection of blood infections like sepsis. Traditional blood culture methods are often expensive, time-consuming, and prone to contamination. The lack of cost-effective and efficient diagnostic tools hampers timely and accurate diagnosis, leading to delayed treatments and higher mortality rates. This study, therefore, addresses this critical gap by developing a semi-automated blood culture CO_2 monitoring device that is low-cost, efficient, and suitable for Low- or Middle-Income Countries (LMICs).

The semi-automated CO_2 monitoring device (SCMD) was developed using an MQ-2 gas sensor, powered by a 9 V battery and a 5 V regulator for the ATmega microcontroller. The device was tested with three positive and two negative sepsis samples in the University College Hospital (UCH) microbiology laboratory. Its performance was compared with traditional blood culture techniques, which are the gold standard, and the BACTEC blood culture bottle. The accuracy of the device and the testing time were evaluated.

The SCMD detected the increased CO_2 produced by the growth of microorganisms in infected blood samples with over 90% accuracy and substantially reduced the turnaround times for detecting bacterial growth, potentially enabling quicker diagnosis and treatment of sepsis, by displaying test results in less than five minutes. Additionally, the device demonstrated a running time of up to 24 h on a single battery charge, making it suitable for continuous use in healthcare settings.

The semi-automated CO_2 monitoring device offers a prompt solution to improve diagnostic capabilities and patient outcomes in resource-constrained environments. Integrating this device into existing healthcare infrastructure could significantly enhance the timely detection and treatment of bloodstream infections, ultimately saving lives.

7.36. Time–Frequency Approaches for Analyzing Electromyographic Bursting Signals with High Non-Stationary Components: Towards Assessing Muscle Function

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Introduction: The contractile dynamics of peripheral muscles are governed by complex recruitment and relaxation strategies optimized by the central nervous system. These dynamics aim to maximize the efficiency of resulting work and are finely regulated by synergies, intermuscular coordination, and sensory feedback mechanisms. When individuals are affected by injuries, trauma, cognitive impairments, or neurodegenerative diseases, among others, such contractile dynamics are altered and often manifested in the musculature through changes in the frequency content of electromyographic (EMG) signals. During rapid contractions, these changes are challenging to study and detect because the time series comprising the EMG exhibit highly non-stationary processes.

Methods: Here, we have proposed an exploratory analysis of the time–frequency characteristics of EMG signals using three different approaches: spectrograms (SPs), Hilbert transform (HT), and empirical mode decomposition. Specifically, for empirical mode decomposition, we employed the noise-assisted multivariate empirical mode decomposition (NA-MEMD). These methodologies were applied to EMG signals obtained from a Parkinson's disease (PD) lesion model to longitudinally study the muscle function alterations.

Results and Discussion: These approaches allowed for determining and characterizing the contraction phases of the biceps femoris muscle in a free movement protocol. The SP of the EMG revealed changes in frequency content in the initial phase of contraction, depending on the progression of the injury. These initial observations were made under certain limitations of time–frequency resolution. The HT revealed subphases at the onset of contraction with significant differences in the frequency content of the EMG signals obtained across different stages of injury progression. Finally, the NA-MEMD of the signals revealed intrinsic mode functions primarily affected by anatomical–functional changes in the animal model over time.

Conclusions: This study allowed for extracting spectral information contained in non-stationary segments of the EMG, thus characterizing changes in contractile dynamics caused by progressive functional alterations in the animal model of PD.

7.37. Transgenic Bioenergy Crops as Sustainable Substrates for Recovery of Cellulosic Sugars and Lipids Using Natural Deep Eutectic Solvents (NADESs)

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Continuous exploitation of fossil resources and increasing energy consumption have urged the scientific community to look for a new alternative feedstock for producing bio-based materials, fuels, and chemicals. In view of this, a newly developed transgenic crop, i.e., oilcane, has been genetically engineered to accumulate vegetative lipids and carbohydrates in plant tissues; thus, it can be considered as an alternative feedstock to cater to the enhanced biofuel yield by providing lipids along with cellulosic sugars for large-scale biodiesel and bioethanol production. However, these components are entrapped in a highly recalcitrant lignin--carbohydrate matrix, which limits the efficient recovery of these components for their downstream processing.

Thus, NADESs, a combination of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), have demonstrated exceptional solvent characteristics as an alternative to conventional organic solvents and have benefited from easy preparation, low toxicity, high biodegradability, and high fractionation efficiency. NADESs can act as adjuvants

for weakening the lignin--carbohydrate recalcitrance matrix at the desired temperature of 100–160 °C. Thus, herein, we have synthesized several choline-chloride-based NADES using bio-derived precursors, i.e., lactic acid, oxalic acid, glycerol, ethylene glycol, and acetic acid by varying the molar ratio of HBD and HBA (1:1, 1:2) at 60–80 °C and they were employed for the pretreatment of oilcane bagasse for the fractionation of lipids and carbohydrates. Compositional analysis showed that oilcane bagasse is enriched with 3.3% of total lipids and 51% of carbohydrates. The initial study showed that the suitable eutectic combination of NADES, i.e., choline chloride and lactic acid in a 1:1 molar ratio at 60–80 °C, could effectively solubilize >80% lignin while enabling high biomass digestibility (>85%) and enhance lipid recovery (>80%). These research findings could further promote the design and fabrication of a low-cost, environmentally friendly, biodegradable, NADES-assisted pretreatment for enhanced lipid and sugar recovery from transgenic oilcane for renewable production in a circular biorefinery.

