Experimental report

29/01/2025

Proposal: EASY-1293 **Council:** 10/2023

Title: EXPLORING THE KINETICS OF PROTEIN ADSORPTION ON A PLANAR BIOPOLYMER SURFACE

Research area: Materials

This proposal is a new proposal

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Samples: Collagen adsorbed onto PVDF substrate

Instrument	Requested days	Allocated days	From	To
FIGARO	48	48	06/03/2024	08/03/2024

Abstract:

Controlling the protein layer plays a crucial role in designing tissue engineering materials. In our study, we formed a protein layer by exposing collagen at varying concentrations to a thin film piezoelectric polymer (PVDF deposited through Langmuir-Blodgett method) for 18 hours to analyse its adsorption kinetics. Previous characterizations revealed differences in protein-surface interaction, conformation, and roughness in the protein layer. NR experiments will provide data on thickness, roughness and protein coverage over time, crucial for optimizing interactions and facilitating the future modification of PVDF surface potential. After several attempts to get beamtime, our former proposal (No 8-05-480) received a high mark (A8.0) but did not receive beamtime due to national balance (the same was with our previous proposal). This NR experiment are crucial for Paula Rodriguez's PhD thesis (3rd year of PhD study) funded by the Basque Government neutron program. Supervised by Viktor Petrenko and Marite Cardenas, our project aligns with the IKUR Strategy to advance neutron scattering in bio-related materials, making this experiment essential for successfully finish our project.

EXPLORING THE KINETICS OF PROTEIN ADSORPTION ON A PLANAR BIOPOLYMER SURFACE

Scientific background

Human life expectancy has increased in the last decades, favouring the appearance of musculoskeletal and related diseases, including bone resorption and formation imbalances, leading to osteoporosis. Tissue engineering (TE) [1] approaches have arisen over the years as valid strategies for bone healing and regeneration and bone TE has shown the need for a specific scaffold-based microenvironment able to reproduce the physiological characteristics of the tissue itself. The importance of smart (active and multifunctional) polymer-based materials has recently increased in this field. In particular, polymeric surfaces with distinct and dynamically varying net surfaces interaction with extracellular matrix (ECM) proteins can comprise the cell environment, strongly influencing cell behavior [2]. Thus, the active control of the conformation of the ECM proteins through electric cues opens promising perspectives toward the controlled cellular responses. However, despite numerous efforts in the field, the influence of electrical stimulation on the material-protein interface remains largely undescribed. Consequently, understanding the protein interaction at basic levels with biomaterials is critical to tailoring systems able to impact cellular growth.

[1] G. L. Koons, et al. *Nature Reviews Materials 2020 5:8*, vol. 5, no. 8, pp. 584–603.

[2] B. Trappmann, *et al. Nat Mater*, vol. 11, no. 7, pp. 642–649, 2012.

Preliminary work already carried out

The neutron reflectometry (NR) experiment represented a crucial advancement in the research previously conducted by our group. It enabled us to complement existing data on the impact of protein concentration on the properties of the adsorbed protein layer.

Investigation of the possibility of tuning properties of proteins adsorbed on well-known piezoelectric polyvinylidene fluoride (PVDF) polymer was already performed. In order to obtain a protein layer, our samples were prepared by placing a diverse concentration of collagen solution in contact at different times PVDF polymer substrates. This system has been thoroughly characterized by different techniques (QCMD, SEM, AFM, BCA, immunofluorescence, FTIR, Force Spectroscopy among others). An example of QCMD data is presented in Figure 1A, and a scheme of the system is shown in Figure 1B.

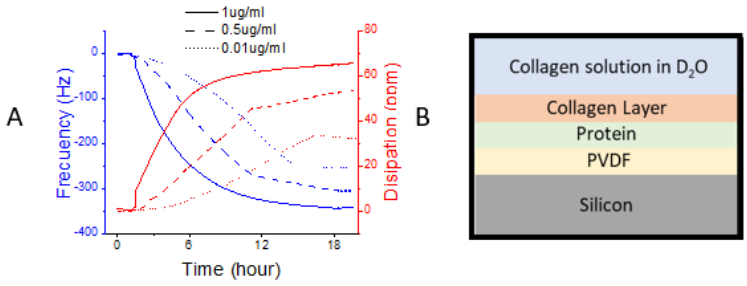

Figure 1: (A) Frequency and dissipation of a QCMD experiment for the protein adhesion at different protein concentrations with time (1 μg/ml,0.5 μg/ml and 0.1 μg/ml). System scheme (B) of the system.

