Multi-scale Films Inspired by Bone

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Multi-scale films inspired by Bone

A Dissertation Presented

by

Benjamin Rhys Davies

Approved as to style and content by:

__________________________________________________________

Name, Department Head or Dean as appropriate
Department Name
DEDICATION

I would like to, in no defined order, dedicate this thesis to:

My family who provided me with a secure childhood, excellent education, and opportunities for international travel and experiences that most children are never given. In particular, I thank my parents, whose tireless efforts to harness my ever questioning mind and deep interest in the world around encouraged me to believe in myself when conventional education challenged me. This unconditional support was incalculable and of everlasting value to me. My sister Sarah has always been caring and supportive despite my appalling record of responding to emails, texts, and phone calls. For all of this I am very thankful.

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ABSTRACT

Multi-Scale Films Inspired by Bone

The mechanical properties of bone, specifically the combination of strength and toughness, when adjusted for the mechanical properties of its constituent materials exceeds the performance of synthetic materials [1]. Synthetic materials suffer from a trade-off between strength and toughness called the “rule of mixtures”. Although the absolute strength and toughness of synthetic materials still exceed that of bone, the possibility of producing synthetic materials that match bone’s proportional improvement in strength and toughness remains tantalizing.

In this thesis we take a step towards developing synthetic materials that replicate the mechanical performance of bone by mimicking the nano and microstructure of bone. We, for the first time, outline a theoretical framework to explain the mechanisms by which the exceptional strength of the nano-scale building blocks of bone are transferred to the macro-scale using the concept of phonon localization. In the second part of this thesis we synthesize using a scalable method, for the first time, films that have a multi-scale structure similar to bone. This was achieved using a transient network to suspend both micro and nanoparticles simultaneously, a novel approach.

Major Findings

1. A theoretical framework for explaining the unexpected toughness of multi-scale materials such as bone (Chapter 1).
2. Validation of a novel photoluminescence technique for measuring the effectiveness of the liquid phase exfoliation process for producing 2D materials (Chapter 2 and 3)

3. Assembling of exfoliated 2D materials into hierarchical composites using a novel transient network based approach (Chapter 4)
PAPERS AND PATENTS

First Author


AU2014903480, ‘NACRE-LIKE COMPOSITES, METHODS OF SYNTHESIS AND METHODS OF USE’. Filed as a provisional patent application, 1 September 2014. Applicant: The University of Sydney. Inventors: Davies Benjamin, Zreiqat H, and Minett A.

Contributing Author

Alice King, Benjamin Davies, Nikan Noorbehesht, Peter Newman, Tamara Church, Andrew Harris, Joselito Razal, and Andrew Minett. “A New Raman Metric for the Characterization of Graphene Oxide and its Derivatives”, 2015, Scientific Reports Online

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No. PCT/AU2013/001148 “Ionic Liquid Carbon Electrodes” 2013, Sydney, Australia
Applicant: Zenogen. Inventors: Chuan Zhao, **Benjamin Davies**.
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LITERATURE REVIEW

1.0 INTRODUCTION

This thesis seeks to rationalize the structure-property relationship of bone and synthesize materials that exhibit a similar structure-property relationship to bone. In order to achieve these aims we focused on improving the theoretical understanding of bone’s structure and on recapitulating bone’s structure synthetically. Consequently, this literature review is split into in three sections:

1. Why study bone?
   a. Bio-inspired composites
   b. Tissue engineering

2. What is bone’s structure-property relationship?
   a. Physics of fracture
   b. Fracture of bone

3. How to synthesize of bone-like composites
   a. Multi-scale synthesis
   b. Liquid phase exfoliation for the synthesis of nano-materials
1.1 Bone: a remarkable combination of strength and toughness

The most remarkable feature of bone and other natural materials from a mechanical perspective is the combination of multiple properties such as strength and toughness [1-5]. Bone’s toughness (the capacity of material to absorb energy before failure), for example, is inferior to many synthetic materials. However, the combination of stiffness and toughness found in bone exceeds, by orders of magnitude, the expected rule of mixture’s performance (Figure 1) [1, 6].

Complicating the process of defining bone’s generic properties is the huge variation of function, and therefore structure, of bones in different parts of the human body [7]. For example, the femur’s primary function is load bearing, the rib cage protects internal organs, and the tailbone serves no clear purpose [7]. The combination of stiffness and toughness, summarized in the Ashby plots (Figure 1), is orders-of-magnitude greater than what one would expect from bone considering the individual mechanical properties of its constituents: hydroxyapatite and collagen [1-6, 8, 9].
Figure 1 Ashby Plot of Bone of selected bio-mimics

Illustrating the unique combination of mechanical properties achieved by natural and biomimetic materials in comparison to the mechanical properties of their constituents [6].

Synthetic materials possess relatively simple structures but have substantial embodied energy (total energy used in its synthesis) while natural materials embody minimal energy yet possess extremely complex structures [10]. Bone is no exception; hydroxyapatite and collagen are made from common elements (primarily Ca, O, C, H) and possess a fiendishly complex microstructure however, their multi-scale structure remains incompletely understood [4, 7, 11, 12]. A key point of difference between synthetic and natural materials is how they are synthesized. Natural materials are synthesized under mild conditions (1 atm, 25°C) by a “bottom up” self-assembly approach. In contrast, synthetic materials are generally made using “top down” methods under harsh and energy-intensive conditions [5]. The different synthesis strategies are best illustrated by the fact that bone forms entirely under ambient conditions, while structural metals, such as steel, require
temperatures in excess of 900 C° [13]. In theory, this means it may be possible to replace currently un-substitutable rare, expensive and energy intensive metals (for example niobium-containing super-alloys) with substitutes made of common elements with intricate and multi-scale structures [13, 14]. Therefore, natural materials are an excellent example of how to “design” and synthesize materials with minimal environmental impact by substituting energy for structural complexity. Neither the synthetic or biological synthesis strategy is superior as each reflects the different constraints under which natural and synthetic materials are made. It is much easier for material scientists to supply energy than to exert control of a material’s structure from the nm to macro-scale [10]. Natural materials are a tantalizing illustration of the mechanical properties achievable through structure control alone. The intense interest in natural materials is primarily due to the, admittedly distant, possibility of combining high-performance and high-energy synthetic materials with the structural complexity of natural materials [2].

Natural materials, such as bone, possess the capacity to self-repair [5], unlike synthetic materials where only a few specially-engineered polymers exhibit self-healing, such as metal-ligand co-ordination polymers [15]. Although outlined in more detail later on in this literature review, bone’s toughness arises due to the stable growth of micro-damage.

A number of properties differentiates bone from synthetic materials, and are detailed in this section. Bone has the combined properties of being tough with
excellent strength and stiffness while synthetic materials suffer from a trade off between strength and toughness[1-5]. It is the fact that natural materials are apparently able to subvert the trade off between strength and toughness that is behind much of the interest from material scientists in bone's mechanical properties [1]. Briefly, toughness arises in bone not because bone does not sustain damage, but rather because the damage is controlled and prolonged to dissipate maximal energy [4, 11, 12, 16]. Therefore, if a material in its normal use accumulates micro-damage, then the ability to heal such damage would be critical for its long-term function.

The inter-relatedness of bone’s structural features highlights the difficulty in ascertaining structure-property relationships in natural materials and attributing a property to a single structure. For example, toughness arises due to energy dissipated during stable fracture however, without the ability to self-heal natural materials would soon fatigue and fracture from everyday use. This synergy between prima facie unrelated features is why it is imperative to consider natural materials at the system level.

The final major feature that separates bone (and natural materials) from synthetic materials is the bone is hydrated; bone is approximately 20% water by weight [5, 17]. In the context of mechanical properties, water is a plasticizer that increases the extensibility of bone (specifically collagen) at the expense of the strength the polymer phase (collagen). In combination with stiff hydroxyapatite crystals this leads to bone’s excellent toughness. Once again natural materials are multi-
functional. It is the author’s opinion that water’s major function is not mechanical but rather biological. Water is critical for cellular function and nutrient diffusion, a function that takes precedence over mechanical properties.

1.2 Bone Tissue Engineering

Understanding bone’s structure-property relationship is critical when the aim is to heal bone and understand its pathology. Although bone fractures heal naturally above a certain critical defect or fracture size, the body is incapable of healing without medical intervention [18-20]. Extensive local bone defects can often arise due to traumatic injury or tumour resection, the repair of which can easily overwhelm the body’s natural healing capacity and lead to non-union [21, 22]. This presents a significant challenge in orthopaedic medicine. Current treatments with bone grafts have major limitations including: second site surgery, donor site morbidity and limited availability. More importantly, the healing of large bone defects remains challenging using standard bone grafting procedures. The demand for bone grafts is constantly growing and now exceeds 2.5 million per year worldwide [23], with $1.3 billion dollars spent on synthetic bone grafts in 2010 in the USA alone [19]. This places a major burden on national health budgets. Given the limitations and complications of auto-grafts and allografts, synthetic bone graft substitutes represent a promising alternative. Over the past 30 years, a wide range of synthetic materials, scaffolds, have been developed to overcome the problems associated with bone grafts. However, successful translation of these scaffolds from research laboratories to clinical applications has been extremely limited due to unaddressed issues [18-20]. These issues include inadequate bone bridging within
implanted defects and an inability to withstand the mechanical stresses present at load-bearing sites.

In this section we outline the precise properties of an ideal bone tissue-engineering scaffold.

**The difficulty in matching bones mechanical properties to synthetic materials**

A bone tissue scaffold must support the mechanical loads borne by healthy bone and as such its mechanical properties must, as closely as possible, match the mechanical properties of bone [18-20]. Specifically this means that the strength, stiffness, fatigue resistance and toughness of bone must be reproduced to good approximation in bone tissue scaffold [18-20]. It is also worth noting that the stiffness of the scaffold is important in signalling cells to begin producing bone [20]. Given that the previous two sections highlighted just how unique bone's mechanical properties are, the mechanical requirements of a bone tissue scaffold are daunting [1-5].

In order for cells in the interior of the scaffold to proliferate and form new bone nutrient transport, oxygen diffusion and waste removal in the interior of the scaffold is critical [20]. In practice this means a highly porous structure is required in the period before the vasculature is formed [18-20]. Unfortunately porosity greatly reduces the strength, toughness and stiffness of a scaffold. As a result there are currently no synthetic bone tissue scaffolds that combine the high porosity and the appropriate mechanical properties that match bone [18-20].
Material biocompatibility

Biocompatibility, defined as a material that does not elicit negative local or systemic responses from tissue, is a key property for any scaffold [18]. The biocompatibility or bioactivity of materials is a function of its surface properties and not its bulk properties. This is because cells only interact with the surface of material and gives some scope in developing mechanically robust and bioactive materials.

A bone tissue scaffold should also ideally be osteoconductive; it must allow the relevant cells (mesenchymal stem cells) to adhere, proliferate and form an extracellular matrix on its surface [20, 24]. An ideal bone tissue scaffold must also be osteoinductive (to provide scaffolding onto which new bone can be formed) [20, 24].

Currently available synthetic bone scaffolds

The existing tissue scaffolds fall into two main classes, inorganic and organic [18-20].

Inorganic scaffolds

Inorganic scaffolds are made from materials with similar chemistries to the inorganic component of bone (hydroxyapatite) [18]. Examples of inorganic scaffolds include calcium phosphate based ceramics (beta tri-calcium phosphate and hydroxyapatite) and bioglass [18, 25, 26]. The major benefits of inorganic scaffolds are their osteoconductivity and high compressive strength. Their major drawback is that they are too brittle for load-bearing applications; over multiple loadings they simply break from uncontrolled crack growth [18-20, 27-29].
**Organic scaffolds**

Organic scaffolds are generally polymers that are derived from natural sources or produced synthetically [18]. Polymers derived from natural sources such as alginate, collagen, and silk have excellent bio-compatibility [18]. However, natural polymers are difficult to process, suffer from batch to batch variability, and can cause adverse reactions with tissue [18]. Synthetic polymers such as polylactic acid, polycaprolactone, and polymethyl methacrylate are easier to produce but have poor biocompatibility. Finally, all purely polymers scaffolds cannot match the mechanical properties of bone and *in-vivo and* lose their already sub-par mechanical properties rapidly [18-20].

Given the fact that bone is an organic/inorganic composite, the combination of organic/inorganic materials is an obvious one. An example of this strategy is the use of chitosan and hydroxyapatite [25]. Unfortunately, composite scaffolds still cannot match the mechanical properties of natural bone [18-20].

The requirement that a scaffold be biocompatible and preferably bioactive severely limits the choice of materials, in practice, to materials with inadequate mechanical properties [26, 27]. The obvious solution would be synthesizing a material with bone's microstructure. However, this is hampered by two large gaps in the literature which include:

Reproducing an entire organism at the molecular level is a daunting and currently insurmountable problem [30]. It is more feasible to abstract the principles behind an organism’s structure and then apply them to synthetic materials. In the case of
bone there are many structural features we do not fully understand (1, 5-9, 11, 12, 18, 19).

**Inability to synthesize multi-scale structures**

As will be expanded upon in section 3 of this literature review, it is currently impossible to synthesize multi-scale structures with the nm fidelity of bone. It is apparent that there is a substantial and unmet clinical need for bone tissue engineering [18-20]. Unfortunately the limited understanding of bone’s structure-property relationship coupled with an inability to synthesize materials with a multi-scale structure similar to bone precludes the synthesis of materials with a bone-like combination of strength and toughness [1-6, 8, 9]. In the following sections we will exhaustively review bone’s structure property relationship (Section 2) and methods for synthesizing multi-scale structures (Section 3).

**1.3 Bio-inspired materials**

Biological systems and materials are unquestionably the most fascinating, unique, remarkable and mysterious area of science; it is, after all, the study of life itself. However even the simplest single cells organisms are extraordinarily complicated and continue to slowly reveal processes that we neither understand nor can duplicate [30]. The field of bio-inspired materials, by necessity, does not seek to reproduce biological systems in full but rather to functionally mimic parts of the system by using the same underlying principals [30]. Successful examples of bio-inspired materials include Velcro, super-hydrophobic lotus leaf-like surfaces, mussel
inspired adhesives and gecko tape adhesives [30]. Still, given the immense progress in synthetic materials over the past century, why turn to biology for inspiration? Surely it is more sensible to start by analysing the simplest and not the most complex system one can find? Although a valid criticism, there are three main counterpoints:

**Unimagined possibilities**

It is one thing to work towards a goal that is supremely difficult, but it is another thing entirely to work towards a goal that may be, as far as we know, impossible. In the context of natural materials the knowledge that both strength and toughness can even be achieved in natural materials is very valuable [1-5]. This is because it demonstrates that strong and tough materials are at least possible. Furthermore this knowledge can be gained and is valuable even with no knowledge of how strength and toughness, for example, is achieved.

**Novel principles**

Natural materials often work on “left field” or novel principles that have not yet been considered or been discarded by scientists. For example synthetic ceramics, such as alumina, manufacturing processes are designed specifically to minimize the presence of flaws. In contrast, bone’s microstructure is full of precisely designed “soft” regions (containing collagen) in which stress and strain is concentrated but is also efficiently dissipated [1-5]. Put simply, bone purposefully includes flaws but controls how the resulting cracks propagate, while alumina’s toughening strategy is
to eliminate flaws entirely. Such dramatically different strategies can then form the basis of new materials whose structure, toughening mechanisms and synthesis may have minimal similarity to bones but reproduce (at least in part) its toughness [31].

Having outlined why bone properties are so unique and remarkable in the next section we will review the structure of bone and attempt to relate bone’s structure to its mechanical properties. We will focus on identifying the design principles underlying bone’s structure that can be translated to synthetic materials.

2.0 THE STRUCTURE OF BONE

2.1 Multi-scale and hierarchal
Bone and natural materials all possesses a multi-scale hierarchal structure that is organized from the nano to macro-scale (Figure 2); no other synthetic structural materials yet possess such a structure [1-6, 8, 9, 32, 33]. Well studied hierarchal materials include nacre [4], dentine [34, 35], spider silk [36], squid beaks [37], stomatopod dactyl clubs [38], and fish scales [39]. This would strongly indicate that hierarchal structures are key to reproducing the mechanical properties of bone. However a large gap exists in the literature regarding the precise physical principle by which multi-scale and hierarchal structures operate to achieve toughness and defect tolerance.
Figure 2 Multi-scale structure of bone.

1st level from top, substituted hydroxyapatite platelets and collagen helix. 2nd level from top, collagen fibrils consisting of mineralized collagen. 3rd Level from Top, Collagen fibrils arranged into lamellae and surrounded by viscoelastic glue. 4th level from top, osteons (consisting of lamellae) surrounded by cement lines (grey). 5th Level from Top, macro-scale bone showing compact bone (that contains osteons) and spongy bone (porous with no osteons). [32]
Bones structure at the nano-scale

Bone consists of an inorganic (50-74%, top left Figure 2), and an organic phase (~30%), with the balance consisting of water [40]. The organic phase consists mainly of collagen type I but also contains proteoglycans and non-collagenous proteins (osteocalcin, osteopontin, bone sialoprotein) that are important for mechanical function [40]. Osteopontin and osteocalcin, in particular, are localized at the nano-scale initiation point of bone fracture and are structurally important [41, 42]. The collagen molecule is 1.5 nm in diameter and approximately 300 nm long; collagens form hydrogen bonds with adjacent collagen molecules [12]. Collagen’s role is to provide elasticity and energy dissipation under mechanical load and also provide a template for mineralization [12]. Hydroxyapatite (HAp) is the inorganic phase of bone. In bone Hap is heavily doped; deficient in calcium, enriched with carbonate, and contains trace elements such as strontium and magnesium [40]. HAp forms thin plates that are 2 to 7 nm thick, 15 to 200 nm in length, and 10-80 nm wide [40]. The nano-scale dimensions of the HAp crystals place these crystals in the flaw-tolerant length scale; and so the presence of a crack does not affect its strength [43]. The role of HAp is to provide stiffness and strength.

There is some disagreement around how collagen attaches to HAp; it is definitely supra-molecular and has been attributed to electrostatic attractions, though the precise mechanism and protein conformation is debated [40, 44]. The strength of any composite is dependent upon to the interface between its two composites. Yet in bone, weak supra-molecular bonds bind the collagen to HAp [40, 44]. The
existence of many weak supra-molecular bonds at interfaces instead of covalent bonds is at first puzzling. Of course, many “weak” supra-molecular bonds can match the strength of a few strong covalent bonds, but that is more elaborate for no apparent gain. The critical advantage of multiple supra-molecular bonds is their ability to spontaneously reform after breaking under ambient conditions; in this way strength and toughness (energy dissipated from bond breaking) can be achieved [5, 33, 45, 46].

**Bones structure at the micron scale**

Collagen and HAp combine to form collagen fibrils that are approximately 100nm in diameter and microns in length [4, 7, 12, 40, 47, 48]. The HAp plates are located between collagen molecules [4, 7, 12, 40, 47]. The collagen fibrils are held together by a matrix of non-collagenous proteins such as phosphorylated serine, osteopontin and bone sialoprotein, with some inter-fibrillar HAp sheets [49-51]. From a mechanical perspective the proteins perform two major roles, adhesion between fibrils and energy dissipation [49-51]. Indeed animal models have demonstrated that otherwise identical bones without osteopontin have significantly reduced toughness [41, 52].

**Bones structure at the 10s of micron scale**

Bundles of collagen fibrils form sheets that are called lamellae; these sheets are arranged at varying angles in a plywood-like arrangement [4, 7, 12, 40, 47, 48]. The plywood structure is a direct response to the fibrillar nature of bone; fibres are strongest in the axial direction. However, the forces experienced by bone can be in
any direction and a plywood design, where lamellae are rotated slightly relative to each other, resist forces from all directions. The angular offsets between lamellae are clearly visibly under an optical microscope with a polarizer producing clear birefringence. The area between lamellae is enriched with non-collagenous proteins, which have a lower elastic modulus than lamellae and contains reduced numbers of collagen fibrils orientated in different directions to adjacent lamellae [53]. In many ways interlamellae areas are a micron scale analogue of the fibril matrix, a compliant energy dissipating matrix [53].

**Bones structure at the 100's of micron scale**

Osteons consist of cylinders of lamellae arranged around a central hole containing blood vessels [4, 7, 12, 40, 47, 48]. Osteons are visible with an optical microscope, being approximately 300 micron across. Osteons are surround by hyper-mineralized/collagen-deficient cement sheaths or cement lines [12, 48, 54]. The function of the cement line appears to be to redirect cracks away from the osteon on a long and tortuous path [48, 54]. Crack redirection occurs because the cement line is more brittle than surrounding bone. Due to the fact cracks grow in the direction of greatest strain energy release rate (or least resistance) they preferentially follow the cement line instead of penetrating the osteon [55]. In a final note, at the 100-micron scale damage cannot spontaneously heal as it can at the nano-scale level. As a consequence, self-healing is achieved through active cellular remodelling by osteoblasts and osteoclast [4, 12, 48]. We note the process of remodelling is not just
a response to injury, but dynamic and continuous [4, 12, 48]. Interstitial bone is normal lamellar bone that is not contained within an osteon; osteons are roughly circular so there is always interstitial bone. Similar to fibraller matrix at the nano-scale most micro-cracks initiate in the interstitial bone [56, 57]. It was also found that once micro-cracks of a sufficient size form they penetrate the osteon and cause catastrophic failure to occur soon after [11, 55].

**Bones structure at the macro-scale**

At the organ scale, bone can either be dense (cortical) or spongy (trabecular) [4, 11, 12, 48]. The most important factor affecting the mechanical properties of bone is porosity; cortical bone is 5-10% porous while trabecular bone is 75% to 90% porous [11]. Long bones, such as the femur, consist of cortical shell and trabecular core containing bone marrow [4, 11, 12, 48]. The local distribution of cortical and trabecular bone is dynamically remodelled in response to local stresses to ensure the needed strength with minimal bone mass [4, 12, 48].

Before outlining the structural features of bone that act as toughening mechanisms, we will review the process of fracture in synthetic materials. This will allow us to highlight the difference between the fracture of natural materials and synthetic materials.