8. Biochemical Engineering

8.1. A Comparative Developed Method for Visible (VIS) Spectrophotometric Determination and Statistical Analysis of Ibuprofen in Two Pharmaceuticals

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Ibuprofen is a propionic acid derivative, a non-steroidal anti-inflammatory drug with a very effective anti-inflammatory and analgesic action and an significant antipyretic effect. It is a non-selective strong inhibitor of both Cyclooxygenase isoforms, Cyclo-oxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). The main purpose of this research was to establish the accuracy of the official Ibuprofen amount calculated on the pharmaceutical tablet, according to the Rules of Good Pharmaceutical Practice provided by the European and Romanian Pharmacopoeias. Ibuprofen real concentrations can also be exactly found from various unknown pharmaceutical samples, according to the Official Pharmacopoeias Rules. Color reaction occurred quantitatively between Ibuprofen with alpha-naphthylamine ethanolic solution 0.1%, sodium nitrite $NaNO_2$ aqueous solution 4–5%, by heating at a high temperature of 70–75 °C, which led to the quantitative synthesis of an intense orange-yellowish azo dye that was spectrophotometrically analyzed at its maximum assigned absorption wavelength, λ = 460 nm. The pure Ibuprofen amount on the pharmaceutical tablet was calculated at λ = 460 nm for both pharmaceuticals and was found to be 611.750 mg, assigned to a mean percentage content of 101.958% active substance for the first pharmaceutical product, very close to the officially declared amount (600 mg), with a average percentage error of only 1.958% in addition to the stated active substance content. For the second pharmaceutical product, pure Ibuprofen content was calculated and assigned to 394,774 mg for a mean percentage content of 98,694%, close enough to the official declared amount (400 mg). The average percentage error was only 1.307% less than the officially declared active substance content. The Ibuprofen amounts found in both pharmaceuticals were within the normal range of values, located below $\pm$5%, which is the official maximum percentage deviation mentioned in European and Romanian Pharmacopoeias. Ultimately, both spectrophotometric methods were statistically validated.

8.2. A Physiological-Based Model of the Incretin Effect on the Insulin Production Related to Intestinal Glucose Absorption

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This work presents a physiological-based model of the process of insulin production in relationship to the presence of glucose in the intestinal tract. Insulin is secreted by pancreatic β-cells in the Islets of Langerhans and it plays a fundamental role in both the proper functioning of the energy metabolism and glucose homeostasis. This process is regulated and influenced by numerous factors, such as glucose, but there are numerous other substances that can influence the release of insulin; among these there are the GIP (glucose-dependent insulinotropic peptide) and the GLP-1 (glucagon-like peptide), hormones secreted by the intestine as a response to the nutrients ingested. These two polypeptides are called "incretins", and they have an "insulinotropic" effect, increasing the insulin production while suppressing glucagon. The effects of these two incretin hormones are the cause of greater insulin blood concentrations during an Oral Glucose Tolerance Test in comparison to what is observed after an Intra-Venous Glucose Tolerance Test. This work presents a mathematical model constituted by eight ordinary differential equations aiming to reproduce both the insulin production modulated by the incretins as well as their time course over time following a bolus of oral-administered glucose. The proposed model adapts very well to the observations and a sensitivity analysis was employed to assess the most sensitive parameters in determining the trend of the variables of interest.

8.3. AI in Nanobot Feature Recognition

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The principles of "green chemistry" establish the definite demands for chemical procedures in carrying out synthetic or analytic methods using compounds extracted from biological resources. Thus, nanoparticle synthesis based on components extracted from bacteria, fungi, algae and plantae has been used for wide applications. Natural proteins, lipids, carbohydrates and unsaturated aliphatic (aromatic) compounds have the capability to act as reducing and stabilizing agents in nanoparticle synthesis. Gold nanoparticles (GNPs) have been known to be characterized by some specific features, viz. plasmonic ones, which are the main factors in the exposure of SPR, LPR and SERS. The formation of a limited area in isotropic and homogeneous media is a necessary condition for the appearance of a new phase (nanoreactor formation), which is the result of kinetic (diffusion) and spatial effects (thepresence of both structural and electrostatic obstacles). Taken together, these lead to some peculiarities in the region where nanostructure growth occurs. A nanoreactor can be viewed as a nanobot, defined as "a controllable nanoscale machine composed of a sensor and motor used to perform specific tasks specified by the appropriate conditions". The specific task is GNP formation, the sensor is the environment condition (temperature and pH) and the motor (driven forces) is the specific medium conformation where the GNPs are formed. In order to recognize a nanobot's features, we determine the factors (environment content, its temperature and pH) influencing its capability to form GNPs. Such recognition is facilitated by the data obtained from TEM, SPR and SERS on the formed GNPs. The main aim of our investigation is to provide the method for nanobot feature recognition using a model of a multilayer fully connected perceptron whose architecture includes several hidden layers with different numbers of neurons to ensure the depth of the learning and the ability to process the complex dependencies in the data.

