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Book of Abstracts

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Materials properties of bacterial biofilms

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As bio-sourced materials are raising interest for their sustainability, using bacteria to produce biofilms made of a protein and polysaccharide matrix has become a new strategy to make engineered living materials. Our group contributes to this emerging field by clarifying how bacteria adapt biofilm materials properties to the environment. For this, we culture E. coli producing curli amyloid and phosphoethanolamine-cellulose fibers on nutritive agar substrates with varying physico-chemical properties and study the growth, morphology and mechanical properties of the resulting biofilms. We demonstrated that changing the properties of the agar substrates with polyelectrolyte coatings or by varying their water content, affects E. coli biofilm growth, morphology and mechanical properties. We also used E. coli producing only amyloid fibers to focus on the matrix structural and functional changes at the molecular scale. To assess the contribution of each matrix component to the macroscopic biofilm materials properties, we compared the characteristics of biofilms produced by a collection of E. coli mutants differing in the matrix they produce. The results indicate that E. coli biofilm matrix is a composite made of rigid and brittle curli amyloid fibers assembled within a mesh of soft and adhesive phosphoethanolamine-cellulose fibers. Finally, we explored how tuning the chemical environment of the bacteria can lead to biofilm biomineralization and expand the range of materials properties reachable with biofilm-based materials.

Morphogenesis of protein/silica skeletal superstructures in demospongiae

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Living organisms form complex mineralized architectures that perform a variety of essential functions. These biological materials are not only responsible for structural support and mechanical strength, but often provide optical, magnetic and sensing capabilities. This diversity in functionality is accomplished using a relatively narrow range of inorganic and organic components arranged into an astonishing variety of highly ordered hierarchical architectures. The control over the shape of the inorganic mineral building units, which in most cases differs significantly from their synthetic and geological counterparts, is executed by the cellular components. Here, growth and form are regulated by generating biochemical and physical boundary conditions that guide the self-assembly of a specific morphology. In the case of crystalline minerals, it is well established that the organic framework secreted by the cellular tissue manipulates the thermodynamics and kinetics of growth to give rise to complex morphologies that contradict the highly symmetrical crystal habit of the pure mineral phase. In essence, incorporated macromolecular content is used to challenge the crystallographic constraints imposed by the physical properties of the mineral. However, the mechanisms by which macromolecular frameworks shape and impose order on inherently disordered amorphous minerals are not well understood. In this work, we describe the formation of skeletal elements of sponges made of amorphous glass. Templated by branched single-crystalline protein filaments, these elements are a paradigm example of symmetry in biological systems. In this talk, I will review all aspects of this extraordinary self-assembly, biomineralization and morphogenesis scheme.

A: Bioinspired/biomimetic materials (inanimate/animate)

3D Printing Conductive Hydrogels with Tunable Properties for Soft Robotics and Biosensing

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With mechanical properties similar to that of living tissue, soft electronics can serve a central role in the development of biosensors, wearable devices, and implantable devices. 3D printing offers a powerful way to fabricate the customized geometries that will be necessary for such applications and provides opportunities for rapid iteration and modification. Because different applications may call for various hydrogel properties, we demonstrate the ability to tune ink viscosity, hydrogel stiffness, and hydrogel conductivity. The demonstrated functional hydrogels are based on acrylamide, due its stability and ubiquity, and PEDOT:PSS, a commonly used conductive organic polymer. The mixed ionic-electronic conductivity of PEDOT:PSS makes it an ideal candidate for interfacing electronics with biological cells and tissue, like neurons or muscle. Using rheometry as well as additional characterization methods, we highlight the advantages of designing conductive hydrogels in this way. Ultimately, we demonstrate the ability to use extrusion 3D printing to fabricate components suitable for soft electronics.

Figure 1. The top and right image show different structures being 3D printed with using conductive hydrogel material. The versatility of 3D printing allows for rapid iteration. The spider web structures are all printed with increasing amounts of methycellulose from left to right, which increases the ink's viscosity and improves the resolution of the printed material. To an extent, this parameter can be optimized without influencing conductivity.

Because PEDOT:PSS is the conductive polymer, it is unsurprising that increased PEDOT:PSS content also increases the conductivity of the hydrogel. However, other hydrogel properties are also influenced by the increase of PEDOT:PSS. Ink viscosity, for example, is important for achieving high-quality printing resolution (Figure 1). We show that methylcellulose content can be adjusted to achieve the desired viscosity without dramatically impacting conductivity. Similarly, hydrogel stiffness can be tuned by adjusting crosslinker concentration without impacting conductivity. When using salts as charge carriers instead of conductive polymers, tuning these properties would also impact the conductivity of those hydrogels, highlighting the advantage of these PEDOT:PSS systems. We show that a diode can be created using three different hydrogel materials to demonstrate the potential of these materials for practical implementations. In future steps, these materials could also provide a good interface with living cells and could maybe be implemented to stimulate neurons or muscle cells.

Alternative strategies for the recombinant synthesis, DOPA modification and analysis of mussel foot proteins – A case study for Mfp-3 of two *Mytilus* species

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Mussel foot proteins (Mfps) possess unique binding properties to various surfaces due to the presence of L-3,4-dihydroxyphenylalanine (DOPA) [1]. *Mytilus* foot protein-3 (Mfp-3) is one of several proteins in the byssal adhesive plaque. Its localization at the plaque-substrate interface approved that Mfp-3 plays a key role in adhesion [2]. Therefore, the DOPA-modified protein is suitable for the development of innovative bio-based binders. Commonly, mushroom tyrosinase (type III tyrosinase) is used for the modification to peptide-bound DOPA in Mfp-biopolymers. However, it is limited and results only in hydroxylation of 15 – 40 % [3]. A promising alternative are microbial tyrosinases (mTyr) [4]. The enzyme from *V. spinosum* was shown to also hydroxylate peptide-bound tyrosines in Mfps *in vivo* [5].

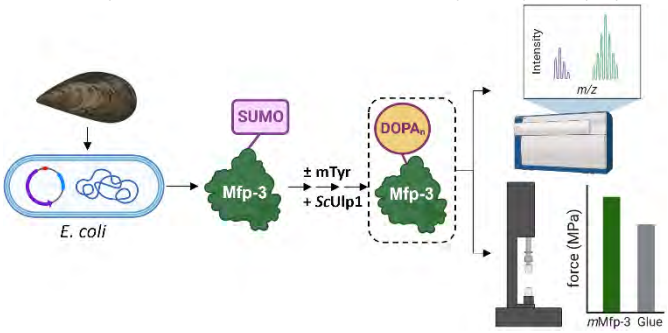

Figure 1. Alternative strategies to produce recombinant and modified mussel foot protein-3 (Mfp-3) using SUMO-fusion technology. The SUMO-fusion-tag is cleaved off using SUMO protease (scUlP1). DOPA-modification is accomplished using microbial tyrosinase (mTyr).

In our study we aimed to adress two aspects. On the one hand we wanted to overcome solubility limitations of previous recombinant production strategies of Mfp. Therefore, we designed Mfp-3s from the genus *M. edulis* and *M. galloprovincialis* as SUMO-fusion proteins. Our protocol for obtaining pure recombinant Mfp-3 only from the soluble protein fraction of *E. coli* is robust, reproducible and demonstrates the possibility of economical mass production (55 mg Mefp-3 and 100 mg Mgfp-3 per liter culture from 7.5 L-scale fed-batch bioreactor). Furthermore, we used recombinantly produced His_6-mTyr from *V. spinosum* to modify Mfp-3 *in vitro*. MALDI-TOF-MS confirmed that up to 12 hydroxyl groups were incorporated. Considering the fact that the Mfp sequences contained 10 tyrosine residues our finding suggests that not only DOPA but also 3,4,5-trihydroxyphenylalanine (TOPA) was formed.
Finally, the highly hydroxylated Mfp-3s exhibited excellent bulk adhesive properties. Modified Mefp-3 resulted in a tensile shear strength of 1.68 MPa, comparable adhesiveness to a commercial glue. Mfp-3 and Mfp-3-based polymers modified with His_6-mTyr have the potential to become a practical bioadhesive for material science as well as for medical and biotechnological applications for future biomaterials.