**2.2 Physics of fracture**
Fracture, the irreversible breaking of an object, a fundamental process that is critical in engineering and science, has been studied intensively since antiquity [58-60]. I will first review our understanding of the physics of fracture before discussing the toughening mechanisms in bone. The first modern studies were undertaken by Leonardo da Vinci, who found that longer wires, with identical cross sections, were weaker than shorter wires [58]. Despite the obvious importance of fracture to society a complete understanding remains elusive, primarily because studying fracture requires interactions across multiple length scales [60]. In this section I will outline the basics of fracture mechanics (Figure 3), its limitations and the resulting difficulties in applying fracture mechanics or statistical physics approach to natural materials.

![Figure 3 Definitions of Mechanical Terminology](image)

**Figure 3 Definitions of Mechanical Terminology**

The definition of stiffness (energy required per unit strain), strength (force required to break), and toughness (energy required to break) in structural materials [40]
**The Griffith criterion**

A.A. Griffith, while studying the strength of glass fibre in the 1920’s, found that he could not reconcile the fact that measured strength of glass fibres (100 MPa) was far below the expected strength based on atomic bond energies (10,000 MPa). He realized that strength of the fibres was not governed by atomic bonds but was limited by the presence of flaws. These flaws, which are practically impossible to eliminate, create areas of locally high stress. This means that although the average stress in the materials is far below the failure stress of a material, a small region of high stress that exceeds the failure strength, can still initiate a crack that causes premature failure of the entire material.

To justify this empirical finding, Griffith used a thermodynamic criterion for catastrophic fracture in combination with linear elastic fracture mechanics. Griffith postulated that when the strain energy released by the growth of crack \( G \) is greater than the surface energy \( \gamma \) required to grow the crack catastrophic fracture occurs. This is because above a critical crack size a positive feedback loop occurs; as the cracks grows more strain energy is released which then in turn grows the crack.

\[
G = 2\gamma \quad \text{Equation 1}
\]

This approach explained the data for brittle solids, such as glass, quite well but for ductile solids produced unrealistically high surface energies. Despite its shortcomings the notion of an energy balance between crack opening and strain energy release remains fundamental to our understanding of fracture.
It was not until Irwin realized in the 1950’s that in structural materials, such as steel, plasticity at the crack tip was an important factor. Plasticity is defined as irreversible (non-elastic) deformation of materials; the process of permanently deforming a material dissipates substantial energy and therefore inhibits crack growth. Irwin found that by accounting for plasticity the Griffith Criteria was also applicable to tough structural materials. His solution was to include an empirical measure for plasticity to the energy balance.

\[ G = 2\gamma + G(V)_{\text{Plasticity}} \quad \text{Equation 2} \]

Although extremely useful for engineering applications the theory had its limits. It is empirical and does not provide any insight into the mechanisms of plasticity or toughness. It is based on linear elastic fracture mechanics; as such it is assumed that an area of plastic deformation around the crack tip is small relative to the crack size. If this area ahead of the crack tip—the fracture process zone—is too large then linear elastic fracture mechanics breaks down.

The notion that a single flaw of sufficient size can cause the catastrophic failure of an entire piece of material, far below its theoretical strength, has fundamental implications. It means that whatever the precise distribution of crack/size (or in strict statistical physics terminology “disorder”) in a material it is the extreme values of the distribution that govern material strength [58, 60-62]. This leads to three important consequences:
The effect of material size ("size effects") on mechanical properties

Marked size effects arise in mechanical properties; the probability of large cracks occurring in materials of dimension L is directly proportional to volume of the material ($L^3$). This means that smaller materials are, on average, stronger than larger materials due the lower probability of a critical flaw existing in smaller volumes of material [58, 60-62].

"Mean field" approaches don't work

Structural materials, like chains, break at their weakest point. As such the fracture of a material is dependent on the extreme values of the distribution of mechanical properties in a material (roughly the variation in strength of constituent materials) [58, 60-62]. This means that mean-field approaches that use a single average value, such as elastic moduli, to characterize the properties entire material are not applicable [59].

Flaw elimination is not an option

The obvious response of the reader might be to simply process materials so that the material free of flaws. This is practically impossible; a single atomic flaw can cause premature fracture.
The importance of interacting cracks

The linear elastic fracture mechanics approach works well for relatively
homogenous materials with a single large flaw, but not heterogeneous materials
with many small cracks [58, 63]. Given that the focus of this thesis is natural
materials, we note that natural materials are highly heterogeneous materials whose
fracture is characterized by the steady growth of numerous stable micro-cracks and
extensive plastic yielding [1, 4, 5, 7, 12, 41, 43, 47]. This means linear elastic
fracture mechanics must be extended. Unfortunately the fracture of heterogeneous
materials and our understanding of them are complicated by two factors:

*Long-Range Interactions in the Elastic Strain Field*

The elastic strain field decays with $\frac{1}{r^2}$. This means that cracks that are physically
distant from each other still interact with each other. This is especially complicating
because depending on the precise spatial distribution of cracks, crack size and
loading condition, an adjacent micro-crack could inhibit or encourage crack growth
[58, 64]. This produces extremely complex and dynamic strain fields and
consequently complex crack growth dynamics.

*The precise arrangement and variation in material properties of the material*

Every material is slightly different, however, using a statistical approach Sethna et al
demonstrated that greater the disorder or heterogeneity in the material the greater
the tendency to fracture in a plastic manner [64]. Conversely, the more homogenous
the material is, the greater the tendency for abrupt failure [64].
The growth of cracks in a heterogeneous material is clearly a complex problem that we will discuss in the context of the microstructure of bones in the next section of this thesis.

### 2.3 Toughening mechanisms of bone

At the core of bone toughness is its ability to undergo significant inelastic (plastic) deformation before failure (Figure 4) [4]. Inelastic deformation allows for the redistribution of stress around areas of strain concentration that would otherwise initiate catastrophic fracture [4, 11, 12, 16].

![Stress-strain curve of bone in tension and compression](image)

**Figure 4 Stress-strain curve of bone in tension and compression**

Stress-strain curve of bone [4]
Toughening mechanisms in bone can be divided into two groups, intrinsic and extrinsic toughening mechanisms [1, 12, 48, 65, 66]. Intrinsic mechanisms operate in front of the crack tip, in the form of plasticity, and can be thought of as a material’s inherent resistance to elastic and inelastic deformation [1, 12, 48, 65, 66]. Extrinsic mechanisms operate behind the crack tip and are only active once a crack has formed. Extrinsic mechanisms shield the crack from full mechanical load applied to the material [1, 12, 48, 65, 66]. Intrinsic mechanisms predominate at the sub-micron scale. Extrinsic mechanisms are more important at the micron scale and above [1, 12, 48, 65, 66]. In this section we review how toughening mechanisms act on each length scale before considering how the different mechanisms work together across multiple scales.

**Nano-scale toughening mechanisms**

Fracture is initiated at the nano-scale and is therefore the most critical length scale in the study of the toughness of bones [67]. At the nano-scale intrinsic mechanisms are dominant. We outline the toughening mechanisms present at the nano-scale [1, 12, 48, 65, 66] below. Mineralized collagen fibrils first deform under tensile loading by stretching the collagen within the fibril, before transitioning to shear deformation of inter-fibrillar matrix [68]. This mode of deformation is called the “stick-slip” model of deformation [43]. Consequently, as tensile stretching of the collagen fibril is the first stage of deformation, we will first discuss the mechanisms within the collagen fibril that maximize strength and toughness [68]:
**Intra-fibrillar Mechanisms**

At the nano-scale, bone is a composite of calcium deficient HAp plates and collagen I. Collagen’s role is to provide elasticity and energy dissipation while the HAp plate’s role is to provide strength and stiffness [12, 69]. The collagen molecule has a triple-helix structure and is held together by hydrogen bonds [12, 70]. The collagen initially deforms elastically but stiffens non-linearly as hydrogen bonds are broken [70]. The presence of hydrogen bonds in the collagen molecule dissipates substantial energy due to the bonds dynamic breakage and reformation [70].

**Strain amplification**

Collagen has a very large strain at failure (~40%) while HAp has a strain to failure of (~3%) [71]. This means that since bone’s failure strain is ~2-3%, a simple composite (in which the strains applied to each component are equal) would not fully deform collagen. Consequently, the dissipated energy would be significantly less than collagen’s potential [71, 72]. One strategy to amplify the strain of collagen is to use HAp particles with large aspect ratios (ratio of length to thickness, ρ).

Indeed, this is the case for bone; HAp has dimensions of 2x50x50 nanometers equating to an aspect ratio of ~25 [65].

\[ \varepsilon_{\text{collagen}} \sim \rho \varepsilon_{\text{bone}} \]  

*Equation 3*

Essentially the strain in the collagen is proportional to the applied strain multiplied by the aspect ratio of the HAp crystals. In this way the collagen molecule can be fully deformed to maximize the energy dissipation under strain [71]. Tai et al found
direct evidence of strain amplification, they found nano-scale regions of comparatively high and low strains in bone under deformation [73]. Large aspect ratio HAp crystals, because they are so stiff, also reinforce the collagen to increase the stiffness and strength of collagen fibrils [69]. In short, large aspect ratio HAp crystals increase the toughness of the fibrils by fully straining the collagen molecules and simultaneously reinforcing the collagen by bearing the majority of the stress [69].

**Nano-scale flaw tolerance**

As a consequence of strain amplification in collagen, the stress is also amplified in the HAp crystals in proportion to the aspect ratio in accordance with the “tension-shear chain” model outlined by Gao et al [71].

$$\sigma_{\text{mineral}} \sim \tau_{\text{protein}} \rho \text{ Equation 4}$$

Although HAp is in theory very strong (3.3 GPa), in practice, its strength is limited by the presence of flaws (65 MPa for a macroscopic crystals) [71, 74]. It is thus not clear why a large aspect ratio is a good strategy, as the strength of bone is still limited by HAp’s brittle nature. Surprisingly, below a critical length scale (which is material dependent), all materials become immune to flaws [43]. This result is implied by the Griffith criterion and is described in following equation:

$$h \approx a^2 \frac{\gamma_{\text{mineral}}}{\sigma_{\text{theoretical}}} \text{ [43]}$$
Where $\alpha$ is a geometric constant, $\gamma$ is the surface energy, $E_{\text{mineral}}$ is the stiffness of the HAp, and $\sigma_{\text{theoretical}}$ is the theoretical strength of HAp (estimate as $\frac{E_{\text{mineral}}}{30}$). For a bio-mineral with $\gamma = 1 \text{ J/m}^2$ and $E_{\text{mineral}} = 100\text{GPa}$ this corresponds to $\sim 18\text{nm}$, well within the size range of HAp in bone. The idea is rather straightforward; every object has a critical crack size that if exceeded, results in catastrophic failure. If the object is smaller than this critical crack size then it is must be flaw tolerant and fails homogenously. Although the concept of flaw tolerance does not have a rigorous theoretical basis, the prescience of flaw tolerance has been confirmed experimentally in synthetic materials and is widely accepted [75]. Nano-scale flaw tolerance is thus critical to the mechanical properties of mineralized collagen and bone. This explains why nano-scale is so important to the mechanical properties of bones.

**Role of the hydroxyapatite collagen interface**

As stated above, the full extension of the collagen molecule is critical to maximize toughness. To achieve full collagen extension (Figure 5a) both the strain and adhesion strength of the collagen/HAp interface must be optimized [74]. If the interface is too weak then the magnitude of load to the HAp crystal (Figure 5b) is below what collagen is capable of supporting (Figure 5C). If the interface is too strong, then collagen the molecule will fracture [74]. However Buehler et al found that if the strength of collagen/HAp interface was at a certain critical value, toughness was optimized. This was attributed to dynamic breaking and the
reforming of collagen/HAp bonds under strain [74]. Fratzl et al found, in deer antler, which is structurally similar to human bone, that mineral/collagen sliding was indeed the dominant form of toughening [76].

![Diagram of collagen triple helix structure](image)

**Figure 5 Nano-scale Toughening Mechanisms**

a) The collagen triple helix structure possesses extensive inter-chain hydrogen bonding, red lines, that break upon extension of the collagen. As the collagen is progressively extended the transition from entropic unfolding to bond breaking stiffens the collagen. This is critical because delocalizing strain in surrounding collagen fibres ameliorates regions of high strain that would otherwise cause early failure. 

b) Carbonated HAp platelets are nm in thickness to achieve flaw tolerance and possess an aspect ratio of 25 to increase the strain in collagen (which has a large failure strain) and increase the stress in the HAp platelets (which have low failure strain). 

c) Under strain the collagen/HAp fail at the interface between HAp and collagen (red lines). The failure of the interface, ideally, occurs just before the failure of the HAp platelet with the collagen almost fully extended and sacrificial bonds broken. In this way both toughness of the collagen and stiffness of the HAp are combined to produce a strong and tough composite.

**Inter-fibrillar sliding**

At first the collagen fibrils are deformed under tensile strain but once the collagen fibrils are sufficiently stressed the inter-fibrillar matrix begins to deform under
shear [68]. Once the fibrils reach a certain strain, collagen fibrils begin to slide or slip past each other inelastically. The “slip” is important because instead of accumulating strain within the fibrils, resulting in the fracture of the fibirls, extra energy is dissipated by relative motion between fibrils. This is called fibrillar sliding. The role of this matrix, which contains elevated levels of non-collagenous proteins such as osteocalcin and osteopontin, appears to be energy dissipation [4, 12, 41, 54]. The matrix essentially acts as a viscoelastic “glue” that holds mineralized fibres together. The proteins, along with inter-fibrillar HAp, utilize predominately ionic sacrificial bonds, which break and reform under deformation [49-51]. Poundarik et al found that the osteocalcin/osteopontin complex is a key component in fibrillar matrix for energy dissipation [41]. They found that failure in these osteocalcin/osteopontin complexes, seen as dilatational bands, are the initiation points of bone fracture (Figure 6) [41]. These dilatational bands eventually combine to produce the diffuse damage present at larger length scales. These are explained in figure 6.
The deformation of osteopontin and osteocalcin complexes in the viscoelastic glue that holds collagen fibrils together [41]. The fracture of the viscoelastic glue results in the formation of dilatational bands [41].

**Sacrificial Bonds**

Sacrificial bonds are defined here as bonds that break and dissipate energy under strain. As outlined in the previous section, sacrificial bonds are integral to bone’s strength and toughness. This is because sacrificial bonds constantly break and then spontaneously reform during inter-fibrillar sliding, (Figure 7). We also note that
synthetic materials overwhelmingly utilize permanent covalent bonds. In this section we outline the advantages of sacrificial bonds.

Sacrificial bonds have to be weaker than covalent bonds that form the “backbone” of a protein, in most cases a C-C bond, to ensure that under strain the sacrificial bonds are all broken instead of the C-C bonds that make up the protein backbone [49]. In the context of bone, sacrificial bonds are also non-covalent bonds, which means they can dynamically reform because their bond energies are sufficiently small for background thermal energy to reform spontaneously [49]. Sacrificial bonds are important on many levels to the mechanical properties of bone. Here we outline their most important effects:

Energy dissipation
In order to break a chemical bond, energy (in this case in the form of strain energy) must be provided. Every bond that is broken consumes energy and therefore increases toughness. Bone, specifically collagen, is full of bonds that are broken during fracture [41, 42, 46, 49, 50, 69]. Sacrificial bonds in bone are non-covalent bonds, for example, hydrogen bonding in collagen and ionic interactions between HAp and collagen [41, 42, 46, 49, 50, 69]. This is counterintuitive because non-covalent bonds energies are between 4-20 kJ/mol vs. ~250 kJ/mol for C-C bonds [77]. If the aim is to maximize toughness then why use comparatively low energy sacrificial bonds? The reason is that by utilizing many non-covalent bonds cooperatively more total energy can be dissipated due to two mechanisms; dynamic reforming and hidden length [78].
**Hidden length**
Sacrificial bonds also affect the structure of the protein by imparting a tertiary structure that unfolds after the breaking of sacrificial bonds [67]. This “hidden length” increases the strain to failure of the protein, and therefore its toughness (Figure 7) [49]. We note that because of the reduction in entropy that occurs when unfolding a protein, additional energy, beyond that consumed by the breaking of sacrificial bonds, is dissipated over-and-above that needed to break a sacrificial bond [49].

![Sacrificial Bond Breaking and Molecular Uncoiling](image1)

![Dynamic Reforming during Mineral or Fibrillar Sliding](image2)

**Figure 7 Hidden length and dynamic reforming**
Hidden length (top) increases the strain to failure of collagen by imparting a tertiary structure that unravels under strain. Due to the fact that sacrificial bonds are substantially weaker than covalent bonds of the protein backbone the tertiary structure unravels first under strain, revealing hidden length and increasing roughness. Dynamic reforming (bottom) illustrates that collagen, when bonded non-covalently to HAp (orange), is able to reform interfacial bonds in locations remote from where it was initially bonded.

**Dynamic reforming**
Covalent bonds do not reform spontaneously after breaking (except in very rare cases), primarily because their energy is too large for thermal fluctuations to overcome their activation energy [78]. Comparatively, non-covalent bond’s much
smaller individual bond energies allow for activation by thermal fluctuations. This can be seen in the strong temperature dependence of supra-molecular systems [78]. This means that non-covalent bonds can break and reform (at different sites) multiple times as the collagen fibrils slide past each other [41, 42, 46, 49, 50, 69]. When bonds are broken multiple times, substantially more energy is dissipated [41, 42, 46, 49, 50, 69]. The importance of dynamically reforming sacrificial bonds is most clearly seen in the substantial decline (~33%) in the toughness of bones. This is attributed to a decline in intrinsic toughening mechanisms as the strain rates increase [54]. This is because as the strain rate approaches bond reformation time the extent of dynamic reforming is reduced, and ultimately ceases when collagen fibrils slide past each other.

Robustness
Robustness is the ability of a structure to tolerate changes without disproportionate effects on the structure's performance. Multiple non-covalent interactions result in better robustness than a single covalent bond [78]. This is because the loss of one, of many non-covalent bonds doesn't result in a total loss of energy dissipating ability, as in the case of a single, strong, covalent bond.

Self-healing
The use of sacrificial bonds means that bone, at sub-micron scale, is self-healing because these bond spontaneously reform due to background thermal energy [67]. Covalent bond energies, with activation energies much greater than background thermal energy, do not spontaneously reform (Figure 8).
Figure 8 Activation energy of chemical bonds

Activation energy of non-covalent bonds (green) and covalent bonds (red) relative to background thermal energy (black horizontal line). Covalent bonds cannot spontaneously reform after breaking and therefore cannot self-heal.

Comparatively at the osteonal length-scale bone, the self-healing nature of bone is derived from active cellular remodelling by osteoclasts [67]. Indeed a recent study found that diffuse damage, such as clusters of sub-lamellar micro-cracks, do not activate a cellular response. Comparatively, larger linear micro-cracks do activate an active response [79], indicating that at this scale and below self-healing is spontaneous.

Interfacial switching
If we refer to the Tension-shear Chain Model of Gao et al, energy dissipation is maximized when failure occurs in the soft polymer phase. However, to maximize strength as well, the polymer phase must fail just before a hard and strong load bearing phase [71, 72]. Tuning the interfacial bond strength is therefore a convenient way to ensure that failure occurs in the soft polymer phase marginally
before the failure of the hard phase. This can be achieved by ensuring the interfacial strength is just below failure stress of the hard phase.

An excellent example of this can be seen when young and aged bone are compared. In young healthy bone, the strength of bond between collagen fibrils and the “glue” layer, which is a sacrificial ionic bond, is such that the “glue” fully deformed and fracture is initiated within the glue [41]. In the case of old bone, supra-molecular bonding between collagen fibrils is replaced by stronger covalent bonds, as a result toughness and strength declines dramatically [80]. This is attributed to the formation of non-enzymatic advanced glycation end products (AGEs) that form both intra- and inter-fibrillar links along the collagen backbone [80]. These covalent bonds are essentially too strong; they restrict fibrillar sliding and hence reduce plasticity and toughness (Figure 9) [80].

![Effect of Covalent Crosslinks on Fibrillar Sliding](image)

**Figure 9 Effect of crosslinking on fibrillar sliding**
Staggered composite covalent bonds (red) restrict the sliding of fibrils (yellow) relative to other fibrils (blue). This limits failure strain and hence toughness of material.

**Micron scale toughening mechanisms**

At the micron scale extrinsic toughening mechanisms, which are active only when a crack has already formed, are the dominant toughening mechanism [1, 12, 48, 65, 66]. Experimental extrinsic toughening mechanisms are best identified by a rising R-curve from non-linear elastic fracture mechanics (NLEFM) based testing.

![Figure 10 Rising R curve.](image)

Note how the fracture resistance of bone, (y-axis) particularly in the transverse direction, increases with crack length (x-axis). This means that extrinsic mechanisms (the increase in fracture resistance from a crack length of 0 to fracture) attribute to approximately 5/6th of bones fracture resistance [12].

In this context the initial value of NLEFM curve represents intrinsic toughening mechanisms, while a rising R-curve shows that bone’s extrinsic resistance to crack growth actually increases as the crack size increases (Figure 10) [12]. A crack
becomes catastrophic when the strain energy released by its growth is greater than the surface energy required to expose the two surfaces of a fresh crack. Extrinsic mechanisms therefore act either by holding the exposed surfaces together (crack bridging), or by reducing the effective stress intensity applied to the crack tip (crack deflection and twists) [65]. The major extrinsic mechanisms are explained below:

**Crack deflection**

Somewhat counter-intuitively a material's toughness is maximized when there is extensive micro-cracking, so it can be very effective to design materials that control how cracks grow. Cracks naturally grow in the direction of greatest strain-energy release rate, or colloquially in the path of least structural resistance [65]. Crack deflection, a major extrinsic toughening mechanism in bone, is based on the principle that cracks only follow the *local* path of maximum strain energy release rate [1, 12, 48, 65, 66]. With proper micro-structural design the *local* path of maximum strain energy release rate can be very much different from the *global* path of maximum strain energy release rate. Theoretically, if one considers the fracture of homogenous material, it fails by the nucleation of a single giant crack that is many times larger than all other cracks in the material. From first principles this is actually quite surprising. If a crack was growing randomly it should fail slowly by the random percolation of multiple cracks into a single large crack [64]. The growth of a single giant crack arises because the growth of a crack also increases the stress intensity at the crack tip. The resulting perturbations in the strain field are long-ranged allowing multiple cracks to interact [58, 64]. This means that multiple cracks
coalesce to form a single giant crack, “guided” by the regions of high strain energy, which connects the two flaws (because their strains superimpose on one another) [63]. In this way, because of the interactions between long-range strain fields and positive feedback cycle of increasing stress intensity with increasing crack-length, a catastrophic cracks nucleates very rapidly [64]. The challenge then becomes to design a microstructure that can prevent long-range interactions in the strain field.