8.4. Alternative Methods for Low-Cost Microfluidic Device Fabrication

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Introduction: Microfluidics refers to the science and technology of fluid flow at the microscale. Nowadays, microfluidic devices are ubiquitous, which makes it crucial to develop rapid and accessible microchip fabrication methods to realize all their potential applications. This work presents three alternative low-cost microfluidic device fabrication methods, accessible for fast prototyping in low-income or non-specialized laboratories.

Methods: Three alternative methods for microfluidic device fabrication that require low equipment investment and commercially available materials are compared: (a) 3D precision cutting on adhesive sheets (simile crystal acetate, PVC film, packaging tapes, etc.) using computer numerical control (CNC) laser engraving (Neje® DK-8-KZ, 405 nm wavelength, 1500 mW)); (b) PDMS chip obtention from a master mold implemented on a chemically engraved copper-laminated board. The mold is obtained using chemical etching after transferring the channel design using laser prints and a heat source. Then, the soft lithography process is carried out on the mold with PDMS. The chip is sealed with a self-adhesive PCV film. (c) 3D digital light processing (DLP) resin printing (Hellbot Apolo Pro printer). The chip is designed in a computer-aided design software environment (Fusion 360) where the printing parameters are configured. A ready-to-use chip is obtained.

Results and Discussion: The three proposed methods successfully achieved microfluidic device fabrication. The chips are capable of transporting fluids by capillarity and by external pumping. Each of these methodologies is low-cost and has advantages and disadvantages when compared to the others. The versatility and affordability of these protocols significantly expand the possibilities for research and experimentation in microfluidics, allowing a broader range of laboratories and professionals to contribute to the advancement of this discipline.

Conclusions: Low-cost microfluidic device fabrication can be achieved with the three affordable methodologies proposed and commercially available materials.

8.5. Antibacterial Effects of the Algerian Tetraclinis articulata Leaf Extract Against Klebsiella pneumoniae

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Medicinal plant extracts, rich in bioactive compounds like alkaloids, flavonoids, and terpenoids, exhibit diverse biological activities and are increasingly researched for pharmaceutical use. Algeria, abundant in medicinal plants, includes *Tetraclinis articulata* from the *Cupressaceae* family, traditionally used to treat various diseases due to its potent bioactive compounds. The leaves of *T. articulata* are especially valued for their therapeutic properties in Algerian traditional medicine. This study aimed to assess the antibacterial activity of an aqueous extract from *T. articulata* leaves against *Klebsiella pneumoniae*, alongside phytochemical analysis. The plant extracts were prepared by decoction; the phenolic compounds and flavonoids were screened using FeCl3 and Shinoda tests, respectively. The antibacterial activity was evaluated using the agar well method with Chloramphenicol as the reference antibiotic, and the Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution method. Preliminary phytochemical screening revealed that the extract from the leaves of *T. articulata* is rich in phenolic compounds and flavonoids. The aqueous extract of *T. articulata* leaves exhibited significant inhibition of *K. pneumoniae* growth at a concentration of 200 mg/mL with an inhibition zone of 11 $\pm$ 0.00 mm and MIC = 25 mg/mL. This extract demonstrated efficacy in inhibiting the growth of *K. pneumoniae*. The use of the

aqueous extract of *T. articulata* as an antibiotic to combat infections caused by *K. pneumoniae* could offer a natural and potentially effective alternative to traditional treatments, while helping to reduce the risk of antibiotic resistance.

8.6. Artificial Intelligence (AI) and/or Machine Learning (ML) Algorithms in Microalgal Bioprocesses

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One novel approach is the usage of artificial intelligence (AI) and/or machine learning (ML) algorithms to monitor, estimate, and manage the risks in microalgal processes. Artificial intelligence (AI) allows computers to simulate the intelligence of humans and machine learning is an artificial intelligence subfield. The basic idea of machine learning is to use inductive analysis to expand the connections between input and output, which are subsequently used to impact decisions in new scenarios. The input variables are pH, carbon dioxide level, inoculum, illumination, temperature, and nutrient level, whereas the outputs are biomass and bioproduct yields in microalgal processes. Enormous quantities of data generated by sensor monitoring systems may be employed as inputs to optimize parameters in AI/ML models. Therefore, AI/ML algorithms can forecast biomass/bioproduct production.

MLAs have recently been used in the research of microalgal processes, although this is still in ithe early stages for industrial applications. Approximately 75% of the energy consumed is wasted during drying step; however, using ML models might dramatically cut costs while improving output. In particular, artificial neural networks (ANNs) are mostly used for predicting microalgal growth, the support vector machine (SVM) algorithm is chosen for microalgal wastewater treatment. On the other hand, the genetic algorithm (GA) is utilized to optimize biomass and bioproduct production and the random forest (RF) algorithm performs better when determining whether microalgal populations are dead or living. In general, the reliability of machine learning models improves as data availability rises.

