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DRAFT TEXT FOR BOOK CHAPTER

There is a growing urgency for architecture to respond to the global environmental challenges. This drive for sustainability has propelled the development of smart materials to the forefront of scientific research (Koyaz, 2018; Lendlein *et al.*, 2019). We are living in a world which is increasingly dictated by climate change and a growing pressure on limited resources, one of which is shelter from environmental extremes. Arguably one of human nature's most successful evolutionary techniques was harnessing the ability to use our intellect to allow us to colonise environments which might not be 'ideal' by building shelter. Whether this shelter is built in the form of an igloo or a modern day skyscraper there are certain requirements it must achieve; to protect the user from extreme temperatures (both low and high), to provide sufficient ventilation, light and moisture control (Banfill, Peacock and Ban¢ll, 2011). If any one of these requirements are not fulfilled it can have a detrimental knock-on effect on the users' health and wellbeing. If, for instance, sufficient natural light is not incorporated, user enjoyment will decrease and if moisture and humidity levels are not properly maintained it can lead to damp, fungal growths, viral proliferation and subsequent respiratory diseases (Seppänen and Kurnitski, 2009).

Current static buildings rely on mechanical systems to adapt and change to their environments to maintain these optimal internal conditions, however this requires a huge amount of external energy input (Augustin, 2018) which cannot be sustained – cities of the future need efficient buildings governed by sustainable architecture (López *et al.*, 2015; Juaristi *et al.*, 2018) External environments are dynamic, with ever changing conditions and organisms must evolve to survive (López *et al.*, 2015). Unlike the natural world, current architecture does not 'evolve' and respond to its environment as a living being would, it is inert and not dynamically influenced by its 'habitat' and therefore is unable to survive in our ever changing world without mechanical systems (Armstrong, 2016; Beesley, 2019). While such mechanical systems, for example air-conditioning units, are highly effective at providing the desired outcome of maintain the internal temperature and humidity, they require huge amounts of limited resources such as fossil fuels (Banfill, Peacock and Ban¢ll, 2011). The buildings of today do not 'give and take' from the environment like a natural organism does, instead they just take - we do not consider our architecture a closed system. Innovation in technological architecture is the key - harnessing smart, passive materials heralds the beginning of bridging the gap between living, responsive materials and artificial, unresponsive materials commonly used within architecture (Armstrong, 2016; Beesley, 2019). These smart materials have the capacity to begin to close the system by allowing our homes to become more self-sufficient (Juaristi *et al.*, 2018). Architecture of the future is beginning to take steps in the direction of smart passive materials of the natural world, producing dynamic, adaptive beings which are capable of evolution over time (Banfill, Peacock and Ban¢ll, 2011; López *et al.*, 2015), with passive adaptive façade prototypes harnessing algal walls being evaluated (Saidam *et al.*, 2017; Mazzucchelli *et al.*, 2018; Wahab *et al.*, 2019).

In this context we will be referring to smart materials as those which 'act' in direct response to their environment. Smart materials encompass those which can be externally activated, either electrically or via another energy source and a small subsection of materials which are passive, meaning they respond without input from an external energy source (Reichert, Menges and Correa, 2014; Holstov, Bridgens and Farmer, 2015). Passive smart materials utilise the minute energy exchange from processes such as evaporation - the concept of 'tiny energies', intrinsic energy production at the molecular level. Currently, these processes are broadly overlooked as an energy source in architecture, we have become muted to observing and utilizing such energy generating processes for productive outcomes and instead actively avoid them in favour of immediate, external, and 'easy', unsustainable sources. Instead of plugging in the electrical dehumidifier should we not be actively seeking out the benefits of that unutilized evaporative energy source?

Smart passive materials include shape memory alloys, shape memory polymers and hygromorphic materials. The specific materials we are interested in here are those which are hygromorphic, meaning they change size in response to water(Holstov, Bridgens and Farmer, 2015) (Sartori and Hestnes, 2007). These materials can respond directly to water or to changes in water content in the air (relative humidity), the latter being highly sensitive and responsive.

