

Construction with Collagen – Insight through Atomic Force Microscopy.

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Collagen monomers form a variety of constructs in solution preferentially, depending upon the conditions to which they are subjected. Depending upon the solution conditions, the length, diameter, and surface properties of these constructs may be controlled. Both transmission electron (TEM) and atomic force microscopy (AFM) have been used to probe these constructs; here, we demonstrate the unique insight that may be obtained by AFM.

In all cases, we start with an acidified collagen monomer or oligomer solution. This solution contains rod shaped monomers with ~1.5 nm diameter. If the collagen solution is gradually increased in pH, long, fibrillar type structures of microns in length may be formed. If this is carried out in an acidified solution containing collagen alone, formation of the standard fibrillar form, native collagen, displaying a 67 nm banding periodicity (Fig 1) may be induced. Subsequent addition of the anionic nucleotide ATP (Fig 2) or addition of a much larger anionic species (α_1 -acid glycoprotein) to collagen with gradual pH increase produces fibrillar species with spacing clearly longer than the 67 nm native spacing (Fig 3) [1]. In the latter case, the fibril surface by AFM is seen to consist of a series of protrusions separated by an extremely reproducible ~ 270 nm (giving rise to the name fibrous long spacing (FLS) collagen), which correlate exactly to the dark bands evident in TEM images [2]. Notably, FLS collagen diameters are typically a factor of 10-20 greater than those of native collagen. AFM has allowed us to unambiguously identify a stable intermediate in FLS collagen formation – something never resolved by TEM [1]. If the monomer solution is kept at acidic pH (~3.8) and incubated with ATP, a highly curious construct forms. Somehow, aggregation is limited to two-dimensions producing rod shaped crystallites with a very definite and reproducible surface morphology visible by AFM (Fig 4) [3]. This is the segmental long spacing (SLS) collagen crystallite – a construct corresponding exactly in length to the collagen monomer at ~280 nm. SLS crystallite diameter may be increased with increased ATP addition up until some critical concentration, beyond which all crystallites display identical diameters on the order of 100x the monomer diameter. Here, AFM has allowed us to unambiguously identify this critical concentration through the ability to quantitatively measure diameters of large numbers of SLS collagen constructs. Generally, in order to produce lengthened constructs with defined surface morphology, a pH increase appears necessary while diameter increase of constructs seems to be generally augmented by the addition of anionic species [4].

References

- [1] M.F. Paige et al. *Biophys. J.* 74 (1998) 3211.
- [2] A.C. Lin and M.C. Goh. *J. Microsc.* (2002) In press.
- [3] M.F. Paige and M.C. Goh. *Micron* 32 (2001) 355.
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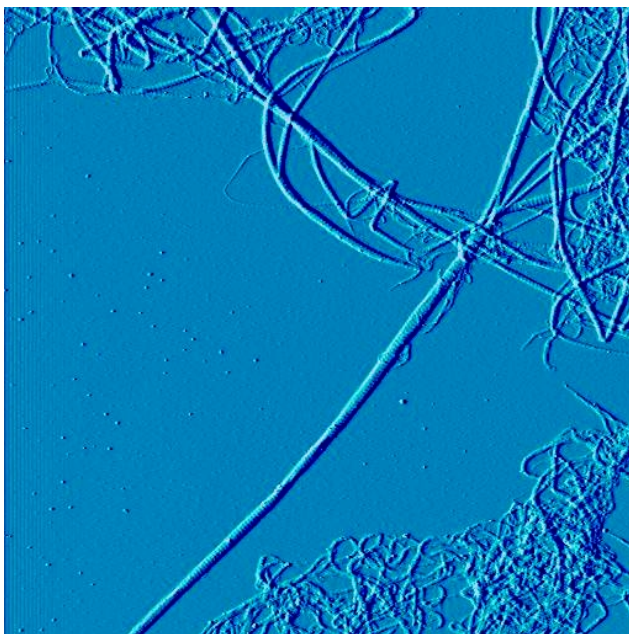


FIG 1. Native collagen fibrils formed by gradual pH increase of collagen monomer solution. Contact mode AFM (deflection image - 15 μm x 15 μm .)

