Nanocomposites based on Self-assembly of Collagen with DNA

Vamsi K. Yadavalli [*] and Ramana M. Pidaparti [**]

Virginia Commonwealth University, Richmond, VA 23284
[*] Department of Chemical and Life Science Engineering, vyadavalli@vcu.edu
[**] Department of Mechanical Engineering, rmpidaparti@vcu.edu

ABSTRACT

Collagen is a major extracellular component that is a key contributor to the mechanical strength of tissue, tendons, skin, cartilage and bones. It is characterized by the assembly and arrangement of suprafibrillar architectures by the assembly of lower order components. Collagen complexes with other (bio) polymers have been used to enhance the properties of collagen as a biomaterial – structural strength, biocompatibility and non-toxicity. Collagen-DNA complexes in particular, represent novel biomaterials that can also be utilized as carriers in drug and gene delivery systems. Controlling collagen/DNA to assemble hierarchical organization ex vivo remains a significant challenge and may lead to development of superior fibers for multiple applications. Here we present some initial atomic force microscopy (AFM) studies on experiments to investigate the self-assembly of collagen with DNA to observe the morphology of the structures that are formed at a single fiber level. These fibers were characterized using nanoindentation techniques to determine their mechanical stiffness.

Keywords: collagen, DNA, self-assembly, nanoindentation

1 INTRODUCTION

Molecular self-assembly is defined as the spontaneous organization of molecules into structurally well-defined and stable arrangements of a number of non-covalent atomic bonds [1]. Molecular self-assembly provides an innovative approach for the design and production of novel materials, which may complement existing alloys and other composites. Many natural and biological systems are formed by the process of molecular self-assembly, and this can provide the basis for new computational and manufacturing techniques at multiple length levels (nano-micro-macro). Understanding the mechanisms and rules in nature from engineering perspectives facilitates the development, synthesis and integration of advanced biomaterials.

Hierarchical collagen fibers and DNA biopolymers are excellent natural examples of self-assembled molecular structures. Collagen is a fibrous protein that undergoes a complex self-organizing process at the nano-, micro-, and macro-scales to form hierarchical fibers [2]. Fiber nano/microstructures are extremely sensitive to biophysical/biochemical environments. Their mechanical properties and anisotropy (macroscale) are affected by the orientation and compositions of fibers, which in turn are directed by the external environment. At the micro- and nano-scales the organization of collagen is greatly affected by cell morphology, migration, proliferation, and gene expression. Biomaterials made of collagen are widely used on account of their high biocompatibility, low-toxicity and high structural strength [3]. Efforts to achieve better control over the mechanical strength of collagen and control its surface morphology have led to attempts at creating synthetic hybrids of collagen with other polymers and biomolecules [4]. In particular, composites of collagen and DNA have been proposed as novel materials for the field of tissue engineering and in regenerative medicine as carriers and reservoirs of genes and growth factors [5, 6]. The analysis of mechanisms for delivering properties of type I collagen triple helices in gene delivery systems is particularly intriguing [7-9]. Collagen is variably charged, while DNA is a negatively charged molecule and is known for its repairing capability with enzymes in response to damage. They can form aggregates through electrostatic interactions both in the presence and absence of uncharged synthetic polymers. The ability of proteins and peptides to self-assemble into aggregates, in normal and pathological processes is of physiological interest. Earlier work has shown that collagen can preserve DNA relatively well [12]. Collagen-DNA complexes may thus have a big role on the development of new bio-nano technologies for wound healing applications as well as new carrier biomaterials for applications in drug and gene delivery systems [13].

Accordingly, it has also become very important to study the principles of organization of the molecular complex between collagen triple helices and DNA double helices. Detailed analysis of the mechanisms of interaction between collagen triple helix and double helix of nucleic acids will lead to possibilities in engineering novel nanocomposite materials.

While the formation of collagen/DNA films has been demonstrated [5, 14], the mechanism of self-assembly and hierarchical reorganization under varying conformations remains the subject of active investigation. Zhao et al. [5] proposed to employ their DNA/collagen complex as a drug carrier , and Murata et al. [15] formed a membrane complex for a reservoir of bone morphogenic protein. Mrevlishvili et al proposed a molecular model for interactions between DNA and collagen [6]. These studies pointed out the

necessity for quantitative investigations on the effects of DNA length and ion concentrations.

