OSTEOGENESIS IMPERFECTA: BRTITTLE BONES FROM STRONGER COLLAGEN FIBRILS

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Introduction

Osteogenesis imperfecta is a group of genetic disorders that ultimately leads to weak bones and therefore is known as the brittle bone disease. Although familiar as such, it mainly originates by mutations at the molecular level of collagens. Hence, the entire range of a body's collagenous tissues is affected. Here, we mechanically assess single collagen fibrils (CF) of an osteogenesis imperfecta mouse model (*oim/oim*) by performing tensile tests with a novel nano-tensile testing instrument and nanoindentation with an atomic force microscope (AFM). CFs of the studied mouse model are assembled by homotrimeric collagen molecules, caused by substitution of the α2(I) chain by a third α1(I) chain.

Methods

CF were extracted from a tail tendon of each a wild-type B6C3Fe-a/aCol1a2$^{+/+}$ (WT) and a homozygous B6C3Fe-a/aCol1a2$^{oim/oim}$ mouse (severe OI model), both 14 weeks old. After drying, CF were prepared for tensile testing by attaching a magnetic bead with epoxy resin. Each prepared CF was then imaged in contact mode at ambient conditions with the AFM to measure dry diameter and assess the existence of d-banding. Subsequently, CFs were hydrated with phosphate buffered saline solution (PBS) and AFM nanoindentation was performed. By that, indentation modulus, hydrated diameter and swelling are measured. Finally, tensile tests are conducted by lifting one end of the CF by applying a magnetic field. This end is picked up with a two-photon-polymerization printed microgripper that is attached onto a fiber-optic cantilever based force probe (Optics11, Netherlands). CFs are then pulled to fracture using a levered z-piezo stage (Physik Instrumente, Germany) at 5 %/s strain rate.

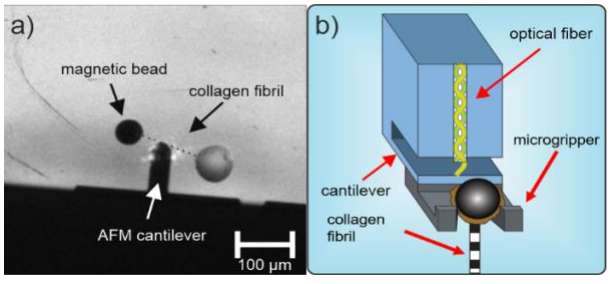

Figure 1: a) AFM measurements on single CF that are prepared for tensile testing. b) Sketch of the force probe equipped with microgripper.

Results

Qualitatively, *oim/oim* CFs exhibit a second phase of stiffening during tensile tests (see figure 2). This leads to about 8-fold higher tensile strength in *oim/oim* CFs. Additionally, the peak tensile modulus within the initial 10% strain regime is higher in the *oim/oim* compared to WT CFs (2.6±0.7 GPa vs. 0.9±0.5 GPa). Concurrently, *oim/oim* CFs swell less when being hydrated in PBS (1.9±0.1 vs 2.1±0.2).

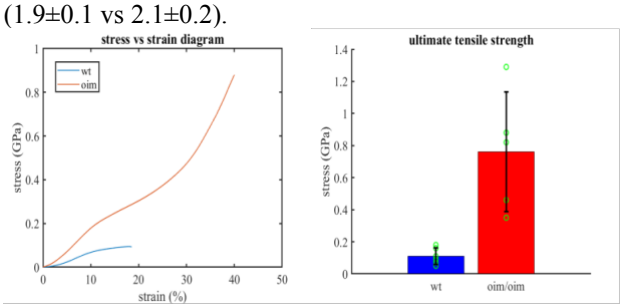

Figure 2: Exemplary stress-strain diagram of a WT and an oim/oim CF and resulting tensile strength at failure of all tested CFs.

Discussion

The overall observation of stronger CFs in *oim/oim* comes as a surprise as fascicles of such mice seem to have lower ultimate strength [1]. Furthermore, molecular dynamics simulations (MD) suggest lower stiffness and less dense packing of *oim/oim* CFs [2]. However, MD does not take into account increased levels of advanced glycation end product cross-links in *oim/oim* [3], which can explain the results reported here. Previously reported *in vitro* cross-linking of CFs resulted in similar mechanical properties [4]. Furthermore, lower swelling potentially impacts stiffness in the initial strain regime by higher molecular interaction [5].

Just recently, force controlled tensile loading and therefore direct viscoelasticity testing was implemented in the novel tensile testing instrument. This is achieved by an error-corrected two-degree-of-freedom controller. As of writing, experimental results of this method are being analyzed.

References

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