Top-down Proteomics and Metabolomics based Profiling and Characterization of Collagen by LC-QTOF-MS

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BRUKER

ASMS 2019, TP663

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Introduction

Collagen is a rod-shaped protein (~285 kDa) made of three left-handed linear polypeptide chains; each chain consists of ~1000 amino acid residues (~100 kDa); five packed collagens form a crystalline microfibril; thousands of microfibrils are assembled into a fibril; and thousands of assembled fibrils form a fiber of tissue (Figure 1). Collagen, which is the most abundant mammalian structural protein and the main protein of connective tissues, serves as a major source of extracellular support for multicellular species. Collagen-based biomaterials in tissue engineering applications have been extensively expanding as they are readily available and bio-degradable. Shotgun proteomics is the most commonly used approach to characterize collagen. Multiple teams have attempted to analyze collagen based on top-down proteomics but have achieving only limited success.

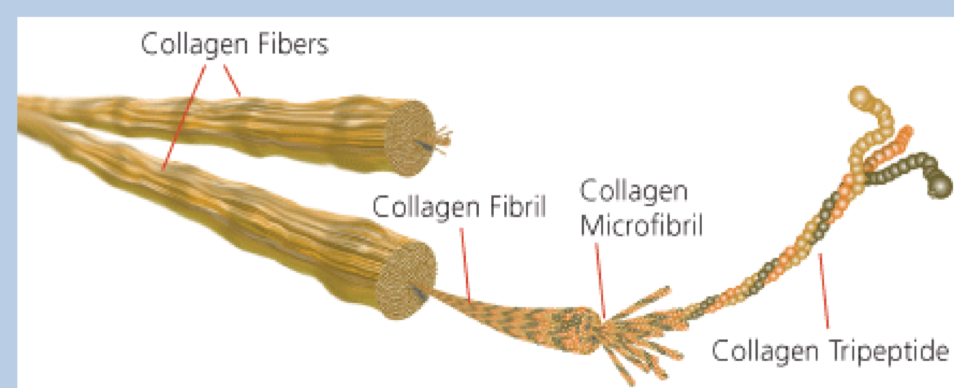

Figure 1. Collagen structure

Methods

Two rat tail collagen Type I solutions were purchased (100mg/via) from commercial vendors. Tissue engineering sample solutions were collected from culture media, centrifuged and filtered. Samples (~50 μL) without further dilution were transferred into insert HPLC vials, and then analyzed using UHPLC (Elute, Bruker, 2μL injection) interfaced with an ultra-high resolution QTOF (maXis II, Bruker; Figure 2). LC separation was performed on C4 columns with 2.1μm packing and 1x150mm dimension for collagen, and 3μm, 3x100mm for small molecules in gradient elution and ESI positive mode. Data was analyzed using software DataAnalysis with Maximum Entropy and MetaboScape 4.0 (Bruker).