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Bioinspired living coating system for architectural surfaces

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Architectural coatings enhance the functional and aesthetical durability of building materials by protecting their surface. Consequently, paints and coating applied on the exterior of a residence, or a commercial building not only add to the aesthetics of the structure but also defend it against heat, UV, harsh winters, soaking rain, and other adverse weather conditions. The commonly used surface treatments consist of mineral oil binders and many environmentally unwanted ingredients, therefore environmentally friendly alternatives are within the interest of architects, constructors, and end-users. Recent progress in biomimetics allows the fabrication of man-made surfaces with similar properties as biological ones. Engineered living materials (ELM) use an alternative (living) set of building blocks compared with conventional man-made materials. ELMs, being a combination of artificial and biological components, are the most relevant contemporary revolution in materials science and engineering. Due to unique functionalities and outstanding properties, they outperform current examples of "smart materials". The convergence of engineering, biology, and materials science allows the integration of unicellular and multicellular organisms into next generation engineered systems. ELM's possess enormous potential as a new generation of active materials in sustainable built environments or as an integrated network of living sensors to monitor the presence and movement of pollutants and pathogens [1].

The ARCHI-SKIN project takes a bio-inspired approach and aims to generate completely new solutions for architectural coatings. A prototype microbial coating based on biofilm built by the ubiquitous, yeast-like, oligotroph fungus, *Aureobasidium pullulans* is under development. The concept presented in Figure 1, is based on a technically applicable, controlled, and optimized biofilm formation that effectively protects the substrate surfaces, assuring optimal service life performance and different functionalities including self-healing. This contribution presents ambitions, challenges, and first results related to the development of a living coating system for architectural surfaces.

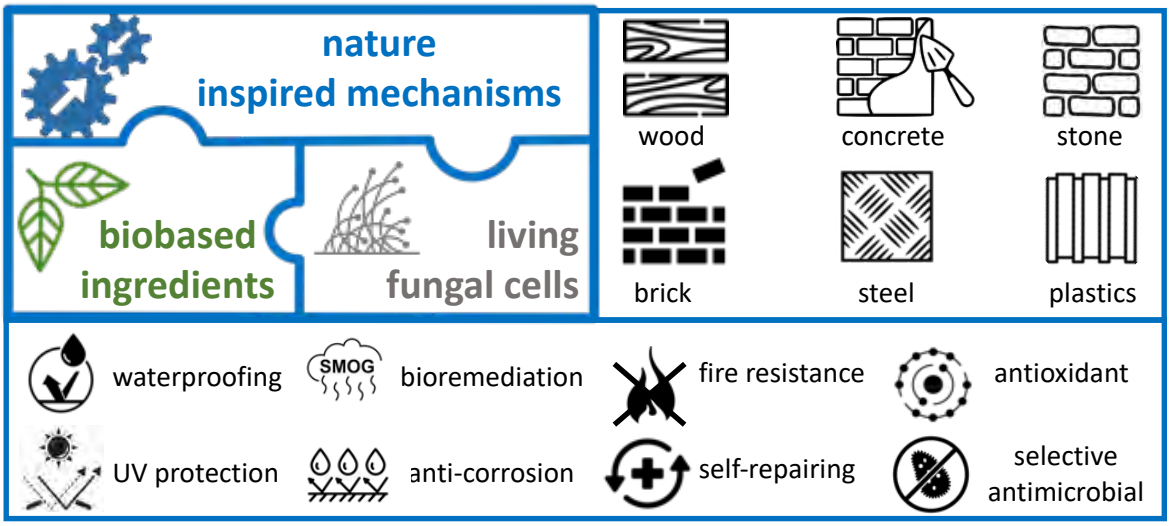

Figure 1. ARCHI-SKIN project concept.

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Bioinspired strain-stiffening design as a mechanical metamaterial

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During straining, soft biological tissue like skin or aortic tissue as well as single cells often show strain-stiffening properties. In single cells, strain-stiffening occurs because actin filaments are crosslinked into stress-fibers as a response to strain. This crosslinking mechanism was the inspiration for a unique, minimalistic design where the intracellular effect is mimicked by structural features: Parallel elastic slats between several backbones form the elements that induce the strain-stiffening effect (see Fig. 1). During elongation of the strain-stiffening unit, the flexible slats first deform and then touch each other, resulting in stiffening of the metamaterial. This structure has a highly nonlinear, adjustable, rate-independent and reversible strain-stiffening behavior that can be implemented into various elastic materials. Changes in geometry affect the point of stiffening as well as the initial and final stiffness of the unit. [1]
The strain-stiffening mechanics can also be used in complex three-dimensional arrangements like sheets or tubes. Strain-stiffening units and complex strain-stiffening arrangements were created in different sizes and from different materials via manufacturing techniques ranging from filament printed molds over SLA 3D printed structures to samples in the micron range produced by 2PP direct laser writing. To achieve strain-stiffening structures in the micron range, new designs and adaptations of the design were explored to ensure printability and overcome the challenges of the tiny features. Examination via tensile testing demonstrated the unique strain-stiffening behavior of the samples in the cm-range, which makes our structure a promising design feature for medical implants that should resemble the mechanical characteristics of soft tissue. Hence, our strain-stiffening metamaterial has vast application potential in the medical field, where a non-linear, tunable, two-stage mechanical response is desired.

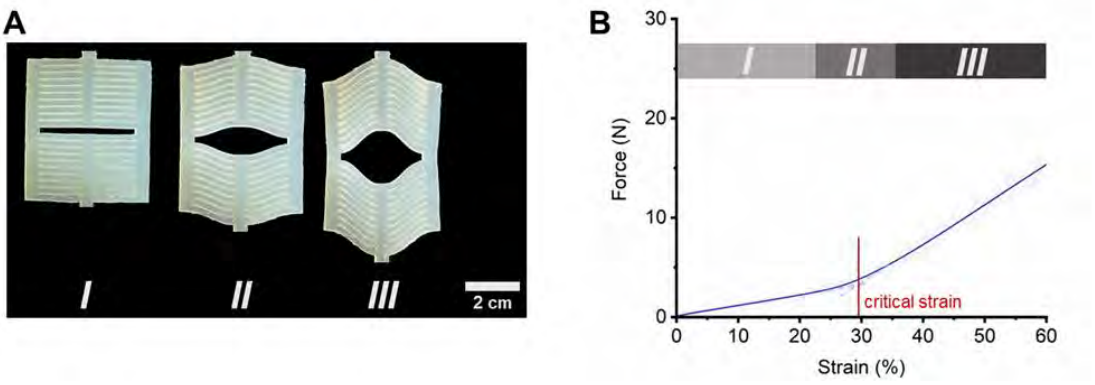

Figure 1. Straining the strain-stiffening unit from PDMS and respective force-strain-curve. (A) Shape change of the strain-stiffening unit from PDMS during a tensile test: During positive strain, the flexible slats come into contact, resulting in mechanical interactions and consequently stiffening of the 3D structure. (B) Force-strain-curve of the strain-stiffening unit shown in A. In the stress-strain-curve three stiffness regimes can be identified: The initial stiffness (I), the transition stiffness (II) and the final stiffness (III) [adapted from 1].

