Collagen at the solid/liquid interface: Self-assembly, supramolecular organization and interaction with inorganic nanoparticles

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Collagen is the most abundant structural protein in mammals, located in the extracellular matrix (ECM) of connective tissues. *In vivo*, collagen molecules have an outstanding ability to self-assemble and to interact with other entities to form highly organized 3D networks surrounding cells as in hard biomineralized tissues, cartilage, tendon, and skin. These architectures provide a physical support to cells and serves as transducer for biochemical signaling. Collagen is also one of the most common proteins used for the design of biomimetic materials with broad applications in drug delivery and tissue engineering. In vitro, collagen plays a pivotal role to mediate cell-material interaction during in vitro tests. It is, indeed, frequently adsorbed to cell culture substrates to provide a protein coating for cell attachment, as cells lose their normal ECM environment.

Nanostructured collagen layers may be obtained at solid/liquid interface by adjusting the adsorption procedure, the characteristics of the medium (pH, temperature, composition and ionic strength), the properties of the substrates (wettability, surface charge, topography) and collagen conformational states (triple-helix or random coil) and fibrillogenesis [1]. Even so, the self-assembly and organization of collagen at solid/liquid interface remain difficult to predict.

Understanding the behavior of collagen at solid surfaces requires coping with the complexity of the interfaces. Herein, examples are given to illustrate key considerations in situations of biomedical interest:

(i) Mechanism of interaction with spheroid- and rod-shaped TiO_2 nanocrystals. These nanoparticles have, indeed, been the subject of a vast literature regarding their toxicity and their interaction with proteins impacting cell behavior [2], rods being explored for their enhanced photocatalytic properties, however with possible consequence on particle aggregation [3]. In solution, results show that nanoparticles do not alter the conformation of collagen (triple-helix), and slightly delay the kinetics of its fibrillogenesis. By contrast collagen layer is strongly impacted by the presence of nanoparticles in the medium (Figure 1, top).

(ii) Mechanism of interaction with hydroxyapatite (Hap) nanoparticles. A comprehensive understanding of this mechanism is a pivotal step for guiding the design of biologically relevant nanocomposites with controlled hierarchical structure. By using a variety of Hap nanoparticles differing by their shape (rod vs platelet) and their size (~30 vs ~130 nm), we showed that collagen strongly interacts with rod-shaped nanoparticles while only a weak effect was observed with platelets. Interestingly, the use of small rods, typically with ~30 nm of length, leads to the formation of assembled collagen fibrils decorated with Hap nanocrystals which, in turn, self-assemble progressively to form larger fibrillar composite (Figure 1, bottom). Through this study, we showed the possibility to design hierarchical collagen-hydroxyapatite nanostructures which may be relevant for various medical applications.

(iii) Methodological development for probing collagen layers both in the dried and hydrated phases. This includes AFM imaging, force spectroscopy, QCM-D, etc. In particular, we developed a powerful yet straightforward method to exploring the dewetting patterns of the collagen layers using AFM imaging. The method allows the extraction of quantitative parameters describing the collagen networks: density and degree of nodes, area and distribution of holes, physical distance between nodes, etc (Figure 1, top). We showed that information obtained from AFM imaging are consistent with the analysis of the protein layers in the hydrated state, regarding the elastic compliance of the film, i.e. its softness (data from QCM-D), the surface density of fibrils and their “length” (data from AFM force spectroscopy). This constitutes a step forward toward reconciling disparate views of collagen layers’ characterization in the dried state and in the liquid phase.

The approaches described here offers new perspectives to examine the interactions between collagen and inorganic nanoparticles at the solid/liquid interface, particularly at the stage of *in vitro* cell culture.

References

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Figures

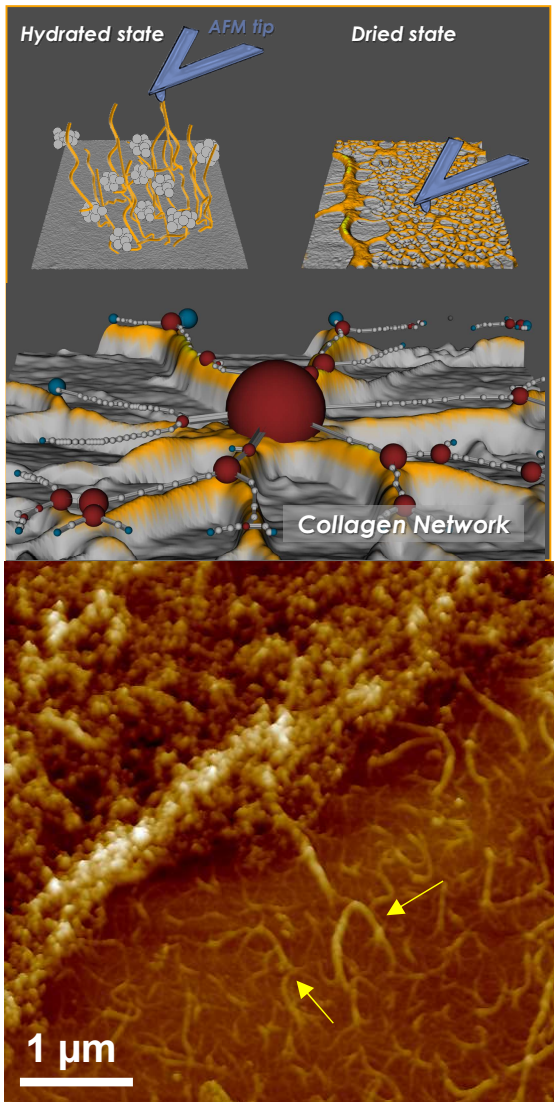

Figure 1. (Top) Schematic representation describing (i) the supramolecular organization of collagen on PS-coated substrate in the hydrated and the dried state in solution containing TiO_2 nanoparticles and (ii) network extraction from AFM images of collagen layers with the degree of nodes (blue = 1 ; grey = 2 ; red > 2). (Bottom) AFM height image showing the formation of collagen-hydroxyapatite composite, and well-defined collagen fibrils (see arrows) remain clearly visible.