Shekhawat et al [64] showed that greater material heterogeneity, which as fully explained in Chapter 1, leads to disruption of the long-range strain field. As a result the material is more likely to fail percolatively (with substantial energy dissipation). However, as the dimensions of the material increases, the more likely brittle failure becomes. (Figure 11) [64]. Bone is a highly heterogeneous material, which explains, in part, its propensity for stable crack growth [64].
Figure 11 Phase diagrams of fracture versus disorder

The effect of decreasing disorder (y-axis), and decreasing size (x-axis) on the type of fracture. Percolative failure (bottom right) is accompanied by substantial energy dissipation (red lines) and occurs in highly heterogeneous materials such as bone.

In essence catastrophic cracks nucleate because there is a correlation between the local and global path of maximum strain energy release rate. However, a highly heterogeneous structure, such as that found in bone, can in theory break this correlation. In a homogenous 3D material the path of greatest strain energy release rate is in the direction of the applied strain. That is, under tensile loading in x direction the path of maximum strain energy release rate is also in the x direction.

The stress as a function of $\theta$ is defined by $\sigma_\theta = \sigma_x \cos \theta$. This means a crack would ordinarily grow in the x-direction. If however the microstructure of the material was able direct a crack to grow in at an angle of $\theta = 45^\circ$ then $\sigma_\theta = \frac{\sigma_x}{2}$. In such a scenario the driving force for crack growth is halved; a very potent toughening mechanism.

In bone, this is an exceptionally potent toughening mechanism and is evidenced by how bone is much tougher the in the transverse than the longitudinal direction [65].

Micro-structurally crack deflection, as outlined by the He and Hutchinson criteria, occurs at interfaces between materials with dissimilar stiffness.

$$\alpha = \frac{E_{Interface} - E_{Osteon}}{E_{Interface} + E_{Osteon}}$$

and strain energy release rates (toughness ratio = $\frac{G_{Interface}}{G_{Osteon}}$)[65]. The presence of alternating stiff and compliant phases occurs at all length scales in bone, indicating that the spatial control of crack growth by bone’s
micro-structure is both ubiquitous and an important contributor to bone toughness [1-6, 8, 9, 32, 33].

The lower the toughness ratio and the larger the stiffness ratio the more favourable crack deflection becomes. Once a crack is deflected away from the direction of maximum stress intensity at an angle $\theta$, then stress intensity at the crack tip is reduced by $\sigma_x - \sigma_x \sin \theta$. These two mechanisms of reducing the driving force and breaking of the global/local correlation are combined in bone very clearly by osteons and cement lines [1, 12, 48, 65, 66]. The cement line has a higher mineral content than osteonal bone and is therefore believed to be comparatively stiff and more brittle than lamellae bone [12, 48, 54]. This means that osteons are very effective at deflecting cracks (Figure 12) [1, 12, 48, 65, 66].

![Crack deflection](image)

**Figure 12 Crack deflection**

Scanning Electron Microscope (SEM) image of a crack growing from a notch (left of image) that is deflected away from the path of maximum applied strength (right of image).
In fact the importance of crack deflection is easy to determine quantitatively as, under longitudinal strain, the cement lines are not in the correct orientation to deflect cracks away from the plane of maximum stress. This means that the difference in strain energy release rate (G), in the longitudinal and transverse direction (12 J/m$^2$ vs. 200 J/m$^2$), provides an excellent estimate of the importance of controlling the micro-structure to ensure the crack takes the most tortuous path possible [65].

**Crack bridging and constrained micro-cracking**

Crack bridging is a common extrinsic toughening mechanism. After the formation of a crack, small pieces of materials, often fibrils in the case of bones, bridge the crack and carry some of the load that would otherwise grow the crack [3, 66]. Crack bridging from fibrils is believed to be a modest toughening mechanism but crack bridging by much larger pieces of bone, called uncracked ligaments, is considered to be far more important [80]. If a uncracked ligament is able to span a crack then it is able to relieve some of the stress at the crack tip, reducing the effective driving force, and increasing toughness [65].

Micro-cracking is a form of inelastic deformation that is present in healthy bone, as an extrinsic toughening mechanism its role is considered minimal [65]. The formation of micro-cracks naturally cause a volumetric expansion which generates a compressive stress that can inhibit crack growth and is important in synthetic materials [65]. Calculations by Nalla et al [81] have shown that constrained micro-
cracking is not a significant extrinsic toughening mechanism by itself but micro-cracking is a prerequisite for more potent mechanisms such as crack deflection and un-cracked ligament bridging [81].

**Spatial variations in stiffness**

Bone possesses a heterogeneous structure of soft and hard phase at all length scales, from the nano to the macro scale [1-6, 8, 9, 32, 33, 65]. What is generally unappreciated is that a material with periodic variations in stiffness ($E$) naturally has an increased fracture resistance compared to its homogenous equivalent [82-84]. This is because at the interface between stiff and compliant phases, the driving forces for crack growth, are reduced [82-84]. This crack growth is arrested at the interface until more strain energy (than is needed in the homogenous material) is available to drive further crack growth[82-84]. This increase in toughness between materials of varying and non-varying stiffness is considerable. For example, the difference in toughness between bone with and without a lamellae structure is estimated to be a factor of approximately 80 [84]. The mechanism for this increase in toughness arises because cracks, as stated earlier in this section, grow following the path of least resistance with crack growth resistance increasing naturally as a materials $E$ increases [84, 85]. The reason crack growth resistance increases naturally as a materials $E$ increases is definitional. For a crack to grow a certain length "$a$" the material must be strained, as $E$ is the strain energy density per unit strain. This means the greater the $E$ the more energy that must be applied to grow the crack. Therefore, in the linear elastic case, a crack in a comparatively compliant
region will require a greater stress (or strain energy) to enter the stiffer region than that for the more compliant region. We note that a crack in a stiff region will also find it easier to propagate into a soft region, provided that the spacing between the stiff and the compliant regions is smaller than that for the critical crack size. Otherwise, a crack will be arrested before it can initiate fracture, elevating the fracture resistance of the entire material.

Kolednik et al [82, 84] analytically derived an expression that relates magnitude and period of the variations in Young’s Modulus (E) to the ratio of toughness in the inhomogeneous and homogenous material [82, 84].

\[
R_{\text{Multiple}} = \Psi \frac{E_{\text{Average}}^{2l_c}}{E_{\text{Min}}\lambda} \quad \text{Equation 5}
\]

Where \( \Psi \) is a geometric constant approximately equal to 1, \( E_{\text{Average}} \) is the average E of the composite, \( l_c \) is the critical crack length of material, \( E_{\text{Min}} \) is the stiffness of the compliant phase, \( \lambda \) is the wavelength of stiffness variation. In short, the greater the magnitude of the stiffness variation as well as the smaller the wavelength of the stiffness variation, the greater the fracture resistance of the material [82, 84].

The most important aspect of this toughening strategy is that it is achieved at the interface between hard and soft phases [82-84]. As a consequence, significant toughness and stiffness can be achieved simultaneously. This is because the fracture resistance arises due the difference in stiffness between and hard and soft phases \( \left( \frac{E_{\text{Average}}}{E_{\text{Min}}} \right) \) and is not dependent on the volume fraction of the soft component.
According to rule of mixtures, the Young’s Modulus of a composite material is an average weighted by volume fraction of the Young’s Modulus of each material. At this point both stiffness and fracture resistance can be achieved [84]. Since fracture resistance also limits strength, a material that is strong, tough, and stiff is achievable [84]. Furthermore this toughening strategy scales effectively to smaller dimensions; thinner compliant layers allow for a smaller material wavelength, \( \lambda \), further increasing a materials fracture resistance [84]. It is therefore no surprise that bone exhibits large spatial variations in stiffness (2 to 30 GPa) right down to the nano-scale level [73].

### 2.4 Design principles

Bone’s structure is exceedingly intricate and beyond what can be recapitulated by existing techniques [6]. It is therefore critical that one identifies the underlying principles behind bone’s structure-property relationship [2]. In this section we will highlight key design principles that can be applied to synthetic materials. Finally, we will outline how the myriad of individual design principles are combined to produce a material “system” that combines the disparate principles synergistically.

**Nano-scale flaw tolerance**

Nano-scale flaw tolerance is an extremely powerful design principle. It allows one to replace expensive, energy dense, weak, ductile, dense, but tough metals with cheap, ubiquitous, stiff, strong, lightweight, but brittle ceramics [13].
For example, the strength of monolithic Hap is 0.065 GPa but increases to 3.2 GPa at the nano-scale (~4nm in thickness) [71, 86]. It is the structural material equivalent of turning water into wine.

The problem is that nano-scale flaw tolerance is a phenomena and not a strategy. The question becomes how to translate this nano-scale strength and flaw tolerance to materials of macroscopic size [6, 87]. One cannot simply combine many nanoparticles together in a random agglomeration, as the material will fail due to weak Van der Vaal bonding between the nanoparticles. The challenge is to find a design in which nano-scale mechanical properties can be translated to the macro-scale.

**Spatially varying heterogeneous elastic moduli**

Bone combines stiff, flaw tolerant HAp nanoparticles into a macroscopic material with a soft but extensible collagen matrix [71, 73]. Collagen itself has a hierarchal structure and is compliant, extensible, tough, and tenaciously binds to HAp [70, 74]. It appears that the lack of stiffness in the collagen is critical in preventing cracks from spreading between HAp crystals. This is due to the fact that cracks cannot enter a stiff material from a compliant region with a sufficiently large (~5x) variation in stiffness [82-85]. Indeed, the existence of periodically varying stiff and compliant regions has been shown, to increase the fracture resistance of material with minimal effect on stiffness (80x in the case of bone) [82-85].
**Strain partitioning**

Although collagen is tough and extensible its strain to failure is much greater than HAp. Therefore, to maximize energy dissipation capacity the strain in collagen must be amplified [70, 74]. This is achieved through the use of HAp particles with large aspect ratio’s [72]. Bonderer et al [72] demonstrated experimentally that by using particles with a large aspect ratio it is possible to amplify the strain in the extensible polymer phase [72]. If an optimal aspect ratio is chosen, below which fracture of the HAp crystal would occur, then the strain in the collagen is maximized [71]. As a consequence, the composite is both tough (because the collagen is strained to failure) and strong (because the large aspect crystal HAp reinforces collagen matrix) [71, 72]. Therefore, aspect ratio control is a simple principle to combine material with widely varying strains to failure.

**Anisotropy of natural materials**

The use of large aspect ratio HAp crystals introduces a strong directional dependence, or anisotropy, in the mechanical properties of bone. Anisotropy is useful because it produces superior mechanical properties to random composites, with the drawback that the mechanical properties are elevated only in one direction [84].
A) Chains that strength is limited by the strength of its weakest link, as is the case for strength in the transverse direction (3). b) The directions of transverse (3) and longitudinal strain (2) in a layered composite (84).

The strength in the longitudinal direction (2) is greater than the strength in transverse direction (3) (Figure 13). In the transverse direction (2) the strength is limited by the weak inter-layers, as shown in the equation below [84].

$$E_T = \frac{100}{\frac{E}{E_\perp}} \approx 0.92E, \sigma_{\parallel} \approx 0.1\sigma$$ Equation 6

In the longitudinal direction (3) the strength is not limited by the weakest element (the thin polymer layer) because the strain in polymer and HAp interlayer must be equal and therefore the HAp bears most of the stress.

$$E_\parallel \approx (99E + E/10)/100 \approx 0.99E, \ \sigma \approx 0.99\sigma$$ Equation 7

In real world multi-axial loading scenarios the anisotropic nature of collagen fibrils presents a problem. This is because non-longitudinal stresses will cause early failure due to buckling [71]. In order to overcome this limitation, lamellae bone
(which is strongly anisotropic) is arranged in layers that are offset by small angles [4, 7, 12, 40, 47, 48]. In this way despite each individual unit possessing strong mechanical anisotropy bone can be made strong under multi-axial stresses [4, 7, 12, 40, 47, 48].

**Sacrificial bonds dissipate more energy than covalent bonds**

Bone utilizes multiple supra-molecular bonds in favour of strong covalent bonds. This is because supra-molecular bonding can dynamically reform and self-heal leading to improved robustness and fatigue resistance [78]. This preference for supra-molecular bonding over covalent bonding is most useful in the design of the tough polymer phase.

**Breaking the global/local correlation**

Breaking the global/local correlation (Section 2.3.2) through microstructural design is a powerful principle, particularly at larger length scales (10’s of microns). Unlike the nano-scale, structural control at the micron scale is readily achievable with 3D printing [88, 89]. A 20x difference in toughness between bone strained in transverse and longitudinal directions is entirely due to the breaking of the global/local correlation at the osteonal scale [65]. This strategy was best demonstrated experimentally by Barthelet et al [90] who showed dramatic improvements in the fracture toughness of glass by using lasers to introduce defect patterns that cause the crack path to follow a tortuous path [90].
The use of weak interfaces (the engraved plane) to redirect the crack at an angle, $\theta$, from the direction of maximum stress ($\theta = 0$) [90].

A major benefit of the use of “weak” interfaces to induce crack deflection is that only very small amounts of “weak” material is needed for tough composites with a high volume fractions of brittle material (93%) with 800x greater in toughness compared to monolithic glass (Figure 14) [91]. In summary, the presence of weak interfaces, which deflect cracks away from the direction of maximum stress, is a potent and effective design principle.

**Engineered strain hardening**

The existence of many individual toughening mechanisms is only useful if strategies are in place to ensure as many individual toughening mechanisms over the widest possible area of the material are activated during failure [92, 93]. In metals this is achieved through strain hardening (Figure 15), the phenomenon where metals that plastically deform, also stiffens, thus relieving locally high stresses. However, HAp and other stiff brittle materials have no intrinsic strain hardening qualities.
Figure 15 Linear vs. non-linear deformation

Under strain a material may (left) or may not (right) harden. This can be seen by the increase in the slope of the stress-strain curve with increasing strain.

When a material that is plastically deformed stiffens then adjacent material, which is not plastically deformed (and is less stiff), is preferentially deformed. As strain increases this area of plastic deformation, the process zone, can grow to be quite large. Indeed the size of the process zone is direct measure of its toughness (Figure 16) [87].

Figure 16 Strain hardening at the crack tip
Plastic deformation is important to tough structural materials such as metals but almost non-existent in ceramics. Strain hardening allows regions of stress concentration, such as at a crack tip, to dissipate strain energy over much larger areas (bottom) than in a material with no strain hardening (top). As the material at the crack tip stiffens under strain, material remote from the crack tip is more easily deformable relative to strain-stiffened material at the crack tip. Consequently, the area of elevated strain is spread over a much greater volume of material and the stress intensity at the crack tip is reduced.

Increasing the size of the process zone is critical because it amplifies the aggregate number of additional mechanisms (crack bridging, crack shielding at soft interfaces, sacrificial bonding, fibrillar sliding) that are utilized during fracture. Strain hardening can be engineered into any composite if the one component of the material exhibits non-linear strain hardening. In bone, this is achieved by the well documented non-linear deformation of proteins such as collagen and osteocalcin/osteopontin [41, 67]. When collagen fibrils begin to slide against each other, the viscoelastic “glue” first deforms elastically before stiffening as sacrificial bonds are broken and covalent bonds become directly stretched [49, 50].

Nacre, also known as mother of pearl, is very similar to bone; it is also a multi-scale, heterogeneous composite consisting of calcite (instead of HAp) and a protein interlayer that is studied because of its remarkable toughness [4]. Nacre also achieves significant strain hardening through a different mechanism than bone; dovetailed platelets [92, 93]. Similar to bone, nacre also consists of mineral platelets in a soft matrix (the mineral plates are an order of magnitude larger in size) that slide against each other [4]. Significant strain hardening occurs when the nacre platelets have a dovetail shape.
In nacre-mineralized tablets, similar in shape to HAp crystals have a slight dovetail. Consequently, when they slide past each they become progressively interlocked, strain stiffen, and produce an expanded process zone.

When platelets are subject to shear strain and glide by each other they progressively impinge on one another resulting in pronounced strain stiffening (Figure 17) [91]. The increase in toughness can be significant, resulting in a material that is 700x tougher than glass (its bulk material) but still 93% by weight glass [91].

**Figure 17 Engineered strain hardening due to dovetails**

The role of structural hierarchy of natural materials

All natural materials are hierarchal yet the precise role of hierarchy in determining the mechanical properties of natural materials is controversial [1-6, 8, 9, 32, 33]. One explanation for hierarchal structures is that it exploits size effects. A material consisting of smaller units of the same material will always be stronger than a single large fibre due to size effects. A simple example of this can be found in the fibre-bundle model, the strength of a fibre is given by the following equation.
\[ \sigma = \alpha \sqrt{\frac{E}{2\gamma r}} \quad [94] \]

Where \( \alpha \) is a geometric constant, \( E \) is Young’s Modulus, \( \gamma \) is surface energy, and \( r \) is the fibre radius. If we consider a fibre of equal area, \( A \), but consisting of \( N \) individual fibres:

\[ \sigma_{\text{Bundle}} = \alpha \cdot A \sqrt{\frac{E}{2\gamma N r}} \quad [94] \]

That is because fibres with small radii are stronger than an identical fibre with a larger radius. A collection of smaller fibres (of the same area) is always stronger [94]. Note that because strength increases with \( \sqrt{N} \) not \( N \), the predicted improvement is substantially less than what is seen in bone.

**Flaw tolerance in natural materials**

Size effects are not the only explanation as to why hierarchal structures can be both strong and tough. In a hierarchal structure a growing crack will encounter successively larger structural features that successively arrest its growth [94]. An enlightening study by Buehler et al demonstrated that structural hierarchy alone was able to generate flaw tolerance [95]. This is remarkable, as Sen et al [95] did not include a tough and extensible material in this model, using only silica and porous silica (which is comparatively compliant) [95].
Figure 18 Effect of Structural Hierarchy on the Fracture Strength

a) Single level Nacre-like structure b) Single level Calcite-like structure c) Two level Nacre-like structure d) Two level Calcite-like structure.

Although the addition of structural hierarchies reduces the strength of the material (From 400 MPa to 300MPa) the materials become tolerant to the presence of large flaws (Figure 18 a vs. c and b vs. d) [95]. This defect tolerance is achieved by the growth of stable micro-cracks that at failure coalesce to form a single dominant crack [87, 95]. The role of hierarchy in this minimal model can be rationalized as follows:

Micro-cracks are arrested at the interface of porous and solid silica [82-85].

Eventually, with greater applied strain, micro-cracks coalesce to form a single larger
crack that is unstable. In a structural hierarchy the larger crack is eventually arrested at a yet larger interface. The process is repeated until all hierarchal levels are exhausted. Essentially structural hierarchies are useful because they trade strength for toughness (in comparison to the base material) [87, 95]. This strategy is particularly useful when combined with a base “unit” that is nano-scale in dimensions. This is because nano-materials are so much stronger than their equivalent bulk material (65 MPa vs. 3.3 GPa) [43, 71, 74].

**Hierarchy is an enabler of extrinsic toughening mechanisms**

Hierarchy does more than trade strength for toughness. We believe that it is helpful to view hierarchy as the “skeleton” that supports all extrinsic toughening mechanisms. A material’s toughness is maximized when the strain and stress is uniform throughout the material. This is because the greatest total volume of material is strained sufficiently to activate both extrinsic and intrinsic toughening mechanisms. Structural hierarchies are able to produce relatively uniform strain distributions and also to generate stable crack growth [88]. This is demonstrated experimentally in Figure 19 using macro-scale models of hierarchal structures [88]. Note how much more delocalized the strain is in the 4 level hierarchal sample are compared to the stiff phase (Figure 19a is least delocalized and Figure 19d to most localized) [88]. In practice this means a much greater total volume of material is strained sufficiently for extrinsic toughening mechanisms to become active.
Figure 19 Effect of hierarchy on stress concentrations at a notch.

Note how the normalized strains become more delocalized with increasing hierarchy [88]. A.) No Hierarchy, B.) 1 Level of Hierarchy C.) 2 Levels of Hierarchy, D.) 3 Levels of Hierarchy

Once a stable crack has formed a hierarchal structure it can support stable crack growth with no additional mechanisms, [95]. The more cracks that form, the longer the cracks grow, and the more energy is dissipated by extrinsic mechanisms such as stiffness modulation [82-85], crack bridging [3, 66] and crack deflection [1, 12, 48, 65, 66]. In the absence of hierarchy and stable crack growth, toughness would be greatly reduced because a single crack would nucleate and undamaged regions would be unable to contribute to energy dissipation. For this reason in bone, for example, samples that do not show extensive micro-cracking are actually less tough than those without micro-cracking [55].

Summary

The myriad of individual toughening mechanisms in bone, and other natural materials, described in this section are now fairly well understood. However, there
is no coherent framework for understanding how the different individual mechanisms work together synergistically. Chapter 1 of this thesis addresses these short coming by outlining a plausible framework for rationalizing how the individual mechanisms work together as a material system.
3.0 SYNTHESIS TECHNIQUES FOR BIO-INSPIRED COMPOSITES

The synthesis of bio-inspired materials with structures organized from the nano to macro scale remains a major challenge in the field; one that is well beyond the current state of the art [6]. At best, with concerted effort, the synthesis and rational design of multi-scale structures is believed to be 10-20 years away [6].