Previous theoretical studies on DNA/Collagen complexes in the literature focused on molecular models in which DNA molecules are predicted to bind collagen through a phosphate backbone [6, 14]. The models also predicted the critical role of water layers surrounding collagen/DNA complexes. It is hypothesized that addition of DNA will contribute to an overall increase in the H-bonding interactions and the mechanical strength of the complex.

Directing the assembly of DNA and collagen into novel architectures based on their assembly is of great engineering interest. However, little is known about conformational possibilities and conditions in which to achieve specific collagen/DNA complexes and characterize their mechanical and fracture properties

The atomic force microscope (AFM) provides the ideal tool for studying the assembly processes of collagen and DNA molecules into nanostructures because of the ability to study the system in real time in an aqueous environment thus preventing denaturation and loss of conformation. The high signal-to-noise and spatial resolution of AFM allow imaging down to a few nm [16, 17]. Here we present initial experiments that investigate the self-assembly of collagen with DNA. We investigate the morphology of the microfibrils formed via AFM imaging during the initial stages of assembly. The mechanical properties of the fibrils were measured using AFM-based nanoindentation techniques. These experiments will be used to direct further studies in the development and engineering of novel composites based on collagen and DNA.

2 MATERIALS AND METHODS

Type I collagen from calf skin as a sterile filtered solution (1 mg/ml in 0.1 M acetic acid), and DNA from salmon sperm were obtained from Sigma Aldrich (St. Louis, MO) and used as received. AFM experiments were conducted on an MFP-3D instrument (Asylum Research, CA) operating in non-contact (tapping) mode. AC240TS cantilevers (Olympus, Japan) with a nominal force constant of 2 N/m were used for imaging in pH adjusted solution. Deionized water (18 MΩ-cm) was obtained from a MilliQ system (Millipore, MA). Image processing was conducted using Igor Pro (Wavemetrics, OR). All experiments were conducted at room temperature on freshly cleaved mica (Ted Pella, CA) in a fluid cell. Experiments were conducted in phosphate buffered saline (1x solution pH 7.4, 137 mM NaCl, 11.9 mM phosphates, and 2.7 mM KCl). Collagen/DNA complexes were prepared by mixing a 10:1 ratio of 1 mg/ml solutions of Type I collagen and DNA and diluting to ~ 1 µg/ml before application to a freshly cleaved mica surface. Images of fibers were obtained both in solution and in a dehydrated state after drying in a gentle stream of N_2. Nanomechanical indentation was performed under dry conditions only.

3 RESULTS AND DISCUSSION

3.1 Formation of collagen/DNA complexes and nanomechanical measurements

Figure 1 shows an image of collagen fibrils prior to lateral assembly into collagen fibers. Image was taken on a mica surface at pH 7.4. It is seen that long fibers several microns long and 3-4 nm in height are formed within a few minutes of assembly at this pH.

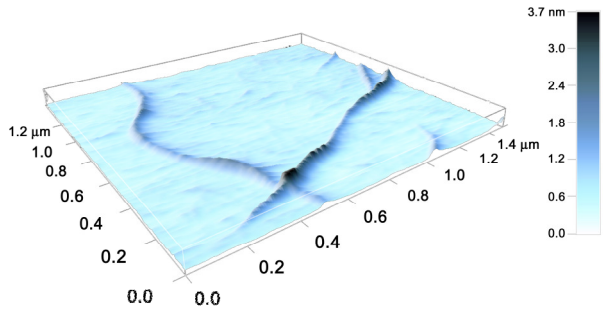

Figure 1: Fibrils of collagen on a mica surface at pH 7.4

Collagen and DNA complex together at physiological pH conditions to form long, twisted fibers within a few minutes. The image of a single fiber complex formed is shown in Figure 2. Fibers around 1-2 µm long and ~10 nm in height were observed during AFM imaging in solution (PBS). Aggregates were also observed at higher concentrations. However the distribution of aggregates decreased at low overall concentrations of both collagen and DNA (~ 1 µg/ml) leading to large numbers of single fibrils as shown in the figure.

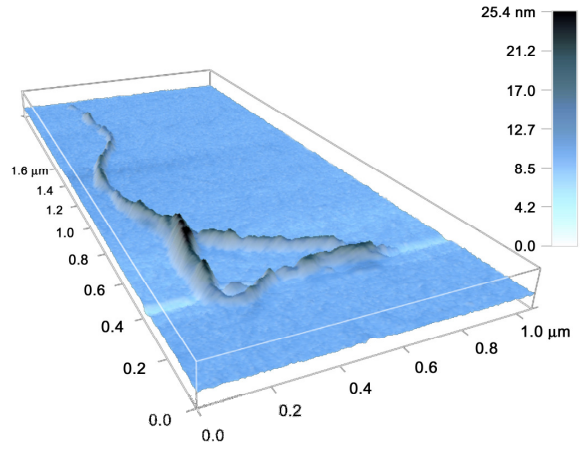

Figure 2: Fiber of DNA collagen complex imaged in buffer at pH 7.4 on a freshly cleaved mica surface.