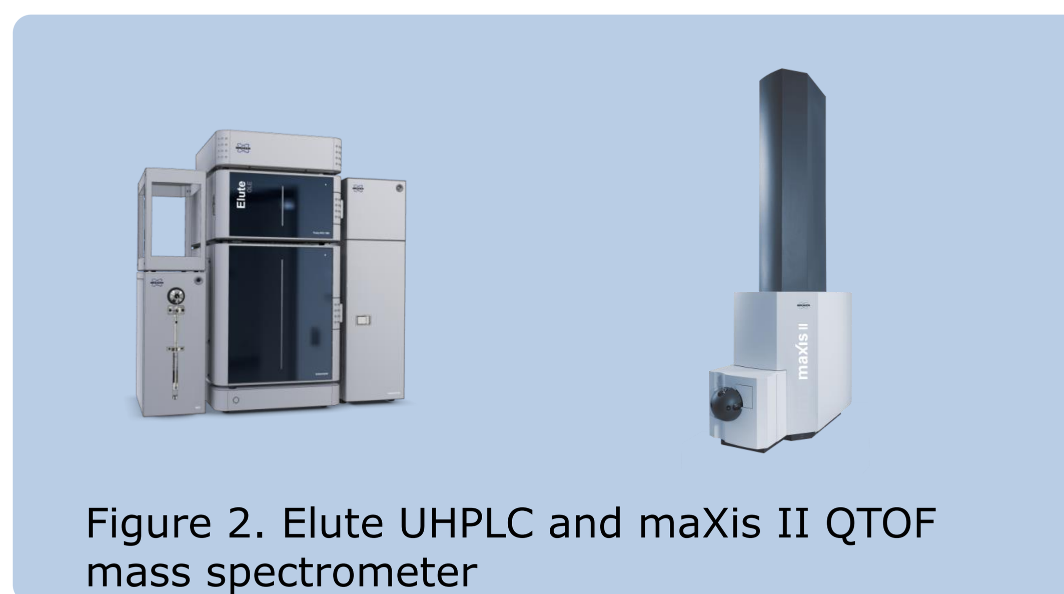

Figure 2. Elute UHPLC and maXis II QTOF mass spectrometer

Intact collagen Analysis

The maXis II QTOF is very well suited for intact protein analysis due to its high resolution with no mass range constraints. It can isotopically resolve intact proteins at ppm level, such as top-down intact antibody (~150 kDa) analysis at 2 ppm mass accuracy. In the current study, the maXis II was used to analyze intact collagens.

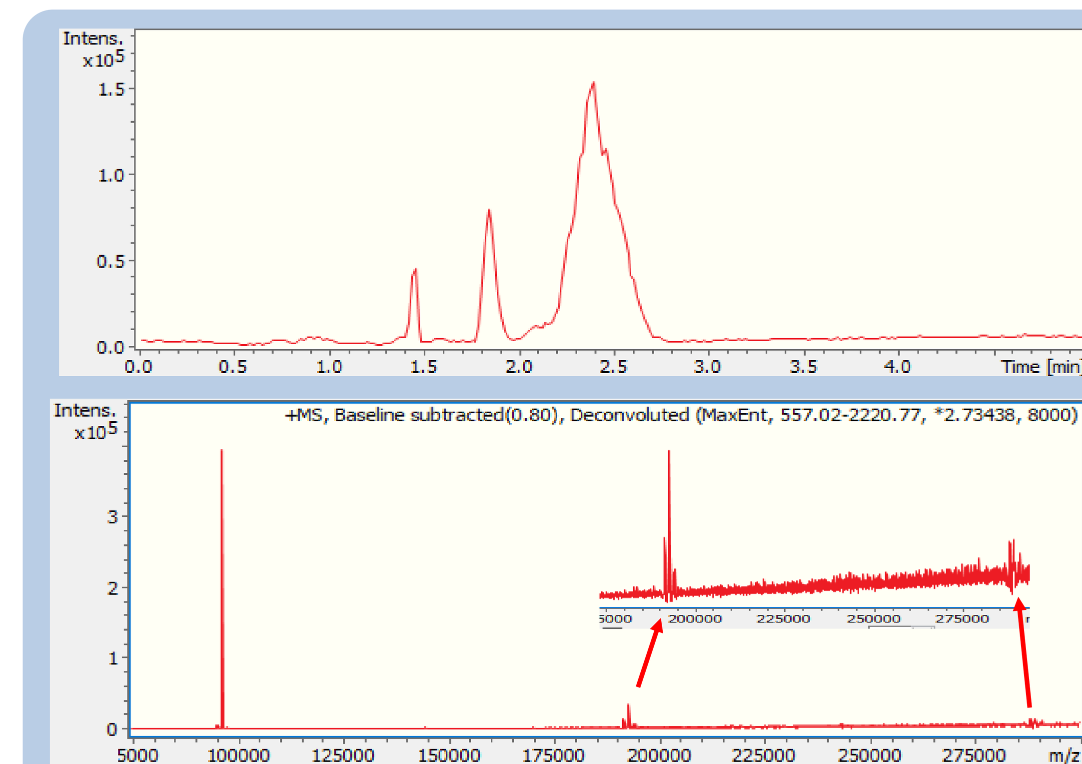

Figure 3. Collagen LC-MS chromatogram and its deconvoluted mass; m/z 300-6000

Three peaks were observed during LC-MS separation of rat tail Type I collagen (Figure 3), which could be used as a fingerprint to evaluate collagen product quality. The deconvoluted three collagen mass at m/z ~100K; ~200K and ~300K might be related to collagen-building units of three polypeptide chains. Native intact collagen experimental results also confirmed the tested collagen standard solutions from commercial vendors are a mixture of multiple proteins.

Collagen evaluation

Collagen protein is cross-linked and arranged with a repeating sequences of (-Gly-X-Y-)n, where Gly is glycine, X and Y can be any amino acid residue. Two commercially available rat tail collagen type I solutions were studied to understand their small molecule composition which can be used as an important parameter by which collagen final product quality is evaluated.

