

The Soft–Hard Tissue Junction

Richly illustrated throughout with an abundance of tissue images, this innovative book shows that the soft–hard tissue junction is best understood in a biomechanical context. The authors describe their pioneering experimental methods, these providing an essential structure–function framework for computational modelling, and thereby encouraging the development of more realistic, predictive models of this important tissue junction.

Covering the three main musculoskeletal junctions of cartilage–bone, disc–vertebra and ligament/tendon–bone, the relevant soft tissues are examined with respect to both their own inherent structures and their mode of integration with the hard tissue. The soft–hard tissue junction is also explored with a focus on structural damage resulting from overloading, and its associated pathologies. Adopting a multiscale approach that ranges in structural resolution from the macro- to the fibril level, this is a must-have guide to the field and an ideal resource for researchers seeking new and creative approaches for studying joint and spine tissues.

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The Soft–Hard Tissue Junction

Structure, Mechanics and Function

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Preface and Acknowledgements

Nature excels in the design of structural discontinuities that serve to integrate tissues of widely contrasting mechanical properties. Some obvious examples from the marine world include the ligamentous hinge of bivalves, the byssus anchoring thread of *Mytilus* (the common mussel) and the squid's rigid beak embedded within its muscular buccal envelope – all are characterised by a functionally robust joining of soft and hard tissues. More relevant to the theme of this book, the mammalian musculoskeletal system provides multiple examples illustrating this same integrative principle; we find in the joint systems of the body a variety of structurally specialised junctions uniquely tailored to achieve the safe transmission of mechanical forces of varying magnitude and directions of action.

In 2014 Dr Michelle Carey, Commissioning Editor at Cambridge University Press, invited one of us (NDB) to consider writing a research monograph aimed at presenting a coherent story of the soft–hard tissue interface. Initial enthusiasm for embarking on such a project was somewhat tempered by our feeling that there were already several works published in this general area, so a concern was whether we could realistically offer the Publisher a manuscript that added usefully to the field.

Reflecting on our own individual and collaborative research activities in the area of experimental tissue mechanics over many years we concluded that there did indeed appear to be a case for co-authoring a unified work focusing primarily on the multi-scale structural anatomy and related micromechanics of the three primary soft–hard tissue discontinuities in the musculoskeletal system, namely the cartilage–bone junction, the ligament/tendon–bone junction and the intervertebral disc–vertebral junction. All three of these tissue systems involve the integration of a highly compliant collagen-rich matrix with a rigid, mineralised substrate. And, given the advances in both biomaterials and tissue engineering strategies, an in-depth exploration of the structure, composition and biomechanics of the soft–hard tissue junction, and its design rationale for these three main systems, seemed to us to have considerable merit.

The transition from the compliant to the rigid state in the three junctions covered in the book is achieved over micro- and nano-scale dimensions and constitutes a mechanically demanding structural discontinuity that is potentially vulnerable to focal disruption and failure when loaded beyond its normal physiological limits. Degeneration and age-related changes can also bring about structural changes in these junctions, further increasing their biomechanical vulnerability.

There is no shortage of books covering the related disciplines of biomechanics and biomaterials within which musculoskeletal tissue mechanics sits as a major subset. This latter field of research has evolved rapidly and is driven, in part, by both the scientific advances arising from new imaging technologies and the need to solve highly complex clinical problems in orthopaedic practice. The merging of engineering, biology and medicine in the area has been particularly impressive, and due largely to the application of both mechanical analysis and materials-science principles to the study of the passive load-transmitting tissues involved, namely bone, cartilage, ligament, tendon and the intervertebral disc. The primary intent of our own musculoskeletal research has been to explore the relation between structure and function for these tissues and their larger-scale biomechanical role in the body.

In writing this book we have ‘approached’ the soft–hard junction from a predominantly experimental perspective and for one simple reason – possessing an in-depth understanding of its structure will provide that all-important ‘guiding light’ for any researcher wanting to develop predictive biomechanical models possessing at least some degree of physiological relevance. Getting the structure ‘as right as possible’ should always precede any attempt to formulate near-realistic models of such tissue systems. As authors it is our hope that this work will encourage and inspire modellers of the soft–hard junction to incorporate an adequate level of structural realism across the dimensional spectrum, from the macro- to micro- to nano-scales.

The *structure–function* theme that is pursued throughout the book reflects, in part, the methodological roots of the authors’ collective and individual research histories. From an earlier research background in metallurgical materials, one of us (NDB) had the opportunity to move into biological materials research, and specifically the area of soft connective tissues. In metallic materials the deformed state is captured relatively easily using conventional methods of structural analysis, principally optical and electron microscopy. In their elastic range of behaviour the strains involved are tiny and hence there is little detectable change in the structural state of the metal when loaded within its elastic limit. Very large deformations can be induced in a ductile metal above its elastic limit, and this is a consequence of the atoms in the crystalline grains comprising the metal matrix moving into new but equivalent and structurally stable positions. Hence it is relatively straightforward to capture structural changes arising from such large strain or ductile behaviour simply by comparing the structure in its ‘before’ and ‘after’ strain states. We can describe this as *static* structure capture.