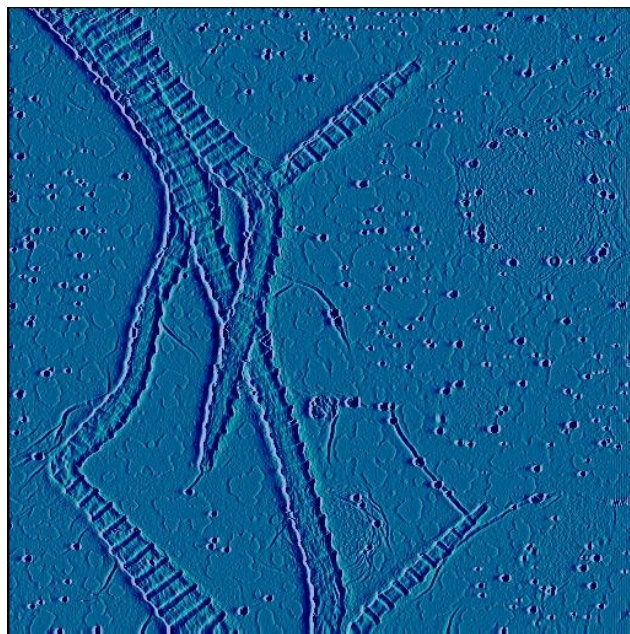


FIG 2. Fibrous long spacing collagen formed by gradual pH increase of collagen monomer mixed with α_1 -acid glycoprotein. Contact mode AFM (deflection image - 9 μm x 9 μm .)

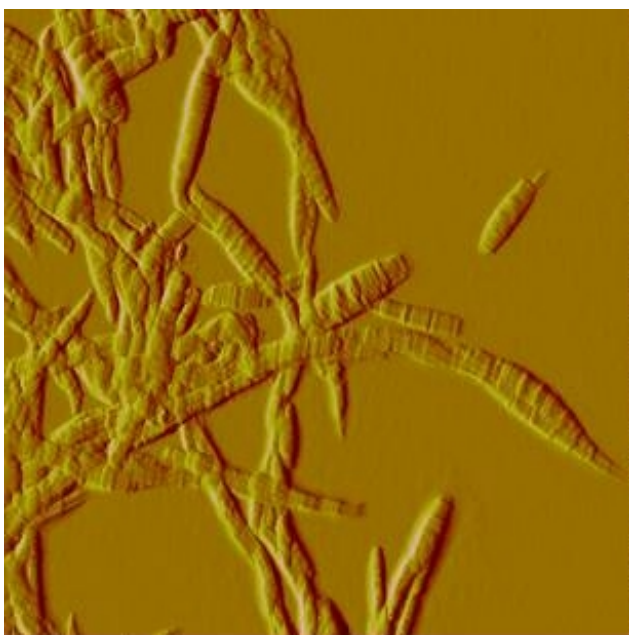


FIG 3. Fibrous long spacing collagen formed by ATP addition to collagen monomer after pH increase. Contact mode AFM (deflection image - 7.5 μm x 7.5 μm .)

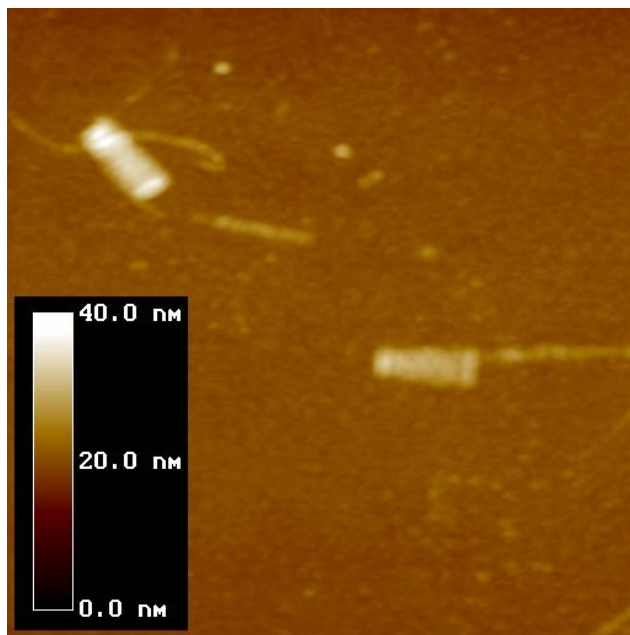


FIG 4. Segmental long spacing collagen formed by incubation of acidified collagen monomer mixed with ATP. Contact mode AFM (height image - 2 μm x 2 μm .)