



Studies on Assembly and De-assembly of Collagen

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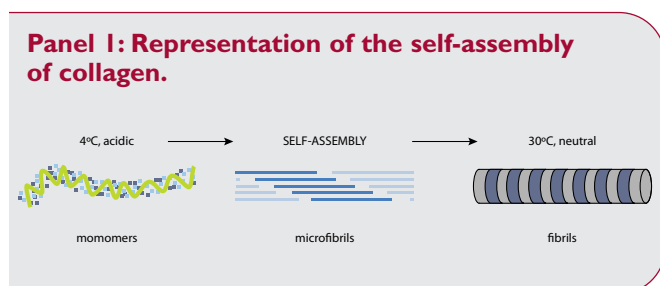
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Collagen type I is the main component of skin. There are still unanswered questions in the mechanism of tanning that make it necessary to continue research on this topic. There is a need to understand tanning more clearly, or rather the interaction between collagen and tanning agent.

The Investigation

The *in vitro* self-assembly of calfskin collagen from monomers to fibrils was studied using UV/Vis spectroscopy and atomic force microscopy (AFM). Fibrillation in an acidic monomer solution is initiated by increasing the temperature to 30°C and the pH to neutral conditions^[1]. The schematic build-up of fibrils from monomers via microfibrils is shown in *Panel 1*:



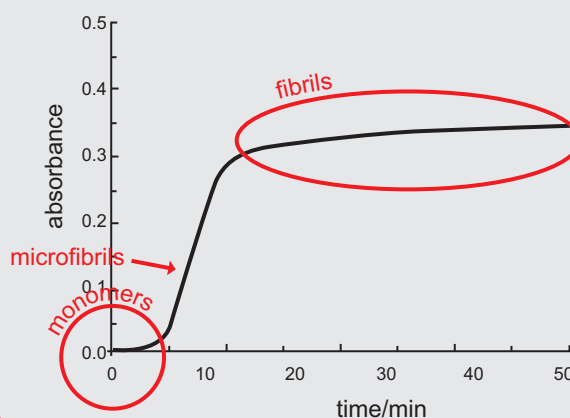
This assembly process is revealed in the kinetic spectra taken by UV/Vis spectroscopy, and shown in *Panel 2*.

This unspecific size effect is measurable at wavelengths between 200 nm and 400 nm. Monomers do not scatter light at 340 nm whereas the fibrils do, and the delayed slope (lag time) proves that there is a transition state between monomers and fibrils.

It can be concluded that changes to the spectroscopic measurements of turbidity may provide information/insight to the interaction between additive (eg. tanning agent) and collagen at different hierarchical levels. Accordingly, changes in the lag-times and in the height of the turbidity plateaus are detected as a function of the amount and type of additive^[2].

These findings have been further investigated by means of a simple model that assumes that so-called microfibrils are an essential intermediate. Changes in the morphology of the

Panel 2: Time dependence of the assembly of collagen from monomers to fibrils.



assembled fibrils are (as complementary information) studied by AFM.

Further, a method was developed to study the de-assembly of fibrils, where monomers are removed from the fibrils at low pH values. The obtained time-dependent turbidity data provide information about the stringency of the binding of additive to collagen^[3].

Tanning agents strongly influence the self-assembly and de-assembly of collagen. Polymers based on acrylates are used in tanning processes, and in particular, the influence of polyacrylic acid and polymethacrylic acid was studied.