Using AI/ML models may help achieve microalgal production objectives through a more sustainable, smart, and economical approach. Using AI/ML-powered smart systems, such as 3D-printed, real-time optical density monitoring instruments and an Internet of Things (IoT) enabled by smartphones, could help microalgal processes make better decisions.

8.7. Biological Treatment of Second Cheese Whey Using Marine Microalgae/Cyanobacteria-Based Systems

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The biological treatment of second cheese whey (SCW) was investigated using two different marine cultures, the microalgae *Picochlorum costavermella* and the cyanobacterium *Geitlerinema* sp. SCW is produced as a by-product in the manufacture of whey cheese and is characterized by a high organic load (d-COD), an acidic pH and high salinity. Seawater from the coastal area of Rio, near Patras, was used for dilution of the SCW to achieve an initial concentration of about 2000 mg d-COD/L in both cases without any external addition of the inorganic nutrients N and P.

Lab-scale experiments were conducted in separate Duran flasks with a working volume of 1 L, under non-sterilized conditions, at room temperature (24 $\pm$ 1 °C), under continuous stirring (150 rpm) and continuous illumination (2000 lux) and without mechanical

aeration. Optical microscopy studies revealed the development of a mixed-microorganism culture, consisting of the dominant microalgae/cyanobacteria biomass and the indigenous bacteria of the SCW.

The growth of the mixed biomass over time was studied, as was the removal of NO_3^--N, PO_4^{3-}, d-COD and sugars. The simultaneous accumulation of bioproducts, such as proteins, carbohydrates and lipids, was also evaluated. The final biomass concentration was similar for both cultures, 710 mg/L for *Geitlerinema* sp. and 800 mg/L for *P. costavermella*, and the d-COD removal was approximately 55% and 65%, respectively. High removal rates were also achieved for sugars, with values of up to 80% and 91%, respectively. The cyanobacteria-dominated culture achieved higher carbohydrate (25.4%) and similar protein contents (19.8%) but a lower lipid (5.0%) content on the last day (10th day) of cultivation than the microalgae-dominated culture (10.7%, 21.3% and 11.1%, respectively).

The biological approach used in this study has demonstrated that marine microalgae/cyanobacteria-based systems can be used as post-treatment steps for the treatment of dairy wastewater, while producing biomass useful in the biotechnology industry at the same time.

8.8. Comparison of the Effects of Essential Oils and Antibiotics on Listeria Monocytogenes Isolates

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Food contamination with *Listeria monocytogenes* can cause health problems of increasing global concern. The resistance of *L. monocytogenes* to antibiotics requires finding alternative solutions to protect human health. This mini study was designed to evaluate the effects of antibiotics and some particularly essential oils on *L. monocytogenes* isolates from food of animal origin and isolates from food-producing surfaces.

This aim was to investigate the effects of seven antibiotics (amikacin, gentamicin, penicillin, cephalexin, ceftriaxone, tetracycline, and nalidixic acid) on *L. monocytogenes* isolated from food and surface samples. Also, the antibacterial activity of four essential oils (thyme, oregano, peppermint, and rosemary) against the growth of *L. monocytogenes* isolates was investigated. *Listeria monocytogenes* isolates were from food of an animal origin and food-producing surfaces as a potential source of food cross-contamination and subsequently a causative agent of listeriosis.

The isolation and determination of *L. monocytogenes* from food samples followed ISO 11290-1 [30], including the API Listeria. Surface samplings were performed following ISO 18593 [31] followed by the isolation and determination of *L. monocytogenes*. The disc diffusion method was used and the tests were performed in triplicate.

The results of this study confirmed the more pronounced antibacterial activity of essential oils compared to antimicrobial drugs. The essential oil of *Thymus vulgaris* showed a bactericidal effect against all tested *L. monocytogenes* isolates.

8.9. Current Perspectives on the Applicability of Lignin Material in the Biosorption Process

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Generally, cheap and environmentally friendly biosorbent materials attract the attention of researchers and become the focus of research. Cellulose ($C_5H_{10}O_5$) and hemicellulose ($C_5H_{10}O_5$) come to the fore among biosorbent materials in biosorption processes. However, lignin is also a very abundant and underutilized biopolymer resource that can be preferred for biosorbent production. Lignin ($C_{18}H_{13}N_3Na_2O_8S_2$) is an amorphous phenolic biopolymer with a structurally three-dimensional branched network structure. Three major

cinnamyl alcohol (p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol) monomers combine to form the lignin matrix. This biopolymer has advantages such as being available in large quantities, high selectivity and sorption capacity. However, one of the biggest disadvantages is that lignin exhibits a heterogeneous structure for the balanced production of biosorbents. The structural behavior of lignin depends largely on the source and the process conditions from which it is isolated. Today, although the use of lignin-based biosorbents is increasing, only 5% of the available lignin globally is used. Therefore, there are still significant opportunities for the development and evaluation of this material in various fields (water/wastewater treatment, industrial sectors, medicine and cosmetics, etc.). This review summarizes the world's current trends, perspectives, and recent developments in lignin-based biosorbents in terms of all properties of lignin.

