Self-Assembly of Collagen Molecules into Fibrils in Solution

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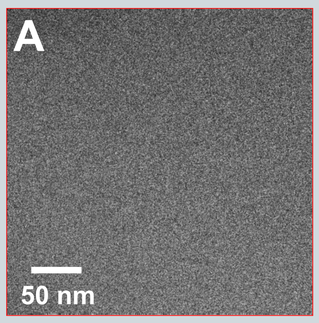
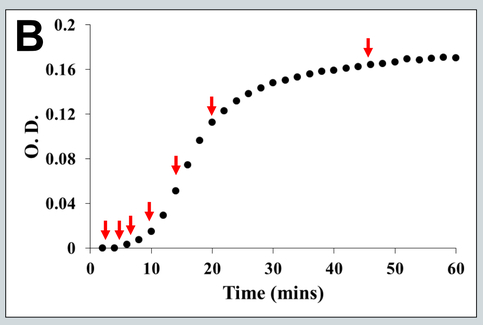
Introduction

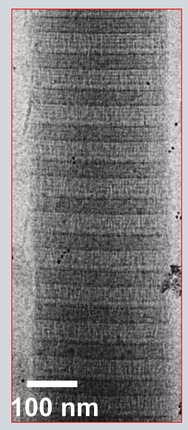
Type I collagen is a major constituent of many biological tissues, including skin, bone, tendon and cartilages. Its main functions are to shape extracellular matrices, promote cell attachment and provide tissues with strength, flexibility and elasticity[1]. At the core these functions is its remarkable ability of collagen to form highly organized fibrils through the self-assembly of the molecules[2,3]. The fibrilogenesis involves the lateral association of collagen triple helices into staggered parallel arrays that give rise to the characteristic D-band periodicity of 67 nm[4]. Currently, the mechanisms of collagen self-assembly are poorly understood Here, we combine the nanometer-scale resolution of cryo-transmission electron microscopy (cryoTEM)[5] with molecular dynamics[6] to investigate the self-assembly of collagen molecules into fibrils in solution.

Self-assembly

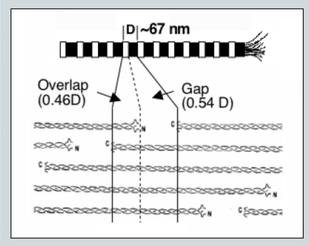




A. CryoTEM image of the collagen molecules prior to the self-assembly. The molecules are not visible due to low contrast. The self-assembly was triggered by diluting type I collagen at pH 2 into tris-buffered saline at pH 7.4 at 37 °C and monitored by UV-Vis at 440 nm (**B**). Samples were collected after different reaction times (red arrows), plunge-frozen in liquid ethane and analysed using cryoTEM.

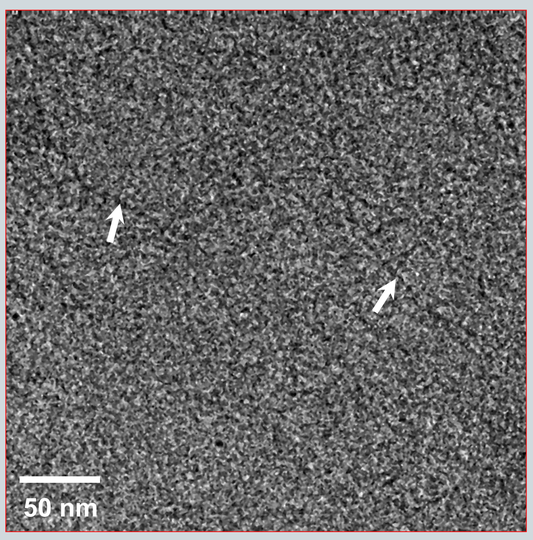


CryoTEM image and schematic representation of a collagen fibril after self-assembly, with the characteristic D-banding of 67 nm.