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Contact Angle Pump Inspired by Load-Induced Fluid Flow in Bone

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Nature includes a vast array of micro- or nanoscopic fluidic networks that perform various biological functions, including the transportation of fluids and nutrients, and regulation of physiological processes. For instance, the lacuno-canalicular network (LCN) in bone plays a crucial role in bone remodeling by sensing the fluid flowing through it and thereby orchestrating bone remodeling. This process helps maintain the strength, health, and flexibility of bone tissue.[1]

Microfluidic channels, which enable transportation of small volumes of fluids, have great potential in mimicking LCN in bone. However, fluid flow in microfluidic channels is typically studied under static boundary conditions without considering the dynamics and responsive nature of the liquid channels. A fluid flow can be directly induced within vascular networks as a result of externally applied deformation inputs.[2] This load-induced fluid flow is believed to transmit information and perform biological functionalities. Therefore, investigating the relationships and potential programmable interactivities between external mechanical loadings, matrix, and fluid flow is an intriguing approach to understand the fundamental mechanisms of information transmission in the venous architecture.

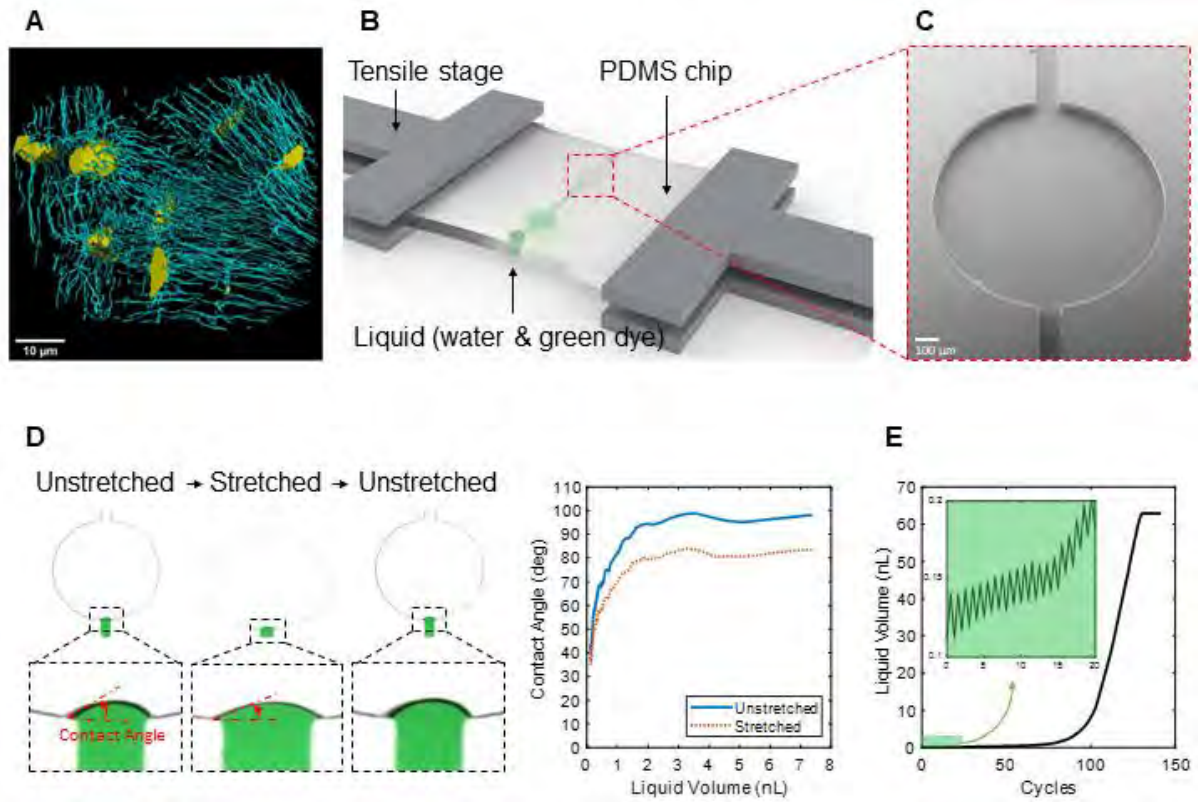

Figure 1. (**A**) Lacunae and canaliculi networks in bone.[3] (**B**) Experimental setup for studying load-induced fluid flow. (**C**) Scanned electron microscopy image of the PDMS microfluidic chip. (**D**) Fluid flow induced by stretching the PDMS matrix. Contact angle changes during stretching in the initial cycling stage. (**E**) Changes in liquid volume with the number of applied stretching cycles.

A polydimethylsiloxane (PDMS) microfluidic chip, inspired by the LCN in bone structure, features channels with a height of 80 µm (**Fig. 1A-C**). Capillary-driven forces enable liquid flow in the channel, but

are impeded by a geometric discontinuity at the reservoir entrance. Upon mechanical loading, the flexible substrate undergoes deformation, causing the contact angle between solid and liquid interface to change repeatedly (Fig. 1D). This stretch-induced deformation initiates a pump-like effect and facilitates reservoir filling (Fig. 1E). Therefore, mechanical loading ensures controlled unidirectional fluid flow, providing insights into fluid dynamics influenced by mechanical stimuli.

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Evaluation of substrate suitability for the production of mycelium-based composites by means of real-time PCR

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Natural systems underwent an extremely long evolutionary optimization process when compared with anthropogenic inventions. Many human-made processes and materials are therefore inspired by these systems. Fungal mycelium is the biggest networker in nature: while mycorrhizal fungi connect plants in the forest, wood-decaying fungi use their hyphal nets to degrade dead trees and plant matter, building up fertile top soil. Combining the ideas of connection and the ability of utilizing biomass for growth, led to the concept of using mycelium as a binder for disperse organic particles or fibers [1]. Fungal hyphae grow into the substrates, reaching every nook and cranny of it to access the nutrients. Terminating this growth process after complete interconnection – but before destabilizing degradation – can lead to a composite material with promising properties. Since the substrate has a big influence on these properties, several different organic materials have been tested already. While the main focus was on forestry and agricultural by-products so far, we investigated several different lignocellulosic waste streams that have not yet been described as substrates for mycelium composites. Apple pomace, sugar beet pulp, spent brewer's grains, cotton fibers, digested biogas substrate, green waste, paper sludge, and beech sawdust were inoculated with *Ganoderma sessile* and some of them also with *Pleurotus pulmonarius* and *Trametes versicolor*. For the quantification of fungal growth, a new method was established. The fungal DNA was quantified in a certain amount of composite and then converted into mycelial biomass based on DNA quantification in pure mycelium. The result gives an insight into how well the mycelium grows on different substrates and allows to compare between fungal species. In future studies, the influence of fungal colonization rate on different material properties can be investigated.

Figure 1. Pictures of sugar beet pulp (left) and apple pomace (right) colonized by *G. sessile* for 21 days.

With the established method, clear differences between the fungal species and the colonization rates on certain substrates were observed. Some combinations of fungi and substrates resulted in very poor growth during the 21 days of incubation whereas others led to firm composites (Fig. 1). Besides the mycelium content, the handling stability of the materials depends on the fungal species and substrate characteristics [2, 3]. However, a higher amount of mycelium is not necessarily indicating good stability

because *Ganoderma sessile* shows stronger surface growth compared to the other two candidates that has only little contribution to particle binding. *Ganoderma sessile* performed best in terms of fungal biomass production for all substrates with the exception of sugar beet pulp, where *Trametes versicolor* grew better. This indicates that not only the fungal species and the chosen substrate are relevant for successful composite fabrication, but also their combination.