To contextualize the difficulties in synthesizing bulk nano-composites with the structural complexity of bone we will contrast this task with microprocessor manufacture. Microprocessors are a triumph of modern engineering; they are the most sophisticated objects that are produced commercially. They are also vitally important, and their exponential improvement in performance is the bedrock of the IT and software industry. Modern commercial microprocessors possess feature sizes in the order of 22nm, which is similar in dimension to mineralized collagen fibrils. The top-down production of a microprocessor on silicon is incredibly expensive, technically demanding, requires hundreds of discrete steps, and is the product of hundreds of billions of dollars in cumulative research and development.

Comparatively the synthesis of artificial bone requires the synthesis of a material with feature sizes smaller (~5nm) than found in commercial microprocessors. Bone requires a smaller level of structural resolution, in 3 dimensions (instead of 2), the integration of two dissimilar materials (a collagen and HAp analogue), in much larger volumes (structural applications require much greater quantities of materials), and at a cost comparable to structural metals. Therefore, and in order to
recapitulate bones structure, we need to synthesize a material with finest structural detail achieved synthetically and do this over areas of macroscopic dimensions (regions with a length scale in the order of a centimetre).

Despite the difficulties in producing materials with structures at the scale and complexity of bone there has been no shortage of innovative and inspiring synthesis approaches. In the following section we outline existing techniques to produce bio-inspired composites and discuss their relative merits.

### 3.1 Methods for synthesizing free standing films

**Layer by layer deposition**

Layer by layer deposition (LbL) is a simple, controllable, versatile (wide range of materials), and precise method for the synthesis of thin films [96, 97]. LbL films are synthesized by the repeated dipping and drying of a substrate in solutions of polymers/colloids with alternating electrostatic charges [96].

**Polyelectrolyte based LbL**

Any charged material, that can be dispersed in solution, can form alternating multi-layer structures with components that possesses an opposite charge [96]. Due to the use of alternating charges, a single layer is formed in each dipping cycle because once a single layer is formed like charges prevent the deposition of a second layer
Consequently the thickness of each layer is precisely controlled. For example, in the case of nacre like LbL clay composites, each layer is 5 nm (the thickness of the colloid) [98]. Furthermore the resulting films are remarkably well ordered consisting of alternating layers of stiff and compliant phases [96]. Multiple dipping cycles, each taking approximately one minute, lead to multi-layered films [96, 99].

**Colloidal LbL**

An alternative approach to make LbL films of micron sized fillers using particles located at the air-water interface was pioneered by Bonderer et al [72]. Alternating layers of polymers and stiff alumina plates were synthesized by dipping a glass slide in solution (to form a thin layer of platelets) followed by spin coating of chitosan (Figure 20) [72].
Figure 20 LbL casting of alumina films

$\text{Al}_2\text{O}_3$ platelets are coated with a hydrophobic silane in ethanol solution (1), because these platelets are now hydrophobic they form a monolayer on the surface of water (2), a glass plate is inserted into the water and then slowly withdrawn (3), the glass is then coated with polymer by dropping a dispersion onto the glass slide followed by spin-coating to form an even film (4). Steps 2-4 are then repeated to form a thick film [72].

Polyelectrolye LbL films are some the earliest examples of nano-scale bio-inspired films [98, 100]. The reported mechanical properties are superior to their expected rule of mixtures performance and superior to that of artificial nacre [98, 100].

| Table 1 Mechanical properties of selected LbL films |
|---------------------------------|----------|-----------|--------------|-------|
| Materials                      | Stiffness (GPa) | Strength (MPa) | Failure Strain (%) | Source |
| PolyVinyl Alcohol              | 1.7       | 40        | 35            | [100]  |
The stiff phase in these LbL films was montromillite, a type of hydrophilic clay that is ~2 nm thick, ~100 nm in length and width which is easily dispersed in water [98, 100]. The strength of the films derived from their “brick and mortar” structure, the highly anisotropic plates reinforce the polymer matrix leading to increased strength and stiffness at the cost of extensibility [98, 100]. Key to the mechanical performance of LbL films is the bonding strength between MTM and PVA, the limiting factor in the mechanical strength of the composite appears to be the interfacial strength [98, 100].

Beyond proof of principle studies LbL have serious practical limitations; principally that it is practically impossible to produce thick films [101]. For example a 1.5 micrometre films requires 300 bilayers and takes approximately 10 hours to synthesize with an automated dipper [101]. Scaling this process up to macro-scale requires a prohibitive amount of time and is not feasible for practical applications.
Size Effects

The reported mechanical properties for LbL films are films that are ~1.5 micrometres thick, far smaller than macro-scale composites. Due to size effects, the tendency for all things being equal a smaller sample to always be stronger than a large sample, the reported mechanical properties overstate the mechanical strength of the equivalent macroscopic films.

Very Low Strain to Failure in Nanoclay Films

Clay LbL films, particularly cross-linked films, exhibit increased strength and stiffness at the expense of toughness [46]. This lack of toughness, manifested by very low strains at failure, is attributed to the reduction of ductility of polymer chains confined between nanoclays [46]. Bonderer et al [72] demonstrated that strength and large strain to failure could be achieved if micron sized fillers were used. This is because the polymer chains were not strongly confined between the alumina plates [72].

Low Volume Fraction

The volume fraction of mineral component in bone and nacre is 60% and 95%, comparatively volume fractions of ~20% are the limit of what can be achieved in strong LbL films [46, 72, 98, 100]. Larger volume fraction cannot be achieved because of the difficulty of avoiding defects, aligning platelets, and fully coating the polymer [46, 72, 98, 100].
3.1.1 Colloidal casting

Colloidal casting partially addresses the major drawback of the scalability of LbL films [101]. Colloidal casting uses the same large-aspect ratio particles and polymer interlayers that LbL methods use. It is also much faster (only one drying step) and produces thicker films. This is a major advantage; LbL films are too difficult to produce for anything but proof of principle studies. Pioneered by Walther et al, colloidal casting consist of preparing and stabilizing a dispersion of the stiff phase (often nanoclays or graphene) with dissolved polymers and then drying the dispersion (Figure 21) [101]. This process is very similar to traditional paper-making, is therefore scalable, occurs at room temperature, and uses water instead of organic solvents [101].

![Figure 21 Colloidal casting of nanoclays](image)

High aspect ratio clay nanoparticles are coated in a single layer of polymer before processing into films. In step 1 nanoclay and water-soluble polymer are mixed thoroughly in water. In step 2 the polymer coated clay left for 1 day to remove excess polymer. In step 3 the polymer-coated nanoclay is dried to form a brick and mortar like film [101].

Colloidal casting consists of simply drying a set volume of the dispersion (solids dispersed in a liquid). As expected, the mechanical performance of such films are lower than LbL films albeit quite substantial [101, 102]. These films are 10’s to 100’s
of microns thick therefore their mechanical properties are not directly comparable with LbL films. Due to mechanical size effects, thicker films are weaker than thinner but otherwise identical films [101, 102]. This means that the mechanical properties of solution cast films can be compared to the reported mechanical properties of bone and nacre [101]. Unfortunately, there is no reliable formula for comparing the mechanical properties of materials with vastly different dimensions such as comparing a single nm HAp crystal to a cm sized HAp single crystal.

<table>
<thead>
<tr>
<th>Table 2 Mechanical performances of clay-based composites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness (GPa)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>PVA/MTM</td>
</tr>
<tr>
<td>PVA/MTM (Borate)</td>
</tr>
<tr>
<td>PVA/MTM/Nano-Cellulose</td>
</tr>
<tr>
<td>Carboxy Methyl Cellulose (CMC)</td>
</tr>
<tr>
<td>CMC/MTM (80/20)</td>
</tr>
</tbody>
</table>

More recent studies using colloidal casting to make bio-inspired films have focused on the use of graphene or graphene oxide (GO) instead of nanoclays. This is
unsurprising given that the estimated strength and stiffness of graphene is 100x and 4x greater than nanoclay respectively. It is hoped this will translate to improved mechanical performance of the composite [105]. This has proven the case as graphene and graphene oxide exhibit improved mechanical properties in comparison to clay films. However, improvements are much lower than expected based on a simple comparison of their reported strength and stiffness [106-109].

Table 3 Mechanical performance of graphene based colloidal films

<table>
<thead>
<tr>
<th></th>
<th>Stiffness (GPa)</th>
<th>Strength (MPa)</th>
<th>Failure Strain (%)</th>
<th>Dimensionless Improvement Ratio</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced graphene oxide and poly(acrylic acid-co-(4-acylamidophenyl)boronic acid Films)</td>
<td>8 GPa (6.6)</td>
<td>350 (160)</td>
<td>5 (2.5)</td>
<td>4</td>
<td>[110]</td>
</tr>
<tr>
<td>Reduced Graphene Oxide and polymerized 10,12-pentacosadiyn-1-ol (PCDO)</td>
<td>1.8 (6)</td>
<td>160 (120)</td>
<td>7 (2)</td>
<td>4</td>
<td>[111]</td>
</tr>
</tbody>
</table>
The major advantage of solution casting approach is its simplicity, versatility and ability to synthesize comparatively thick large area films [101, 102, 104, 113]. Stable dispersions can also be vacuum filtered to rapidly produce nacre-like films [114, 115]. Vacuum filtered films form more rapidly than films that are formed by evaporation and, due the additional ordering from the suction force, are more ordered [115].

**Table 4 Mechanical properties of vacuum cast films**

<table>
<thead>
<tr>
<th>Source</th>
<th>Stiffness (GPa)</th>
<th>Strength (MPa)</th>
<th>Failure Strain (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTM-Chitosan Films Vacuum Filtration</td>
<td>11</td>
<td>76</td>
<td>.97</td>
<td>[115]</td>
</tr>
<tr>
<td>MTM-Chitosan</td>
<td>7</td>
<td>100</td>
<td>3.3</td>
<td>[115]</td>
</tr>
</tbody>
</table>
The advantage of vacuum filtration over solution casting in terms of mechanical properties is clearly limited as minor increases in stiffness are matched by a decline in strength [115]. Practically speaking, vacuum filtration is useful to reduce drying times, but appears to offer no significant benefits in terms of mechanical properties or structural organization.

A major challenge in synthesizing bio-inspired films is achieving ordered structures over large areas [116]. This is due to the fact that very small imperceptible defects at the nano-scale level can accumulate leaving pockets of local order that are not macroscopically ordered [116]. Liquid crystals are a state of matter that exhibits long-range order due to their anisotropic molecular structure. 2D materials with large aspect ratio, such as graphene oxide, can exhibit liquid crystal behaviour at very low concentrations and in turn be formed into films with long-range order [116, 117].

<table>
<thead>
<tr>
<th>Solution Casting</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly Exfoliated MTM-Chitosan Film (No Brick and Mortar Structure)</td>
<td>1.6</td>
<td>37</td>
<td>3.98</td>
<td>[115]</td>
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</tbody>
</table>
Table 5 Mechanical performance of liquid crystal based films

<table>
<thead>
<tr>
<th>Source</th>
<th>Stiffness (GPa)</th>
<th>Strength (MPa)</th>
<th>Failure Strain (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Crystal GO/ PVA Films</td>
<td>10</td>
<td>250</td>
<td>2.5</td>
<td>[116]</td>
</tr>
</tbody>
</table>

No Mechanical Properties of PVA were reported in the references [116, 117]

Key considerations of colloidal casting

Colloidal casting works because the large aspect ratio stiff phase (clay, graphene, or cellulose fibres) naturally aligns face to face as the liquid is removed [105]. This is due to energetic considerations; bonding is stronger face to face than edge to face. As a consequence, solution casting is a very flexible process; any material/polymer combination that can form a stable dispersion can in theory be used to a bio-inspired film. Despite the apparent simplicity of colloidal casting a stable dispersion is a necessary but not sufficient condition for forming a strong and tough bio-inspired film [105].

There are four key critical factors in synthesizing strong, tough and stiff bio-inspired films using colloidal casting:

Building Block Selection
The strength, stiffness, and aspect ratio of the stiff “building block” phase is critical to mechanical performance of solution cast films [105]. The stronger and stiffer a “building block” then the better the mechanical properties of the composite. For example, carbon nanotubes and graphene produce both the strongest “building block” and strongest composite (Figure 22) [111, 118]. In practice the majority of research papers utilize nanoclay because of their low cost, availability at the 10’s of grams scale, and ease of dispersion in water. Comparatively, graphene and carbon nano-tubes are expensive, only available in commercial quantities after late 2014, and more difficult to disperse in water. Due to the increased availability and high performance of graphene and graphene oxide, research is now directed towards the use of graphene or graphene oxide in bio-inspired composites [106-109]

Figure 22 Strength vs. Stiffness for common building blocks of bio-inspired films
The mechanical properties of bio-inspired films made from different building blocks [105]

*Polymer Selection*

The polymer phase must be both tough and when dissolved in water stabilize the building blocks [105]. This is achieved in nacre and bone through structural control of the polymer and the use of reversible bonds [41, 42, 46, 49, 50, 69]. However, almost all colloidal casting approaches utilize commercial polymers followed by the post-infiltration of reversible cross-links such as Ca\(^{2+}\), Cu\(^{2+}\), glutaraldehyde and borate [46, 100, 113, 119]. To the candidate’s knowledge, only a single recent study uses rationally designed polymers with supra-molecular bonding; 2-ureido-4-pyrimidinone, grafted to poly (oligoethylene glycol methacrylate). The study created a dynamically self-healing polymer phase that exhibited both increased stiffness *and* strain at failure [113].

*Interface Engineering*

The interfacial strength between the polymer phase and the “building block” can be the limiting factor in mechanical performance of the composite [105]. This is because even a composite made of the stiffest “building block” and toughest polymer inter-layer mechanical performance is compromised by insufficient interfacial bonding [105]. Covalent bonds do produce very stiff and strong composites but severely compromise toughness [105]. Comparatively, supra-molecular bonds, primarily hydrogen and ionic bonds, are not as strong and stiff as covalent bonds but exhibit greater extensibility [113, 120]. Strong interfacial bonding, often
requiring surface modification of fillers, is a key factor in the mechanical performance of alumina films [72].

Quality of Dispersion

It is non-trivial to fully disperse nano and micron sized particles in a liquid. This is problematic because any aggregation of particles upon drying compromises the structure of the resulting film [104]. To maximize the mechanical properties of the composite great care must be taken to disperse and stabilize the nanoparticles. However this approach is often challenging; it requires the selection of compatible polymer/filler pairs, pH control, purification steps to remove aggregates, and intense mechanical mixing by either high-shear or sonication [104].

Limitations of the colloidal casting literature-

Inability to produce multi-scale structure
The major drawback of colloidal casting for synthesizing bio-inspired films is the inability to produce multi-scale films; this is critical because natural materials are almost universally multi-scale [1-6, 8, 9, 32, 33]. The difficulty in producing multi-scale films arises because micron scale particles, such as alumina, do not easily form stable dispersion in comparison to nanoparticles. This is because particles are stabilized by electrostatic repulsion due to the electric double layer at the particles surface. As micron sized particles have inherently smaller surface to volume ratios than nanoparticles, they are more difficult to stabilize. The inability to readily include multi-scale structure in colloidal casting is a major drawback to creating truly bio-inspired films.
Opaque mechanical comparisons
The mechanical data of colloidal cast films presented in the literature is often misleading and not truly comparable between studies. Recently Peng et al [108] developed graphene/chitosan composite with excellent strength (550 MPa) and toughness (10% failure strain) in a [108]. However the Young’s Modulus was only 5.5 GPa. Considering that the stiffness of graphene is estimated to be 1000 GPa, the reinforcement efficiency of this developed composite is quite poor [108]. In contrast, natural materials such as bone display an excellent combination of all three properties (strength, stiffness, and toughness) [1], though replicating these combined properties in a synthetic material remains a major challenge.

Aggregation of nanoparticles
Aggregation, the tendency of dispersed materials to form large agglomerates, is a major problem in colloidal casting and nano-materials processing. It has three major consequences:

1. **Low concentrations of nanoparticles dispersions**
   In order to prevent aggregation, the concentration of the dispersion needs to be low, typically < .1% by weight, to prevent agglomeration. This means that large volumes of water needs to be either dried or filtered to produce even relatively thin 50 micron thick films. This adds time and, in a commercial setting, cost to the process.

2. **Limited Volume Fractions**
The need to stabilize nano-materials against aggregation makes it difficult to produce volumes fraction of reinforcement greater than 0.4 in dried films. This is lower than volume fraction of bone ~0.6 [40]. The issue is particularly important for micron size fillers. Bonderer et al [72] reported volume fractions of 0.2 for LbL films [72]. Further increases in filler content resulted in a deterioration of mechanical properties due to brittle failure. This is a problem because the mineral content of bone is typically much higher (~.6) than achievable for colloidal films (~.2).

3. **Initiation of Defects**

Upon drying, aggregates form weak points that act as stress concentrators. Under strain these points initiate premature fracture, compromising mechanical performance. Care must be taken to avoid introducing defects into the film.

**3.1.2 Bio-mineralization**

Bone, and other mineralized tissue, grow by depositing minerals onto a polymeric template [6]. In principle this is an obvious and effective technique for producing synthetic materials. Unfortunately progress in this field has been disappointing [6]. Synthesis by bio-mineralization is slow, painstaking, and produces only micron-sized samples [6, 121-123]. Furthermore, the resulting materials possess inferior mechanical properties compared to their natural counterparts [6, 121-123]. While we do not believe that bio-mineralization is a viable path to synthetic composites, they do provide an excellent model system to study the still poorly understood process of bio-mineralization [18-20].
3.2 Bulk composites

Freeze casting

Freeze casting, or ice templating is an ingenious method to form bio-inspired composites that are both ordered at multiple length scales and macroscopic in size [6, 97]. The key steps of the freeze casting process are outlined below and in Figure 23:

1. Directional freezing of a ceramic suspension to produce lamellae ice crystals with ceramic particles and dispersants concentrated between the lamellae [31, 124].

2. Sublimation or freeze drying of the ice to leave continuous and oriented ceramic structure ready for sintering [31, 124].
   a. For a “bricks and mortar” structure the sublimed ceramic structure can be compressed before sintering [124].

3. Sintering of the ceramic phase to fuse the orientated ceramic structure at ~1500 C° [31, 124].
4. Infiltration of a “soft” polymer or metal phase [31, 124].

**Figure 23 Freeze Casting**

Left, the directional freezing apparatus for freeze casting. Note how the slurry freezes from the bottom to the top, to achieve the desired lamellae structure [6]. Right, ice particles are concentrated between the growing ice lamellae.

Freeze casting is a complicated multi-step yet flexible process that has been extensively developed by the Ritchie and Deville groups [6, 125]. It can produce bulk composites with ~80% ceramic content. For example the thickness of lamellae can be controlled by the freezing rate (down to a thickness of 40 μm) [6]. A nacre-like “bricks and mortar” structure can be developed by compressing sintered lamellae to produce an extremely tough material [124]. The roughness of the lamellae can be controlled by varying the interfacial tension of ceramic suspension through the addition of sucrose, which leads to the formation of mineral bridges [6, 124]. The infiltrated “soft” phase can be polymers, metals, or even glass [6, 125]. The ceramic
phase can be alumina, hydroxyapatite, silicon carbide, or any other particle that can be sintered and dispersed in solution [6]. Interfacial bonding, critical for mechanical strength, between the ceramic and soft phase can be enhanced by the use of a grafting agent [124]. Although a flexible process, freeze-casting also suffers from the large number of variables that must be controlled; it is difficult to achieve reproducibility [126].

The outstanding feature of freeze cast ceramics is that they have a remarkably ordered structure similar to natural materials such as bone and nacre, albeit at much larger scales (Figure 24 a, b, and c are nacre and d, e and f are freeze cast).

**Figure 24 SEM of nacre (top row) and freeze cast ceramics**

Comparison of nacre structure and freeze cast structure at similar length scales [125]. Scale bars, 10 μm (a, d); 500 nm (b, e); 250 nm (c, f).

Freeze cast composites produce materials that not only exhibit similar structure to nacre, but also have outstanding combination of strength and toughness, (Figure 25) and below [6, 125]
**Figure 25 Strength vs. toughness for bio-inspired alumina**

A plot shows the trade-off between strength and toughness for various alumina ceramics. Note how the nacre-like alumina, red, lies outside the beige area defining the combination [125]

The improvement in toughness seen in freeze cast alumina, 300x in energy terms, is still an order of magnitude less than the toughness in nacre (3000x), but a significant achievement [124]. The mechanism of the improved toughness is believed to be similar to that of natural materials with multiple mechanisms acting over the entire volume of the material. Posited mechanisms include crack deflection, polymer shielding and uncracked ligament bridging [124]. Experimentally, this is evidenced by distinct rising R-Curve behaviour, indicating the presence and effectiveness of extrinsic toughening mechanisms [31, 124, 125].

Freeze casting, like all processes, has some significant drawbacks:

*Lack of Nano-scale Control*
The major drawback of freeze casting is that it does not allow structural control at the nano-scale level, a key feature of natural materials [31, 124, 125]. There appears to be no obvious route to decrease the thickness of the lamellae as this thickness is dictated by kinetics of ice crystal formation. Using nanoparticles instead of microparticles leads to, for unknown reasons, a dramatic but well documented decline in the strength of the composite [126]. This difficulty in integrating nano-scale structural features is a major drawback and there has not been sustained improvements in material’s mechanical performance since the initial freeze casting publications [124, 125].

**Complexity and Energy**

The freeze casting process is extremely complex. A recent review remarks that its complexity is beyond what can be communicated in a single academic paper [126]. One needs to control the formulation of the suspension (nature of the solvent, particle size of the starting powder, solid loading, binder, surfactant, nature of the material, pH, viscosity), and freezing conditions (setup, temperature, cooling rate) [126]. Only a few of these variables have been systematically studied [126]. When factored in with the need for multiple sintering steps (at 1500 C°) the complexity and cost of freeze casting is a major issue.

In summary, freeze casting is an effective technique for producing multi-scale structures but the lack of nano-scale structural control prevents it from being an
effective tool in synthesizing composite materials that replicate the structures of bone and nacre.