Performed experiments

Polymer thin film layers were deposited onto silicon substrates using the Langmuir-Blodgett technique at the Partnership for Soft Condensed Matter (PSCM) laboratory. Subsequently, the thickness, roughness, and scattering length density (SLD) of the polymer layer were determined at the neutron beamline using three different contrasts: D2O, silicon-matched water (SMW), and H2O, as illustrated in Figure 2A. A protein solution in D2O was then introduced into the cell at a flow rate of 0.5 ml/min using a peristaltic pump. The adsorption process was monitored in real-time by acquiring scans at two different angles every 40 minutes. Finally, the thickness and SLD of the final collagen layer were measured using the same three contrasts (Figure 2B). This procedure was repeated for three different substrates and three distinct protein concentrations: 5 µg/ml, 1 µg/ml, and 0.1 µg/ml. At the lowest concentration, the protein amount was insufficient to observe any significant adsorption. At the intermediate concentration, protein adsorption occurred and was measurable, as depicted in Figure 2C. At the highest concentration, an air bubble entered the system, rendering the data unusable.

Regarding the adsorption kinetics, slight variations were observed, and further analysis is required to determine the feasibility of fitting the data to a kinetic model. However, upon collagen layer saturation, discernible differences in reflectivity were observed, as depicted in Figure 2C.

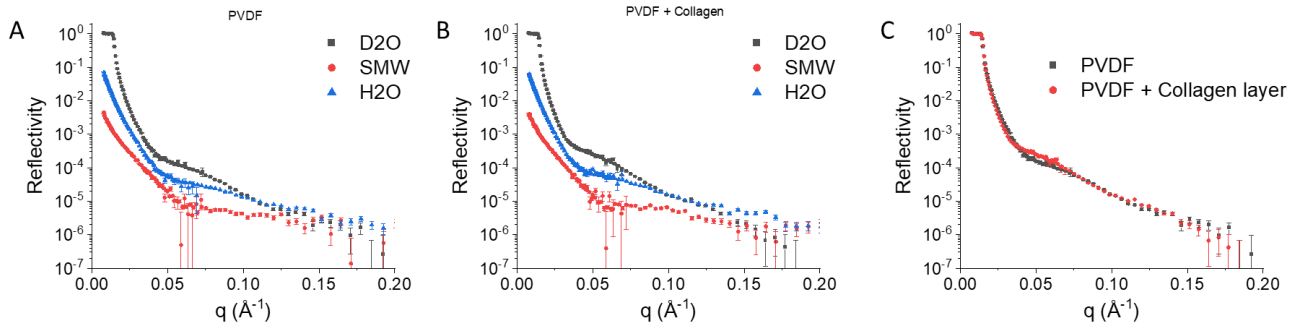

Figure 2: Reflectivity data for (A) the bare PVDF layer and (B) the PVDF layer with an adsorbed collagen layer. (C) Comparation of the reflectivity of the bare PVDF layer with that of the PVDF + collagen layer for the D2O contrast.

Data fitting is being conducted using the refnx Python library, with recent improvements to the fitting process evident in Figure 3. However, further analysis is necessary to comprehensively characterize the system.

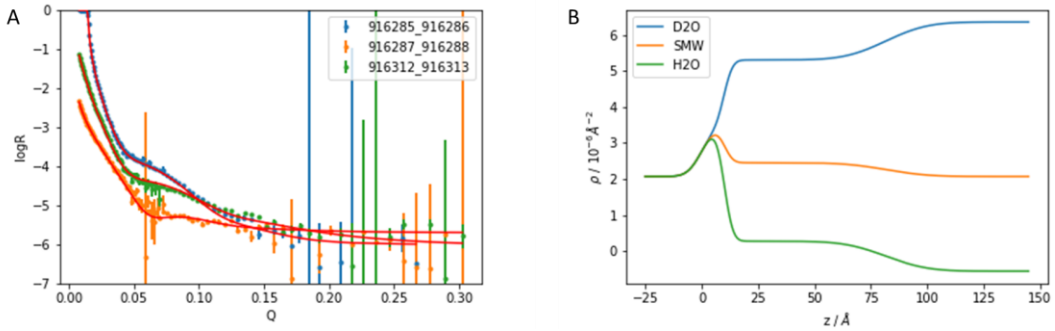

Figure 3: (A) Fitted reflectivity curves for the PVDF layer in different contrast media. (B) SLD profiles derived from the fitted curves.