on the extracellular matrix composition and biomechanical properties, including collagen content, fiber orientation, stiffness, and glycosaminoglycan content was assessed. Particularly, crosslinking or denaturation of proteins was studied using two-photon microscopy, Picrosirius Red (PSR), H&E, and Collagen IV staining. Furthermore, the biocompatibility of the sterilized matrices was assessed by analyzing cell viability after recellularization with intrahepatic cholangiocyte organoids (ICOs) (N=3) and HepG2 cells.

***Results:** Exposure to Gamma radiation, $sCCO_2$, and 10% Antibiotic-Antimycotic were effective in removing micro-organisms from decellularized liver scaffolds, while exposure to UV radiation and Peracetic acid were not found sufficient. Micrococcaceae, S. Maltophilia, Bacillus species, and fungi were detected in these scaffolds. Visualization of the overall structure of collagen fibers with two-photon microscopy and protein staining showed alterations in the protein matrix and modifications in fiber-to-fiber orientation of collagens after exposure to UV and Gamma radiation. Recellularization with ICOs and HepG2 cells resulted in viable and proliferating cells in all scaffolds, confirming that the sterilization methods did not affect the biocompatibility of the decellularized scaffolds.

***Conclusion/Significance:** Exposure to UV radiation and Peracetic acid did not result in the complete removal of micro-organisms. Sterilization by Gamma radiation and UV radiation resulted in alterations in matrix protein composition and fiber orientation. The biocompatibility of the scaffolds was not altered after sterilization. Going forward, we suggest $sCCO_2$ or Antibiotic-Antimycotic treatment as it limits damage to the matrix proteins. This study demonstrates the importance of sterilization of decellularized liver matrices for tissue engineering purposes and potential clinical applications.

A281 - DECELLULARIZATION OF BOVINE SPINAL CORD MENINGES VIA SUPERCRITICAL CO_2 AND EVALUATING THE EXTRACELLULAR MATRIX PERFORMANCE FOR NEURAL TISSUE ENGINEERING APPLICATIONS

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***Purpose/Objectives:** One of the main goals of modern tissue engineering is the invention of novel decellularization methods that reduce the immunogenicity and risk of rejection of the obtained acellular tissue. Current decellularization methods generally use chemical detergents that can damage tissue structure and cannot be well purified from the tissue, thus causing toxicity. To upgrade decellularization results while reducing contamination and time costs, utilizing natural, environmentally friendly, and non-toxic supercritical CO_2 ($scCO_2$) has recently emerged as a promising alternative decellularization technique to harsh detergents. Herein, we offer a fast, effective, and detergent-free decellularization technique for bovine spinal cord meninges.

***Methodology:** Bovine spinal cord meninges were decellularized by a combination of $scCO_2$ (at three different pressure values) and enzyme (DNase/RNase) methods. Then, dsDNA content analysis, glycoseminoglycans (GAGs) assay, hydroxyproline (HYP) content, and hematoxylin and eosin (H&E) stain, which are traditional analyses for decellularization, were performed. Additionally, tissues were digested with pepsin enzyme and incubated at neutral pH and temperature to achieve 3D hydrogel. A compression test was conducted in a micromechanical testing device to ascertain the hydrogels' strength and elastic modulus. Finally, cellular adhesion, survival, proliferation, and morphology were confirmed with glioblastoma cells via XTT and Live/Dead assays.

***Results:** The tissue was successfully decellularized, which was confirmed by the aforementioned studies. The $scCO_2$ method reduced the decellularization period from 1 week to only 3 days. The DNA content analysis of decellularized tissues was found to be below 50ng/mg dry tissue in all three methods. Besides, there seemed to be no significant differences in GAGs and HYP contents between native and decellularized tissues. In the H&E studies, there seemed to be no nuclei in decellularized samples. This result was also compatible with dsDNA content analysis. The mechanical strength of the hydrogel obtained by decellularizing with detergent was significantly lower than that of the hydrogels decellularized with $scCO_2$. Finally, XTT and Live/Dead assays showed that the cytoviability of the glioblastoma cells on $scCO_2$-decellularized matrices more than the control group.

***Conclusion/Significance:** The results clearly demonstrate that the combination of $scCO_2$/enzymatic methods is highly effective in decellularization of tissues. Furthermore, the findings suggest that these detergent-free techniques may replace the conventional detergent-based decellularization methods. We believe that the use of $scCO_2$ in the decellularization of human and/or animal tissues is important, and the technique fits green and sustainable agreements. This work was supported by the Health Institutes of Türkiye (TUSEB) (Project ID: 2022-B-03-24533).