### 3.3 Additive manufacturing

Additive manufacturing or three-dimensional (3D) printing, as opposed to traditional subtractive manufacturing such as milling, is a diverse set of techniques that build an object LbL under computer control (Figure 26). Additive manufacturing has the ability to synthesize 3D objects from plastics (3D printing and photo-polymerization), ceramics (Figure 26a), and metals (Figure 26b) in precise structures directly from a computer model [6].

![Additive manufacturing](image)

**Figure 26 Additive manufacturing**

a) Nozzle directed deposition of ceramics to form a porous glass object. The resolution of nozzle directed deposition is typically limited to 100 micron; resolutions are limited by the rheology of the ink that contains the solid material. b) Droplet deposition followed by the laser sintering of the droplets to form a solid object, often called selective laser sintering [6].
In theory, the ability to precisely synthesize similar objects with minute changes in structure is ideal for establishing structure-property relationships by rigorously testing the theory of bio-inspired composites in real materials. Additive manufacturing is particularly suited for structure-property relationship studies because of the ease at which such theoretical designs can be synthesized. This aim is very similar to that of mechanical meta-materials where nano-scale micro-structural control has allowed the synthesis of rationally designed structures with unique combinations of mechanical properties [75, 127, 128].

The use of additive manufacturing to produce bio-inspired materials is in its infancy. 3D printing of bio-inspired macro-scale models has confirmed the ability of bio-inspired composites to delocalize damage but has yet to be extended to nano-scale work [88, 89].

Recent work by the Greer group has extended the resolution of 3D printing to ~100 nm using two-photon lithography to produce intricate structures that are subsequently coated with alumina using atomic-layer deposition [127]. Greer et al were able to produce hierarchal nano-composites, rationally vary their structure, and correlate enhanced resilience of the material to structural hierarchy (Figure 27 bottom row) [127].
**Figure 27 Two-photon lithography synthesized multi-scale structures**

Top row: images of hierarchal structure of struts starting from a normal strut (0\textsuperscript{th} order) to a single octahedral strut (1\textsuperscript{st} order) followed by subsequent structural levels. Middle row: CAD images of the rationally designed hierarchal structures. Bottom row: SEM Images of the 3D printed structures (scale bar is 20μm). [127]

The impressive resolution of two-photon lithography comes at the cost of speed. A reported write speed of 50μm/s means that this process is currently too slow to produce macroscopic materials for real life applications. Unfortunately, additive manufacturing techniques which can produce macroscopic materials, such as robo-casting and stereolithography is limited to the tens of microns [6].
**Drawbacks of additive manufacturing**

Despite the obvious benefits of additive manufacturing there are substantial drawbacks outlined below:

*Nano-scale control and scalability*

There is a complete lack of techniques for producing materials with nano-scale resolution that are macroscopic in size [6]. The current ability of additive manufacturing to produce micron scale features suggests that if this could be achieved then ordered structures from the macro to the nano-scale would become possible.

*Limited Material Selection*

Bio-inspired composites consist of two dissimilar materials; this presents substantial problems in material processing. For example, ceramic and metal-based structures require a sintering step (from a laser or as part of post-processing) to solidify the material and this inevitability destroys the polymer phase. Although post-processing, such as atomic layer deposition in two photon-lithography, can combine two differing materials ideally elaborate post-processing should be avoided [127]. The ability to print both soft and hard phases simultaneously would dramatically include the utility of additive manufacturing.

**3.4 “Top down vs. “Bottom up”**

All natural materials possess a multi-scale structure organized from the nano to the macro scale, a feat that has not been matched in synthetic materials [1-6, 8, 9, 32,
The inability of any existing technique or combination therein to achieve multi-scale material remains the “Achilles heel” of bio-inspired composite design. Multi-scale structures are achieved in nature by self-assembly and we believe that “bottom-up” self-assembly is the only viable approach to produce nano-scale bio-inspired materials [1-6, 8, 9, 32, 33].

“Top-down” approaches, such as 2-photon lithography, are simply too slow and tedious to produce macro-scale materials [127]. The ability of 2-photon lithography to accurately reproduce structures that have been rationally designed from first principles to experimentally test structure-property relations is, however, extremely valuable but only, we believe, for academic research [127]. Mechanical metamaterials, materials, whose property derives from structure rather than intrinsic material properties of the unprocessed material, highlights how mechanical properties, such as mechanical cloaking and negative Poisson ratios, can be achieved through only structural control [129, 130]. We believe, however, that “top down” 3D printing will be critical in linking the first principles design of mechanical meta-materials to the structural features of natural composites.

“Bottom up” colloidal casting can produce a large area (5cm by 5cm) of nano-scale thick films using a wide variety of materials by simple drying. “Bottom up” approaches require tight control of all processing steps but are uniquely capable of producing nano-scale materials in large quantities. As a consequence, we believe that “bottom up” self-assembly is the only potentially scalable and cost-effective
approach for producing bio-inspired films. This thesis will focus on the use of colloidal casting due to the simplicity and flexibility of this procedure. The major drawback of colloidal casting is adding structural hierarchy to films, which will be a major focus of this thesis.
3.5 Synthesis of 2D layered materials

In order to synthesize bio-inspired materials using “bottom up” colloidal casting it is necessary to produce dispersions of 2D materials that are stable, have a high monolayer content, and can be produced in sufficient quantities for mechanical testing. This is non-trivial, as commercially available dispersions are both costly and, as we found, are produced in low quality [131, 132]. Therefore in this thesis we developed our own dispersions method to synthesize the bio-inspired materials.

In this section we briefly review methods for producing layered materials for use in colloidal casting.

Layered materials, such as graphene, consist of covalently bonded sheets of atoms held together by comparatively “weak” Van der Vaal's forces [133], and when exfoliated into a monolayers these sheets are exceptionally strong. Graphene is the strongest and stiffest material known to science [133]. Unfortunately the “weak” Van der Vaal's forces (in comparison to covalent bonds) are still difficult to overcome; it was not until 2003 that graphene was isolated from graphite by repeated stripping by Scotch Tape [134]. The famous “scotch tape” method is completely useless for composites because the quantity of material produced is so miniscule [131, 132]. As a consequence, a crucial focus of applications based research into layered material is developing scalable synthesis routes. Table 6 summarizes and rates the most established synthesis approaches.

Table 6 Synthesis routes for the production of 2D layered materials
<table>
<thead>
<tr>
<th>Method</th>
<th>Simplicity</th>
<th>Yield</th>
<th>Sheet Size</th>
<th>Monolayer Content</th>
<th>Scalability</th>
<th>Presence of Defects</th>
<th>Cost</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Exfoliation [131, 132]</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>Sheet size in the 10’s of μm</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>Only suitable for academic studies</td>
</tr>
<tr>
<td>Scotch Tape Method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical Vapour Deposition</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>Produces sheets in the cm size range</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>Suitable for large area monolayers for electrical applications such as touchscreen but too expensive for composites</td>
</tr>
<tr>
<td>Electrochemical Exfoliation [131, 132]</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Mixture containing some monolayers</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>Only usefully for conducting materials e.g. graphene</td>
</tr>
<tr>
<td>Requires a potentiostat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid Phase Exfoliation [133, 135]</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>&lt;5μm</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>Produces a heterogeneous dispersion of multilayers but is simple and scalable.</td>
</tr>
<tr>
<td>A kitchen blender is sufficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionic Liquid Exfoliation [136]</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>~2 μm</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>Promising but only 1 report and bespoke ionic liquids are too expensive</td>
</tr>
<tr>
<td>Requires bespoke ionic liquid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive use of strong oxidants and concentrated H₂SO₄ leads risk of explosions</td>
<td>4</td>
<td></td>
<td>4</td>
<td>Large Sheets that form liquid crystals</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>Graphite specific and chemically quite different to its precursor.</td>
</tr>
<tr>
<td>Graphene Oxide [131, 132]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1=poor, 5=excellent
We believe that liquid phase exfoliation is the most suitable method for synthesizing layered materials for use in composites. It easily produces sufficient quantities of layered material, requires no specialist equipment or hazardous chemicals can produce a wide variety of materials, and causes no chemical damage to exfoliated material [133, 135]. Furthermore, liquid phase exfoliation is the most established route; a recent and exhaustive paper by Coleman et al (137) clearly established the key processing parameters and demonstrated the technique at the pilot plant scale [135].

**Table 7 Synthesis routes for 2D layered materials**

Comparison of synthesis methods for layered materials suitable for use in composites.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pro's</th>
<th>Con's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical Exfoliation [131, 132]</td>
<td>Large sheets, high yield</td>
<td>Inconsistent product due to varying voltage. Oxidative damage</td>
</tr>
<tr>
<td>Liquid Phase Exfoliation   [133, 135]</td>
<td>Simple, scalable, no oxidative damage</td>
<td>Low yield and heterogeneous product</td>
</tr>
<tr>
<td>Graphene Oxide             [131, 132]</td>
<td>High yield, water soluble</td>
<td>Batch to batch variation, risk of explosion, highly oxidized product, only for graphite</td>
</tr>
</tbody>
</table>

Electrochemical exfoliation is limited to the exfoliation of graphite because the exfoliated material must be conducting. This is a significant drawback as graphene is but one of hundreds of 2D materials most of which are not conductors [133]. While the graphene oxide route is also limited to graphite, it possesses the additional
disadvantage of significantly reducing the mechanical performance of the graphene sheets [131, 132]. Therefore, liquid phase exfoliation was the method used in this thesis due to its relative simplicity, flexibility, and existence of more established synthesis protocol.

Liquid phase exfoliation is by no means a perfect technique. Its major drawback is the heterogeneous nature of the produced dispersion consisting of a mixture of multi-layer and monolayer sheets of differing lengths. It is both challenging to purify the dispersion and to measure the quality of the monolayer content of the dispersion. The goals of Chapter 2 and 3 of this thesis are to develop methods to purify the dispersion and to develop methods to accurately and rapidly characterize the dispersions.
4.0 GAPS IN THE LITERATURE - ORIGINS

Having extensively reviewed the literature regarding the structure-property relationship of bone and approaches to synthesizing multi-scale composites, it is clear that there are significant gaps in the literature. We believe that such gaps are not solely the result of poor scholarship or research interests. Instead, we believe that these gaps in the literature arise because of more general limitations from other scientific fields (such as microscopy). In this section we identify some of these systemic shortcomings and explain their significance and origin.

4.1 Multi-scale nature of fracture and bone’s structure

Fracture is a multi-scale process that arises due to complex interactions from nano to the macro scale [60]. As a consequence bone’s structure, which resists fracture, is also multi-scale [1-6, 8, 9, 32, 33]. This makes the fracture of bone a difficult problem to study for two major reasons:

Multi-scale imaging is challenging

Ideally one would simply observe a single nano-sized crack and watch it grow to a macroscopic crack under increasing strain. Unfortunately this is not possible with current imaging techniques [137].
Figure 28 Length scale of structural features and imaging techniques

The length scales of biological features (top row) matched with the achievable resolution of light and electron microscopy (bottom row) [138].

No technique exists that can resolve nm sized “cracks” and has the field of view necessary to image an macroscopic entire sample (Figure 28) [137, 139]. This is particularly limiting for the study of fracture, which is by its nature is random and its exact path is extremely sensitive to rare events. This means that the location of the critical crack cannot be known a priori and therefore the entire sample must be imaged at very high resolution to follow the growth of the critical crack. Currently techniques such as confocal microscopy allow micron scale resolution but lack nm resolution [139]. Meanwhile powerful techniques such as atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission x-ray microscopy allow nm resolution but only over very small areas [139]. No single technique can currently bridge the nano and micron length scale.

The lack of appropriate techniques that can link the nm and micron scales represent a major problem; as a result it is very hard to link the myriad of toughening
mechanisms into a single coherent “system level” picture [139]. Consequently structure-property papers tend to use a single technique and compare mechanisms on the same length scale [53, 73, 80]. Without the development of multi-scale imaging technique, it is impossible to conclusively demonstrate how the different toughening mechanisms, acting on different length scales, work synergistically.

Fortunately, driven in part by the desire of the semi-conductor industry to image entire wafers, the emergence of the multi-beam SEM made it possible to produce nm images over square centimetre areas [140]. By using up to 91 electron beams in parallel (current SEM’s use 1 beam) the Zeiss Multi-SEM 505 was able to image an entire human osteon at 11nm resolution by stitching together multiple high resolution images [140]. Combining a Multi-SEM with in-situ tensile testing will yield novel insights into the fracture of bone and likely shed light on the interplay between the nano and macro length scales. The ability to image large-area samples using electron microscopes (EM) also opens the possibility to correlate the EM with fluorescence microscopy; combining spatial location of specific proteins with crack growth data [141]. Currently the expense, limited availability of machines, and limited throughput hampers the use of multi-scale SEM imaging. We believe in time it will prove to be an exceptionally valuable technique for multi-scale fracture mechanics.
**Multi-scale simulations are too complex**

Computer simulations of multi-scale composites that include nano-scale features are simply too computationally intensive to simulate macroscopic composites [95]. As a result, multi-scale modelling generally simulates a nano-scale unit cell, evaluates its mechanical properties, then scales the calculated properties to a larger length-scale [142]. This approach works well for properties that can be meaningfully averaged or “homogenized”, such as stiffness, but not for properties, such as fracture resistance, that are a function not of the average but the extreme values of an ensemble of similar objects. The dependence of fracture on the extreme values of properties, such as strength, means that typical multi-scale simulations are of questionable accuracy. That is not to say that multi-scale simulations are not extremely valuable, but they must be rigorously tested by experiments, as practiced by Buehler [143]. In summary, current computer simulations cannot reliably account for emergent effects, which arises at the system level, and can most therefore be taken in generalizing their results.

**Difficulty of multi-scale synthesis**

In this literature review we have stated repeatedly that multi-scale synthesis is challenging. Fundamentally, this is because the relative importance of forces (inertial, electromagnetic, electro-static, Van der Vaal’s, capillary etc.) varies with length scales [144]. Therefore, using a single force, such as the electrostatic force, is not sufficient for multi-scale synthesis.
Consider the change in the surface area of a sphere \((4\pi r^2)\) to the volume of a sphere \(\left(\frac{4\pi}{3} r^3\right)\) as \(r \to 0\) the relative importance of surface forces (such as capillary action) increases, compared to body forces (gravity). For example, the papermaking route exploits the drying of stable colloidal dispersions (stabilized by electrostatic surface forces) but cannot currently incorporate micron scale fillers to add multi-scale structure because large particles sediment out in solution due to gravity. The need to manipulate different forces (often non-orthogonally) is a fundamental reason why multi-scale synthesis is so difficult.
4.2 Gaps in the literature - thesis focus

The field of natural and bio-inspired composites is relatively new and immature, consequently substantial gaps exist in the literature. In this section we focus on the gaps in the literature that we will address in this thesis.

Structure-property relationship for bone’s toughness

The origin of bone’s toughness is still unclear, specifically the role of hierarchy in transferring nano-scale flaw tolerance to the macro-scale. In the first chapter of this thesis we seek to explain the mechanistic origin of flaw tolerance and how/why it can be translated to the macro-scale.

No mechanism for nano-scale flaw tolerance

Despite its general acceptance there is no sound theoretical or mechanistic underpinning behind the concept of nano-scale flaw tolerance [43]. Rather an observation exists that if we apply the equations of linear-elastic fracture mechanics (LEFM) at some point any object must eventually become flaw tolerant [43]. This is tenuous at best; a key assumption of LEFM, that the propagation of elastic waves is not impeded by material microstructure is manifestly false at the nano-scale.

Thesis contribution

In chapter 1 we outline a plausible mechanism for nano-scale flaw tolerance, the localization of elastic waves, and resulting reduction in redistributed elastic strain energy available to drive crack growth.
The role of hierarchy

Bone is a material with exotic and unexpected mechanical properties (both strength and toughness) that possesses an internal structure that has dimensions similar to that of elastic waves. During the past 15 years there has been a realization that structural control of length scales of the same dimensions as propagating waves (be they elastic or electromagnetic) can lead to exotic meta-materials with properties such as super-resolution [145], invisibility cloaks [146], mechanical “unfeelable” cloaks [147], and resilient hierarchal structures [75, 127, 129]. Chapter 1 demonstrates how the principles of meta-material design can be used to explain bone’s unique combination of strength and toughness.

Thesis contribution

In chapter 1 we also explain from first principles, how nano-scale flaw tolerance is transferred to the macro-scale through a multi-scale and heterogeneous structure that localizes elastic waves of all wavelengths. Specifically, we demonstrate that the interface between “stiff” and “soft” phases is critical to ensuring elastic wave localization.

Multi-scale films

It is currently impossible to synthesize multi-scale materials with both nano and micron feature size in a scalable manner [1, 5]. In this thesis we sought to address these limitations.
Thesis contribution

In chapter 2 we apply a novel photo-luminescence based technique for measuring the monolayer content of nanoparticle dispersion. The synthesis and characterization of nanoparticle dispersions is a perquisite for the “bottom up” synthesis of nano and multi-scale composites. In chapter 2 we outline a novel method for characterizing nanoparticle dispersions.

In chapter 3 we use the photo-luminescence based technique, developed in chapter 2, to assess the efficacy of novel liquid phase exfoliation synthesis techniques. Current exfoliation techniques give low yields (.01) and we develop an effective methodology for comparing the efficacy of different methods (sonication, shear mixing, laser cavitation etc.).

In chapter 4 we develop a novel process, using transient networks, to prepare multi-scale films with a bone like structure. We utilize the nanoparticle dispersion optimized in chapter 2 and 3 as the nanoparticle phase.
CHAPTER 1: HYPOTHESIS: BONES TOUGHNESS ARISES FROM THE SUPPRESSION OF ELASTIC WAVES

Statement of novelty:

Under what physical principle nano-scale flaw tolerance and multi-scale structures operate to form a strong and tough composite, such as bone, is an open question. In this chapter we outline, for the first time, how both nano-scale flaw tolerance and multi-scale structures can be understood to produce strength and toughness through phonon localization. We explain how bone’s hard-soft structure (necessary to produce strongly scattering interfaces) and multi-scale structure (necessary to scatter phonons (packets of strain energy) of all wavelengths) localizes or suppress the propagation of strain energy. We then outline a modification to Griffith’s criterion to show how strain energy localization produces a strong and tough composite by preventing the transfer of strain energy to the crack tip, therefore preventing unstable, catastrophic crack growth. We then argue that bone should be considered a mechanical meta-material because bone consists of repeating units designed to manipulate the propagation of waves in a manner to suppress strain energy transfer to crack tip.

Research output

1. Introduction

Natural structural materials such as bone and nacre can have a resistance to fracture (toughness) that profoundly exceeds their major components. These materials appear to subvert the conflict between strength and toughness that limits the mechanical performance of synthetic materials [1-3, 5, 87, 148]. Flaw tolerance and toughness arise in natural materials due to local regions of high strain that undergo controlled deformation (plasticity) without initiating catastrophic fracture, as is the case with brittle but strong synthetic ceramics [1, 4, 5, 7, 12, 41, 43, 47].

Numerous individual extrinsic and intrinsic toughening mechanisms have already been identified experimentally in bone; in fact one of the major differences between bone and synthetic materials is the presence of many different toughening mechanisms [1]. What is lacking is a framework or principle that explains how each length scale and toughening mechanism acts synergistically at the system level. We propose that the hierarchical nano to macro-scale structure of natural materials has specifically evolved to attenuate phonons, which are the elastic waves that propagate strain and thermal energy. As a direct result, bone and other natural material demonstrate surprising toughness because phonon attenuation allows multiple mechanisms to operate separately by limiting the transfer of elastic energy between areas of locally high stress. Preventing such interactions allows for greater energy dissipation and toughness by delaying the formation of a catastrophic crack [5, 41, 87, 149].

Bone is a nano-scale composite and the nano-scale is accepted as vitally important
to bone’s toughness [43]. It is also known that below a critical length scale, HAp becomes flaw tolerant and that bone’s structure can “transfer” this flaw tolerance to larger length scales [43]. The existence of nano-scale flaw tolerance has been validated by the appearance of flaw tolerant behaviour in atomistic modelling [43], experimentally, [150] and through scaling arguments [43, 64]. Current explanations use the concept of hierarchy to explain how a flaw tolerant nano-scale can be “transferred” to the macro-scale [43, 87, 149]. However, they cannot explain the physical origin of this flaw tolerant length scale and therefore cannot provide a mechanistic explanation as to how and under what conditions hierarchy transfers flaw tolerance to the macro-scale [43, 87].

Ordinarily in linear fracture mechanics, the interface between collagen and HAp acts as a weak point for the initiation of a catastrophic crack leading to brittle fracture. Bone, however, is much tougher than a homogenous sample of hydroxyapatite, its major component [1, 4, 5, 7, 12, 41, 43, 47]. The difficulty in understanding arises because linear fracture mechanics cannot be applied at the atomic and nano-scale level. This is due to the effective medium assumption, where propagating elastic waves are not affected by the medium they travel through ($\lambda \gg MFP$, Wavelength and Mean Free Path Respectively) being violated at small length scales by the existence of atoms, nanostructure, and surface reflections. Conversely, atomistic modelling is currently too computationally intensive to be applied to simulations of macro-scale materials. In this article, we propose a novel explanation as to why heterogeneous materials can in fact lead to a tough material by invoking the non-
linear wave concept of phonon (elastic waves) localization [87, 151, 152]. We assert that multi-scale hierarchy, material heterogeneity, and the nano-scale nature of bone are the root causes of the plasticity seen in natural materials and explain how bio-inspired tough mechanical materials can subvert the conflict between strength and toughness [1].

This paper is structured to first introduce the concepts of phonons and describe how wave localization and attenuation can explain how strain energy is localized in an inhomogeneous medium. Developing this idea further, we explain how changes to the strain energy balance that underlies catastrophic crack propagation, changes when $\lambda \approx MFP$ (meaning a heterogeneous medium such as bone) in comparison to $\lambda >> MFP$ (a standard homogenous medium) to increase toughness. We then argue that bone’s heterogeneous and multi-scale structure is built on the principle of phonon localization.