Water is absorbed in high humidity environments causing expansion, and expelled in environments of low relative humidity causing an overall shrinkage (Holstov, Bridgens and Farmer, 2015). Hygromorphic materials utilise the 'tiny energies' released through evaporation and in fundamental terms this movement is understood as 'movement through expansion'. This hygromorphic property of certain materials has been harnessed as a passive energy technology for centuries. For example in ancient Egypt swelling wooden wedges were used to split stone and marble for construction (Le Duigou and Castro, 2016), and wooden barrels the world over were self-sealed by adding wine to swell the curved planks (Arends, Pel and Huinink, 2017). While there are many examples of natural hygromorphic materials, including wood and cellulose, this chapter specifically focusses on *Bacillus subtills* spores as hygromorphs for a myriad of reasons. They are highly resilient to decay, dramatically increasing its long term durability as a hygromorphic material for use within material architecture (Driks, 2003) (Correa *et al*, 2013). They are microscopic in size and are genetically identical so produce a homogenous material (Reyssat and Mahadevan, 2009; Ramirez-Figueroa *et al.*, 2016) which facilitates predictable movement. In addition their unique spore-coat architecture has specifically evolved to maximise passive water transport which promotes a rapid and powerful hygromorphic response to changing environmental humidity (Driks, 2003; Yao, Ou, Cheng, *et al.*, 2015). This promises a resilient, responsive and programmable hygromorphic material which is capable of generating considerable hydraulic force when applied in a simple bilayer structure (Le Duigou and Castro, 2016; Birch *et al.*, 2019). These hygromorphic bilayers work through the principle of differing coefficients of expansion between materials, the *B. subtills* spores are the smart passive material and so expand and contract at a much faster rate than the inert substrate material therefore causing an bending motion to accommodate the difference between the two materials (Arends, Pel and Huinink, 2017). In more complex hygromorphic systems such as those adopted by plants, this system is augmented though incorporation of hygroscopic macromolecules (peptidoglycan and polysaccharides), cellular ultrastructure (utilising vacuole turgor pressure) (Burgert and Fratzl, 2009) and complex tissue architecture (cellulose and hemi-cellulose fibres) to not only harness the hygromorphic response but to program it to have a directional and functional outcome (Le Duigou and Castro, 2017). Harnessing the power of evaporation in this fashion offers a huge and potentially neglected opportunity in human engineered systems (Burgert and Fratzl, 2009; Li and Wang, 2016). In plants hygromorphic actuation creates movement without muscles; in architecture it proposes motion without motors with embedded responsive capacity within the material (Menges and Reichert, 2012).

This biomimetic, hygromorphic actuator research aims to develop novel active materials for architecture in a truly multi-disciplinary effort, the success of which is underpinned by combining expertise from the fields of engineering, microbiology, materials science, biophysics, design, physiology and architecture (Geitmann, Niklas and Speck, 2019). The distinctive advantage of this integrative, multi-functioning approach is that it offers the opportunity to foster a significant performance revolution in the field of kinetic architecture and sustainable buildings design (Li and Wang, 2016).

*Bacillus subtilis* morphs into a robust, dormant 'spore' to protect their genetic material when their immediate environment becomes hostile e.g. pH, temperature, toxin build-up or most crucially inadequate nutrient supply (Arora, Kumar, Yadav, & Raghu, 2016; Henriques & Moran, Jr., 2007). These bacterial spores can survive hostile environments for thousands of years(Henriques and Moran, Jr., 2007), spores from lake sediments over 7000 years old are still viable and spores dating back 25 million years have been isolated (Henriques and Moran, Jr., 2007). Bacteria form spores through the process of sporulation which is a stepped process not dissimilar to mitosis. This begins with replication of the bacteria's DNA before a spore septum forms, enclosing one complete DNA strand at either side (Arora et al., 2016). At this point the cell appears physically divided by this internal membrane, then one of these halves is engulfed by the other forming two complete cells encapsulated within one membrane. At this point the multiple protective coats begin to form around the engulfed pre-spore. This forms a complex layered structure of the spore; the central inner core contains the DNA and is protected by the innermost layer – the cortex. This cortex layer is vital to the hygromorphic behaviour of the spore, in an evolutionary sense this layer is what protects the spore's genetic material by ensuring the core remains desiccated. By ensuring the core remains dehydrated so does the DNA, therefore inactivating enzymes and reducing metabolic activity to a sustainable dormant state, mineralising the core, and creating thermo-resistance. To generate this desiccation within the cortex layer, multiple specialised spore coats form, with an inner and outer coat comprised of a complex protein structure which control water and substance movement into and out of the spore.  It is this specific coat protein structure which provides the spore with resilience to environmental stresses (Abhyankar *et al.*, 2011) and the ability to rapidly move water across the membrane generating substantial hydraulic pressures.

The spores' ability to be robust, resilient, and durable throughout infinite contraction cycles due to their unique structure provides the opportunity for their application in architecture that vegetative cells could not provide. As these spores are non-metabolically active, they are unable to divide, which would ensure that the concentration applied to a surface would not increase, nor would there be viable bacteria which could interact negatively with internal environments. Despite this it is important to note that this specific strain of *B. subtilus* (*Bacillus subtilus,* 168 wild type) is a safe and native soil bacteria found in many environments across the world and indeed, is incorporated into probiotics and yogurts for human consumption (Elisashvili, Kachlishvili and Chikindas, 2019).