8.10. Development of Intraoral Sensors for the Continuous Measurement of Clinically Relevant Parameters in the Oral Cavity

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A strong understanding of bio-adhesion, bio-mineralization, and metabolic processes in the oral cavity is crucial for maintaining oral health. Metabolic processes in the oral cavity are influenced by the diverse environment of oral fluids with its complex composition. Real-time monitoring is challenging due to heterogeneous composition variations across sampling volumes and secretion variability within different regions of the mouth.

The development of a multi-sensor devices for continuous monitoring within the oral cavity aims to integrate flexible sensors into dental splints to monitor glucose, pH, lactate, calcium, phosphate, and fluoride concentrations in saliva.

The sensing device, embedded into a personalized splint features four electrochemical sensors and one open circuit potentiometry sensor for pH measurements. Controlled by a low-power microcontroller handling data collection and control.

For pH-sensors, a potentiometric measurement mode was chosen. Ag/AgCl reference electrode (RE) was covered with a solid-state layer of polyvinylbutyrate (PVB) and the working electrode (WE) was functionalized with polyaniline (PANI) via electrodeposition.

The pH measurements were conducted by open circuit potentiometry. A linear response with -53.8 mV/pH was achieved. Unstimulated saliva samples from different volunteers were applied and the pH values determined by our sensors fitted well the pH values measured by a commercial electrode.

Continuous in vitro measurements of glucose and lactate followed amperometric sensing approaches. Electrochemical sensors were fabricated by applying oxidases and Prussian blue onto the WE while incorporating chitosan. The prepared sensors were tested in solutions with varying analytes and were conducted with the developed intraoral electronics.

The detection of additional parameters such as calcium, phosphate and fluoride are subject of current research. The collected data can be displayed in real-time on a mobile device and forwarded to cloud storage for post-processing. This approach enables real-time monitoring of important parameters for oral disease development.

8.11. Embracing Fear: The Role of Refuge in a Three-Species Biological System

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In this study, we explore a three-species ecological model incorporating the concept of refuge alongside fear, focusing on a non-delay scenario in the intricate dynamics of the food web within the biological system. The prey population is modeled to exhibit logistic growth until it reaches a predefined carrying capacity, mirroring the typical pattern of population dynamics in the absence of predators. Within this framework, diseased prey is postulated to consume healthy prey utilizing the Holling's type II functional response, elucidating how predators interact with their prey based on specific parameters. Furthermore, the predators are assumed to engage in consumption using the Beddington–DeAngelis and Crowley–Martin response functions. Our analysis aims to ascertain the non-negativity of the solutions, ensuring that they remain within biologically realistic boundaries and exhibit stability over time. By exploring every biologically feasible fixed point of the system, we seek to unravel the stable states of the ecosystem. Local stability is evaluated through the distribution of eigenvalues, providing insights into the system's equilibrium behavior. Additionally, we conduct a thorough examination of Hopf bifurcation concerning the fear factor b, shedding light on potential dynamic transitions. To validate our theoretical findings, numerical solutions are meticulously scrutinized using the MATLAB software package. Through this comprehensive approach, we offer a practical understanding of the model's behavior under specific conditions, bridging the gap between theoretical analysis and a real-world biological system. By validating the theoretical findings through numerical solutions, researchers can develop predictive models to forecast the behavior of ecological systems under different scenarios. This predictive capability can be valuable for assessing the impact of environmental changes or human interventions on ecosystems. Overall, this study will provide a solid foundation for future research endeavors in ecological modeling and biological system and ecosystem management.

8.12. Enhancing Biomanufacturing Efficiency: A Model-Based Plug-and-Play Hybrid Fed-Batch Process Using ATF Perfusion for High-Yield Drug Substance Production

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The biopharmaceutical industry is witnessing a rapid growth, with the therapeutic antibody market expanding significantly due to the increasing demand for targeted therapies and personalized medicine. Traditional fed-batch processes for monoclonal antibody (mAb) production, while effective and well established, necessitate the use of large bioreactors and extensive supporting infrastructure. These requirements translate to high operational costs and complex logistics, which pose significant challenges for small and medium-sized enterprises (SMEs) that may lack the resources and capital to invest in such expansive setups. This study proposes a new single-step concentrated fed-batch process leveraging Alternating Tangential Flow (ATF) perfusion of the inoculum integrated to the production bioreactor to achieve high initial cell densities. A model-based approach was adopted to rationally optimise the process parameters. By integrating high seeding densities and optimised process strategies, this approach enhanced bioreactor efficiency and product yield by 4 to 6-fold (conventional 1.9 g/L to 10–12 g/L in intensified process), without compromising protein quality. The intensified process was further validated using different territory CHO cell lines and was found to yield similar results. An analysis of conventional fed-batch process to the new plug-and-play hybrid process suggested a 60% reduction in costs, improvement in production efficiency, and consistent product quality, including post-translational modifications and glycosylation profiles. This scalable method is adaptable

to various cell lines and biopharmaceutical products, offering a promising alternative to conventional methods and enabling faster, more cost-effective biomanufacturing.