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Fiber reinforced plant organs as inspiration for carbon fiber reinforced concrete constructions

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Carbon fiber reinforced concrete components (CCC) are regarded as a promising technology for the development of high performance and material-efficient buildings. Due to the material properties of carbon fibers, the amount of concrete in constructional components can be reduced. Therefore, new design strategies are needed for which inspiration can be derived from biological models.
Plants are exposed to a variety of forces and have to resist those without failure. Non-woody plants can be considered as a fiber reinforced materials consisting of lignified fibers embedded in parenchymatous tissue. In nodal elements, such as junctions between adventitious roots and shoots or the connection between petiole and lamina in peltate leaves, fibers are responsible for a reliable connection and efficient load distribution. Since the requirements for joints in CCC are quite similar, the fiber arrangement in such junctions may serve as inspiration for innovative bioinspired CCC.
In a multidisciplinary approach (Figure 1), we first characterized the anatomy and mechanical properties of respective plant structures and transferred the identified fiber structures into abstracted models. Additionally, material and growth models were developed and used to simulate the influence of differences in the fiber organization and load adaptations under growth and external stresses. Finally, promising structures will be scaled up to the dimension of building components and manufactured. The manufacturing process combines the robot-assisted placement of the textile reinforcement and 3D concrete printing technology.

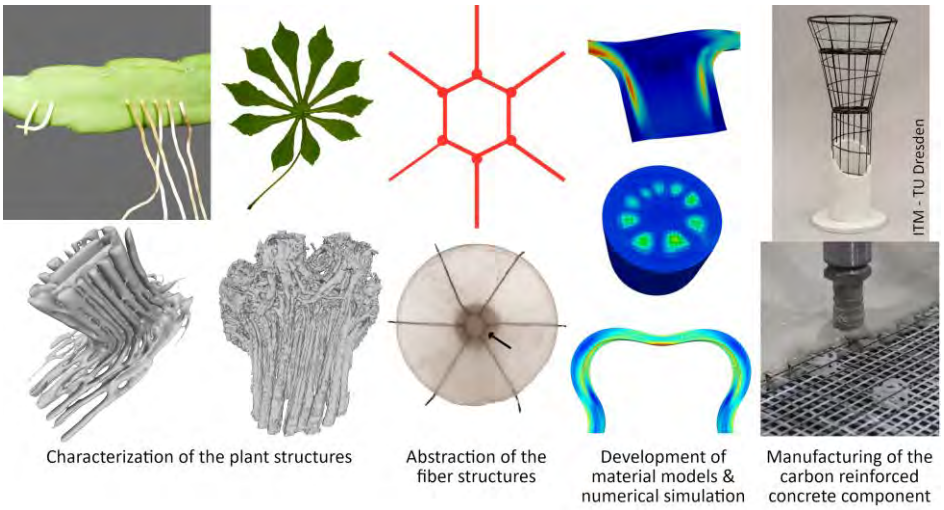

Figure 1. Multidisciplinary approach towards a plant structure-inspired carbon fiber reinforced concrete component (partially adapted from [1]).

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One Pot Synthesis of Ceramic Reinforced Hydrogels with Bioinspired Complex Microstructures Using Magnetic Assisted Slip Casting

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Bioinspired composites exhibit well-defined microstructures, where anisotropic ceramic particles are assembled and bonded by an organic matrix. However, it is difficult to fabricate these composites where both the ceramic particles and organic matrix work together to unlock toughening mechanisms that lead to stiff, strong, and tough mechanical properties. Here, we produce composites inspired by seashells, made of alumina microplatelets assembled in complex microstructures and are physically bonded by a small amount of interpenetrating polymer network (IPN) consisting of poly(*N*-isopropylacrylamide-co-acrylamide) and polyvinylpyrrolidone (PVP). The fabrication employs magnetically assisted slip-casting to orient the microplatelets as desired, and *in situ* gelation of the IPN, followed by drying. The process was successful after carefully tuning the casting and gelation kinetics. Samples with horizontal, vertical, and alternating vertical and horizontal microplatelets orientations were then tested under compression. It was found that the IPN threads bonding the microplatelets acted as sacrificial bonds dissipating energy during the compression. Paired with the alternating microstructure, the IPN significantly enhanced the compressive toughness of the composites by 205% as compared to composites with horizontal or vertical orientation only, and less than 35% reduction on the stiffness. This study demonstrates that microstructure control and design combined with a flexible and tough matrix can effectively enhance the properties of bioinspired ceramic polymer composites. Further research on the microstructured alumina-IPN composites in dry and hydrated states or varying different parameters (e.g., ratio between the hard and soft IPN-forming components, amount of crosslinker used, etc.) could unlock toughening mechanisms to generate bioinspired composites with outstanding mechanical properties and biocompatibility. [1]

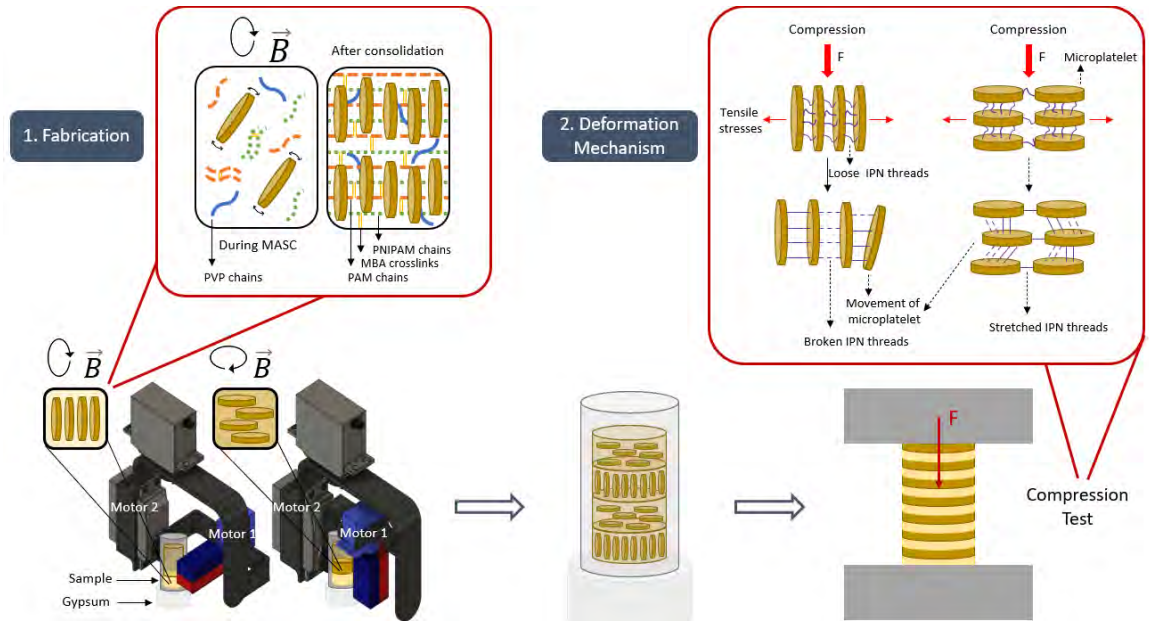

Figure 1. Schematic of the (1) fabrication principle using Magnetically Assisted Slip Casting (MASC) under a rotating magnetic field *B* and the (2) deformation mechanisms under compression of microstructured composite.