Between the inner spore coat and the cortex there are points of adhesion which are proposed to be scaffold-like proteins which may function to shorten the diffusion pathway for water entering the spore, reducing the desiccation response time (Ghosal *et al.*, 2010) such that the spores launch a hygromorphic response almost immediately when relative humidity changes.  The unique reversible folding design of the outer spore coat means that spore volume can therefore change in response to fluctuations in environmental humidity without the coat material properties resisting this change in morphology (Sahin *et al.*, 2012). This hygromorphic response generates a force due to expansion, which if kinetically harnessed, can be applied to mechanical work (Arora et al., 2016; Chen et al., 2015; Garg, 2014). The magnitude of this force was measured at the microscopic scale using an automatic force microscope cantilever experiment.  A force was exerted downwards on a single spore, which was then exposed to a high RH (>95% RH) to trigger expansion. Since the force required to resist this hygromorphic expansion is equivalent to the force exerted by the spore it allowed the energy density produced by this expansion to be measured as more than 10MJ/m$^3$ (Chen, Goodnight and Gao, 2014).  These are molecular level forces, 'tiny energies' generated by water movement into individual spores  which, if a mechanism could be designed to harness it on a large scale, would be sufficient to lift a car (Arora et al., 2016; Chen et al., 2015).

The building blocks of development for this potential smart material have begun, although some of these foundations are in fields as diverse as nanoengineering and reactive clothing development. To date all these studies have incorporated the bacterial spores within a bilayer structure to form a spore bimorph. The first of these utilised an inert substrate layer of 0.02mm thick polyimide tape (Chen *et al.*, 2014) which was highly flexible , with a low elastic potential but yet ultra-light weight in nature to create the fast action deflection required to produce a biohybrid nanogenerator. These polyimide bimorphs demonstrated a high deformation but low force output due to the low elastic potential properties of the highly flexible substrate. Whilst this study was successful in producing a nanogenerator by orientating the bimorphs in such a fashion that the momentum created by their 'flick' motion forward and backwards we would need to use a substrate with a higher elastic potential to provide a higher force output.

With this in mind other possible substrate materials were investigated, such as latex elastomer sheeting (0.5mm thick) which was calculated (Chen, Goodnight and Gao, 2014) to have optimum elasticity to generate maximum force from the change in hydration state of the spores (Young's modulus of elasticity of 3.0MPa) (Wang *et al.*, 2017). These bimorphs were capable of deflection despite the significant increase in substrate thickness and mass. Wang *et al,* based at MIT have applied these preliminary studies to incorporate this bimorph technology within fashion textiles to produce 'Biohybrid Wearables'. Here, the *B. subtilis* spores have been sandwiched into a textile-based bilayer and incorporated into fabrics for sports clothing. Following the heat map created of an athlete during exercise the spore bimorphs have been situated to allow opening of a myriad of ventilation 'fins' when humidity levels increase (influenced by the athletes sweat) and close again when humidity drops (as the athlete is cooled and sweating reduces). This is a fantastic example of the practical application of this technology at the small scale and provides a baseline for the progression of this smart material.

All these studies utilise the bimorph concept as the simplest level – they do not require any further outcome than a bend of the bimorph. No greater force output is required than to 'lift' one end of the actuators to produce that bend. If this technology is to be successfully applied to architecture, significant force output is expected to be required. While a conclusion of Chen *et al's* work (Chen *et al.*, 2015) shows that altering the substrate can affect the potential force output of the bimorphs (a change from the fast acting but weak polyimide tape to the more resistant natural latex) no consideration was given to amplifying the hygromorphic response of the spore layer to increase the performance of the bilayer.

Studies from our own group(Birch *et al.*, 2019) have begun to explore factors to enhance the performance and investigate the potential programmability of the spore bilayers. We began by investigating the effect of spore concentration on deflection angle, which has indeed shown that an increase of *B. subtilis* spore concentration (monolayer number) increased the magnitude of deflection at any given relative humidity. This study also used 0.5mm natural latex (following (Chen *et al.*, 2015)), isolating this variable to ensure that the change in deflection could be attributed to the change in spore concentration. These bimorphs deformed rapidly in response to a changing environmental humidity which supported previous findings (Chen, Goodnight and Gao, 2014; Chen *et al.*, 2015; Yao, Ou, Cheng, *et al.*, 2015) where responses occurred in under 5 minutes. The unique ultrastructure of the spores has evolved to facilitate rapid water movement and this property offers encouraging signs for the development of a very responsive smart material. A detailed study(Sunde *et al.*, 2009) of water movement in *Bacillus* spores showed that this water movement was biphasic; the first, across the coat, was rapid and the second transversing the cortex was slower. Interestingly, this study also showed the importance of the spore coat morphology. They used *B. thuringiensis*, which has an additional layer as part of the spore coat (the exosporium) meaning the diffusion distance across the spore coat layer is greater, predicting an increase in rehydration time compared to *B. subtilis*, which was supported by our study. These findings all support the notion that *B.subtilis* spore based hygromorphs would respond with a sufficiently rapid hygromorphic response to be appropriate for application in an architectural setting.