8.13. Exopolysaccharide Production by Rhizobium Radiobacter Using Constant Impeller Tip Speed Methodology

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Exopolysaccharides (EPSs) have many potential uses in several fields. However, the cost of production of many exopolysaccharides is disadvantageous and the labour requirement is costly. Apart from the use of exopolysaccharides as a food additive, they also have increasingly important applications in sectors such as medicine and cosmetics.

This study aimed to scale up exopolysaccaride production by *Rhizobium radiobacter* from 500 mL Erlenmeyer to 2000 mL in a stirred tank bioreactor using constant impeller tip speed methodology. *Rhizobium radiobacter*, obtained from the American Type Culture Collection (ATCC) in a total of 19,358 strains, was used to produce exopolysaccarides. The cells were grown in 30 °C with a pH value of 5.5 for 96 h at 238 rpm in the bioreactor and at 180 rpm in Erlenmeyer. The 3.5-dinitrosalicylic acid (DNS) assay and phenol-sulphuric acid method were used to determine the reducing sugar and total sugar levels, respectively.

The biomass produced in the bioreactor was found to be 5.48 ± 0.02 g/L, which was 13% better than that produced in the flask. On the other hand, an exopolysaccharide yield of 12.38 ± 0.01 g/g was obtained in the bioreactor, and an increase of 7% was recorded when compared with Erlenmeyer production.

For EPS production, cost-effective conditions were determined according to this process in order to both increase product efficiency and reduce production costs. In addition, in order to increase product quantity and efficiency, processes with shorter production times can be designed and appropriate environmental conditions can be provided.

8.14. Functional Characterization of Monoclonal Antibodies

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The development and deployment of monoclonal antibodies (mAbs) for therapeutic applications hinge on meticulous functional characterization to ensure efficacy and safety. This critical review delves into the characterization processes for a GLP-1 analog used in diabetes treatment and a high molecular weight mAb for arthritis. Despite promising clinical outcomes, these biologics encounter significant challenges, such as proteolytic degradation and immunogenic responses. Binding assays, activity assessments, and in vivo studies offer insights into their therapeutic mechanisms, yet discrepancies between laboratory and clinical responses highlight the need for rigorous assay optimization. Addressing these challenges involves innovative approaches and robust validation protocols. For GLP-1 analogs, detailed binding assays are essential to confirm receptor specificity and activity, while in vivo studies are critical for understanding pharmacokinetics and pharmacodynamics. For high molecular weight mAbs targeting arthritis, overcoming immunogenicity requires advanced engineering techniques to enhance biocompatibility and reduce adverse reactions. The continuous advancement in mAb development is propelled by these innovative methodologies, ensuring that new therapeutics meet the stringent requirements for clinical success. Ultimately, this progress enriches the treatment landscape for various diseases, offering improved outcomes for patients and expanding the potential of biologic therapies.

Through ongoing research and development, the characterization and optimization of mAbs remain pivotal in translating laboratory discoveries into effective clinical treatments.

8.15. Impact of Food Intake on Exhaled Breath VOC Profiles Using a Self-Developed E-Nose

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The analysis of volatile organic compounds (VOCs) in exhaled breath offers promising insights into metabolic processes and their changes in response to food intake. This study investigates the impact of food intake on exhaled breath VOC profiles using a custom-built electronic nose system. The primary objective was to differentiate the exhaled breath profile before and after food intake at various time intervals. A cohort of 35 healthy, non-smoking individuals was selected for the study. Breath samples were collected and analyzed at four key time points: before food intake, ten minutes post intake, one hour post intake, and two hours post intake. The collected VOC data were subjected to analysis using a Support Vector Machine (SVM) classification model. The SVM model successfully differentiated breath samples taken before food intake from those taken ten minutes after with an accuracy of 71%, and from those taken one hour after with an accuracy of 69%. However, no significant difference was observed in the breath profiles between the baseline (before food intake) and two hours post intake. These findings suggest that the exhaled VOC profile is significantly influenced by recent food intake, particularly within the first hour. The lack of significant differences between the baseline and two hours post intake indicates a potential stabilization of metabolic changes induced by food. This study demonstrates the potential of using electronic nose technology for monitoring dietary impacts on metabolic processes through VOC analysis in exhaled breath.

8.16. Large-Scale Production of α-Ketoglutaric Acid by Yeast Yarrowia lipolytica

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Introduction: In industry, α-ketoglutaric acid (KGA) is produced by chemical synthesis. A more promising approach is the microbiological synthesis of KGA using the yeast *Yarrowia lipolytica*. This approach allows for the production of a high-quality product, which is of significant interest to the food and pharmaceutical industries. Ethyl alcohol, glycerol, vegetable oils, and n-alkanes can be employed as the carbon sources for KGA production by *Y. lipolytica*. Despite the extensive body of literature on the biosynthesis of KGA by *Y. lipolytica*, there is no information on scale-up experiments. Concurrently, the approbation of the technology for the synthesis of KGA in pilot bioreactors is a prerequisite for the transition to industrial production.

Methods: The yeast strain *Y. lipolytica* VKM Y-2412 was cultivated in a 1000-L SGI bioreactor (Setric Genie Industriel, Toulouse, France) with 400 L of Reeder medium at 30 °C.