Keywords: Supercritical CO_2, decellularization, spinal cord meninges, neural tissue engineering

A282 - 3D HYDROGELS FROM DETERGENT-FREE DECELLULARIZED SPINAL CORD MENINGES REINFORCED WITH HYDROPHILIC SILK FIBROIN FOR REGENERATIVE MEDICINE APPLICATIONS

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***Purpose/Objectives:** Decellularization involves removing cell components from the extracellular matrix (ECM) using different methods. The use of detergents is the most common of such techniques. The drawbacks of using detergents include a degraded matrix, prolonged processing, and unwanted residuals. Furthermore, removing cellular components during harsh decellularization often results in softer tissues with reduced mechanical stability compared to natural tissues. In the current study, we have developed a detergent-free sonication method for the decellularization of bovine spinal cord meninges. The ECM was reinforced with hydrophilic silk fibroin (hSF) fragments to increase the mechanical properties of 3D hybrid hydrogels.

***Methodology:** Bovine spinal cord meninges (SCM) were subjected to a series of decellularization protocols, including 125 watts of ultrasonic power and nuclease treatment. Decellularized SCM (dSCM) were digested with an acidic pepsin solution to obtain a pregel. After decellularization, DNA content analysis was performed using Qubit™ 4 Fluorometer to determine dsDNA residues. The hydroxyproline (HYP) and glycosaminoglycans (GAGs) content in native SCM (nSCM) and dSCM were determined using spectrophotometric techniques. On the other hand, hSF from B. mori silk was degummed in boiling sodium carbonate solution and then dried. The dried silks were dissolved in $CaCl_2$:H_2O:EtOH. This aqueous solution was dialyzed and lyophilized. Lyophilized hSF was then added to the pre-gels of dECM at different ratios. Horseradish peroxidase and H_2O_2 were added to initiate the crosslinking and generate mechanically stable 3D hybrid hydrogels. A micromechanical testing device with a 10N load cell was used to evaluate the mechanical characteristics of these hybrid hydrogels, and their rheological characteristics were determined using a DHR-2 rheometer in the 0.1-100 Hz range. The angiogenic properties of 3D hybrid hydrogels were investigated using the In Ovo chorioallantoic membrane (CAM) analysis.

***Results:** The DNA content analysis revealed that dsDNA in dSCM was <50 ng/mg dry weight. Furthermore, HYP and GAGs contents showed no significant difference between nSCM and dSCM, as determined by one-way ANOVA analysis. Then, mechanical test results indicated that the mechanical strength of the hydrogels with hSF increased compared to the dECM hydrogel alone. Based on the step-strain rheological characterization, it was observed that the hydrogels exhibited recovery following deformation. Finally, In Ovo CAM assay showed that there were more blood vessels at the time point of explantation compared to the initial time point.

***Conclusion/Significance:** The results of related analysis showed that the proposed decellularization method was satisfactory compared to conventional ones because no damage seemed to the matrix structure while removing cellular components. The findings from micromechanical testing and rheology analysis demonstrate that incorporating SF into dECM enhances the mechanical robustness and flexibility of the resulting hydrogel. The In Ovo Cam analysis shows that the hybrid hydrogel produced is biocompatible and has an angiogenesis-inducing effect. In conclusion, we believe that the

3D hybrid hydrogel from dSCM/hSF has the potential to be used in regenerative medicine applications. The financial support of the Canakkale Onsekiz Mart University Scientific Research Projects Coordination Unit is acknowledged (Project ID: FYL-2023-4519).

Keywords: Sonication assistant-decellularization, spinal cord meninges, silk fibroin, enzymatic crosslinking.

A283 - Scalable And High-throughput In Vitro Vibratory Platform For Vocal Fold Tissue Engineering Applications

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***Purpose/Objectives:** Pathologies leading to dysphonia, such as vocal fold (VF) cysts, paralysis, and scarring/fibrosis, are estimated to affect millions worldwide. The development, maintenance, and regeneration of VFs following injury are heavily influenced by phonation-induced mechanical stimulation. Thus, various in vitro systems, speakers, actuators, and airflow-based bioreactors have been developed to closely mimic the mechanical environment of the VFs. These bioreactors incorporate relevant biomaterials, biochemical cues, and cells to enable faster, more controlled, and affordable characterization of potential VF therapies. Even so, there needs to be standardized parameters for oscillatory regimes, mechanical forces, experimental units, and biomaterials used in the system. This study aimed to design, fabricate, and characterize a high-throughput, easy-to-construct, and affordable platform that simulates the VF microenvironment in vitro.

***Methodology:** The platform described in this study comprises a commercially available bottomless 24-well plate fitted with a flexible membrane atop a custom-designed waveguide equipped with a set of piezoelectric speakers for micron-scale vibrations. Frequency and amplitude precision were evaluated using Laser Doppler Vibrometry (LDV) by independently measuring the displacement of the flexible membrane and the piezoelectric speaker during various oscillatory routines. Human Vocal Fibroblasts (HVOX) and Human bone marrow-derived mesenchymal stem cells (hMSCs) were cultured on top of the flexible membrane and vibrated for 1 and 2 hours each at 100% volume at 100Hz prior to pro-fibrotic and pro-inflammatory gene expression being analyzed via quantitative real-time polymerase chain reaction.