2. Theoretical Background

Phonons are quanta of elastic waves that are responsible for strain and thermal energy propagation. Because phonons are waves, they can be scattered, attenuated, and impeded by the medium they travel through (when $\lambda \approx MFP$, Figure 29). Sufficient confinement and scattering of phonons can suppress strain propagation because the phonon amplitude decays with distance and becomes insignificant when the amplitude is smaller than the thermal and mechanical noise (Figure 1) [152].
This figure demonstrates how a inhomogeneous material can scatter waves through spatial variations in material properties and how a hierarchal structure scatters multiple wavelengths simultaneously [153]. Note that with sufficient distance, on the order of a few wavelengths, the scattering of the elastic wave reduces to amplitude to the point it is smaller than thermal and mechanical noise.

Size effects and phonons are intimately related because the wavelength of phonons possible within a material is a function of the material’s dimensions. In a material, the minimal resonant frequency is given by the atomic spacing, 2a, therefore any phonon in the material must be larger than 2a whilst also less than the dimension of the sample (2l).

\[2a<\lambda<2l\]

Multi-scale structures are therefore required for phonon localization in macro-scale materials because a phonon can only be scattered by a structural feature that is similar in size to its wavelength and a broad spectrum of possible wavelengths are
inherent in any macro scale material. In fact, bone’s hierarchical structure consists of superimposed periodic variations of differing lengths (Figure 29), resulting in superimposed bandgaps that suppress a range of phonon frequencies; orders of magnitude wider than a single scale microstructure [153]. A way to express the degree of phonon localization in a material is to use $\Psi$, defined below, as the proportion of phonon energy that propagates freely throughout a material.

$$\Psi = 1 - \frac{\sum_{\lambda=2a}^{2l} \lambda h D(\lambda)_{\text{heterogeneous}}}{\sum_{\lambda=2a}^{2l} \lambda h D(\lambda)_{\text{homogenous}}}$$

Equation 8 Phonon localization

$D(\lambda) =$ Density of States, $\lambda =$ Wavelength, $h =$ Planks Constant

Phonon localization links directly to crack propagation because it dramatically affects the energy balance that underlies fracture mechanics. This is the ubiquitous Griffith criterion. The Griffith criterion states that when redistributed strain energy released by an advancing crack is greater than energy required to open the crack, crack growth becomes self-sustaining and catastrophic fracture ensues ($G_{\text{Critical}} > 2\gamma$, Figure 30). Due to the fact that crack growth is self-sustaining, no further external input of mechanical energy is required for fracture and toughness is comparatively low. As the strain energy released from the crack tip are phonons, then as $\Psi \rightarrow 1$ some, and potentially all of the strain energy released during crack propagation, is stopped from reaching the crack tip and sustaining further crack propagation. Strain energy that ordinarily drives catastrophic crack growth is dissipated as heat and deformation, effectively increasing the critical strain energy release rate and
therefore material toughness. Previous studies have found that energy losses between the process zone and crack surface can be significant and have been observed to limit the maximum speed of crack propagation [154, 155]. As a result, Atrash et al argued that in the case of a moving crack, the Griffith criteria should be modified to account for phonon transfer losses [154, 155]

\[ G(V)_{\text{Critical}} = 2\gamma + G(V)_{\text{Phonon Loss}} + G(V)_{\text{Plasticity}} \]

**Equation 9 Griffith criterion modified for phonon localization**

\( \gamma = \text{surface energy}, \ G(V)_{\text{Phonon Loss}} = \text{energy lost due to phonon localization}, \ G(V)_{\text{Plasticity}} = \text{energy lost due plasticity}. \)

Therefore if \( \Psi = 1 \), once a crack begins to propagate, it is dynamically arrested as \( G(V)_{\text{Phonon Loss}} \) is increased due to the disruption of energy flow between the process zone and crack tip. In practice, this is the same as utilizing materials with a large \( G(V)_{\text{Plasticity}} \). In fact, we have demonstrated in this paper, through nothing more than a simple energy balance, that a material of sufficient heterogeneity can become tough precisely because it is sufficiently heterogeneous.

This is a counter-intuitive and novel result. Consider an otherwise homogenous material where the existence of a single crack and associated regions of locally high stress will quite obviously weaken the material. Yet in the case of a highly heterogeneous material with many regions of locally high stresses, such as bone, we demonstrated above that if \( \Psi \rightarrow 1 \), then a material with many “defects” can be tougher than a homogenous material with a single defect. We stress that energy is
not lost, but rather dissipated as heat, elastic deformation and plastic deformation. The key difference is that released strain energy is no longer able to drive self-sustaining crack growth that leads to brittle fracture, instead strain energy is dissipated locally.

Beyond a single crack energy balance, phonon suppression also eliminates the long-range elastic field and hence the self-organizing avalanche-like dynamics of multiple cracks [61, 64, 156]. Fracture can be treated as a dynamic phase transition characterized by a continuum of two universality classes: at small scales (and large disorder) by the percolation universality class and at larger scales (and small disorder) by the pinned elastic line universality class [61, 64, 156]. The difference between both universality classes is the absence and presence of long-range interactions respectively [61, 64, 156]. Physically, the origin of the long-range elastic interactions is the phonon. Thus a flaw-tolerant, Ψ=1 material eliminates long-range interactions between many cracks because the propagating elastic waves are suppressed [64]. This corresponds to the percolation universality class: a continuous phase transition characterized by extensive and diffuse localized damage and increased toughness (Figure 2b) [64].
Figure 30 Effect of phonon localization on crack growth
Phonon suppression leads to both stable crack growth of individual cracks (part a) but also eliminates the interaction of multiple cracks (part b) leading to extensive diffuse damage (black) before the final fracture (red) [64]

The concept of phonon localization is also elucidating because it explains why the disconnect between linear fracture mechanics and atomic modelling exists (Figure 30) [87]. This disconnect arises because materials are made of atoms but continuum mechanics assumes that $\lambda >> MFP$. This assumption is made quite rationally to ensure that the resulting equations are analytically tractable. Naturally, at the atomic length scale when $l \rightarrow a$, this assumption is not valid and $\Psi \rightarrow 1$. The physical mechanism is that defects in the atomic structure of the material and reflections at the surface of the material surface are sufficient to localize all permissible phonons. Intriguingly, $\Psi \rightarrow 1$ and flaw tolerance is possible at the macro-scale for hierarchical materials if multiple length scales attenuate the extra phonon wavelengths that are...
permissible when $l \rightarrow$ macro-scale [151, 153]. The suppression of larger wavelengths is critical because as the size of the material increases, the number of possible phonons wavelengths increase, and without a larger scale structure these phonons can propagate strain energy freely.

We note that hierarchal structures approximate the case of infinite disorder in the random fuse model used by Shekhawat et al on their analysis of the effect of material disorder and size on fracture. Thus, theoretically we show that through phonon attenuation a hierarchal and heterogeneous structure can increase toughness. This is done by dynamically by arresting cracks, and preventing existing cracks from acting co-operatively to cause catastrophic fracture.

3. Results

What evidence is there that bone actually suppresses phonon propagation? Bone exhibits the generic microstructure required to localize a broad wavelength of phonons: a large reflectance between collagen and HAp(Reflectance =.83, S3) and a hierarchical structure capable of localizing multiple phonon wavelengths simultaneously (Figure 31) [5, 152, 153] (Figure 31). We have estimated the propagating elastic energy by calculating the transmission of a wave through 3 and 5 consecutive interfaces with the same reflectance as collagen and HAp(Reflectance =.83, S3) being .5% and .014% respectively. This corresponds to $\Psi = .995$ and .99986, for a single wavelength over a distance of 3 and 5 wavelengths respectively. We note that a collagen matrix when hydrated is viscoelastic, leading to the adsorption of elastic waves. Though not included in the above analysis for simplicity, this
property would only serve to increase phonon attenuation because elastic energy is dissipated by the viscous component of the collagen matrix.

The attenuation of phonons over distances only 5x the phonon wavelength is very relevant to fracture mechanics. The process zone is the region in front of the crack in which released strain energy (from crack growth) is used to grow the crack in a self-sustaining and catastrophic manner. Phonon attenuation or suppression places a limit on the size of the process zone that can contribute to self-sustaining crack growth and therefore the energy available for catastrophic crack growth. This is because released strain energy is not available to appreciably propagate energy to the crack tip and therefore do work to drive crack propagation if it is more than 5x the phonon wavelength from the crack tip. Instead the strain energy is dissipated locally as increased temperature, elastic deformation, and stable plastic deformation.

Achieving flaw tolerance is possible with homogenous structures at small length scales due to edge reflections alone, but it can be achieved at the macro-scale if structural features are present similar in size to the longer wavelengths that become permissible in larger materials. Although data for bone or HAp doesn’t exist, an estimate based on crystalline semi-conductors shows that 50% of phonon energy is propagated by wavelengths greater than 2500 (+/-800) nanometres at 300K [157]. This indicates that for materials larger than a few microns in dimension, over half the phonon energy can propagate freely. Consequently, structural features to
suppress the propagation of phonons with wavelengths greater than 1 micron (which also have large mean free paths) are required for flaw tolerant macro-scale composites such as bone.

This mirrors the findings of a multi-scale computer model by Sen et al who found that increasing structural hierarchies increase the flaw tolerant size of a material [151]. Bone possesses the required features: a large reflectance between collagen and HAp(R=.83, S3) and a multi-scale structure to localize multiple phonon wavelengths [5, 152, 153]. If only an atomic-scale microstructure is present, larger wavelength phonons are not suppressed and thus elastic interactions (and hence self-organizing crack growth) are restored in bulk materials. In bone however, HAp and collagen form fibrils that form fibres, that in turn form lamellae, which are then arranged to produce osteons [12, 16, 41, 53, 73, 153]. This provides reflective interfaces and scattering at multiple length scales. Thus the heterogeneous nature of bone and other natural materials is rationalized by the need for large impedance mismatches to produce phonon scattering. Hierarchical structures in turn contain multiple length scales of variation in mechanical properties super-imposed upon one another to allow for much broader bandgaps than a single-scale periodic structure [87, 153]. If this band gap encompasses a large proportion of the permissible phonon wavelengths then flaw tolerance, even in macro-scale materials, is achieved [87, 153].

In terms of toughness, extrinsic mechanisms that operate only when a crack has
formed are believed to be responsible for the majority of bone toughness; our analysis does not dispute this. Instead, we argue diffuse and stable crack growth enabled by hierarchy *amplifies* the effect of extrinsic toughening mechanisms—because more diffuse micro-cracks means more crack bridging, deflection, twisting, and ligament bridging—and therefore increased energy dissipation before failure [1, 151].

![Multi-scale phonon confinement and localization in bone](image)

**Figure 31 Multi-scale phonon confinement and localization in bone**

Note the red arrows (incident waves) and dashed red arrows (reflected waves) are scattered at the interface between soft and hard phases. The large number of highly reflective interfaces of differing length scales allows multi-scale structures, such as bone, to localize a wide range of phonon wavelengths [87, 153].
4. Discussion

We point out that the idea of a periodically varying stiffness inhibiting crack propagation is not novel but accepted; as demonstrated by Kolednik in the case of an individual crack using linear fracture mechanics [82, 158]. These assumptions do not hold at the nano-scale because the assumption of a homogenous and isotropic medium breaks down. Consequently, this work can be viewed as extending the work of Kolednik. Our approach, unlike Kolednik’s, is valid at the nano-scale and therefore allows us to explain directly how nano-scale flaw tolerance is fed to the macro-scale. Furthermore our analysis can explain multiple interacting cracks, which Kolednik’s work cannot; by utilizing the scaling arguments of Shekhawat et al, we can show that cracks are unable to grow co-operatively in a highly heterogeneous material [64].

From a phononics perspective, we have described bone’s structure as a periodic system with bandgaps combined with added scattering; this is an effective strategy to achieve at least partial suppression of 3D classical waves [152]. A wave scattering strategy is advantageous compared to a flaw minimization strategy because unavoidable defects—be they from accumulated damage, introduced by manufacturing, or purposely introduced for other functions (e.g. Haversian canals for nutrient transport)—are integral to phonon suppression [152]. The flexibility and robustness of this strategy may explain the widespread use of multifunctional hierarchical materials in mammals, molluscs, and plants [5].
The seemingly separate field of thermo-electrics provides direct experimental evidence that nano-structured materials do suppress phonon propagation and that multi-scale structures can affect large and small wavelength phonons simultaneously to produce previously unobtainable performance [159]. In fact, a multi-scale structure with interfaces present at the atomic, nano, and mesoscale (reminiscent of natural materials) reduced the thermal conductivity (phonon propagation) by 50% without affecting electrical properties [159]. This ability to change phonon propagation while minimal effecting other material properties (in this case thermal conductivity) is remarkable. Given that phonons are also the agent of propogation of mechanical energy, it would be surprising in the extreme if such dramatic changes in thermal conductivity at the nano-scale did not result in dramatic changes in strength and toughness seen at the nano-scale and at larger scales by hierarchical materials [151].

Although this is strictly a hypothesis paper it is important to discuss the experimental evidence for and how this hypothesis can be tested. Gomopoulos et al used Brillouin light scattering to measure phonon dispersion in spider-silk, which like bone is a hierarchal material renowned for its toughness, and found that a wide phononic band gap existed [160]. They concluded that multilevel structural organization has a major effect on the flow of the elastic energy and, to the best of their knowledge, similar band gaps are not present in synthetic semi-crystalline polymers [160]. We stress that Gomopoulos et did not correlate band gap size to toughness but simply found a broad bandgap in silk, a material known for its
toughness and multi-scale structure. We note that bone theoretically has been demonstrated to possess similar bandgaps [153]. Testing of our hypothesis is difficult; it would require fabricating controlled hierarchal structures [75, 148], measuring their phonon dispersion [36, 160], and mechanically testing toughness. We point out each individual experimental step has been demonstrated in principle and that it is only the combination of techniques that is novel.

5. Conclusion

The ultimate aim of biomimetics is to discern the design principles behind natural structures and apply them to produce superior synthetic materials. In this work, we have outlined how phonon suppression is a plausible principle to explain, at the system level, how bone’s multi-scale structure and multi-scale extrinsic and intrinsic toughening mechanisms can operate synergistically.

Perhaps the most important aspect of this work is that techniques developed in thermal and acoustic phononics can, in theory, be directly applied to the rational design of tough bone-like mechanical metamaterials [161-163]. Conversely, the hierarchal structure of natural materials could also serve as inspiration for thermoelectric materials [164]. We hope work presented in this paper may spur the rational design of strong and tough materials with the diversity of form and function equal to that found in nature [5].
6. Future work

Future work should focus on theoretical modelling using metamaterial-based approaches. The use of two-photon lithography to produce, as pioneered by the Greer group, the designed structures will allow for testing of the in silico modelling [75, 127, 148, 165].
CHAPTER 2: NOVEL PHOTOLUMINESCENCE METRIC FOR THE SIMPLE AND HIGH THROUGHPUT MEASUREMENT OF MONOLAYER CONTENT

Statement of novelty:

Liquid exfoliation (LPE) is a powerful synthesis route to produce defect-free nanosheets in large quantities. The quantities of nanosheets produced are sufficient for reinforcing the strength and stiffness of bio-inspired mechanical composites. However, LPE’s usefulness is limited by broad thickness, distributions and low monolayer contents of the produced dispersion. An approach is needed to improve the monolayer content of LPE’d dispersion. A particularly vexing bottleneck is the difficulty in measuring the monolayer content of dispersion. Current techniques use statistical transmission electron microscope imaging of >200 flakes (~4 hrs) to measure the monolayer content, an exhausting process. In this chapter, we use a novel photoluminescence metric, developed at Trinity College, Dublin, to measure the monolayer content of a WS$_2$ dispersion. Using a Raman spectrometer and in combination with existing UV-Vis metrics we rapidly measured the monolayer content, concentration and average sheet size of a dispersion in ~10 minutes. We then used this metric to measure the effect of key sonication/shear mixing variables (initial powder WS$_2$ concentration, sonication/mixing time, and surfactant concentration) on monolayer yield. We note that the use of photoluminescence to measure monolayer content can be applied to any layered material with a bandgap such as layered dichalcogenides.
Research output:

Claudia Backes, Beata M Szydlowska, Andrew Harvey, Shengjun Yuan, Victor Vega-Mayoral, Benjamin Davies, Pei-liang Zhao, Damien Hanlon, Elton Santos, Mikhail I Katsnelson, Werner Josef Blau, Christoph Gadermaier, and Jonathan N. Coleman. “Production of Highly Monolayer Enriched Dispersions of Liquid-Exfoliated Nanosheets by Liquid Cascade Centrifugation”, 2015, ACS Nano Work completed at Trinity College, Dublin under Jonathon Coleman and Claudia Backes

1. Introduction

Liquid phase exfoliation (LPE) of 2D layered materials is a versatile technique for synthesizing dispersion of 2D materials in sufficient quantities (80mL at 5 mg/mL) for use as a nano-filler in bio-inspired films. LPE uses shearing or sonication of the unexfoliated precursor material in liquids to separate and stabilize the individual nano-sheets [133, 135, 166]. A broad range of materials including graphene, transition metal dichalcogenides (WS$_2$) and boron nitride- have been exfoliated with LPE [133]. The ability of LPE to produce large quantities of stable dispersions is extremely valuable. Dispersions can be easily processed into films and coating to form composites with excellent mechanical properties [167, 168]. The other major benefit of LPE is that it is readily repeatable once conditions are optimized; a one-pot process that requires minimal equipment (i.e. a kitchen blender) [166].

Layered materials, most famously graphene, exhibit record breaking mechanical, electrical, and thermal properties [133]. Despite the potential uses of layered material a major bottleneck is the difficulty of synthesizing them at sufficient scale, low cost, and acceptable quality. In this chapter we will WS$_2$ as a model system to assess the effectiveness of various synthesis methods. WS$_2$ was chosen because it exhibits a bandgap that is dependent upon layer number and the raman peak related to photoluminescence is well separated from WS$_2$’s fingerprint region and the raman peak of water. This allowed simple and unambiguous measurement of the monolayer content.
Unfortunately LPE suffers from important drawbacks where the resulting dispersion is highly heterogeneous [168]. LPE leads to a broad range of lateral dimensions (50-500nm) and layer numbers (1-10) and the monolayer content is generally less than 5% [169, 170]. This is a drawback because monolayers exhibit both the greatest strength and reinforcement efficiency due to their larger aspect ratios. Furthermore, the absolute size of the nanosheets is smaller than that produced by other methods such as chemical vapour deposition and the graphene oxide route leading to reduced reinforcement efficiency [131-133, 135].

The heterogeneous nature of a LPE’d dispersion could theoretically be balanced by the ability of LPE to produce large quantities of dispersions (mg to g scale). It is the ability to produce large amounts of heterogeneous dispersion that separates LPE from competing methods [131-133, 135]. Large quantities of heterogeneous dispersions make subsequent purification steps and the accompanying loss of yield feasible even in light of the added complexity and decline in yield associated with each processing step [169, 170]. The major bottleneck to purifying dispersion remains to be the lack of a simple method to measure monolayer content and length. This is currently achieved by time consuming statistical transmission electron microscope imaging of >200 flakes (~4 hrs). A rapid test would allow for measurement and therefore optimization of the efficiency of the synthesis and purification of the LPE’d dispersion.
In this Chapter, we utilise salting out method; a simple and scalable process, that
utilizes the ability of different salt concentrations to differentially sediment particles
of different size, to for the first time purified LPE’d dispersions [171, 172]. As salting
out only requires the addition of salt, mixing the salt, and collecting the sediment is
simple, cost effective, and scalable.

In this chapter we use a novel photoluminescence metric and UV-Vis to rapidly
characterize WS$_2$ dispersions produced by LPE. We then use the insights gleaned by
this novel characterization technique to measure the effect of synthesis (sonication
time, surfactant concentration, WS$_2$ concentration etc.) and purification
(centrifugation, salting out) conditions dispersion yield and monolayer content.

2. Methods

Sample preparation

Sonication

Variable amounts of WS$_2$ (1-30mg/mL) and sodium cholate (0.01-30 mg/mL) were
mixed together in deionized (DI) water. The samples, 15mL total volume in 50mL
plastic vial, were then sonicated for 1 hour in a Vibra-Cell Tip Sonicator at 30%
Amplitude for 1Hr in pulsed mode (6 seconds on, 2 seconds off) for a total time of 80
minutes. The vials containing the samples were placed in an ice bath during
sonication, with ice refilled every 2 hrs. This sonication protocol was chosen based
on previous studies as giving an acceptable yield in a minimal time period ([135]).
Our own studies confirmed this.
**Blender**

Variable amounts of WS$_2$ (1-30mg/mL) and sodium cholate (.01-30 mg/mL) were mixed together in DI water. 400mL of the dispersion was added a 1-liter capacity Kenwood blender, sheared at 4000 rpm (30 seconds on, 200 seconds off) for 2hrs. The pulsing of the blender was done using a computerized auxiliary timer and was necessary to prevent overheating of the dispersion.

**PL Measurement**

A 30 micro-litre drop was dispensed onto a glass slide and the microscope was then focused onto to the edge of the drop at 10X magnification. The lens was then switched to 100x magnification and the focus adjusted so that laser spot collapses to its smallest possible area. A Horiba Jobin Yvon LabRAM HR800 with 532 nm excitation laser in air was used to take measurements in ambient conditions. The Raman/PL emission was collected using 100× objective lens (N.A. = 0.8) and dispersed by 600 gr/mm with 10% of the laser power (~2 mW). The sample was then irradiated with a 532nm Laser with spectrums taken from 100cm$^{-1}$ to 4000cm$^{-1}$ with a 10% filter and 600 grid. The presence of aggregates, which can reduced by centrifuging the sample at 1.5K (800g) for 1hr, leads to erroneous spectra. Long imaging times increase the chance of aggregates blocking the laser, this can be seen by large changes in the baseline value of the spectra. Short imaging times (~10 sec) were the most effective in reducing erroneous spectra of reasonable quality.

Samples were placed in a quartz vial (0.4cm pathlength) where upon the extinction spectra were recorded in Varian Cary 500 with 1nm increments. The extinction
Spectra were used to calculate concentration, average flake size, and average flake length.

