Chapter 1: Introduction

* 1. **Background**

Load-bearing biomaterials are typically structured as composites comprised of rigid, elastic crystalline reinforcing material and a more compliant and energy-dissipating biopolymeric phase. The biopolymeric phase is found as a matrix with interfacial regions between adjacent crystalline elements, and/or as a film coating in near–surface layers overlaying much more massive bulk material [1–4]. Mineralized biomaterials, for example, are structured as arrays of microstructural reinforcements of diverse shapes and forms, bonded by nano–scale proteinic interfaces. Specifically, the spicule of sea sponges is structured as a concentric array of micro–scale silica cylinders [5–7]; the nacreous layer of sea shells is structured like a brick and mortar array comprised of microscale mineral tiles [8–14]; and the calcite layer of sea shells is structured as an array of prisms, oriented toward the surface of the shell [15–16]. Sutural interfaces, which are structured as zig–zag regions and function as compliant joints that allow biomaterials a certain degree of deformability, represent another arrangement of the biopolymeric phase in biomaterials. This deformation is followed by a lock–in effect that substantially stiffens the biomaterial. Such suture regions exist between adjacent scutes in turtle shells [17–18], between adjacent keratin scales in bird beaks [19], and between epidermal tiles in plant seedcoats [20]. Biopolymeric–coated biomaterials are yet another form of biological composites in which the biopolymers serve as a buffer from local contact loadings. Examples of these include arthropod exoskeletons, such as those of insects, spiders, lobsters and crabs. These exoskeletons are composed of a hard mineralized lamellar architecture of chitin nanofibrils arranged in helical lamellar patterns resembling twisted plywood and covered by a softer proteinic coating (epicuticle) [21–29]. Armored osteoderms, such as armadillos and alligators, as well as turtle shells, are characterized by a keratin-collagen, bi-layer skin that coats a bulky boney core [18, 30–41]. Similarly, plant leaves are made of hard parenchyma rod-shaped cells (mesophyll core), covered by a thin protective soft layer or epidermis [42–44].

Both interfacial and coating biopolymers facilitate the diverse mechanical functioning of biomaterials. Specific examples for *interfacial functions* include providing fracture resistance by arresting crack development, crack deflection, and stress reductions at the crack tip [2, 10, 45–47]. Interfacial biopolymers also allow deformability through locally compliant suture regions [17, 40, 48] and reduction of bi-material mismatch stress concentrations through functional gradients [49–52]. In addition, interfacial biopolymers absorb energy and provide mechanical signal filtering through visco-elasticity [45, 47, 53–57]. Examples of *coating functions* include spreading localized surface tractions by increasing contact area [58–61] and providing energy absorption and signal filtering through surface viscoelasticity [55,62–65]. Coating biopolymers also provide a load barrier by confining high-stress fields to the scale exterior and screening the indentation effects from the inner regions [56, 66–70]. In addition, they improve fracture toughness due to debonding at the substrate-coating interface [41]. While the mechanical properties of biopolymers play a major role in the mechanical functioning of biomaterials, many of the relationships among them remain unknown. Moreover, due to the small dimensions and irregular shapes of the biopolymers within biomaterial (either as interfacial or coatings),measuring their mechanical properties is a key challenge for biomaterials science.