Small molecule composition in collagen from two vendors demonstrate multiple differences (Figure 4). Targeted- and non-targeted metabolomics data analysis were processed in Metaboscape 4.0, and 1,475 de-replicated small molecular feature compounds were identified. The most abundant component in both vendors' collagen solutions was found to be m/z 226.9512. T-test analysis verifies the differences in small molecules between the two rat tail collagen solutions (Figure 5) and amino acids in these two vendors' collagen present at different levels (Figure 6)

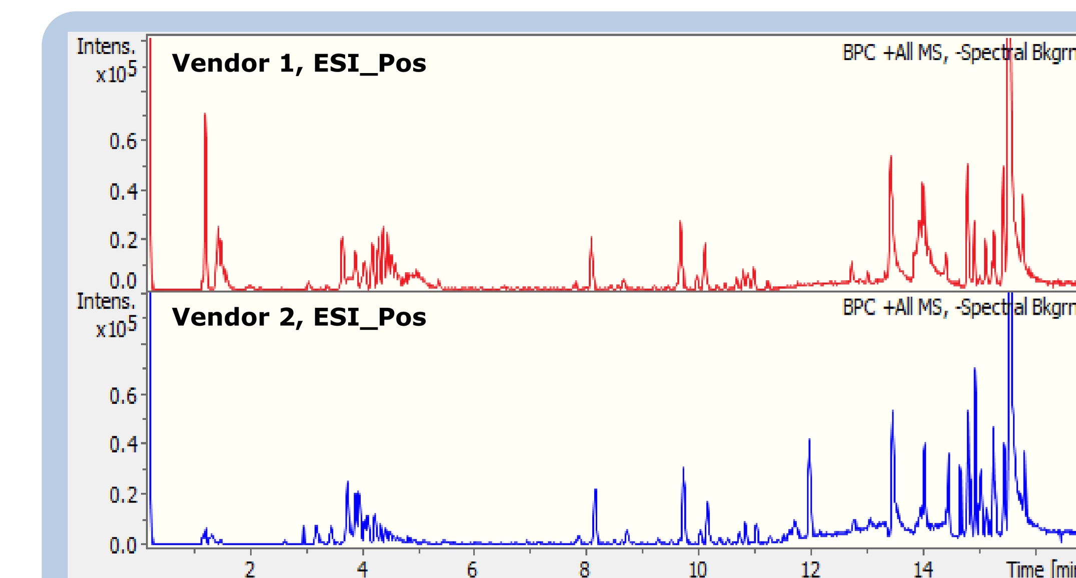

Figure 4. Collagen LC-MS chromatogram on small molecule application, m/z 50-1300

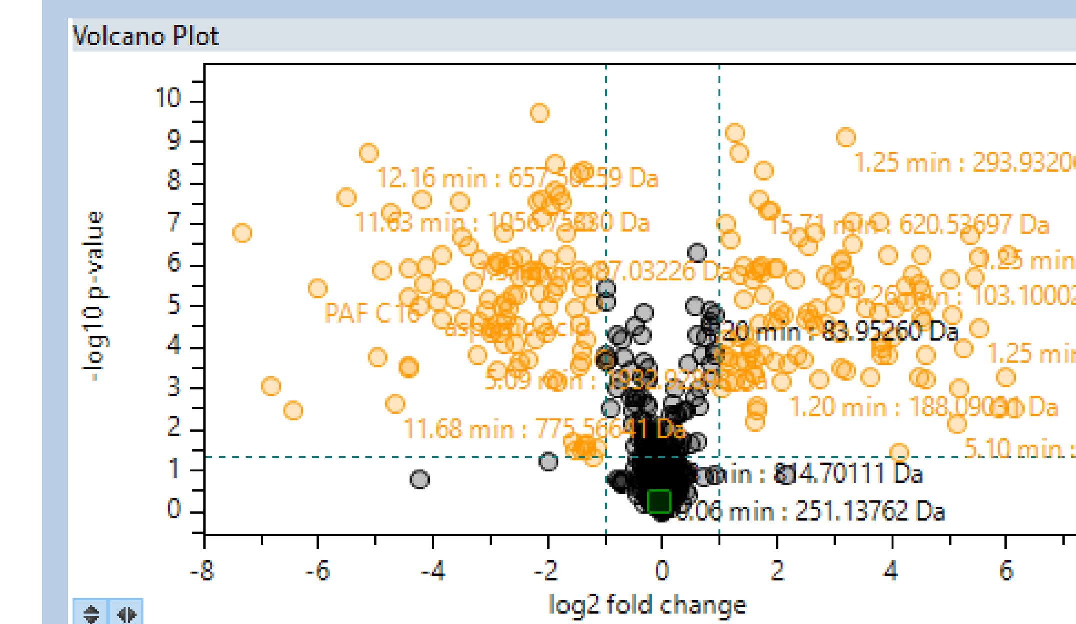

Figure 5. T-test statistical analysis Collagen between two vendors; m/z 50-1300