*Spectra metrics*

Dichalcogenides, such as WS$_2$, exhibit photoluminescence (PL) in the monolayer form but not as multi-layers or in the bulk form [173]. In WS$_2$, the PL peak (at ~2460 cm$^{-1}$) is well separated from Raman fingerprint peak (~355 cm$^{-1}$) when measured with a green laser ($\lambda_{\text{exc}} = 532$ nm). Furthermore, unlike other dichalcogenides the PL peak of WS$_2$ does not overlap with either the broad H$_2$O peak (~3500 cm$^{-1}$) or the Raman fingerprint peak allowing for unambiguous measurement of both the of PL and Raman peak. By fitting the ratio of PL peak to the Raman fingerprint peak with samples with monolayer contents determined by statistical transmission electron microscope imaging PL ratio can be related to monolayer content by the following empirical equation:

$$V_f = \frac{I_{\text{PL}}}{17 I_{\text{raman}}}$$

**Equation 10 Monolayer fraction to PL intensity ratio**

In order to ensure UV-Vis measurements taken from extinction spectra in a Varian Cary 500 in a quartz vial. Metrics were taken from Backes et al (171) and work presently in submission.

Samples were left to settle overnight or centrifuged for 2 Hrs at 1.5k rpm Hettich Mikro 220R with a fixed-angle rotor 1016 (240g) for remove impurities unless
otherwise stated. For rotation speeds above 4K a 1195-A fixed angle rotor was used with vials of 1.5 mL.

**Centrifugation**

Centrifugation is a simple but time consuming method for separating nano-sheets that increases the effect of gravity by spinning a sample at high speeds. As that relative magnitude of gravity and hydrodynamic drag vary for particles with different size, density and shapes. Monolayers tend to be smaller in lateral dimension than multilayers as such they settle more slowly, for a given g force, than multilayers.

**Salting Out**

A 3M KCl stock solution was added in the requisite proportion to produce the desired KCl concentration. In order to speed up settling of exfoliated WS₂ samples were centrifuged at .5K for 5 mins. Sedimented samples were washed in an 8mg/mL sodium cholate 2x before redispersed by bath sonication (15 mins). We found samples completely sedimented by 1.5M KCl could be redispersed with identical monolayer content and 95% yield. “Cuts” were prepared by adding the desired amount of salt to concentration X, collecting the supernatant, increasing the salt concentration to Y, and redispersing the sediment to produce a “cut” named X to Y.
3. Results

**Monolayer content is not affected strongly by sonication conditions**

In agreement with previously published work, we found that the initial sonication conditions identified by previous authors as important for overall yield matched the trend of monolayer content [133, 135, 166, 170]. The initial concentration of exfoliated WS$_2$, surfactant concentration, and sonication time did not significantly affect the proportion of monolayers synthesized (Figure 32, 33, 34) [133, 135, 166, 170].

![Figure 32 Variations of monolayer content with initial WS$_2$ Concentration](image)

A higher initial monolayer concentration causes a sub-linear increase in the final monolayer yield.
The initial concentration of WS$_2$ has no significant effect on the proportion of monolayers (blue); however increasing the initial concentration of WS$_2$ increases the exfoliated concentration WS$_2$. Therefore, adding more WS$_2$ did not increase the yield of the monolayers (total amount of monolayers) but rather increased the overall yield of monolayers (total amount of monolayers multi-layers).

![Figure 33](image)

**Figure 33 Effect of sonication time on monolayer proportion**

Increasing sonication time does not affect the proportion of monolayers produced. After 1 hour there was no significant change in monolayer proportion therefore for further studies the sonication time of 1 hour was used at surfactant concentration of 10mg/mL.
Variations in surfactant concentration

The increase in monolayer content with increasing surfactant concentration is counterbalanced by the dramatic decline of overall WS2 yield with surfactant concentration. The decline in monolayer proportion (top left) is supported by the decrease peak position, which corresponds to average layer number (bottom right) and a decline in the length metric (top right).

Figure 35 demonstrates that overall monolayer yield is at first stable and then declines with increasing surfactant concentration. This can be attributed to depletion interactions between surfactant micelles (which form above the critical micelle concentration (CMC) of sodium cholate ~8mg/mL) and exfoliated WS2. The decline in overall yield accelerates above CMC, when micelles first form, and are attributable to depletion interactions[133].
Figure 35 Monolayer yield vs. surfactant concentration

The monolayer yield (green) is locally maximum at 8mg/mL of sodium cholate but declines dramatically above the critical micelle concentration. This is attributed to the depletion force caused by the formation of micelles above 8mg/ml.

Purification by centrifugation

LPE produces a broad distribution of flake thickness and size therefore for the production of high monolayer content dispersions subsequent purification is critical [170]. Given the invariance of the monolayer proportion of dispersions to previously characterized processing variables (REF [135]), an alternative approach
is to purify the dispersions. In this section we assess centrifugation as method for increasing the monolayer content of the dispersions.

Figure 36 Effect of centrifugation speed

Note the centrifugation time for each sample was fixed for 2 hrs and measurements were taken on the supernatant. (Top Left) PL ratio increases with respect to time. Top Right, Length metric decreases as RPM increases. Bottom Left, thickness metric declines with increasing RPM. Bottom Right, Concentration declines with increasing RPM

The ability of centrifugation to select monolayers is demonstrated by the increase in monolayer proportion with increasing centrifugation speed (Figure 36). Our results indicated that centrifugation, unlike the increase in monolayer proportion seen with increasing surfactant concentration (Figure 35), is not accompanied by a decline in monolayer yield.
Figure 37 Effect of centrifugation on monolayer yield

The monolayer content is significantly increased (6x at 4K), compared to the unprocessed dispersion, without a decline in the monolayer yield. Higher centrifuge speeds were not attempted because increased speeds were only possible in a micro-centrifuge (sample volume 1mL). To avoid the presence of aggregates and without increasing the monolayer content, samples in this graph were not pre-centrifuged at 1.5K for 2 hrs.

Although centrifugation is an effective technique for increasing the monolayer content (Figure 37), it is not particularly scalable; centrifuging large volumes of dispersion requires expensive centrifuges’ and is time consuming (2hrs per run). A major drawback of centrifugation cascades, which require 2-5 centrifugation and redispersion cycles, is the long processing times and reduced yields.

**Purification from salting out**
Samples were fractioned into “cuts” by adding an initial concentration of KCl (to remove unwanted WS$_2$) and then further KCl whereupon the sediment WS$_2$ was captured and redispersed in 8mg/mL SC.

Salting Out (Figure 38), is an effective method for the purification of LPE’d WS$_2$ monolayers which is comparable to centrifugation. The .1M to .2M KCl “cut” produces the highest monolayer proportion (6x 0 to .05M). This is, within error, the same enrichment (6x) seen in simple centrifugation (6x, Figure 38). Salting out has major advantage over centrifugation in that the process is inherently scalable (just add KCl) and we found the time necessary to separate fraction’s is less than $\frac{1}{10}$th compared to that of the the centrifugation.
4. Discussion

Purification of monolayers is difficult because of a broad distribution of lateral dimension

The data unambiguously shows a correlation between declining average lateral dimension (as measured by UV-Vis) and monolayer content (as measured by the PL ratio) (Figure 34). This is important because it indicates that size selection techniques, such as centrifugation and salting out, are effective in increasing monolayer content. However even at the highest photoluminescence ratio (monolayer content) measured (post salting out or by centrifugation), the proportion of monolayers is still only in the order of 0.1. This indicates that the relationship between lateral size and WS$_2$ layer number is not particularly strong.

To address the potential scalability issues, we have utilized the centrifugation salting out method, which is a scalable alternative. We demonstrated that this method (salting out) is equally as effective as that of the centrifugation method in increasing the monolayer content of a dispersion [172]. A further advantage is that salting out is much less labour intensive and time intensive than the centrifugation method. Salted out samples need only be centrifuged at 2K rpm for 5 minutes for complete fractionation (but will settle on a bench top after 1hr), while centrifuging alone would require at least 2 hrs.

5. Conclusion:

In this chapter we demonstrated that the use of novel PL metric in combination with a previously reported UV-Vis metric [169] allows for the measurement, and the
production of monolayers with unprecedented accuracy and in a high throughput manner. We found that the proportion of monolayers in the dispersion was relatively insensitive to the initial processing conditions. This indicated that the purification was the most effective approach to producing high monolayer content dispersions. In the case of LPE, the viability of multiple purification steps is due to the relatively large yields of LPE compared to competing methods [135, 166].

Our study demonstrated that both centrifugation and salting out were equally effective in increasing the monolayer content of the dispersion. In particular, salting out was promising because of its simplicity and scalability to the large volume of dispersions produced by shear mixing [174]. For the first time we have directly measured the monolayer content of dispersion using a simple test. Compared to previous methods which only measured the “average” layer number of a dispersion and were too time consuming for high-throughput studies. This is important because only monolayers exhibit the excellent mechanical, electrical, and optical properties that have driven research into 2-D layered materials. We then used this novel measurement technique to simply, accurately, and directly compare of the monolayer content of different synthesis and purification methods.

6. Future work

Results obtained in this Chapter demonstrate that both the PL and UV-Vis metric prove particularly useful in the study of novel purification and synthesis techniques.
We believe that repeated centrifugation or salting out cycles may prove effective in producing high monolayer content (>0.5) dispersions, and that centrifugation cascades may be capable of producing monolayer contents of ~0.75.
CHAPTER 3: WS$_2$ AS A MODEL SYSTEM FOR STUDYING THE EXFOLIATION OF LAYERED MATERIALS

Statement of novelty:

Liquid phase exfoliation (LPE) of WS$_2$ produces large quantities of defect free sheets but the resulting dispersions suffer from a low monolayer content (~1%). The monolayer content, as shown in Chapter 2, does not vary significantly with the initial processing parameters such as surfactant concentration, initial WS$_2$ concentration, or processing time. In this Chapter, we will use the novel PL metric from Chapter 2, to assess the effectiveness of established exfoliation techniques such as sonication and high-shear mixing, in producing high volumes of monolayers for subsequent purification. Our results, surprisingly, demonstrate that the humble kitchen blender produces the highest monolayer production rate. Finally, we used our sensitive PL metric to assess whether novel exfoliation techniques such as extensional flow, laser steam cleaning and AC electro-kinetic flow, can improve the monolayer yield of WS$_2$ dispersions. We found categorically that these novel methods do not improve monolayer yield but outlined a simple an effective test to compare future exfoliating methods using WS$_2$ as a model system.
1.1. Introduction:

Liquid based exfoliation (LPE) of layered materials produces defect free sheets of relatively small lateral dimensions (<1μm) at a relatively low cost compatible with methods used to produce electrodes, solar cells, conductive inks, coating and composites for fillers [167, 168]. Recently high shear mixing, as opposed to sonication, was shown to be suitable for the production of grams quantities of graphene and other layered material using nothing more than kitchen blender [135, 166]. This is an important innovation because sonication is not amenable to scale up into an industrial process. A drawback of high shear mixing is that compared to sonication the monolayer yield is lower (typically ~1% of the overall yield). A process that can increase the monolayer content of shear mixed dispersion would be very useful. Unfortunately, it is very difficult to directly compare the ability of different synthesis processes to produce monolayers. This chapter details our approach in addressing this long-standing problem by using WS$_2$ as a model system. We also, with less success, tested novel processes to determine if they are capable of increasing the monolayer yield.

LPE itself suffers from two major drawbacks; the process has a very low overall yield (<0.1% of raw powder is exfoliated) and a low monolayer yield (~1% of the overall yield) [135, 175]. This drawback is not pivotal; the low cost of unexfoliated WS$_2$ and graphite ($.03 per gram) and the ability to recycle unexfoliated WS$_2$ left over from previous exfoliations means that low yields are not a major drawback.
[175]. However, a major hurdle in developing a process to increase the monolayer content of the dispersion is the difficulty in measuring it in the overall yield. Current characterization methods TEM (transmission electron microscope) or AFM (atomic force microscope) are extremely time consuming. They require measurement of the thickness of greater than 200 flakes for statistical significance and takes ~4 hrs per sample. In this Chapter we circumvent these characterization issues by using the photo-luminesce (PL) of WS₂, a property only exhibited by WS₂ monolayers, to directly measure the monolayer content. This was achieved by measuring the ratio of the Raman fingerprint region to the PL peak in a dispersion of WS₂.

In chapter 2 we showed that the monolayer content does not vary significantly with sonication processing conditions such as surfactant concentration, initial WS₂ concentration, or processing time. The monolayer content broadly follows the trends from UV-Vis data, which is an “average” of the entire dispersion while the photoluminescence data is a direct measure of the monolayer content. We hypothesized that the exfoliation force and/or how it is applied to raw WS₂ is the limiting factor in monolayer yield. In this chapter we outline the mechanisms for LPE and attempt to exfoliate using novel experimental setups.

2. Mechanisms of proposed monolayer exfoliation methods

We surveyed the literature for novel methods to apply large forces on nanomaterials using the movement of liquid. We systematically identified a number of potential methods using scaling arguments from the underlying equations.
The physics of micro-mechanical exfoliation

The traditional “scotch tape” method is able to produce single layer graphene sheets >10μm, however the major drawback is that it is time consuming and is not scalable. This method is able to produce sheets with large lateral dimensions of sheets because unlike the hydrodynamic forces, as elaborated below, the applied shear stress does not vary with the number of layers in a graphite crystal (Figure 39). This is because, in micro-mechanical exfoliation, one side of the crystal is always fixed to a surface and a monolayer is always left at that surface. Comparatively in liquid phase exfoliation, for a given shear rate, the applied force is ~t². The force applied by micro-mechanical exfoliation scales is significantly better than that for the liquid-phase exfoliation.

\[ F = \eta \gamma \tau L^2 \]

\[ F = \eta \gamma t^2 \]

**Figure 39 Mechanism for the exfoliation of layered materials**

a.) Shows how the micro-mechanical exfoliation force is a function of applied shear stress from the user’s hand and surface area of the sheet and in now way a function crystal thickness. b.) Demonstrates how the exfoliation force is a function of t² and is strongly dependent on layer number or crystal thickness. Black lines with diamonds at both ends represent the length (L) in section a) and the crystal thickness (t) in section b).
The physics of liquid phase exfoliation

Coleman et al [135] outlined a simple model for liquid exfoliation that related the required force for the exfoliation of graphene by shear, to the lateral dimension (L) and to the difference between surface energy of solid (\(E_{PP}\)) and energy of the solvent-solvent interaction (\(E_{LL}\)) [135].

\[
F = \eta \gamma t^2
\]

Equation 11 Force applied by liquid shear

\[
F_{min} = L \times (\sqrt{E_{LL}} - \sqrt{E_{PP}})^2
\]

Equation 12 Force required to exfoliate a layered material

The force applied by liquid moving relative to a graphene sheet is \(F = \eta \gamma t^2\) (equation 1). Not discussed by Coleman et al [135] but implicit in equation 8 is that fluid shear stress scales to the inverse square of sheet thickness; as the sheets get thinner (monolayer sheets, the thinnest possible, and their production is the aim of the exfoliation process) halving \(t\) requires quadrupling of shear rate (\(\gamma\)). Coleman et al reported graphene exfoliation, with some monolayers, at shear rates greater than \(2 \times 10^4 \text{ s}^{-1}\) [135].

The inverse square scaling of applying large forces on nanosheets by agitating a fluid is a major problem because the required force to exfoliate 2D layered material is only proportional to L (lateral dimension) and does not decline with \(t\). Therefore, exfoliation of the sheet becomes 10x harder for every order of magnitude decline in
the nanosheet thickness. As a consequence, producing monolayers and/or sheets of large lateral dimensions (our aim) is challenging. This matches experimental observations that liquid-phase exfoliation produces relatively sheets of small lateral dimension (sub-micron) that are typically 2-5 layers thick.

The physics of nanosheet exfoliation in extensional flow
Extensional flows arise from a difference in velocity between the front and back of a particle. Therefore a particle under extensional flow experiences a stretching force. This is in contrast to a shear force that arises due the difference in fluid velocity between the bottom and top of a particle. An example of strong extensional flow is in a nozzle. In supporting information (SI) we showed that anisotropic particles, such as a 2D material, experience an exfoliation force that is \( \frac{L}{t} \) greater in extensional flow than that for the shear flow. This scaling improvement is particularly important for sheets of large lateral dimensions, and thus may be better suited for producing large sheets. Consequently we seek to experimentally assess the effectiveness of extension-dominated flow in increasing monolayer yield.

The physics of nanosheet exfoliation in electric fields
The electroacoustic effect can apply a force on colloids because of the charged electric double layer present on the surface of all colloids [176]. An electric field moves the ions at the particle surface that in turn causes movement of the fluid and potentially exfoliation. In a reciprocal effect an acoustic wave can also induce a measureable electric potential [176]. Consequently the electroacoustic effect in is
very similar to ultrasonication, instead of an oscillating acoustic field and oscillating electric field generates fluid movement [176].

As the electroacoustic force acts on a surface (~L²) this force doesn’t scale well for exfoliation requiring voltage on the order of 1*10⁶ V across a 1cm gap. Fortunately the same electric field strength (E), $E = \frac{\text{Volts}}{\text{meters}}$, can be achieved with ~1000V across a 100 micron channel to produce an E of 1*10⁷ V. This can be achieved using standard microfluidics approaches.

**The physics of nanosheet exfoliation using laser steam cleaning**

An alternative approach to ultra-sonication is to use a focused high-power laser to induce cavitation, as it has proven more effective than ultra-sonication in removing adhered nanoparticles from computer chips without damage to the chip [177, 178]. High-power laser cleaning of semi-conductors causes the nucleation and collapse of bubbles generating flows that remove nanoparticles without damaging the underlying material. Therefore, laser steam cleaning maybe an effective method to use in exfoliating graphene like materials.

**The physics of nanosheet exfoliation using megasonication**

Ultrasonication (~20kHz) is the standard method for the liquid phase exfoliation of graphene. However megasonication (>850kHz) is the preferred method for removing nanoparticle contaminants, bonded by weak Van der Vaal's forces, from surfaces because of it efficiency and minimal damage to sensitive surfaces [179]. In fact megasonics is the favoured method in the semi-conductor industry for the removal of nano-particulates [179-181]. Given the similarities between nanoparticle
contaminant removal and graphene exfoliation megasonication it is surprising that megasonic exfoliation has not been reported in the literature for liquid phase exfoliation.

Megasonication is theoretically more suitable for producing monolayers than ultrasonication for two major reasons. Firstly, at higher frequencies violent transient cavitation is suppressed but stable “soft” cavitation of smaller bubbles is still able to dislodge particles [179-182]. Secondly, the thickness of the acoustic boundary layer is reduced. The acoustic boundary layer ($\delta$), defined below, decreases with the square root of frequency [179-181].

$$\delta = \frac{2\nu}{\sqrt{2\pi f}}$$

This is important because a thinner boundary layer, the layer of fluid attached to a solid surface in a liquid, the more exposed the nanoparticles are to hydrodynamic flow. Since hydrodynamic flows apply the force needed for exfoliation a thinner boundary layer should mean larger applied exfoliation forces. For comparison, the typical boundary layers thickness for a flat plate in 10ms$^{-1}$ hydrodynamic flow is 1600 $\mu$m vs. 0.5 $\mu$m for megasonic flow [183]. Consequently, megasonic exfoliation is, in theory, much more effective in producing monolayers than the ultrasonic exfoliation.
3. Materials and Methods:

3.1. Dispersion preparation

WS\(_2\) was sonicated, purified and characterized to produce a stock solution using the same procedure and the same initial conditions (surfactant concentration and initial WS\(_2\) concentration) as in Chapter 2. This stock solution of WS\(_2\) monolayers was then further exfoliated using where a custom R script was written to automate the measurement of the PL ratio of large number Raman spectra (~170 spectra) which was used in this study.

Extensional flow

A Waters 515 HPLC Pump, up to 400 Bar at <1mL/min, was used to pump the stock solution at high pressure. A PeekSIL\textsuperscript{tm} 50 micron inner diameter capillary tube was cut to a length of 2cm. A 15mL stock solution was pushed through the PeekSIL\textsuperscript{tm} tube, at a pressure of ~350Bar and flow rate of 0.5mL/min. This setup also allowed the WS\(_2\) solution to be recirculated through the pump up to 50x. According to this system, a flow rate of 0.5mL/min is achievable in the current setup, the extensional strain rate is \(\sim 1*10^6\) s\(^{-1}\). This is far above the critical strain rate and energy density reported by Coleman et al as being necessary to exfoliate graphene[135].

Microfluidic porous bed preparation

A Waters 515 HPLC Pump, up to 400 Bar at <1mL/min, was used to pump the stock solution at high pressure through a Pyrex chip. The microfluidic porous bed was prepared at Australian Nanofabrication Facility at Macquarie University from laser
cut Pyrex glass. The porous bed has pillars 100µ in diameters, a void fraction 0.45, and is 1.5mm wide, 2cm long, and 100 in depth. The shear rate at the maximum flow rate 3mL/min is 2.5*10^7 according to Kozeny-Carman equation.

**Steam laser exfoliation**

A dispersion of WS₂ in a glass vial was irradiated with a Nd:YAG Continuum Mini-light Green Laser with a pulse duration of 5 nanoseconds and up to .1mJ focused midway down the vial. A Thorlabs LA4236 Uncoated Piano Convex Lens (D=25.4, F=125.0 UV-Fused Silica) was used to focus the beam. Laser power was measured using a laser power meter between 82 and 20 mW (minimum power), each sample was irradiated for 1 hr.