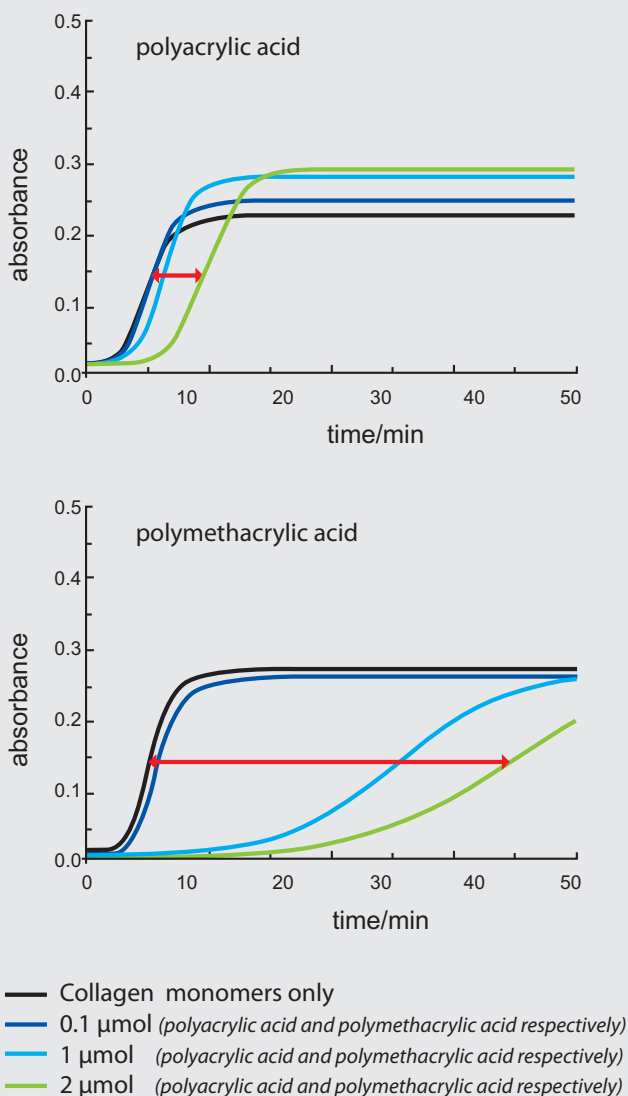
Results and discussion

Polymethacrylate and polyacrylic acid only differ by one methyl side chain in their structure, but this influences their tanning properties. Polymethacrylate has a large impact on the kinetics, whereas the use of polyacrylate leads to considerable changes in the morphology of the assembled fibrils^[2].



The assembly kinetics of collagen carried out with additives of up to 2 μmol polyacrylic acid and polymethacrylic acid is shown in the graphs presented in *Panel 3*:

Panel 3: UV/Vis spectra of collagen assembly in presence of polyacrylic acid and polymethacrylic acid.



With reference to *Panel 3*, the standard curve for collagen assembly is shown in black. The longer lag times caused by both polyacrylate and polymethacrylate can be observed, and this effect is identified by the red arrows. When adding polymethacrylic acid this time-lag effect is more pronounced.

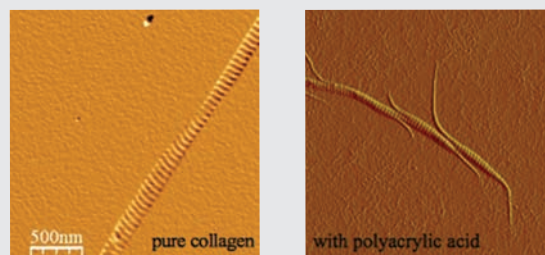
These assembly products can be revealed using AFM, and these are presented in *Panel 4*.

With reference to *Panel 4*, the fibril shown in the left image was assembled under standard conditions. The fibril shown in the right image was formed in the presence of polyacrylate, and shows "tails" associated with the fibril^[3].

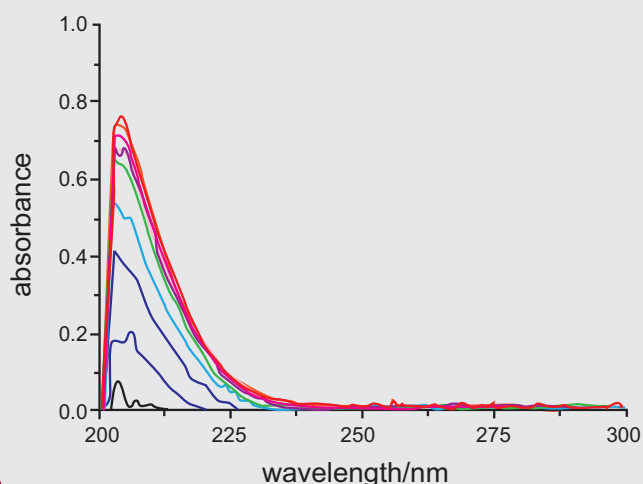
The use of polyacrylic acid causes repulsion or defective assembly, and this causes additional scattering centres. This explains the enhanced plateau level in the UV/Vis spectra.

Data obtained from de-assembly experiments using

Panel 4: AFM images of collagen fibrils assembled without and with 1 μmol polyacrylic acid.



Panel 5: UV/Vis absorbance spectra during the de-assembly of collagen



hydrochloric acid is displayed in *Panel 5*.

The concentration of collagen increases with time as more and more monomers are removed from the fibril matrix when 100 mM hydrochloric acid is added. This rise in the concentration is measured via the intensification of the absorbance at 200–220 nm caused by the peptide bond.

Conclusions

Study of the assembly and de-assembly of collagen, and the influence of additional compounds provides insight into molecular level interaction between tanning agent and the collagen matrix. This alternative approach provides additional insight to help us to better understand the mechanisms involved in tanning. ☺

References

- [1] J.-H. Bradt, M. Mertig, B. Winzer, U. Thiele, W. Pompe: Collagen assembly from acid solution to networks on solid surfaces and to fibrils, *Proc. SPIE*, Vol. 2779, 78-82 (1996)
- [2] D. Naumburger, N. Haufe, S. Garnier, T. Taeger, V. Bach, M. Mertig: The influence of non-covalently binding polymers on the *in vitro* fibrillogenesis of collagen type I, submitted
- [3] N. Haufe, D. Naumburger, S. Garnier, V. Bach, M. Mertig: De-assembly of collagen type I, submitted

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