Applying a Solid-state NMR Approach to Probe Atomic Changes in Collagen Matrices in Health and Disease

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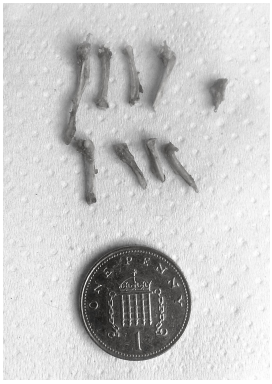
Collagen proteins form the bulk of many structural tissues, such as skin, tendons, cartilage, the wall of arteries, and bone. In structural tissues, the collagen proteins form large, heterogeneous and insoluble matrices which house and interact with the cells, while contributing to mechanical integrity and function. Recent developments in solid-state NMR (ssNMR) provide a means of elucidating atomic structures of insoluble proteins and macromolecules. Therefore, instead of working on isolated and purified proteins, our study tackled tissue samples that contain a high proportion of collagen, such as bone, or extracellular matrix (ECM) generated in vitro.

By feeding a mouse on an isotopically-enriched diet, we obtained ^{13}C and ^{15}N-enriched bone tissues that facilitated 2D ssNMR experiments. From the increased resolution of these 2D spectra, we can make assignments to many more residue types for collagen proteins in native tissue than was possible prior to this study. These assignments serve as parameters against which we can compare synthetic attempts at generating collagen matrices.



We can compare the ssNMR spectrum of synthetic collagen-like peptides directly to bone. From our spectra, it is clear that the model peptides only mimic a subset of structural variations that exist along the collagen triple helix in bone. Using these peptides, for which atomic structures are already available, we can identify regions which correspond to proline-rich and likely more ordered regions in bone collagen.

Mammalian osteoblast cell cultures which lay down collagenous ECM enable us to probe a biologically-generated source of collagen proteins with more targeted isotopic-enrichment schemes. After fine-tuning the cell culture conditions so that we can generate isotopically-enriched ECM that yield ssNMR spectra that closely resemble bone, we can go on to test different models for disease. In aging and diabetes, collagen proteins are known to undergo irreversible changes, such as increased crosslinking and change in material properties. By isotopically enriching the sugar component of the cell culture samples, we can probe the atomic structures formed in both healthy glycosylation and unhealthy glycation of collagen proteins. From these spectra, we identify signals which correspond to sugar phosphates, which may act as a facilitator in bone tissue mineralisation, or an aggressive glycating species if produced in other tissues.

In conclusion, we now have a method of validating atomic-level structure of collagen matrices, which can be used by tissue engineers looking to improve their collagen-based implants. We can also begin to elucidate the atomic-level mechanisms by which age-related diseases and processes affect the structure and function of collagen proteins in our tissues.