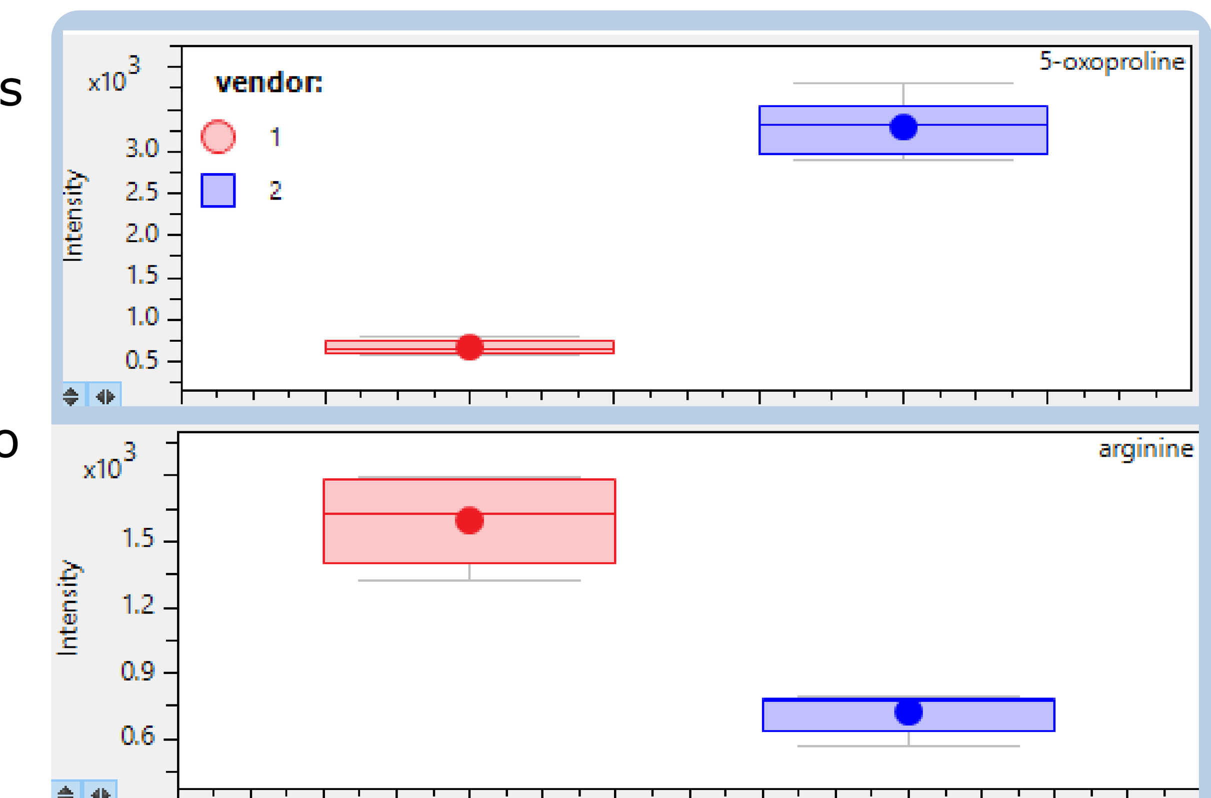

Figure 6. Amino acid levels between vendors

Conclusions

- Established a reliable and high throughput LC-QTOF-MS top-down proteomics methodology to analyze intact collagen and a metabolomics workflow to profile and characterize collagen small molecule composition.
- MetaboScape 4.0 was applied for metabolomics analysis based on integrated HRAM, true isotopic pattern, MS/MS fragmentation, and retention time to boost confidence in compound ID.
- Higher sensitivity is expected with the use of further purified collagen standards.

Acknowledgements

- Special thanks to Barry Steiglitz, Carolyn Sympson, Michael Greig and Jonathan Osborn for their support on this research.
- Funded by Mallinckrodt Pharmaceuticals and Bruker Scientific LLC.

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