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Simple Sol-gel Fluorescent Protein Stabilization Towards Energy-related Optoelectronics

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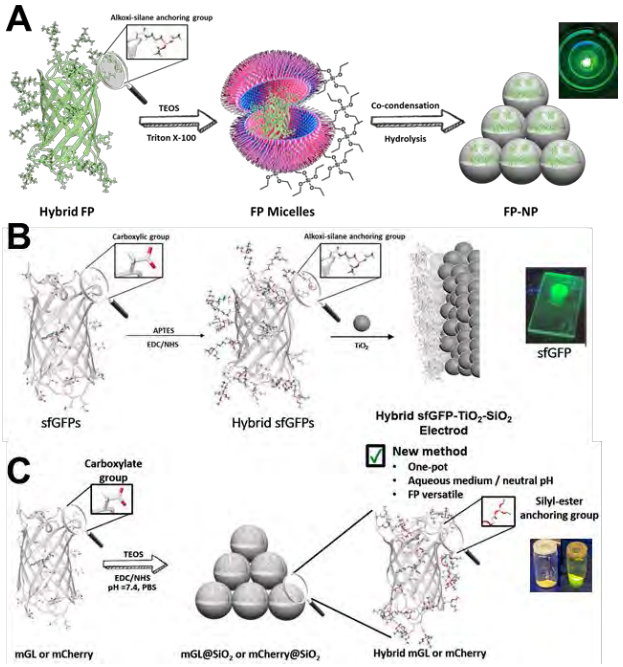
Fluorescent protein (FP) based optoelectronics is a frontier emerging technological concept aiming at replacing environmentally unfriendly and/or toxic components with a new class of eco-efficient and highly performing biogenic and/or bio-hybrid materials/components. Here, the paramount question is how to preserve the unique chemical diversity and hierarchical structure already programmed to optimize their bio-functionality when interfacing with our technological requirements (i.e., fabrication using organic solvent, high temperatures/irradiations operation conditions, inert atmospheres, organic/inorganic interfaces, and much more demanding the need of water-free environments).

Among the stabilization methods, classical sol-gel is the most effective in terms of absolute stability values under different conditions and protein-based device performance. Our team has stablished synthesis methodologies for core-shell FP@SiO_2 nanoparticles, realizing first-class Bio-hybrid light-emitting devices (Bio-HLEDs; Figure 1A). [1] What is more important, the FP@SiO_2 nanoparticles feature outstanding stabilities over 1 year in organic solvents and over thousands of hours under device operation conditions. Prior results showed stabilities of <24 h in polymer nanoparticles and devices with a few hours stability using the respective native FPs.

This approach was further adapted to fabricate stable FP based dye-sensitized solar cells (DSSCs; Figure 1B). Here, *in situ* sol-gel chemistry on metal oxide electrode surfaces allowed a stronger sensitizer attachment onto the electrode through alkoxysilane linkers. This resulted in devices with >2000 h of stability under continuous irradiation without reducing solar conversion efficiency, stablishing a new record for Bio-DSSCs. [2]

Although these results highlighted the great of sol-gel, the biggest drawback is the poor versatility due to the need of harsh reaction conditions that not all FPs can stand (Figure 1C). By covalently bonding FP to the silica precursor, a one-pot water-based, pH neutral and surfactant-free reaction was achieved. Here, the FP acts as a seed for the formation of the silica, auto-catalysing the formation of a network in which all pores were occupied by a FP unit. The simplicity of this method allowed us to take advantage of the stability improvement of the encapsulation in the fabrication of multiple component FP@SiO_2 nanoparticles. By encapsulating two FPs together in the same nanomaterial, white emitting Bio-HLEDs were prepared keeping the excellent performances previously reported.[3]



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Snake-skin inspired 3D structure as a candidate for mechanically adaptive scaffold for cells

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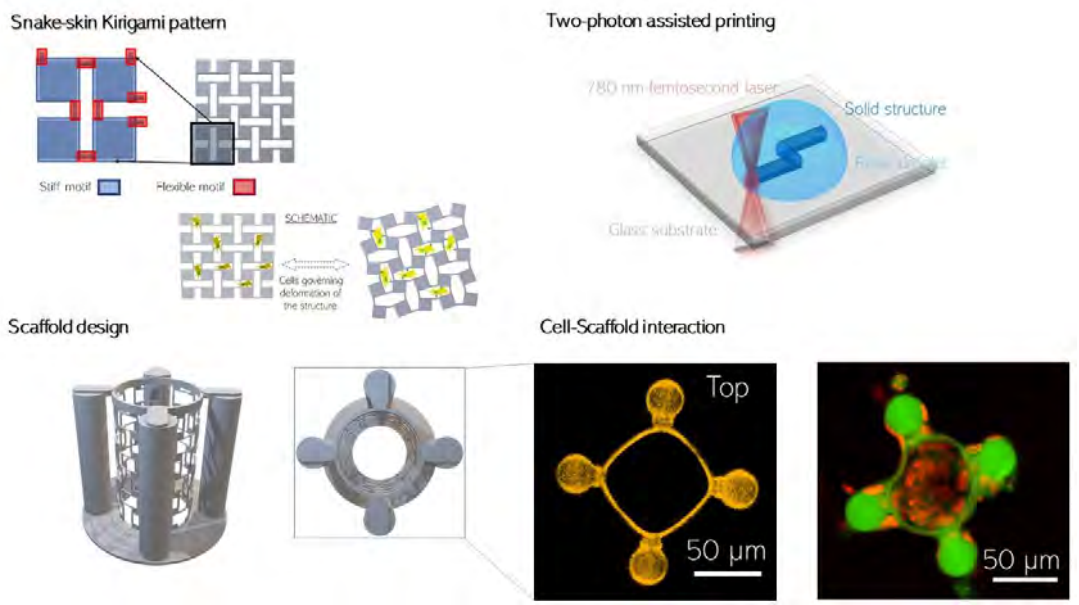
Cells are known to have an active participation in shaping their environment. It has been shown that there is a two-way communication between the cells and the scaffold/extra-cellular matrix. However, most of the approaches have been towards manipulation of cells via either chemical modification of surfaces or static 3D patterns. The aim of this project is to design and fabricate structures which can be actively deformed by traction forces applied by cells.[1]



In the current work, a scaffold design inspired from snake-skin Kirigami based meta-material has been showcased. A snake-skin is flexible, yet it protects the creature on multiple harsh terrains. Similarly, the scaffold is made of tiles that provide surface area, and thin joints connecting the tiles that provide flexibility to the whole structure.[2] The whole structure is 3D printed of the same material. The structures are printed with the help of two-photon-polymerization based printing. This technique uses a near-infrared laser to achieve photopolymerization and can produce feature sizes as small as 100 nm. This allows us to fabricate structures in the size range of what can be perceived as truly 3D environments for cells. The auxetic design not only decreases the force needed for deformation in contrast to bulk material but also notably surpasses the conventional 'beams-and-columns' scaffold design in this aspect. Mechanical characterization of Kirigami pattern at macro-scale (mm range) shows 80-90% drop in stiffness compared to the bulk material. Such low structural stiffness allows us to use wide range of materials since the mechanical properties can be modulated by changing design parameters rather than material chemistry. Preliminary cell studies show the scaffold is structurally soft enough to have visible deformation after cells have attached to the structure.

Study of cell-scaffold interaction in such a system will give us an insight into how cells behave in a 3D auxetic environment, and the kind of forces cells exert in an individual as well as collective form on their surroundings.

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Standardized test methods for performance assessment and lifetime prediction of bioinspired composite materials

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To stop climate change and to protect our natural habitat as well as biodiversity the whole society has to take action. Especially the building and automotive industries are characterized by huge resource and energy consumption als well as pollution of air and environment due to harmful emissions from production processes or the technical use of non-biodegradable and non-recyclable resources and materials. [1] These critical discussions already started in the 1990s. Since then, intensive research work has been done internationally, to investigate the utilization and processability of renewable and biodegradable resources, e.g. the use of natural fibers like wood or bast (hemp or linen) as reinforcements in composite materials again or the direct use of technical biopolymers, e.g. cellulose, which are basically the components of plant fibers and tissue. [2,3]

Besides the very important material science to utilization and processability of new raw materials, the development of standardized test methods for performance and lifetime prediction based on the identification of material-specific defects and the interpretation of their evolution due to service loading is mandatory for the assessment of the technological potential and the scientific basis for technical implementation. [4] This study presents experimental approaches on three different material systems (cellulose composite, wood composite, bast fiber-reinforced plastic) for the reproducible acquisition of characteristic material values, which are needed to estimate the suitability for the intended application.