An improvement of spore bilayer performance has also been demonstrated by using a pre-incubation technique. We observed an increase in the deflection angle response post 24 hour incubation at 95% RH which was also previously noted in the methodologies published by Chen *et al*, (Chen *et al.*, 2014). This phenomenon could be due to the high humidity environment allowing the spores to realign themselves once in their swollen state, forming much greater regularity within the monolayer structure and therefore more coherent net expansion and contraction of the monolayer leading to a greater deflection angle of the hydromorphic actuator, but currently there is no experimental evidence of this being investigated in the literature.

Another crucial performance indicator for a smart material to be used in an architectural setting is that of durability. Our development investigations (Birch *et al.*, 2019) have shown promise in that we have demonstrated that deflections of the hygromorphic actuators were reversible and repeatable over multiple hydration/dehydration cycles. This supports and extends previous work in the field. Chen *et al* (2014) reported this same phenomenon for *B.subtilis* spores in a microcantilever study with silicon wafers as the substrate. This was echoed by Yao *et al*, (Yao, Ou, Wang, *et al.*, 2015) in a study investigating the hygromorphic performance of *B.subtilis* vegetative natto-cells, and by Chen *et al*, (Chen *et al.*, 2015) with *B.subtilis* spores incorporated into polyimide tape as hygromorphic bilayers for potential use in developing artificial muscles. In our studies the hygromorphic actuators have showed no indication of degradation or reduction in deflection angle when tested multiple times. The maximum cycles of low to high relative humidity tested on one strip was 10 cycles, with the starting and end maximum deflections being identical. This suggests there was no deterioration in actuator performance, however 10 cycles would be insufficient to conclude that it could be maintained over an infinite number of cycles. However, in the study by Chen *et al*, (Chen *et al.*, 2014) deflection was recorded over 1 million cycles without performance change, which bodes well for predicted performance of these type of actuators over extended use which would be critical for application in an architectural setting, where the actuators would be designed to last for many years. This durability factor would exclude the possibility of using *B.subtilis* natto cells as potential hygromorphic actuators because these vegetative cells would not be sufficiently resilient in an architectural application and have poor adherence characteristics (Xu Zhou *et al.*, 2017).

To harness this hygromorphic response in a novel smart material we need to develop a bilayer or actuator which is capable of generating sufficient force in a programmable manner. An overwhelming conclusion drawn from our (Birch *et al.*, 2019) study is that increasing the number of *B. subtilis* spore monolayers results in an increase in maximum deflection angle of the actuator. This suggests not only that is there tight adhesion between the spores in each monolayer, but also that there must be an adhesion between the monolayers. This highlights that individual monolayers must be contributing towards a combined force instead of each 'pulling' separately suggesting that the adhesion between the monolayers is sufficient to transfer force from one layer to another. Furthermore, it was shown that the force output of these hygromorphic actuators has a strong linear relationship with the number of monolayers (spore concentration). With each increase of number of monolayers there was a predictable and therefore programmable force response given by these hygromorphic actuators. It is not clear whether this force response is linked to total spore concentration or reflects the number of monolayers adopted in the fabrication process. Further preliminary studies were conducted to investigate if the calculated force was equivalent to actual force output. The (Birch *et al.*, 2019) study demonstrated for the first time that *B.subtilis* biohybrid actuators are capable of doing work (lifting mass over a distance). This work was quantified by measuring the deflection angle when a known load was added, and it was observed that during a pilot study (despite an overall decreased deflection) the *B. subtilis* bimorph actuators were capable of lifting a mass of 150% of the mass of their own substrate layer. This force study (Birch *et al.*, 2019) highlighted the importance of adhesion within the monolayer. When higher loads were added to the actuators, cracking of the spore layer was observed. This is supported by the observations of Chen *et al*, (Chen *et al.*, 2015). However, it was unclear if this was directly related to load or extremes of contraction seen at low relative humidity. There has been some suggestion that adhesion properties could be improved between the individual spores by changing the sporulation conditions to influence the formation of the rodlet layer on the inner spore coat (Driks, 2003). This potential impact of these adhesion factors in the fabrication and performance of spore hygromorphic actuators has not been reported in the literature.

Developing these bimorph spore actuators to be capable of performing complex shape change and high force output with programmable, repeatable responses to environmental relative humidity would produce an intelligent smart material for sustainable architecture. Work has begun to develop beyond the simple unilateral deflections to produce twisting and folding elements with the goal of a component driven prototype to highlight the potential of this novel smart material. By harnessing the miraculous simplicity of a spore - an evolutionary adaptation to maintain moisture levels without using energy, we have the potential to control the humidity of our built environment without wasting precious energy resources.

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