Direct-contact nano-mechanical methods are the benchmark approach for determining the mechanical properties of biopolymeric interfaces in biological materials. Techniques, including nanoindentation, atomic force microscopy, and modulus mapping, can reveal these biopolymeric mechanical properties at a spatial resolution of up to a few tens of nanometers [71–74]. In brief, these methods apply local indentation loadings at the specific regions of interest within the biomaterials, probe the force-depth data during indentation, and quantify the indentation modulus of the tested region applying customary indentation-mechanics theories [75–77]. To determine the mechanical characterization of small-scale viscoelastic materials, dynamic nanoindentation and nanoscale dynamic mechanical analysis (nano-DMA) are employed [74, 76, 78]. These methods apply local contact loadings to certain locations within the interfacial region, analyze their mechanical response upon harmonic forces, and determine the elastic stiffness and viscous damping characteristics of the underlying reinforcement or matrix materials within the interfacial region. When testing biomaterials regions that are substantially distant from the interface, stresses arise only within the reinforcement element of the biomaterial, such that the force-depth data reflect the isolated mechanical response of the reinforcement, and the indentation modulus corresponds to the modulus of the reinforcement. However, when testing an interfacial region of the biomaterial, comparable stresses arise in both the reinforcement and matrix elements, such that the force-depth data reflect the integrated mechanical response of the matrix-reinforcement complex, and the indentation modulus reflects the moduli of both the matrix and reinforcement elements [79]. While these qualitative characteristics of interfacial indentations have been demonstrated for specific biomaterials [80–82], their explicit connections to the interfacial elastic properties of the biomaterial, the dimensions of the interfacial region, and the indentation parameters, such as depth and tip-shape type, have yet to be characterized. Previous studies examining the indentation characteristics of analogous synthetic composite systems with nanoparticle inclusions within rigid matrix mediums [83–86] employed axisymmetric numerical simulations to analyze the indentation modulus of these composites, draw connections between their indentation modulus and their constituents’ underlying moduli , and formulate their connections via analytical expressions. However, while the architecture of these composite systems are usually two-dimensional, the current interfacial architecture of biomaterials is essentially three-dimensional. To the best of our knowledge, the indentation characteristics of such three-dimensional interfacial architectures have yet to be analyzed for either biological or synthetic composite systems. Thus, the relationship between indentation modulus from direct-contact nanoindentation testing in biomaterials and the elastic properties of the underlying matrix and reinforcement components represents a gap in current knowledge.

Various biomaterials interfaces are geometrically confined by surrounding reinforcement phases. Assessing the mechanical properties of these confined configurations via direct-contact nanomechanical methods is therefore largely impossible [15–16, 79]. These biopolymeric mechanical properties connect to the mechanical response of the interfacial region as a whole through shear-lag mechanisms, which transfer axial loads between adjacent reinforcements through tensile-shear loadings of their intermediate matrix material [53, 87–88]. While recent studies on planar interfacial morphologies (e.g., staggered, triangular, and trapezial) have found analytical relationships between the overall mechanical properties of the interfacial region and those of its underlying reinforcement and matrix materials [89–93], these analytical relationships cannot account for non-planar, irregularly-shaped, or unmarked interfacial morphologies (which are usually present in natural material). These relationships can be characterized only through direct interfacial experiments. Practically, such direct interfacial experiments are largely impossible due to the small dimensions and confined locations of the interfacial regions within the biomaterial complex. Consequently, the interfacial mechanical characteristics must be analytically extracted from far-field experiments on a larger-scale biomaterial segment [94–98]. Notably, even small variations in the interfacial characteristics, i.e., material properties or relative content within the biomaterial, may substantially affect the mechanical response of the biomaterial segment [62–64]. There remains a gap in knowledge regarding back-calculation ofthe interfacial dynamic modulus of biomaterials from their far***-***field dynamic mechanical analysis.