Test method design was focused on the definition of application limits with regard to mechanical (quasi-static , fatigue) and functional (humidity, temperature) loading, the definition of optimization potential with regard to the manufacturing process (parchmentizing, bonding, organic sheet production) as well as the definition of guidelines for component design (Figure 1).

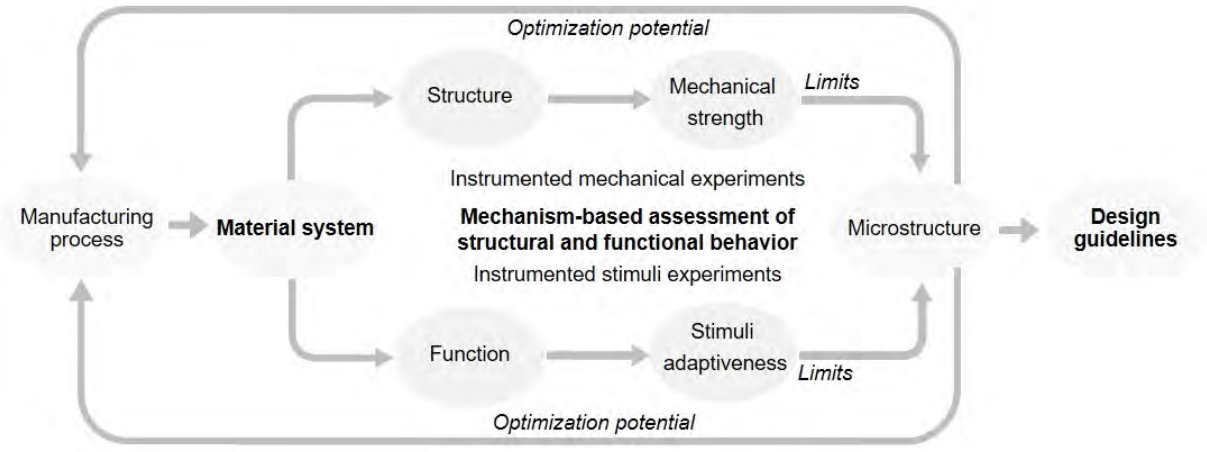

Figure 1. Test method design.

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The wet adhesive wood interface - A biomimetic approach towards surface modification

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The evolving demand for not only recyclable, but also biosourced functional materials drives research in sustainable materials chemistry. Nature sets several examples where the general key factors for adhesion, topography and surface chemistry, result in functional bonding of different materials [1]. Emulating those principles, we aim to combine both in the manipulation of a biosourced and environmentally benign material towards a wet adhesive system.

While the functionality and topographical advantages of artificial nanopillars on surfaces are a known topic of research, the fabrication of such small structures is highly energy-intensive [2]. Wood on the other hand is a renewable and intrinsically anisotropic material which provides an uniquely ordered hierarchical cellulose scaffold. In this work, we present a process involving the controlled fibrillation of a wood surface and subsequent application of a coating to overall mimic the composition and adhesion of mucilagenous seeds [3,4]. A novel and efficient protocol was established to modify only the desired delignification depth of the wood specimen. Following the principal of mucilagenous seeds, the fibre framework is then coated by ductile polymers. To obtain further control over the adhesion strength under varying conditions, a thermo-responsive polymer is introduced. The combination of renewable materials with a biomimetic coating promises wet adhesion through engineering on the microscopic level while providing an environmentally friendly solution.

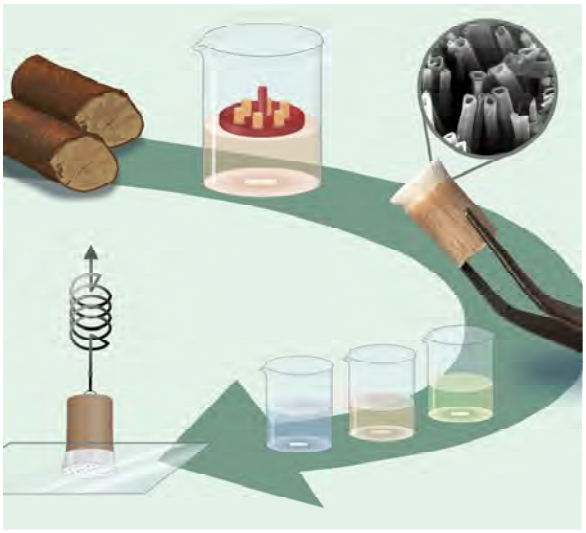

Figure 1. Processing of wood by chemical modification and coating to obtain a wet adhesive system.

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B: Hierarchical materials and structures (inorganic/organic)

Effect of microplatelet orientation in 3D printed microplatelet reinforced composites with bioinspired microstructures

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Natural structural materials have evolved complex microstructural designs over billions of years of evolution, giving rise to a diverse range of intricate hierarchical microstructures that yield exceptional performance in terms of strength, resilience, toughness, and adaptability.[1,2] Amongst the many natural microstructures,[3] the microplatelet-based brick-and-mortar arrangement found in the nacreous layers of seashells is the most studied.[4] However, synthetically reproduced nacre composites always show local misalignment of the microplatelets,[5] and little is known about the effect of this misalignment on the final properties. Furthermore, there exist a number of more complex microstructural alignments,[6] but it is difficult to understand the role of the minerals orientations due to the lack of processing methods to reproduce them in artificial composites. Understanding the effect of microplatelet orientation on the macroscopic properties of reinforced composites would be interesting to then design microstructures specific for an intended application.

This study investigates the influence of microplatelet orientation in composite materials, utilizing the magnetically assisted direct ink writing method (M-DIW) to create microstructured microplatelet-reinforced composites as depicted in Figure 1. Experimental and computational approaches are employed to explore the critical role of microplatelet orientation on the flexural properties of these materials. Horizontal microplatelets are found to significantly enhance the composite's flexural toughness 17% by promoting overlap and increasing fracture energy during crack propagation. Vertical microplatelets contribute to increased flexural modulus 13% and strength around 10%. Perpendicular microplatelets facilitate straight crack paths and smoother fracture surfaces. Moreover, complex microstructural designs were introduced by strategically combining microplatelet orientations to optimize mechanical properties. These findings emphasize the vital role of microplatelet orientation in composite materials, offering potential for tailored materials with superior performance.

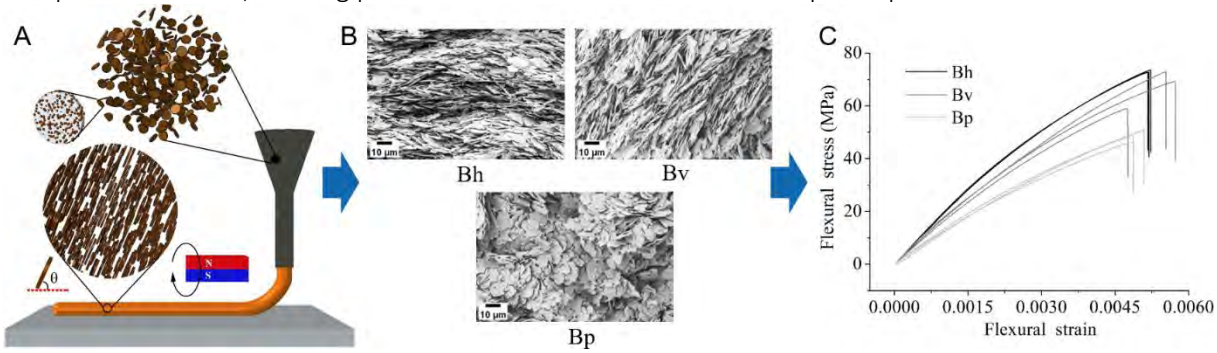

Figure 1. Influence of microplatelet orientation on flexural properties in composite materials. (A) Magnetically assisted direct ink writing method. (B) SEM microstructures and (C) flexural mechanical properties under three different magnetic configurations: Bh (horizontal), Bv (vertical), and Bp (perpendicular).

