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Confocal microscopic imaging of sperm cells: a step toward optimised directional freezing protocols

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The EU-funded project *CryoStore* aims to enhance long-term cryopreservation of reproductive cells through the development of innovative, less invasive freezing protocols. Within this framework, coordinated by the Institute for Multiphase Processes at Leibniz University Hannover, focuses on advancing imaging techniques to better understand structural and biochemical alterations during cryopreservation. A central component of this work is the development and validation of a multiphoton-cryo microscopy platform, enabling the real-time visualization of sperm cell integrity throughout the directional freezing process.

Computer-aided design (CAD) models of a copper cooling block were created with three different channel geometries (Zigzag, Serpentine, and Spiral) and evaluated using thermal simulations in AutoCAD. Among the tested designs, the spiral channel configuration exhibited the most efficient cooling performance, providing a strong basis for its implementation in directional freezing applications. This optimized geometry ensures uniform thermal gradients across the sample, critical for consistent cryopreservation conditions. For viability assessment, ram sperm cells received from the Small Cloven-hoofed Aminals and Forensic Medicine and Outpatient Clinic, University of Veterinary Medicine Hannover, were dual-stained using Calcein AM (green) and Propidium Iodide (red), enabling the differentiation between viable and compromised cells based on membrane integrity and metabolic activity. Confocal microscopy revealed distinct subpopulations: cells exhibiting green fluorescence throughout the head and tail were identified as viable, while those with a red-stained nucleus and green-stained tail indicated compromised membrane integrity. Future work will focus on using the validated copper block to construct a cryostage to investigate effects of freezing on sperm cells, aiming to improve the precision of cryopreservation protocols. Further experiments with fresh samples will enable detailed subpopulation analysis using fluorescent viability staining, supporting objective evaluation of cryodamage and refinement of freezing strategies.

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From traditional to sustainable: new materials for polymer electrolyte membranes

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Polymer electrolyte membranes (PEMs) play a crucial role in diverse technological applications, including fuel cells, ion exchange processes, and biosensors. Conventionally, these membranes are composed of perfluorinated hydrocarbons, with Nafion™ being the most prominent ionomer. However, the inherent drawbacks of these materials such as limited recyclability and environmental concerns arising from their fluorine content necessitate the exploration of alternative materials. This study examines the potential capability of the modified biopolymer cellulose acetate, derived from sustainable sources, to provide comparable or superior mechanical and chemical properties.

The present work introduces a model utilization process wherein cellulose is obtained from pineapple plant residues, a byproduct of fruit harvesting, and is transformed into an ionomer. This biopolymer is designed to incorporate proton-conductive functionalities akin to those in commercially available sulfonated and perfluorinated hydrocarbons. Initially, the cellulose undergoes chemical modification to become a polymer with enhanced resistance to degradation in moist environments, coupled with improved chemical and mechanical stability. Cellulose acetate is used for this purpose, given its robustness obtained by substituting hydroxyl groups with acetyl groups. Post-modification, proton-conducting agents are covalently attached to the polymer backbone, and membranes are fabricated using a surgical blade casting method.

Subsequent to membrane fabrication, a comprehensive characterization is conducted using techniques such as ion exchange capacity (IEC) analysis, electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM), tensile testing, Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy, permeability assessments, contact angle measurements, and evaluations of degradation behavior.

The findings from these characterizations will show the potential applicability of modified cellulose acetate from pineapple residues in medical and energy technologies. By mimicking the distinctive properties of advanced ionomers, modified cellulose acetate sourced from pineapple residues may finally offer a sustainable alternative to perfluorinated hydrocarbons in the development of polymer electrolyte membranes. After proving the functionality of the new membrane material the transferability to technical application has to be proven.