Results and Discussion: For the first time, it was demonstrated that it is feasible to produce KGA under pilot conditions. Additionally, technological approaches to realisation of the process were developed. The producer demonstrated optimal growth at a pH of 4.5 and a dissolved oxygen (pO_2) level of 25% saturation during the first two days. Following a two-day period, the production of KGA commenced. Throughout the acid production process, the pH and aeration levels were maintained at 3.5 and 50–55%, respectively. Additionally, rapeseed oil was added at a concentration of 20 g/L when the pO_2 value increased by 10% above the stable level. After 140 h of cultivation, *Y. lipolytica* VKM Y-2412 produced 42 g/L of KGA, representing 30% of the rapeseed oil consumed.

Conclusions: This research constituted the first attempt to produce KGA in a large-scale bioreactor. Further research could be conducted to optimize the cultivation conditions at the pilot scale in order to increase the product yield.

8.17. Prediction of Drug Transport, Distribution, and Absorption by a Multicompartmental Physiologically Based Pharmacokinetic Model

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The objective of this study was to develop a multicompartmental mathematical model that allows for the reproduction of the function of the gastrointestinal system in silico. This model was used to test the bioavailability of drugs, which is defined as the fraction of a drug administered orally that reaches the systemic circulation. This study employed an innovative approach that considered the individual variability of patients in order to calibrate the therapy using non-invasive preclinical data and accessible measurements. The model is a physiologically based pharmacokinetic (PBPK) model, which aims to overcome the simplifications typically adopted in the literature. It employs the typical tools of chemical engineering, transport phenomena, and human physiological and anatomical knowledge. The developed pharmacokinetic model is not limited to representing the transport of drugs and their interactions with ingested foods; it also describes several physiological aspects that quantitatively regulate the distribution, absorption, and elimination of drugs. Nevertheless, the model only contains a limited number of parameters: the permeability constants of jejunum, ileum, and colon membranes and the drug removal rates in both the blood and cellular compartments. The model was validated by testing it on two drugs, ketoprofen and ibuprofen, which yielded satisfactory results in accordance with the existing literature.

8.18. Role of M6 Membrane Segment in Structure-Function Relationships in the Yeast Plasma-Membrane H^+-ATPase (PMA1)

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Introduction: PMA1 H^+-ATPase is the key enzyme of yeast metabolism. It belongs to P-ATPases, also including mammalian K^+, Na^+-, H^+, K^+-, Ca^{2+}- and other metal-transporting ATPases. This pump couples ATP hydrolysis to transport H^+ ions across the plasma membrane, thus generating electrochemical proton gradient. The enzyme is anchored in the lipid bilayer by ten hydrophobic segments (M1-M10), which form a membrane domain that carries H^+-translocating sites. This work aimed to study the role of M6 amino acid residues in the structure-function relationships in PMA1 ATPase.

Methods: We used Ala-scanning mutagenesis to examine the functional role of amino acid residues throughout M6 of the PMA1 H^+-ATPase. The yeast strains SY4 and NY13 were employed for the enzyme expression in secretory vesicles (SV) and plasma membranes (PM), respectively. In SV, mutant proteins were expressed from plasmid *pma1* gene under the heat shock-inducible promoter at 39 °C and in PM, from the chromosomal *PMA1* gene at permissive temperature. SV and PM were isolated to measure the expression and ATPase activity.

Results and Discussion: Nearly half of the SV mutants possessed sufficient activity and expression levels for further investigation. The majority of them exhibited abnormalities in kinetics and/or H^+-transport. The rest of the mutations led to a loss of activity and/or

blockage in biogenesis. Given that heat shock may affect PMA1 biogenesis, the inactive mutants were integrated into the chromosomal copy of the *PMA1* gene. All but one mutant (F728A) were unable to support growth. F728A was expressed and exhibited activity close to the wild-type level. However, the F728A ATPase ability for glucose-dependent activation dropped almost twice.

Conclusion: M6 is a very important segment in maintaining enzyme structure-function relationships. Further studies of the substitutions' effect will help to reveal details of the mechanism of PMA1 H^+-ATPase regulation/functioning.

8.19. Sustainable Bioprocessing of Acid-Treated Rice Straw Residue for Canthaxanthin Production

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The viability of utilizing biomass as a substitute for energy has been the subject of research recently due to the increasing focus on the circular economy. The complex and resistant structures of lignocellulosic waste materials, in particular, need efficient pretreatment and enzymatic saccharification in order to liberate the necessary saccharides, which can then be further fermented by pigment-producing microbe *Dietzia* sp. The isolated-strain-grown glucose was obtained from acid-treated rice straw residue. Acid treatment removed the hemicelluloses, and the lignin and cellulose remained intact within the rice straw. Hemicelluloses are used for the production of different products [32,33]. Alkali treatment separates cellulose [34] from lignin, and further cellulose is saccaharified by cellulase enzymes to obtain free glucose for fermentation. The pigment was extracted in absolute ethanol. The colour values L*, a*, b*, and c* and the hue of the fermented pigment were also determined. Column chromatography was performed to purify the extracted pigment. The extracted pigment was identified by thin-layer chromatography. The pigment was characterized by a UV–Vis spectrophotometer and UPLC. The pH, temperature stability, and antimicrobial activity of canthaxanthin were determined. The isolated pigment has industrial potential, which can be used in food, pharma, and beverages as a colorant and also in nutraceuticals.