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

To measure the mechanical properties of the complexes, an AFM cantilever was used as a nanoindenter. The stiffness of dehydrated fibers was measured as shown in Figure 2. A conical tip with a half-angle of 36° was assumed and a maximum indent of 5 nm was applied via a silicon nitride AFM tip (k = 1.97 N/m) to the DNA-collagen complexes in a dehydrated state. The tip was moved to several points including the hard mica surface for comparison. The indentation curves were then fit using the Hertz model as shown in Figure 2 [18]. Only the retraction curve is shown for clarity. The Hertz equation (shown in inset in Figure 2), while valid for non-adhesive elastic spherical particles under normal loads has also been successfully applied in the analysis of elastic modulus of collagen fibers [19] and was used to fit the force vs. displacement data. The measured value of stiffness is E=4.9 ± 1.1 GPa (n=20 fibers). In comparison, the reported value of stiffness for a collagen fiber is 1-2 GPa [20]. A recent computational study using steered molecular dynamics estimated the Young's modulus of a tropocollagen molecule at 4.8 ± 1.0 GPa [21]. DNA in comparison, modeled as a uniform elastic rod, has an elastic modulus of 0.3 GPa for the native form and an increased elastic modulus of 2 GPa for the stretched state [22]. It is to be noted that the stiffness data are also a function of physical environment and hydration state of the fibrils. Even accounting for differences in hydration as well as stage of assembly, it is observed that addition of DNA tends to increase the mechanical stiffness of the collagen fibers. This promises the development of novel structures with enhanced mechanical strength for use in applications such as wound healing and smart membranes. We are currently working on studying the variation in the stiffness and elasticity of the fibers during the assembly process as well in a hydrated state in the presence of different environments.

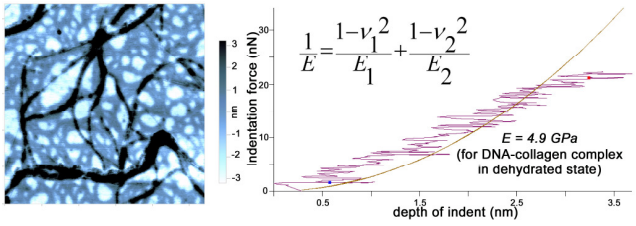

Figure 3: (Left) – Network of fibers of DNA/collagen in the dehydrate state on a mica surface. (Right) Hertz model fit to nanoindentation of complex fiber using an AFM cantilever.

3.2 Proposed mechanism of formation of collagen/DNA complexes

The electrostatic interaction between DNA and collagen molecules exhibits a strong dependence on the patterns of molecular surface groups, adsorbed counter ions, and collagen triple-helix amino–imino acid dipole moments. As a result, it is affected by such structural parameters as the helical pitch (both DNA double helix and collagen triple helix), groove width, etc. Variation of charge along the collagen molecule (sometimes negative, sometimes positive, sometimes apolar) largely depends on the content of the collagen. So the DNA–collagen interaction in that case is described as an overlap of Poisson electrostatic potentials of collagen (electrostatic potential induced by amino–imino acid dipole moments) and the negatively charged DNA surface [11]. The similarity of collagen and DNA hydrations induce additive electric field formation around the molecules as a part of natively formed electric field by the Poisson potentials. As a result, torque raised from summary electric field of hydrations tries to rearrange water molecules situated in the hydration shell of collagen triple helix and DNA double helix. These formatted water clusters act as bridges between the macromolecules. Electrostatic interactions between collagen and DNA then stimulates the complex formation by making water bridges, but the final complex formation and stability is determined not only by water bridges but by the possible existence of H-bonds between donor groups of collagen and phosphate groups of DNA [6, 23]. The net effect of these interactions is to result in materials of enhanced mechanical stability and strength as observed in our initial studies.