**Electroacoustics**

![Diagram](attachment:electroacoustic_diagram.png)

**Figure 40 Electroacoustic experiment**

An applied voltage of up to 1000V (Alternating at 20kHz), with a measured output power up to 10W, flow rate of 1.2mL/min, at a pressure of 1.2 Bar was achieved. The voltage was applied across 130 µ thick insulating polycarbonate microchannel
(Microflexis, Hamburg) sandwiched between a custom designed circuit board with copper electrodes located directly below the polycarbonate microchannel. An Ultrasonicator power supply (Sonics Vibra-cell 700W) was used to provide a potential difference and was connected between circuit board and the conducting metal block. Pressure was maintained by using threaded holes for the fluidic connectors and 4 screw/nuts were used to clamp the metal, circuit board, polycarbonate chip, and metal block together. The voltage was limited by the dielectric strength of the polycarbonate chip and flow-rate was limited by tendency of polycarbonate to fail above 0.8 mL/min (or ~2.5 Bars). Experiments were performed inside a closed box because of the substantial risk of electrocution in case of a leak. Multiple cycles could run recycling the outputted fluid through the device (the output tube is placed in the input reservoir). We compared over 20 samples with their own internal control (WS₂ dispersion passed through the sample without an active electric field) and measured on average 7 spectra per sample.

**Statistical Methods**

Spectra were analysed using the R statistical software. PL and UV-Vis spectra were base-lined manually. At least 5 samples were used to determine the average PL and UV-Vis ratio’s (see Chapter 2, Section 2 for a full explanation). Statistical significance was measured by comparing using a t-test to compare the unprocessed dispersion with the processed dispersion. A p-value of 0.05 is used to define significance.
4. Results:

Comparison of a high shear mixer and kitchen blender

We have demonstrated for the first time that the use of novel PL metrics allows us to accurately, precisely, and easily compare different liquid phase exfoliation methods head to head. We prepared WS$_2$ solution from 10mg/mL WS$_2$ and 8mg/mL sodium cholate and exfoliated them using a sonic tip, high shear mixer [135], and blender [166]. The as produced concentration of the raw dispersions was 9.2mg/mL, 11 mg/mL, and 5.3mg/mL for sonication, high shear mixing, and blender, respectively. Surprisingly the HSM did not produce detectable amounts of WS$_2$ monolayers, even at the maximum possible shear rate, while a standard blender did. This was indeed an unexpected result because the HSM of graphene produced a smaller amount of monolayers than a kitchen blender. Sonication produced the highest monolayer yield and hourly yield, compared to the blender. However as pointed out by Coleman et al [135], sonication cannot be scaled up to larger volumes while the blender can be easily scaled up to the industrial scale (~300L). Therefore, the kitchen blender is the best production method for producing industrial quantities of dispersions and therefore the greatest monolayer yield (Figure 41).
The monolayer yield of three LPE synthesis methods. Both the blender (Kenwood) and high shear mixer (Silverson L5M) used 400mL of 10mg/mL WS$_2$ for 4 hrs of mixing. The sonic tip used 80mL of 10mg/mL WS$_2$ (maximum usable volume) for a six hrs of total operation. The p-value is ~.001 for monolayer yield. No monolayer signal was detected for the HSM hence why the column is empty. Note that the y-axis is a log-scale.

**Figure 41 Production of monolayers using different exfoliation techniques**

Extensional Flow to further exfoliate WS$_2$ dispersions –

In theory, extension dominated the flow scales better than that for the shear-dominated flow (Chapter 2, section 2.3). To experimentally reproduce extension dominated flow we pumped exfoliated WS$_2$ (we found that raw powder immediately caused clogging) through a 25 micron PeekSIL™ capillary tube at 300 Bar 60 times. The calculated shear rates ($\sim 1 \times 10^6$ s$^{-1}$) and energy density ($3 \times 10^7$ Jm$^{-3}$) (Figure 42) were far above the critical values reported by Coleman et al [135]. Larger pressures and flow rates were limited by the availability and cost of ultra high-pressure pumps capable of sufficient flow rates.
Figure 42 Extensional dominated flow exfoliation

The PL and UV-Vis data, shown here, is statistically identical. $10^{-N} > 5$. Note that y-axis is a log-scale.

**Electroacoustics to further exfoliate WS$_2$**

As shown in Figure 43 exposing the WS$_2$ dispersion to very high electric field for one cycle, or up to 120X, led to no a significant increase in the monolayer proportion of the dispersion. We applied the maximum AC electric fields strength (3.85 MV/m, a stronger electric field causes dielectric breakdown of the polycarbonate) to a WS$_2$ dispersion. The UV-Vis indicated that concentration, length and average thickness of the WS$_2$ dispersion was unchanged which was further confirmed with the PL confirms.
Figure 43 AC electro-exfoliation 1x cycle

A sonicated WS2 dispersion was split into 4 groups (2K, 3K, 4K, 5K), each the supernatant from different centrifugation speeds, and then cycled once through an electric field. The monolayer proportion of the different fractions is compared above; they are either not significantly different or significantly lower than the control dispersion. Over 50 spectra were taken to construct this figure. Note that the y-axis is a dimensionless ratio hence no units are listed.

Laser Exfoliation-

Even at the lowest achievable laser power the WS$_2$ appeared to be degraded instead of exfoliated (Figure 44). At the lowest power the quantity of monolayers declined significantly because of the decline overall yield and monolayer proportion. This technique is therefore not effective and instead degrades the dispersion.
The change in Photoluminescence Ratio (PL) and concentration due to exposure to a Nd:YAG laser for 1 hr at the listed laser power. Even at the lowest power, 20mW, there is a significant decline (p<.001) in the concentration (initial concentration of 12g/L) and smaller decline in the PL ratio (initially 0.5).

5. Discussion

The superiority of the kitchen blender to the high shear mixing in monolayer yield was indeed surprising to us. High shear mixer costs ~$10,000 while the cost of a kitchen blender is $35. The fact that high-shear mixing was effective for graphene but not WS$_2$ can be attributed to greater surface energy of WS$_2$ compared to graphene [166]. What is puzzling is that a high shear mixer achieves larger laminar shear rates than kitchen blenders, which according to LPE equations (Chapter 2, Section 2), should be the governing factor in monolayer exfoliation. A persuasive explanation is that of Varra et al who argued that turbulence, which is present in blenders but not in high shear mixers, causes large spatial and temporal variations in shear rate [166]. The “spikes” in these variations are presumably significantly
larger than laminar shear rates present in the high shear mixer, hence the improved monolayer yield. These large spatial and temporal variations are also present in sonication (as a result of cavitation). The high monolayer yield of sonication highlights the importance of the temporal and spatial variation of shear rates in the efficient exfoliation of monolayers. We note, however, that although variation of shear rate for the temporal and spatial existed, it is not a prerequisite to exfoliation; it merely greatly enhanced it [135].

The lack of spatial and temporal variation in shear rates may also explain the failure of the novel approaches to the exfoliation attempted in this chapter, which are all microfluidic. Microfluidic flow is highly laminar, preventing the development of turbulence characterized by the Reynolds number. In the small dimensions of the experimental setup this means that the Reynolds was always <<1 and therefore non-turbulent. For example the flows induced by AC fields, though strong, were not turbulent and hence unable to produce the large spatial and temporal variations in shear rate seen with turbulence. In the case steam laser exfoliation, the induced cavitation was too strong and degraded rather than exfoliated the WS$_2$. We believe that the difficulties of applying the large forces (greater than 1$\mu$) necessary to exfoliate monolayers from the macroscopic agitation, (outlines in section 2 in this chapter), makes direct increase in the monolayer yield a challenging exercise. A more fruitful approach may be the use of chemical methods, such as the intercalation of aggressive chemicals like hydrofluoric acids, to provide the energy
necessary for exfoliation [136]. A very recent report claimed an overall yield of 94%, an extremely high yield 100x greater than reported LPE methods [136].

6. Conclusion:

In this work we demonstrated that the combination of UV-Vis and Raman PL is a very sensitive and rapid approach to measure changes in the composition and monolayer content of WS$_2$ dispersion. We believe that the WS$_2$ system is an ideal “model” system for measuring and comparing the effectiveness of different liquid phase exfoliation methods. For example, we showed that WS$_2$, produced using a blender, possessed a substantial number of monolayers while, surprisingly, high shear mixed WS$_2$ did not produce any monolayers [135]. We attribute the superior performance of kitchen blenders to HSM to the turbulence generated in the kitchen blenders, as shown by others [166]. We assessed novel extensional flow, electro-acoustic, and steam laser exfoliation approaches and, unfortunately, showed conclusively they are unable to improve upon the monolayer yield of sonication in liquid phase exfoliation.

Future work

We believe that megasonic exfoliation, due to its favourable scaling compared to ultra-sonication, is a promising candidate for further evaluation. Unfortunately, the cost of megasonic equipment and difficulty in gaining access to this equipment both at Trinity College, Dublin and at the University of Sydney precluded us from evaluating megasonic cleaning in this study. We believe such studies would be enlightening. This work also highlights the limits of “top-down” exfoliation by even
the most intense mechanical energy. A recent study using chemical energy, in the form of Hydrofluoric acid released by microwaves, reported a 94% monolayer yield and chemical approaches may be required to achieve such remarkable high yields [136].
CHAPTER 4: TRANSIENT NETWORKS FOR THE SYNTHESIS OF MULTI-SCALE FILMS

Statement of novelty:

The ability to produce multi-scale films by colloidal casting is prevented by the inability to form stable dispersions of nano and micro particles. For this reason the literature reports only LbL methods for producing multi-scale films [184, 185]. This chapter addresses this issue by outlining a novel stabilization technique, transient networks, to form stable dispersion of nano and micro particles. We then validated that these dispersions can form mechanically strong and tough bio-inspired films.

Research output:


AU2014903480, ‘NACRE-LIKE COMPOSITES, METHODS OF SYNTHESIS AND METHODS OF USE’. Filed as a provisional patent application, 1 September 2014. Applicant: The University of Sydney. Inventors: Davies Benjamin, Zreiqat H, and Minett A.
1. Introduction

Natural materials such as bone and nacre possess the unique combined properties of being strong, stiff and tough. Scientists and engineers have embarked on promising research to replicate this structure in the form of synthetic materials albeit with limited success [1, 2, 5, 94, 186, 187]. The mechanistic origin of the mechanical properties of bone is not conclusively known but the unique multi-scale structure of natural materials is a critical element [2, 5, 94, 186]. The difficulty in synthesizing bio-inspired composites is that producing a material with a controlled nano, micro, and macro structure is extremely challenging [1, 5]. A particularly promising approach is to use anisotropic nano-scale materials, like nanoclay and graphene, dispersed in solution that allows for self-assemble during subsequent drying or filtering to form a “bricks and mortar” structure with excellent mechanical properties [101, 121, 188-195]. However, the challenge remains to produce materials with bioinspired multi-scale structures. Only recently researchers have attempted to synthesize films that mimic the multi-scale “bricks and mortar” structure of nacre using solution based methods [192, 193]. However, both reports utilized a tedious LbL dip coating approach that, due to the inherently slow sequential synthesis process, is unsuitable for all but small scale demonstrative models of thin films [101].

A prerequisite for multi-scale film formation, using solution based self-assembly methods, is that both macro and nano fillers must form a stable and non-aggregated dispersion. In the case of nano-materials such as graphene and nanoclays, this is
readily achievable due to the steric stabilization by adsorbed polymer chains and electrostatic stabilization due to surfactants, or solvent selection (Figure 46b) [135]. Unfortunately, micro-particles cannot be dispersed using the solution-based methods reported by other groups ([184]) due to their inherently lower ratio of surface area to volume compared to nanoparticles. This roadblock is the reason why complicated LbL approaches are currently the only reported methods to form multi-scale bio-inspired films [72, 192, 193].

In this study we developed an entirely novel stabilization approach (Figure 45); the transient networks approach to disperse and stabilize both micro and nano filler (Figure 45). Transient networks are a dynamically cross-linked interpenetrating polymer network; or more simply a type self-healing gel [196]. We developed a novel, simple, flexible, and scalable method to produce thick multi-scale films using a simple approach of drying nano and micro fillers dispersed in a transient network [197]. This allows, for the first time in the literature, to produce multi-scale films consisting of nano and micro scale fillers using a simple, scalable, and general methodology that is suitable for practical applications [197].
Figure 45 Dispersion versus transient networks

a.) Multi-scale structure of composites such as bone and nacre compared to our synthetic multi-scale structures using alumina and WS2. b.) The traditional approach of bio-inspired composites; dispersed “brick” (yellow blocks) are stabilized due to the steric hindrance of adsorbed polymers. c.) Transient networks stabilize micro and nanoparticles using an entirely different principle. The dynamically cross-linked polymer chains (blue dots) form an interpenetrating polymer network that stabilizes and prevents the aggregation of nano and micron sized building blocks.

2. Materials and methods

Film synthesis
Films were prepared by adding defined amounts of WS$_2$ (from a dispersion made through sonication) and alumina oxide (Al$_2$O$_3$) (from powder, RonaFlair, Whitesapphire) and mixing them with a PVA solution using a high-shear mixer for 10 minutes (Silverson L5M-A Mixer, 12mm head, 4000rpm) (Figure 46). Borate was then added and samples heated to 50 C followed by shear mixing for 10 minutes to ensure a homogenously cross-linked PVA-Borate gel. The samples were sonicated for 10 minutes in a bath sonicator (Branson) to remove gas bubbles that would
otherwise reduce mechanical performance of the film and increases variance in the data.

Figure 46 Process diagram for the synthesis of multi-scale films

Nanoparticles were prepared using liquid phase exfoliation; liquid phase exfoliation was chosen due to its ability to produce a wide variety of 2D nano-materials in large quantities (Chapters 2 and 3). Plate shaped alumina (RonaFlair WhiteSaphire) was chosen as a micro-particle because of its previous use in bio-inspired composites [72].

3. Results

Synthesis of multi-scale films

In agreement with the literature, we found that changing the borate concentration, temperature and pH to produce a vast range of viscosity and elasticity of the fluid could easily vary the rheology of the PVA-Borate system. Figure 47a illustrates the stability of PVA dispersions over a 24 hr. period; the vials are from left to right Al₂O₃ in PVA without borate, Al₂O₃ in PVA with borate, Al₂O₃ in PVA with borate and 0.5%
WS₂, Al₂O₃ in PVA with borate and 2%WS₂, and PVA with 2% WS₂ without borate. Note how, without borate to crosslink the PVA micrometre sized Al₂O₃, the dispersion is not stable; this is problematic because Al₂O₃ settles before it dries into a composite film. SEM images confirm a clearly defined “bricks and mortar” structure. Figure 47b shows the layered structure of the film at a fracture surface. Figure 47 c and d show the platelets firmly embedded in PVA matrix. The presence of voids left from the pull out of intact platelets on the fracture surface indicate that their composites strength could be improved by increasing the interfacial bond strength between platelet and polymer [72].

Figure 47 SEM of multi-scale WS₂ and alumina films
(a) Uncrosslinked Alumina and PVA, 25% Alumina, 25% Alumina and .5% WS2, 2% WS2 and 25% Alumina, and 2% WS2). Note the Alumina dispersion is not stable for >20 minutes and crosslinking is required for a stable dispersion. b) c) and d) SEM images showing the “bricks and mortar” structure of the dried films after fracture.

Mechanical Properties

Films were prepared with varying weight percentages of Al$_2$O$_3$ and WS$_2$ as single scale control and PVA-borate films without fillers as controls (Figure 48). We found that films above 25% Al$_2$O$_3$ by weight and WS$_2$ 2% were the stiffest and strongest films and that any further addition of fillers led to a decline in mechanical performance. We note that, as typical for nano-materials, the reinforcement efficiency of the WS$_2$ is greater than Al$_2$O$_3$ (41.9 vs. 2.39 $\frac{MPa}{\% \text{ Weight}}$ vs. $\frac{MPa}{\% \text{ weight}}$). Tensile testing of single scale WS$_2$ or Al$_2$O$_3$ showed that 7.5x and 5.5x increase in the fracture strength (respectively) compared to the borate-PVA control. The increase in Young Modulus (E) was 5.4x and 6.4x respectively for Al$_2$O$_3$ and WS$_2$.

Furthermore, the increase in toughness of both WS$_2$ and Al$_2$O$_3$ films was 4.1 and 3.5 times, compared to the PVA/borate control. The multi-scale WS$_2$/Al$_2$O$_3$ composites exhibited a 15% and 52% increase in strength compared WS$_2$ and Al$_2$O$_3$. The increase in Young’s Modulus was significantly greater (p<.01) for the multi-scale WS$_2$/Al$_2$O$_3$; a 12x increase compared to unreinforced PVA-Borate control.
Figure 48 Representative stress strain curves- additional length scales.

Note the mechanical data quoted below is not based on these curves but on data averaged over multiple samples (10>n>5).

4. Discussion

PVA polymer was the material of choice in our study because of its good film forming abilities, ductility, and widespread use in nano-composites ([101, 191, 193]). Furthermore, PVA solutions form transient networks by the addition of borate ions at pH > 8. [198]. We used Al$_2$O$_3$ plates because of their similar dimensions (200-400nm thick and 10-30 µ in length) to the “brick” in natural nacre as previously described [72]. WS$_2$, in particular, was utilized because of a combined UV-Vis and Raman/Photo-luminescence metric, developed by the Coleman’s group, Trinity College, Dublin, that allowed accurate and rapid characterization of both sheet size and monolayer content and therefore confidence in its quality [199]. WS$_2$ is
representative of the wide range of novel materials that can be synthesized through liquid phase exfoliation with an inherent size that is 1-4 nm thick and ~500 nm long; an order of magnitude lower than that for Al$_2$O$_3$ plates.

The increase in mechanical properties can be interpreted as synergistic because it is greater than the expected rule of mixtures behaviour between WS$_2$ and Al$_2$O$_3$ single scale films and multi-scale WS$_2$/Al$_2$O$_3$. The major advantage of bio-inspired combination is not their strength or toughness, but rather the combination of strength, toughness, and stiffness [1, 2, 5, 94, 186, 187]. In the case of 0.2 alumina and 0.01 WS$_2$, the multi-scale films were 0.35x in stiffness, 1.3x in strength, and 1.63x in toughness based on the average of single scale films. Furthermore, the combined change in strength, stiffness, and toughness is 1.09x greater than the expected rule of mixture performance. We note that by simply adding more filler does not improve the mechanical properties, as further addition of either Al$_2$O$_3$ or WS$_2$ led to decline in the mechanical properties in the single scale films. We attributed this to aggregation. We conclude that a multi-scale strategy is beneficial for increasing the stiffness and strength in a manner that was not possible for single scale films.

Purely nano-scale bio-inspired composites were initially pioneered using LbL methods because of the controllability and excellent mechanical properties of the films. However simplicity and scalability of producing these nano-materials remain to be a challenge in the field [46, 101, 188, 200]. While solution based routes offer
less structural control, it offers simplicity, scalability and as a result the potential for uses outside of the proof of concept work [101, 201]. For this reason, solution based routes have largely superseded LbL methods when available. We believe that our novel transient network strategy extends solution based methods to multi-scale films that hitherto have been synthesized using tedious layer by layer methods [192, 193]. The major finding of the present study is that we can produce multi-scale films using transient network based approach.

In a direct comparison with LbL approaches to multi-scale films, we demonstrated that our multi-scale composite matched the performance of LbL methods. Wang et al are the only example of multi-scale films made using layer-by-layer methods. Wang et al., [191] demonstrated that multi-scale films outperformed the rule of mixtures by 1.05x versus the expected rule of mixture performance which is slightly less than the 1.09x we found in this work [191].

Our results were unexpected as LbL approaches generally significantly outperform solution-based approaches in terms of mechanical properties [101]. Our reinforcement efficiency, when combined with the simplicity and scalability of our synthesis method (in comparison to LbL), demonstrated superiority of our transient network approach.

The major benefit of our novel transient network approach is that it allows for the stabilization of micron sized particles in a suspension, a critical step in ensuring that
the particles are evenly dispersed in the dried film. The suspension stability achieved in our approach is due to the transiently cross-linked polymer network and not to the surface forces such steric or electrostatic stabilization. This is a major advantage over permanent gels (which permanently break under shear) because transient gels self-heal, allowing for transient network to be processed as if they were liquids. In contrast, traditional covalent gels are irreversibly damaged by small mechanical forces and consequently must be processed in moulds and require precise control of the gelation mechanism (Figure 45) [202]. The resulting simplicity of the transient network approach is a major benefit, as it allows for the production of large area multi-films scale with no change to established techniques suitable for real world applications.

Furthermore, the polymer phase PVA, can be modified with supra molecular (metal ions) and covalently cross-links to further improve the mechanical properties in an identical manner to those used on LbL films [46, 200]. The self-healing property of the borate-PVA system is by no means unique; the range of polymers could be expanded by using supra-molecular organogels allowing for the use of non-water soluble polymers with polyurethanes. Finally the interfacial properties of all constituents are vitally important to composites overall performance; surface modifications can be made to each individual component before mixing to tune the interfacial strength of the polymer and fillers [72].
The simplicity of transient network based approaches does not come at the expense of flexibility. Any nano-material that can be dispersed in water and has the appropriate shape (for self assembly) is fully compatible with our approach. This includes carbon nanotubes, graphene, graphene oxide, cellulose nanofibrils, and layered dichalcogenides. To this end, we demonstrated that liquid phase exfoliation is a simple and scalable method to prepare stable dispersion of a vast variety of nano-materials as fully compatible with our approach by using WS$_2$ in our system [135]. The ability to utilize such a diverse array of materials allows for a degree of multi-functionality as nano fillers can add electrical, magnetic and optical, properties along with mechanical properties.

5. Conclusion

We have demonstrated a novel, simple, and scalable method for producing multi-scale films that can be used to explore the underlying physics and synthesize materials that emulate the multi-scale structure of natural materials. Our resulting multi-scale composites, as hoped for in bio-inspired materials, exceeded the expected rule of mixtures performance.

6. Future works

Hierarchal structures are complex and the existing mechanical models are still developing [94]. Furthermore, testing samples experimentally is challenging because existing techniques don’t allow one to independently vary the properties of the nano-scale and micro-scale simultaneously in a manner that can produce the
large number of samples required for proper testing. The simplicity, flexibility, and ability to compare different scales separately and then as a multi-scale films means our approach is well suited to fundamental studies into the origin of excellent mechanical properties of multi-scale biological materials. Finally, our technology can be used to synthesize multi-scale films for specific applications by leveraging the mechanical performance of multi-scale films and functional properties of nano and micro fillers.
BIBLIOGRAPHY