8.20. The Impact of Different Chitosan Viscosities on the Proliferation and Production of Naphthoquinones in Rindera graeca Hairy Roots Cultured on Hybrid PLA–Chitosan Scaffolds

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Plants are considered prospective sources of a wide range of pharmaceutical substances. Plant biomass can be applied to the secretion of desired secondary metabolites with biological activity. In nature, the amount of bioactive compounds received from plants is relatively low, and their extraction is problematic. To overcome all of these problems and increase biomass proliferation, as well the production of secondary metabolites, in vitro techniques could be applied. Furthermore, elicitation is a well-known method to stimulate the secretion of bioactive substances. Studies show that immobilization could also be beneficial for increasing plant growth and productivity. Elicitor-coated polymeric-based scaffolds are a combination of both of these methods. Such platforms could be used as easy and cheap bioengineering tools for increasing the production of secondary metabolites.

The scope of this study was to examinate the influence of the fungal chitosan's viscosity on the biomass growth and the secondary metabolite production in *Rindera graeca* hairy root cultures. The transgenic roots were immobilized on hybrid PLA–chitosan scaffolds. The surfaces of the scaffolds were modified using different chitosan viscosities, i.e., 10–120 cps,

100–300 cps, or 2000–3500 cps. The average concentration of elicitors in the platforms was 25% m/m. As a control culture, transgenic roots cultured on unmodified PLA scaffolds have been applied. The increase in the fresh biomass and the amount of naphthoquinones produced in the *Rindera graeca* hairy root cultures were determined quantitatively.

Increasing the viscosity of the fungal chitosan had a great impact on the plant biomass proliferation, as well as on the secretion of secondary metabolites. Increasing growth of the hairy roots was observed with increasing chitosan viscosity, while the effect on the production of the naphthoquinone derivatives was quite the opposite.

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8.21. Transfected Nicotiana tabacum BY-2 Cell Cultivation in a Bioreactor with Wave-Induced Agitation

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Plant biotechnology has presented the scientific world with a plethora of challenges and possibilities for decades. One of the most meticulously studied features of plant biomass is callus—unorganised, undifferentiated cells with the ability to heal wounded plant tissues or even regenerate whole organisms. *Nicotiana tabacum* Bright Yellow-2 (BY-2) is one of the most commonly used callus cell lines in research. These cells are able to grow as a suspension, and given their eukaryotic nature, with genetic modifications, they are able to produce mammalian proteins. This feature makes them extremely interesting, as they are likely to be used in biosimilar product manufacturing in the future. They are, however, prone to shear stress, which is the reason a bubble–free, wave–agitated bioreactor was used in this study.

ReadyToProcess WAVE 25 was used, along with a 2 L culture vessel, and cultures were performed, monitoring the effect of changing the angle (α) and frequency (ω) of the oscillations on the BY-2 cells. The dissolved oxygen level was monitored, and the biomass concentration was measured. Moreover, Western blotting was conducted to determine the presence of a protein product.

It was determined that the highest product activity and biomass proliferation was obtained at 0–2 days at $\alpha = 6^\circ$ and $\omega = 20$ min^{-1}, 3–5 days at $\alpha = 8^\circ$ and $\omega = 26$ min^{-1}, and 6–10 days at $\alpha = 12^\circ$ and $\omega = 30$ min^{-1}.

In conclusion, a correlation between the mixing conditions and the process's efficiency was observed. However, further research is needed to establish the exact nature of the observed effects.

8.22. Valorization of Lignocellulosic Materials for the Biosynthesis of Volatile Fatty Acids Using Clostridium Strains

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Volatile fatty acid (VFA) is a crucial intermediate molecule for numerous industrial applications and can be synthesized by *Clostridium* strains through optimized fermentation pathways. However, the industrial-scale biotechnological production of VFAs remains a significant challenge, requiring highly efficient and cost-effective methodologies. The contemporary shift towards environmentally and economically sustainable production techniques, such as the conversion of lignocellulosic materials (LMs) into high-value

chemicals and fuels, is exemplified by the consolidated bioprocessing (CBP) strategy. This innovative approach amalgamates enzyme production, saccharification, and fermentation into a single, streamlined process. Despite its potential, the realization of the high yield and productivity of VFAs from LMs through CBP remains challenging. There exists a critical need for research that establishes robust and economically viable strategies for the application of *Clostridium* strains and the utilization of LMs within CBP frameworks. This study aims to critically evaluate the current advancements in employing LMs as substrates for *Clostridium* strains and the development of integrated fermentation processes for VFA production via CBP. The outcomes of this research hold the potential to significantly advance the sustainable production of high-value chemical products and biofuels, thereby contributing to the development of more efficient and cost-effective industrial processes. By enhancing the understanding and implementation of these CBP techniques, can move closer to achieving industrial-scale VFA production while advancing the concepts of bioeconomy and circularity.

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