4 CONCLUSIONS

Collagen/DNA complexes were prepared by mixing a 10:1 ratio of 1 mg/ml solutions of Type I collagen and DNA. Long fibers around 1-2 μm long and 4-6 nm in height were observed both in the hydrated and dehydrated states. Nanomechanical measurements of the fibers using indentation to estimate the stiffness showed a significant enhancement of mechanical strength as compared to single collagen fibers or DNA alone. In addition to varying morphology, it is expected that altering ionic strength, pH and other environmental conditions can result in engineering the fibers formed for different applications. Currently more results are being obtained and will be presented in the future.

REFERENCES

1. Whitesides, G.M. and M. Boncheva, Beyond molecules: Self-assembly of mesoscopic and macroscopic components. Proceedings of the National Academy of Sciences of the United States of America, 99(8), 4769-4774, 2002.
2. Lodish, H., et al., Molecular Cell Biology. 4th ed. 2000, New York: W. H. FREEMAN.
3. Ramshaw, J.A.M., J.A. Werkmeister, and V. Glattauer, Collagen-based biomaterials, in Biotechnology and Genetic Engineering Reviews, Vol 13. p. 335-382, 1996
4. Lee, C.H., A. Singla, and Y. Lee, Biomedical applications of collagen. International Journal of Pharmaceutics, 221(1-2), 1-22, 2001.

5. Zhao, B.D., et al., DNA-collagen complex as a carrier for Ag+ delivery. International Journal of Biological Macromolecules, 37(3), 143-147, 2005.
6. Svintradze, D.V., et al., Collagen-DNA complex. Biomacromolecules, 9(1), 21-28, 2008.
7. Hanai, K., et al., Potential of atelocollagen-mediated systemic antisense therapeutics for inflammatory disease. Human Gene Therapy, 15(3), 263-272, 2004.
8. Honma, K., et al., Atelocollagen-based gene transfer in cells allows high-throughput screening of gene functions. Biochem Biophys Res Commun, 289(5), 1075-81, 2001.
9. Ochiya, T., et al., New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet. Nature Medicine, 5(6), 707-710, 1999.
10. Prockop, D.J. and K.I. Kivirikko, Collagens - Molecular-biology, diseases, and potentials for therapy. Annual Review of Biochemistry, 64, 403-434, 1995.
11. Klug, A., The discovery of the DNA double helix. J Mol Biol, 335(1), 3-26, 2004.
12. Salamon, M., et al., Relatively well preserved DNA is present in the crystal aggregates of fossil bones. Proceedings of the National Academy of Sciences of the United States of America, 102(39), 13783-13788, 2005.
13. Sano, A., et al., Atelocollagen for protein and gene delivery. Advanced Drug Delivery Reviews, 55(12), 1651-1677, 2003.
14. Mrevlishvili, G.M. and D.V. Svintradze, DNA as a matrix of collagen fibrils. International Journal of Biological Macromolecules, 36(5), 324-326, 2005.
15. Murata, M., et al., Bone induction in subcutaneous tissue in rats by a newly developed DNA-coated atelocollagen and bone morphogenetic protein. British Journal of Oral & Maxillofacial Surgery, 40(2), 131-135, 2002.
16. Engel, A. and D.J. Muller, Observing single biomolecules at work with the atomic force microscope. Nature Structural Biology, 7(9), 715-718, 2000.
17. Muller, D.J., et al., Observing structure, function and assembly of single proteins by AFM. Progress in Biophysics & Molecular Biology, 79(1-3), 1-43, 2002.
18. Withers, J.R. and D.E. Aston, Nanomechanical measurements with AFM in the elastic limit. Advances in Colloid and Interface Science, 120(1-3), 57-67, 2006.
19. Wenger, M.P.E., et al., Mechanical properties of collagen fibrils. Biophysical Journal, 93(4), 1255-1263, 2007.
20. Heim, A.J., W.G. Matthews, and T.J. Koob, Determination of the elastic modulus of native collagen fibrils via radial indentation. Applied Physics Letters, 89(18), 3, 2006.
21. Lorenzo, A.C. and E.R. Caffarena, Elastic properties, Young's modulus determination and structural stability of the tropocollagen molecule: a computational study by steered molecular dynamics. Journal of Biomechanics, 38(7), 1527-1533, 2005.
22. Vinckier, A. and G. Semenza, Measuring elasticity of biological materials by atomic force microscopy. Febs Letters, 430(1-2), 12-16, 1998.
23. Brodsky, B. and J.A.M. Ramshaw, The collagen triple-helix structure. Matrix Biology, 15(8-9), 545-554, 1997.