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Exploring Spider Silk: X-ray Nanobeam Investigation for Medical Advancements

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Spider silk, with its impressive hierarchical structure, is a fascinating material that nature has developed over nearly 400 million years. Furthermore, spider silk can be used for a range of potential medical applications. Specifically, spider silk exhibits promise as a constituent of nerve conduits and a material for nerve regeneration. Silk provides support for the adhesion and movement of Schwann cells (SCs), which are critical for peripheral nerve regeneration [1]. Our recent findings show that even small variations in silk properties affect SCs. For instance, various silk sterilisation procedures have shown significant impact on the motility of SCs along the major-ampullate (MA) silk obtained from *Trichonephila edulis* spider. Autoclaving has been observed to impede SC movement along the fibre, whereas ethanol treatment increases their speed. These observations may be related to stiffness modification, mainly in autoclaved fibres, and ultrastructural changes in ethanol and autoclaved fibres, as concluded from previous nanobeam X-ray diffraction experiments at ESRF, ID 13 [2].
It is established that SCs demonstrate mechanosensitive behaviour [3,4]. Since they appear to be sensitive to small changes in structure as well, our study focused on analysing strain-dependent ultrastructural changes by conducting x-ray nanobeam experiments at MAX IV, Nanomax. We utilized Tubuliform (TU) and MA silk, which coexist in the protective layer that safeguards *Trichonephila inaurata (T. inaurata)* spider's eggs. Both types of silk were used to investigate strain-dependent ultrastructural changes. Previous nanobeam X-ray diffraction measurements at ESRF, ID13 showed that β-sheet poly-(L-) alanine nanocrystals for TU silk had a larger d-spacing in intersheet direction compared to MA silk (Fig. 1). These differences in ultrastructure may also be connected to findings from single fiber tensile tests, which indicated that TU silk had lower strength and higher extensibility than MA silk. To study spider silk in a close-to-natural environment, a series of in-situ tensile humidity cell experiments were performed at ESRF, ID 13. Experiments were conducted in varying relative humidities, and nanobeam scanning technology was used to gather position-resolved data on individual spider silk fibers. Initial results show pronounced variations in spider silk ultrastructure as a function of stretching rate and humidity. A more comprehensive set of results will be presented at the conference.

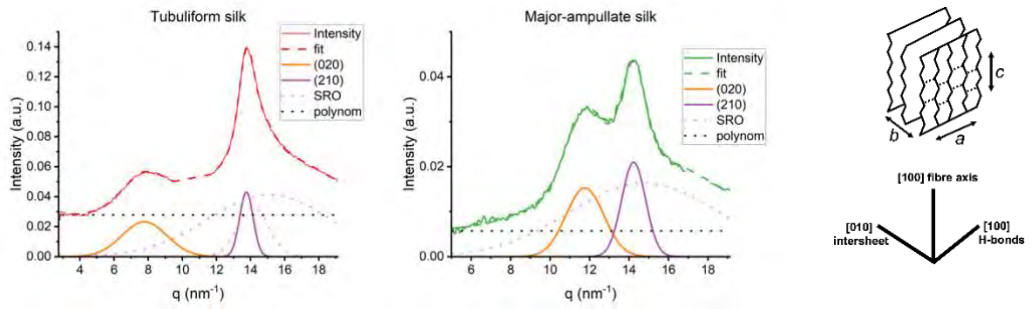

Figure 1. XRD patterns (1d Integration) for MA and TU silk. Bragg peaks and short range order (SRO) Peaks are fitted by Gaussians. Directions in the crystalline lattice are indicated on the right.

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On the wear resistance index for anisotropic biological materials

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Biological materials are largely anisotropic regarding their microstructure and mechanical properties. The contrasting properties in different axis creates extraordinary materials with intricate characteristics such as wood, where the alignment of the microstructure enhances stiffness on the longitudinal section rather than the radial, and bone, with not only elastic modulus but also fracture toughness highly dependent on the orientation of osteons. There is extensive research regarding the anisotropic mechanical characteristics of materials such as elastic modulus and hardness. However, our study aims to bring wear resistance correlation to microstructure to the spotlight. "Wear" is a complicated phenomenon that emerges from contact stresses and involves damage by localized yielding and cracking thus it is not an intrinsic property but rather influenced by the two in-contact bodies.[1] Considering stresses during blunt contact, the index of H^3/E^2, where H is hardness and E is the elastic modulus of the studied material, has been shown to well correlate with its capacity to resist wear damages.[2] Nevertheless, this index is only valid for frictionless contacts and homogeneous isotropic materials. As biological and bioinspired materials are mostly heterogeneous and anisotropic, there is a great need to understand their behavior under wear loads. In this study, we analyzed the wear resistance of the wandering spider (*Cupiennius salei*) claws (Fig 1A). The structure is made of a cuticular material, a biocomposite where chitin fibrils are embedded by a proteinaceous matrix. The cuticle is formed by three regions, epi-, exo- and endocuticle (Fig 1B), each with contrasting orientations of the chitin fibrils. For example, in the exocuticle, the chitin fibers are oriented parallel to the claw longitudinal section. Nanowear tests, where a 1 µm conospherical tip abrades a longitudinal section of the claw, showed contrasting results depending on the tip movement direction, despite a local constant H^3/E^2 index (Fig 1C). This highlights the inconsistency in the wear index for anisotropic materials and indicates the need for understanding wear on heterogeneous structures.

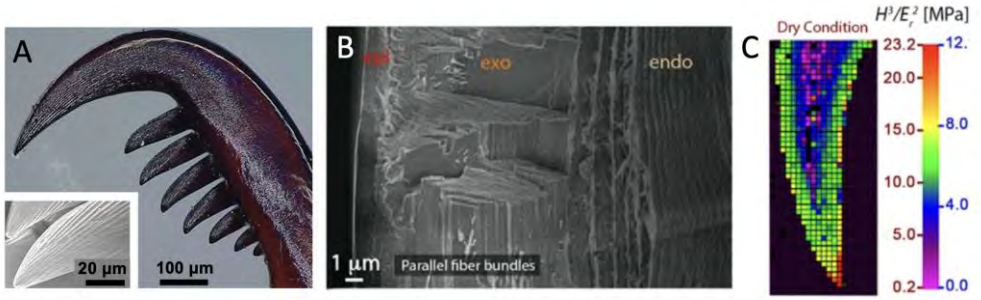

Figure 1. (A) The *C. salei* spider claw. (B) SEM micrograph of a longitudinal section of the spider claw, revealing the chitin fiber architecture in the exo and endocuticle. (C) The distribution of H^3/E^2 ratio values, extracted from elastic modulus and hardness indentation maps. Adapted from [3].

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Structural characterization of centric diatoms through lab-based nanoCT

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Diatoms are single-celled microalgae with unique cell walls (frustules) composed of amorphous hydrated silica composites and an organic layer. Due to their interesting structure and morphology, consisting of uniform and symmetrically arranged pores, they can inspire the development of new materials for nanotechnology applications.

The 3D structure characterization of a centric diatom frustule of the species *Actinoptychus senarius*, with a diameter of about 60 µm, was obtained using the lab-based X-ray microscope Xradia 810 Ultra, here referred to as nanoCT. The nanoCT is a resourceful tool for the 3D characterization of samples down to 150 nm of resolution within a field of view of 65 µm. Due to the low energy X-ray source (5.4 keV) and Zernike phase contrast, this X-ray microscope is ideal for the analysis of low-density samples such as soft tissues and polymers. In addition to structural characterization, a loading stage setup mounted on the sample rotation stage allows for *in situ* mechanical experiments and for the observation of microstructural changes in the sample as a function of the loading and time.