The dynamic mechanical properties of viscoelastic films (coatings) play a critical role in the mechanical function of biological and bio-inspired materials [Gunda 2017, Díez–Pascual 2015, Lazarus 2020]. The dynamic modulus of these viscoelastic films indicates their energy storing and energy dissipating capabilities for continuous, periodic or instantaneous mechanical loadings, which characterize their capabilities for adsorbing impact loadings, filtering mechanical signals, and preventing crack propagation [Erko 2015, Haung 2020, Xu 2020]. Measuring the dynamic modulus of films is typically achieved by nanoscale dynamic mechanical analysis (nano–DMA) via dynamic nanoindentation or force modulation atomic force microscopy techniques [Hey 2013, Hebert 2015, Cohen 2013, Zlotnikov 2017]. While the nano-DMA analysis yields the pristine dynamic modulus of the film for substantially thick films, as the film thickness decreases, these measures are inherently affected by the presence and the mechanical properties of the underlying substrate of the film. This raises an important materials science question of whether we are actually measuring the dynamic modulus of the pristine film, or, rather, the effective dynamic modulus of the film–substrate laminate. Correspondingly, the integrated mechanical characteristics of the film–substrate laminate can, in principle, also be altered by the film thickness and the substrate properties, such that the laminate may demonstrate substantially different functional capabilities than those of the pristine film. This raises another important materials engineering question of whether we should adapt the effective dynamic modulus of the film–substrate laminate to achieve specific functional capabilities. Addressing these questions will help resolve current challenges in various synthetic and biological materials science disciplines and can serve as a foundation for future designs of functional mechanical coatings in advanced materials. The indentation mechanics of film-substrate laminates has been extensively analyzed through various theoretical, numerical, and experimental methods. Thin-film nanoindentation studies have proposed modeling the laminate as a pair of springs in a series, representing the film and the substrate, weighted by a shape function that accounts for the geometrical ratio between the film thickness and the contact area between the indentation tip and the film [Menčík 1997, Hay 2011, Fischer–Cripps 2004]. These studies, analyzing a wide range of film–substrate laminate types, have found that the shape function follows an exponential form. These analyses have revealed semi-empirical relationships, based on the inverse rule of mixtures, that link the elastic moduli of the film, the substrate, and the overall laminate. Contact mechanics studies of elastic films on rigid substrates have focused on small indentations into highly compliant films and have expanded the classical contact mechanics theory via perturbation methods. This has yielded modified analytical formulations for the contact force-depth relationships that account for film thickness [Dimitriadis 2002, Santos 2012]. These relationships were recently adapted and extended to determine the elastic modulus and relaxation modulus of living cells [Garcia 2018a, Garcia 2018b]. Importantly, all the studies referenced above have focused on slowly varying indentation loadings, which cannot directly probe the dynamic modulus of the viscoelastic film or the film-substrate laminate. Nano-DMA studies on various materials have yielded direct measurements of the dynamic modulus for viscoelastic films [Igarashi 2013, Yablon 2014, Chyasnavichyus 2014, Wang 2018]. In these studies, the film thickness was substantially greater than the indentation depth that allowed use of the classical nano-DMA approach [Hey 2013, Hebert 2015, Cohen 2013, Zlotnikov 2017]. In accordance with the outcomes from the thin-film indentation models and contact mechanics formulations described above, the film thicknesses and the mechanical properties of the underlying substrate will become more and more dominant in the case of equivalent nano-DMA measurements on progressively thinner films. Concurrently, the dynamic modulus of the film-substrate laminate will progressively deviate from that of the pristine film. To best of our knowledge, these thickness-dependent effects on the dynamic modulus of viscoelastic films have yet to been analyzed. The relationships between the dynamic modulus and thickness of a viscoelastic film, the elastic properties of its underlying substrates, and the overall dynamic modulus of film-substrate laminate have yet to be established, thus also representing a gap in current knowledge.

