

Introduction

Description

The self-organisation of macromolecules into dynamic and hierarchical supra-molecular assemblies is a key feature of biological systems. Among those systems, soft structures, made of flexible polysaccharides, play a key role in the formation of biological hydrogels, as well as in the constitution of cellular membranes as extracellular/pericellular matrix. Even though pericellular coats play a crucial role in many fundamental biological processes, the understanding of their internal organization and functionality is still limited. The issue of delineating those processes which are physically driven from those which are biologically driven is key step in the understanding of how these polysaccharides mediate basic physiological functions. Consequently, it is desirable to move from living cells/tissues to well-controlled models with tunable complexity, and turn attention to biomimetic systems. To investigate such systems, appropriate tools need to be used and developed ; they can be directed to the characterization of self-assemblies of purified components on solid substrates. A toolbox of physicochemical in situ characterization techniques needs to be applied to study macromolecular systems which are typically highly hydrated and display a very low imaging contrast, while undergoing constant reorganization and lacking fixed three-dimensional structures. An overview of methodological approaches used to study the organization of structure of soft biological matter, together with the limitations of each method, is presented in Table 1, with Hyaluronic Acid (HA) used as a typical example as a key constituent of the pericellular matrix (PCM).

Methodology	Gained information	Limitations
<i>Particle exclusion assay</i>	Thickness of HA matrix around cells. ^{a,b}	Lack of detailed structural information.
<i>Fluorescent HA labeling</i>	Presence of HA gradient –higher concentration on the cellular membrane. ^{c,d}	Lack of detailed structural information. High risk of matrix perturbation by labeling.
<i>High resolution electron microscopy</i>	Fibrillar formation. ^e	Upon drying matrix is destroyed. Fibers might be artifact structures.
<i>Optical tweezer</i>	Measure mechanical properties of the HA matrix and the mesh size. ^{f,d}	The measurement are influenced by the cell status.

The surface-based model of the HA matrix allows to use state of art surface-sensitive techniques for their characterization. Each method by itself is powerful and can provide quantitative information about a few parameters. However no single method alone gives a comprehensive picture of the architecture

of the HA film, but the information combined from the different techniques is required. With a toolbox of methods, presented below, many parameters of HA model systems can be quantified, including film thickness, hydration, permeability, amount of incorporated molecules, their orientation and the kinetics of self-organization.

The following chapters will describe : (1) Quartz Crystal Microbalance ; (2) Spectroscopic Ellipsometry ; (3) Colloidal Probe Reflection Interference Contrast Microscopy