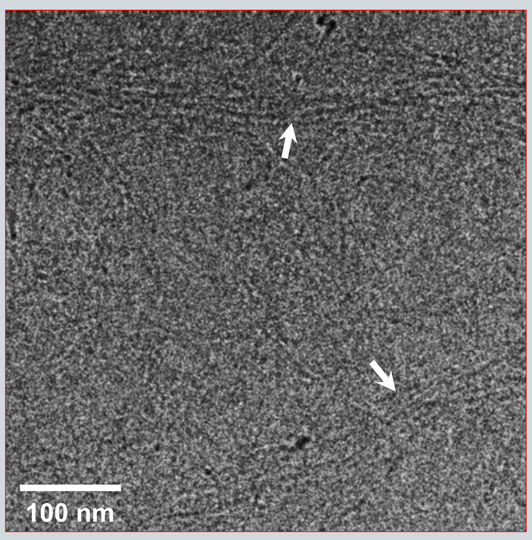


Time-resolved CryoTEM

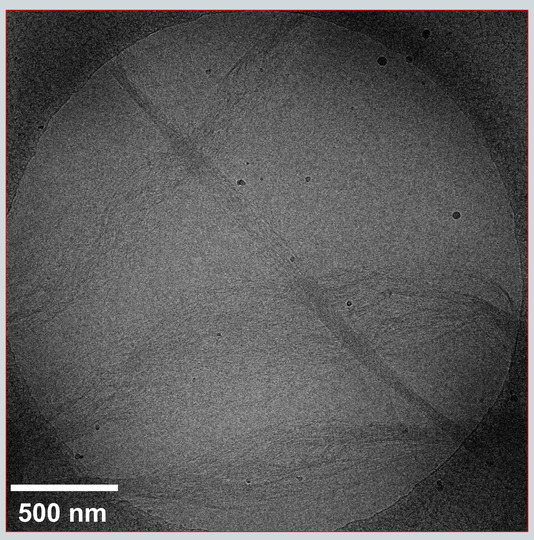
5 min: Molecular aggregates

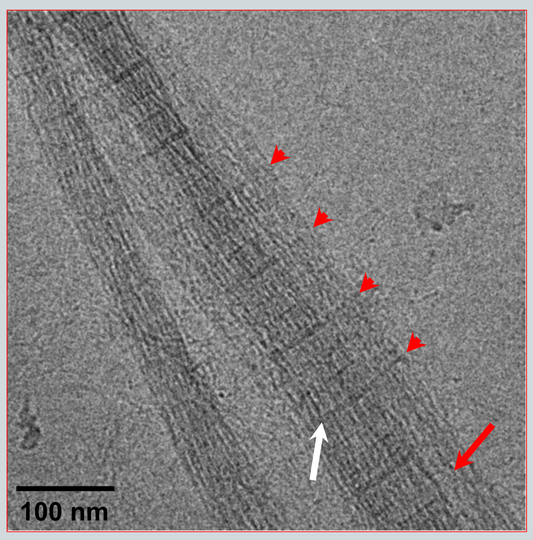


7 min: Disordered strands

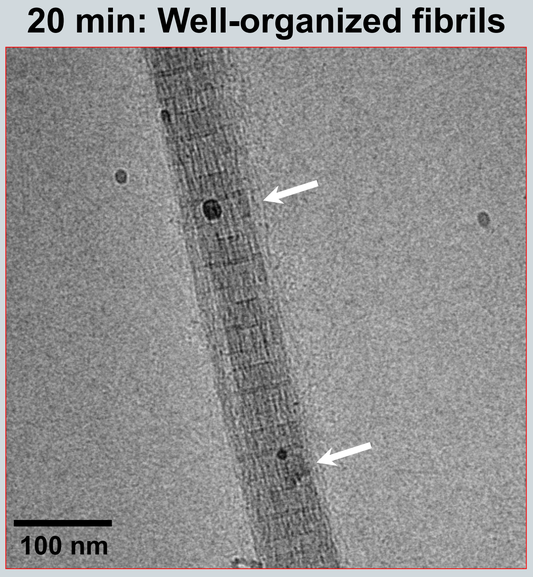


10 min: Disorganized, loosely packed fibrils; development of the D-banding

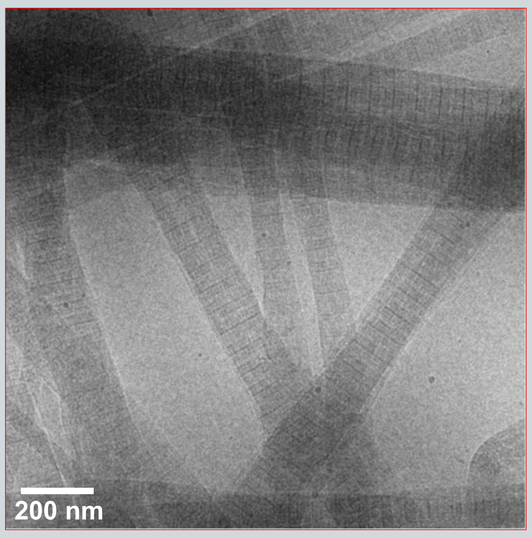




20 min: Well-organized fibrils



45 min: Mature fibrils



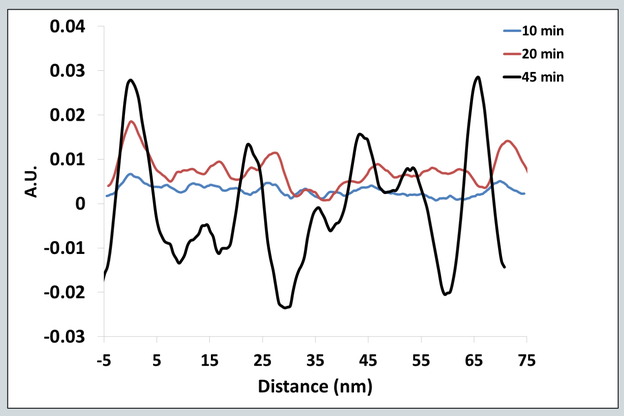
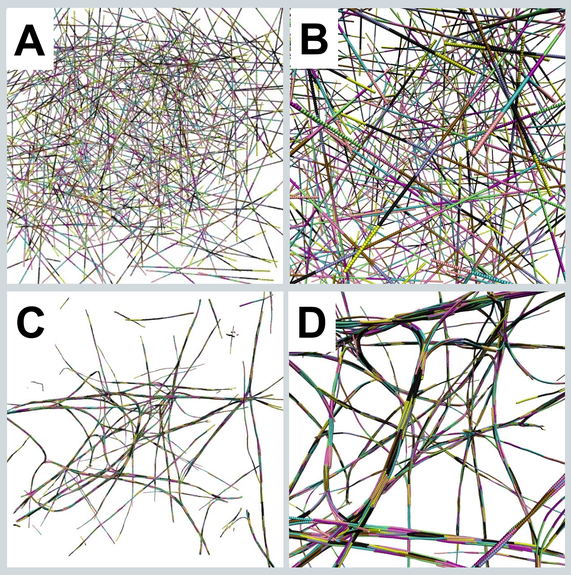


Image analysis of a single D-banding repeat over time, showing the development of the gap and overlap regions and the decrease of the spacing from 70 nm to 66 nm.

Molecular Dynamics Simulations



Initial (A, B) and final (C, D) configurations of the simulation run. Fibrils have clearly formed, and the collagen particles are ordered with an offset between them. Some fibrils branch off to form other fibrils in Y-junctions, but this is a result of the model used. Reducing the strength of the attractive Lennard-Jones potential should results in these Y-junctions disappearing and in only a single stable fibril forming.

Conclusions

Collagen self-assembly occurs by:

- Formation of molecular aggregates;

- Assembly of the strands into disordered, loosely packed structures;

- Further organization into compact, ordered fibrils with the development of the D-banding;

- As the fibril matures, the spacing of the D-banding decreases from 70 to 66 nm. This indicates the self-organization of the molecules within the fibrils during the self-assembly.

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