***Results:** Our vibrational platform produced a maximum volumetric strain on the surface of the Tegaderm membrane of up to about 0.25%, according to our LDV measurements. Gene expression indicated significantly lower MMP1 expression in HVOX at high displacement ranges and in hMSCs at low displacement ranges. Lower ACTA2 expression was detected compared to the static control in HVOX.

***Conclusion/Significance:** Our team has created a unique vibratory platform that can generate frequencies within the range of human phonation. What sets our platform apart is that the wells experience varying displacement magnitudes. This feature allows for greater repeatability of these displacements and could prove to be advantageous for future studies focused on understanding cellular responses to the phonatory regime in different anatomical positions of the vocal fold, such as medially or laterally, as well as specific cell types within the different layers of vocal tissue.

A285 - A Deep Learning Model To Predictsuitable Water Contents For Sustainability Decm-based Corneal Biofabrication

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***Purpose/Objectives:** The global scarcity of donor corneas cannot fulfill the rising need for transplantation; therefore, creating high-throughput alternatives is imperative. Decellularized corneas have been investigated as an option due to their structural and functional capacities. A key aspect of the decellularized extracellular matrix (dECM) is its mechanical performance, which relies on the retention of glycosaminoglycans post-decellularization, influencing the water content of the corneal analogs and, ultimately, their mechanical performance. In this project, we first created various corneal substitutes with five types of water content. We then conducted intraocular compression, tensile, and stress-relaxation studies to examine the impact of water content on mechanical performance. Finally, we applied a deep learning model to precisely predict the mechanical properties of scaffolds under any given water content as we aim to devise a large-scale ophthalmic tissue bioengineering platform.

***Methodology:** Using a sustainable approach, we obtained roughly 500 ovine cornea samples from our main slaughterhouse partner. The samples were then sectioned to a consistent size. We created acellular scaffolds using various combinations of surfactants (1% and 4%) and decellularization periods (2 days and 4 days), i.e., 1%2days, 1%4days, 4%2days, and 4%4days, which would be compared with native corneas. All the samples were freeze-dried and divided into five subsequent conditions to vary water content (2%w/w, 38%w/w, 78%w/w, 150%w/w, and 200%w/w) and subjected to multiaxial intraocular compression, tensile, and stress-relaxation studies. Before mechanical testing, each sample was coated with a consistent layer of mineral oil to prevent evaporation during experimentation. A multilayer perceptron (MLP) algorithm, based on the collected data, was employed to establish the predictive model based on 78%w/w native physiological content.

***Results:** The intraocular compression characteristics of scaffolds with a water content of 150%w/w and 200%w/w were similar to those of native corneas (78%w/w in sheep and humans). Within the acceptable displacement range (0-1.5mm), the maximal intraocular pressures endured by the corneal scaffolds were higher than those in humans (10-21 mmHg), and ranged from 15-40 mmHg. The Young's modulus of human corneas falls in the range of 0.17-0.40 MPa. By comparison, the 1%4D group, with a water content of 78%, via vertical and horizontal tests, achieved a Young's modulus of 0.5 MPa.

***Conclusion/Significance:** Using sustainable materials in tissue engineering and regenerative medicine approaches, we devised an artificial intelligence model that defines the mechanical features of corneal analogs under different water content. Stress-relaxation tests were conducted to explore the viscoelastic properties of the decellularized alternatives. Currently, there are no reported data on this feature of the cornea. However, our studies concluded that scaffolds with physiological water content that were examined in the vertical plane showed trends similar to those of native corneas. The presentation of stress plateaus indicated that the scaffolds possess appreciable viscoelasticity, and by applying the MLP model, we can predict the water-dependent mechanical performance with an error margin of less than 0.0001. This algorithm can help identify the most suitable water content for dECM-derived kerato-prostheses that can be used as substrates for corneal biofabrication.

A286 - Bioprinting Cellularized Constructs Using A Cardiac Tissue Hydrogel And A Low-cost Perfusion Decellularization System

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***Purpose/Objectives:** Cutting-edge technologies like tissue engineering and 3D bioprinting can address medical needs in regenerative medicine effectively, such as the creation, reconstruction, and transplantation of human tissues and organs. In Peru, research has yet to be conducted in these areas, despite being ranked as the penultimate country in South America for organ donation rates in 2022. According to its Health Minister, the transplant waiting list reached more than 6,000 patients, and 732 transplants were performed, which represents only 12% of Peruvians who had the opportunity to participate in this type of procedure. Decellularization of organs and tissues combined with 3D bioprinting provides a rapid and robust approach to assembling functional tissues *in vitro*. Therefore, the following study proposes the creation of an affordable bioink for 3D bioprinting applications derived from decellularized cardiac tissue.

***Methodology:** We design and manufacture a low-cost perfusion decellularization system, that requires the assembly of pressure and temperature indicators, a decellularization chamber, a pump driver, and reagent reservoirs. Subsequently, we standardize the decellularization