The soft connective tissues relating to the dominant theme in this book – namely cartilage, intervertebral disc, tendon and ligament – are all strong and highly flexible biological materials such that their normal physiological function in load-bearing can involve very large ranges of recoverable (i.e., elastic) deformation or strain. Meaningful insights into their governing structure–function relationships therefore require methods that allow direct observation of structure over the entire range of physiological deformations. Ideally, we need to be able to observe in real time the structural response of these tissues to mechanical stimulation and in a way that approximates their behaviour in the body; this is active or *dynamic* capture, rather

than *static* capture, of their structures – the latter being entirely appropriate for conventional engineering materials such as metals, ceramics and rigid plastics, etc.

There are two essential conditions that must be met in order to achieve the kind of *dynamic* capture of the structure–function relationships governing the behaviour of soft connective tissues. First, these tissues must be maintained in their fully hydrated, unstained, unfixed physiological condition. Second, as these tissues are subjected to mechanical loading it must be possible to observe their response at a level of resolution sufficient to image the changing configuration of those primary structural elements that actually control the mechanical response.

At the time when the first author ‘migrated’ from metallic to biological matrices the experimental tools available for micro- or sub-micro structural analysis mostly permitted only *static* capture of the response to loading of the biological matrix over its functional range of strains. These tools were conventional histology (involving fixation, dehydration and the embedding and staining of thin sections cut from the bulk tissue), and scanning and transmission electron microscopy. It was this earlier, somewhat restrictive, range of structural tools that provided the incentive to explore the advantages of using differential interference contrast (DIC) microscopy as a means of imaging these compliant connective tissues in their fully hydrated, functional state.

Also known as Nomarski microscopy after its invention by the Polish-born French physicist Georges Nomarski in the 1950s, the DIC imaging technique provides high-resolution micro-level imaging of relatively thick sections of tissues and, importantly, while maintained in their natural, fully hydrated state. And when used in combination with appropriately designed micromechanical devices it becomes possible to examine these soft connective tissue systems *dynamically* at relatively high resolution over their large range of functional strains, thereby giving more direct access to the governing structure–function relationships. It should also be noted that chemical fixation of these tissues under steady-state loading also permits *static* capture of the deformed structure, thereby offering an additional means of exploring the structure–function relationship.

One further point needs to be emphasised concerning the methodological benefits of employing DIC optical microscopy for the imaging of soft connective tissues: it is entirely feasible to dissect a fresh mammalian articulating joint, remove a sample of articular cartilage and the investigator be able to examine it fully hydrated and at high structural resolution within minutes. Contrast this ‘speed of access’ with that provided by conventional histological procedures which, at the very least, take days or even weeks because of the lengthy processing involved. Of course the latter offer advantages in terms of selective staining of individual matrix components such as proteoglycans, one that DIC on its own does not provide. But we cannot emphasise enough the advantages of DIC in minimising the temporal separation between fresh-tissue collection and its high-resolution structural imaging in a functional, hydrated state. That feeling of disconnect between the original bulk tissue sample and its final microscopic imaging several weeks later, following receipt of a folder or box of histological slides, will probably strike a familiar chord with many researchers.

Of course, there is now available to the researcher a range of highly sophisticated optical imaging techniques such as, for example, confocal laser scanning microscopy permitting the researcher to stack section images for high-resolution three-dimensional reconstructions, multiphoton imaging, and also powerful immuno-histological staining procedures that allow identification of a wide range of matrix components as revealed by molecular labelling. However, in much of our own work with soft connective tissues we have been concerned primarily with imaging the principal fibrous components and seeking to understand how they determine the mechanical properties of these tissues. To this end, being able to image the fully hydrated matrix at high resolution has proved invaluable.

However, the integrating theme of this book is the soft–hard tissue junction and not just that of the compliant tissues alone. With a more-focused research background in musculoskeletal biomechanics, the co-author (AT) had earlier drawn attention to the considerable interpretive advantages in being able to analyse the structural responses of these soft connective tissues while still integrated with their rigid mineralised substrates. Indeed, to examine articular cartilage, intervertebral disc, tendon and ligament isolated from the rigid tissues to which they are functionally attached is to miss so much of their respective structure–function stories. And our desire to understand better these stories provided the stimulus for our collaborative research to begin exploring those complex structural relationships that prevail within the soft–hard junction and determine its mechanical properties.

Methodologically, the authors' dominant approach has been to subject the still-intact cartilage–bone, intervertebral disc–vertebra or ligament/tendon–bone tissue systems to defined patterns of loading and then capture their structural responses by chemically fixing the tissues while maintaining the loaded state. The composite tissue samples are then decalcified and cryo-sectioned for structural analysis either in their fully hydrated state using, in most instances, DIC optical microscopy, or further processed for imaging at the sub-microscopic level using either scanning or transmission electron microscopy.

The procedures used by the authors to investigate the soft–hard junction do, of course, provide a *static* rather than *dynamic* analysis of this region's structure. However, despite this methodological limitation we believe the approach has added considerably to the 'horizon' of structural detail that can be seen, thereby strengthening our understanding of those structure–function relationships governing this biomechanically demanding region. Readers will, of course, make their own assessment of this claim.

No apology is offered for the image-rich nature of the book for how else could we have done justice to the structure of the soft–hard junction in its diverse manifestations? But we also hope that many of the structural images we have incorporated will provide the interested reader with an inspiring glimpse of the sheer elegance of form characterising each of the three main tissue systems explored.

Finally, this preface would be incomplete without paying tribute to the following postgraduate research students, postdoctoral fellows and clinical collaborators who have, over many years, contributed to the research in our experimental tissue mechanics group and made the writing of this book possible:

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