As previously discussed, biomaterials employ mechanically adopted surface regions in the form of film-coatings, which provide the biomaterial’s critical functional capabilities. Specifically, these film-coatings may serve as bioshields, which can be either harder and stiffer or softer and more compliant, than the underlying substrate. Hard-coated bioshields have been extensively analyzed in a wide range of biological systems, including fish scales, teeth, and seashells [8, 25]. Fish scales, for example, are composed of a highly mineralized, hard, and brittle exterior, underlaid by a less mineralized softer layer [66, 99–101]. Experimental and numerical studies have analyzed the indentation resistance of fish scales, which correspond to their bioshielding function against predators’ bites. These studies demonstrate that low-force indentations, that do not cause coating failure, produce shallow penetrations which damage only the hard surface layer. Higher indentation forces, beyond the coating failure point, severely fracture the hard surface layer and damage the softer underlying material. Consequently, these hard-coated bioshields provide a load barrier by confining the high-stress fields to the scale exterior and screening the indentation effects from the inner regions. Notably, other hard-coated biological and bio-inspired shielding elements have demonstrated similar effects [56, 67–70, 102]. Soft-coated bio-shields also appear in a wide range of biological systems, among which are the osteoderms, such as turtle shells [17–18, 30–31, 33–34, 36–37, 39–41], armadillo and alligator skins [32, 35, 38], the epicuticle of arthropods [21–29, 103–106] and the epidermises of plants [42–44]. Whereas the surface protection capabilities of the hard-coated, bioshielding architectures are straightforward, the mechanisms whereby soft-coat architectures promote surface protection, if, indeed, they do, are more complex and difficult to understand. Several studies on synthetic materials have implied that a soft skin coating overlaid on a rigid substrate may protect against surface damage [58–60] and even deter near-surface crack propagation [107–108]. Nevertheless, these functions have not been investigated within the scope of biological materials, and the relationship between soft-coated bioshields and indentation resistance has therefore not yet been analyzed.

* 1. **Research objectives and methodology**

The aim of this work is to identify the mechanical properties of biopolymers, either interfacial or coating, and to link them to the mechanical function of the biomaterials in order to provide new conceptual insights into the structural-mechanical adaptations of biological systems.

This thesis contains four chapters, which are organized as follows:

Chapter 2 establishes an analytical framework with which to extract the local elastic properties of interfacial indentations in biomaterials, using Finite Element (FE) simulations, from the standard results of nanomechanical testing methods. Mechanical modelling is used to: isolate the basic parameters of interfacial indentations in biomaterials (Section 2.1); analyze the connections between these parameters and the indentation force-depth relationships, and the stress morphologies within the biomaterial (Section 2.2); and obtain a compact analytical formula that connects the interfacial indentation modulus of the biomaterial to the underlying moduli of its matrix and reinforcement parts (Section 2.3). Two case-studies of interfacial indentations in specific biomaterial models, the prismatic and nacre parts of sea shells, are then analyzed to demonstrate their force-depth relationships and stress morphologies, and to extract their interfacial elastic properties applying the proposed analytical framework (Section 2.4). The results of these analyses have been published in a peer-reviewed article [\*].Chapter 3 employs composite-mechanics modelling, theoretical approximations, and numerical simulations to identify simple analytical relationships between the dynamic modulus (i.e., modulus magnitude and loss coefficient) of a confined interfacial region within a biomaterial to that of its larger-scale, enclosing biomaterial segment (Section 3.1). Based on these relationships, we propose an analytical-experimental methodology that allows for back-calculating (linear scaling) the interfacial dynamic modulus from far-field DMA results on the biomaterials segment (Section 3.2.1). Finally, the usability and adequacy of our methodology, via numerical experiments, is demonstrated on a class of sutural interfaces that are abundant in natural materials (Section 3.2.2). Chapter 4 analyzes the dynamic indentation modulus of viscoelastic films and presents a and theoretical modelling that can help reveal analytical relationships between the dynamic modulus of the pristine film, the film thickness, and the overall dynamic indentation modulus of the laminate (Sections 4.1–4.2). These relationships are then used to propose a methodological approach to back-calculate the film dynamic modulus from dynamic indentation measurements on the laminate (Section 4.3). Chapter 5 focuses on the specific case study of a turtle shell, representative of the large family of soft-coated, bioshielding elements, analyzing it for its resistance to surface damage upon extensive indentations. First, experimental measurements were used to establish a numerical structural mechanical model for the turtle shell (Section 5.1). Then, we investigated the role of each individual skin layer in protecting the turtle shell against extensive indentations. Finally, we studied the effect of the difference in the mechanical properties of the two layers comprising the turtle-shell skin and analyzed the effect of indenter sharpness and the physiological hydration conditions on the resultant damage patterns (Sections 5.2–5.3). These results have been published in a peer–reviewed article [\*].