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Super-resolution imaging has revealed that most proteins at the plasma membrane are not uniformly distributed but localize to dynamic domains of nanoscale dimensions. To investigate their functional relevance, there is a need for methods that enable comprehensive analysis of the compositions and spatial organizations of membrane protein nanodomains in cell populations. However, super-resolution methods are limited to analysing small, preselected subsets of proteins, at very low sampling fractions, and multiplexing analysis remains challenging. Here we describe thedevelopment of a non-microscopy based method for ensemble analysis of membrane protein nanodomains. The method, termed NANOscale DEciphEring of membrane Protein nanodomains (NanoDeep), is based on the use of DNA nanoassemblies to translate membrane protein organization information into a DNA sequencing readout. This method allows for detection of the inventory of proteins that forms the nanoenvironment of any reference membrane protein in cell populations. Using NanoDeep, we characterised the protein nanoenvironments surrounding Her2, a membrane receptor of critical relevance in cancer. We found that the occupancies of Her2, Her3 and EGFR in the nanoenvironments surrounding Her2 were similar in two cell lines with vastly different expression levels of Her2. Further, we found that adding Heregulin-β1 to cancer cells led to increased occupancy of Her2 and Her3, and to a lesser extent EGFR, in Her2 nanoenvironments. Importantly, we were able to modulate by design the inventory of proteins analysed by NanoDeep, including in the analysis of Her2 nanoenvironment two membrane proteins that have been reported to interact with Her2 (integrin α5β1 and CD63) and a protein unrelated to Her2 signalling (CD71). NanoDeep has the potential to provide new insights into the roles of the composition and spatial organization of protein nanoenvironments in the regulation of membrane protein function.

Posters: Biomaterials

1319-Pos
Optimization of Protocols for Frustule Extraction and their Characterization
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The research consists mainly about founding a suitable protocol to optimize the digestion of frustules in different diatom species. We want to find the protocol that extracts the organic material from the frustule samples, by using two different methodologies, one of them consists in heating extraction and the other one correspond to a chemical extraction. In addition, we characterize the resulting material with electronic scanning microscopy and Energy-dispersive X-ray spectroscopy (EDX) to assess the diatom species that produce more amount of silica and also to compare the efficiency of both extraction methodologies. We hypothesized that the chemical extraction would be more effective than the heating extraction. The results have showed that both methodologies are pretty similar and do not present significant differences between them regarding silica dioxide contains, however this research revealed that one sample had significant differences in relation with elimination of organic material (Carbon). Subsequently, we want to implement this knowledge the preparation of composite biomaterials that can be used as a filament in 3D printers to print biocompatible scaffolds in order to apply it in studies of cellular biophysics and bone tissue regeneration engineering, with future applications in treatments of Cleft Lip Palate patients.

1320-Pos
The Degradation and Tissue Integration of Hydroxyapatite Implants Analysed using Energy Dispersive X-Ray Spectrometry
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Cell and tissue interactions with biomaterials at ultrastructural resolutions can be challenging to investigate. Multi-colour electron microscopy using energy dispersive x-ray spectrometry (EDS) is presented here to enhance the interpretation of SEM data and provide additional analytical capabilities in the study of tissue-biomaterial interactions. We used SEM and EDS to examine the degradation of hydroxyapatite implants and subsequent growth of new bone using minipigs as an animal model. Tissue samples were collected at 1 and 3 months after implantation, fixed, embedded in resin, and ground to a thickness of approximately 1mm before being stained using uranyl acetate and lead citrate. EDS data was collected with an Ultim Extreme detector using accelerating voltages of between 5 and 10kV and a beam current of 1na or less. Data was collected as single area scans and large area maps. We found that it was not possible to determine the difference between cells and the implant material using electron signals alone. EDS was instrumental in identifying cells located within voids inside the hydroxyapatite. Cells within the implant at 1 month were stellate, a morphology usually observed in osteocytes embedded in bone matrix. EDS was also used to identify hydroxyapatite particles that were ingested by cells, determine regions of new bone growth, and revealed variations in nitrogen concentration (often used as a marker for peptides) in areas immediately next to the implant. The 3-month-old samples showed a greater quantity of cells inside the implant. The hydroxyapatite was also observed to be more fragmented. There were areas within the 3-month old implants that displayed high concentrations of nitrogen and sodium, while nitrogen levels surrounding the implant material were lower compared to 1-month old samples. EDS provided data that facilitated interpretation of structures.

1321-Pos
Investigation on the Effects of Diluted Synovial Fluid Adsorption to Model Surfaces
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Synovial fluid (SF) plays a crucial role in joint lubrication, responsible for the ultralow coefficient of friction and protection of cartilage. A healthy knee and other synovial joints contain several surface-active biomolecules, such as lubricin (concentration of 0.05-0.35 mg/mL), hyaluronic acid (concentration of 1-4 mg/mL), phospholipids (concentration of 0.1 mg/mL), among others. To our knowledge, there have been several studies investigating the adsorption of full SF or individual SF molecules to model joint surfaces. In this study, we are investigating the formation of SF films and its tribological behaviors by exposing model silica surfaces to different dilutions of SF. Using quartz crystal microbalance with dissipation (QCM-D), we quantified SF adsorption and film viscoelasticity. We characterized the tribological performance of the diluted SF films using a combination of Surface Forces Apparatus (nano-tribology) and micro-tribometer (micro-tribology). Initial data indicates that SF is highly saturated with surface active biomolecules, as 1:20 dilutions show very similar films on model silica surfaces as compared to non-diluted SF. These results suggest that our model oxide surfaces reach full SF saturation at the incredible low dilution of 1:20, based on Langmuir-isotherm models. These results can have important implications to further understand the role of SF interactions with oxide surfaces, as found in artificial hip or knee implants.

1322-Pos
Adhesive Properties of Synthetic Cement-Derived Biomaterials from the Barnacle *Amphibalanus amphitrite*
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Barnacles, a ubiquitous biofouling organism, produce a micron-thick layer of long-lasting, proteinaceous, amyloid-like adhesive. Though studied for over a century, there is still no clear understanding of sequence traits that directly relate to the adhesive properties of the bulk material. In this work, we separate effects of chemistry and structural hierarchy by using model barnacle cement peptides (BCPs) that form nanomaterials through patterned sequence motifs. To understand the role of structure, we control the solution aggregation state of BCPs to produce films for testing that have similar chemistry but varied peptide structure. From pull off measurements, we obtain a dry adhesion value which includes interfacial and bulk components. From depletion assays, we determine solely interfacial behavior of solution state BCPs. These techniques provide us with a basic understanding of the adhesive properties of synthetic cement-derived biomaterials from the barnacle *Amphibalanus amphitrite*. This work establishes a protein library that defines the relationship between adhesion and structure, shedding light on one of the most tenacious biofouling organisms in the ocean.