The structural characterization of the frustule enabled the quantification of the pore's sizes, proportion and distribution within the specimen (Figure 1a). Furthermore, the *in situ* experiment allowed the observation of fracture evolution at different levels of loading. The cracks originated in the porous diatom frustule and propagated along a path of connecting pores, eventually fracturing the center of the frustule at a later stage (Figure 1 b).

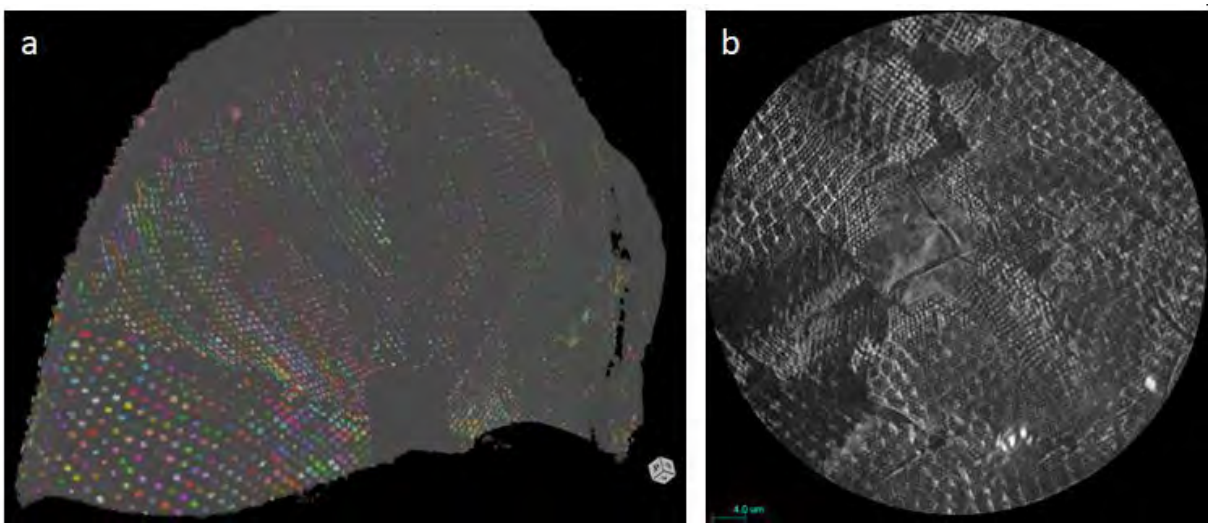

Figure 1. a) Diatom segmented for the structural quantification. Colourful spots correspond to the pores in the frustule. b) Diatom frustule after compression showing fractures in the pores and central regions.

Tailoring mechanical properties of 2D structures using additive manufacturing

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Biological systems often have multi-scale structures to achieve their functionality, e.g., tendons have a multi-scale structure composed of collagen fibers arranged hierarchically parallel to the long axis of the tendon. A tendon's J-curve usually represents the mechanical behaviour of such structures, which is divided into four regions: (I) toe region, (II) linear region, (III) plastic region, and (IV) failure region. The toe region represents the alignment of the collagen fibers. At 2% tension, all fibers are already out of their crimped state. In the linear region, the collagen fibers respond to the load in a linear fashion. The two subsequent regions (plastic and failure) represent the beginning and the total failure of the collagen fibers[1].
Inspired by the J-curve generated by the stretching of a tendon as a reference, 2D TPU structures have been created using additive manufacturing. Parallel elastic slats form the elements that induce the strain-stiffening effect (see Figure 1). Three designs: set of waves, waves, and tape were analysed, with the same thickness and length, but varying the design and the number of slats. As the structures are stretched, there is a longitudinal change in the structures, creating a unidirectional orientation, which causes them to become stiffer and more difficult to stretch at greater elongations similar to collagen fibres in the tendon. This work explores the relationship between the structural design and mechanical properties to enable mimicking the tendon motion.

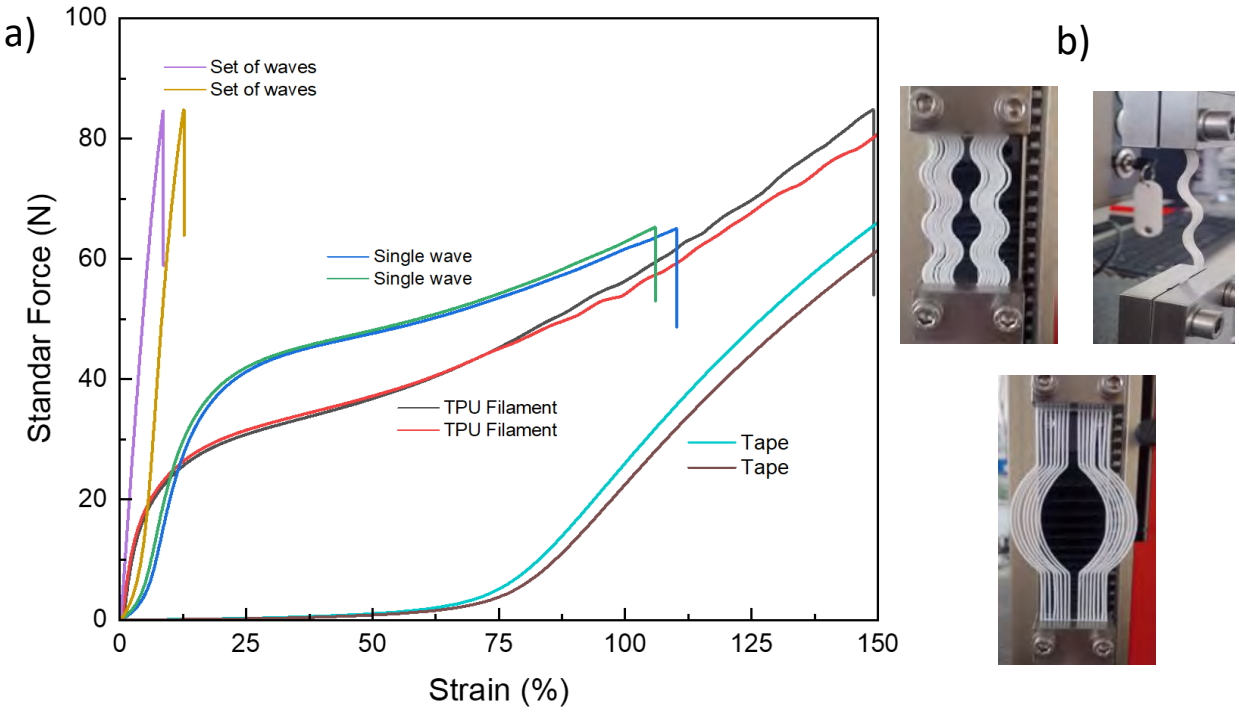

Figure 1. a) Three different mechanical response stress-strain curves of 2D TPU structures. Structure I (waves set) shows that such an arrangement leads to a decrease in stiffness. On the other hand, structure II (single wave) shows a slight delay and an increase in stiffness compared to the measured filament without any arrangement. Similarly, structure III (tape) shows a longer delay in the stiffening response. b) TPU structures (I, II, III)

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Threedimensionally patterned, hierarchically and anisotropically structured bacterial cellulose for immobilisation, degradation and biosensor applications

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Among the most inspiring communities in nature are biofilms. They are a rich source of different biopolymers. In particular, water-insoluble bacterial cellulose (BC) has become the subject of materials science studies due to its mechanical properties [1, 2].

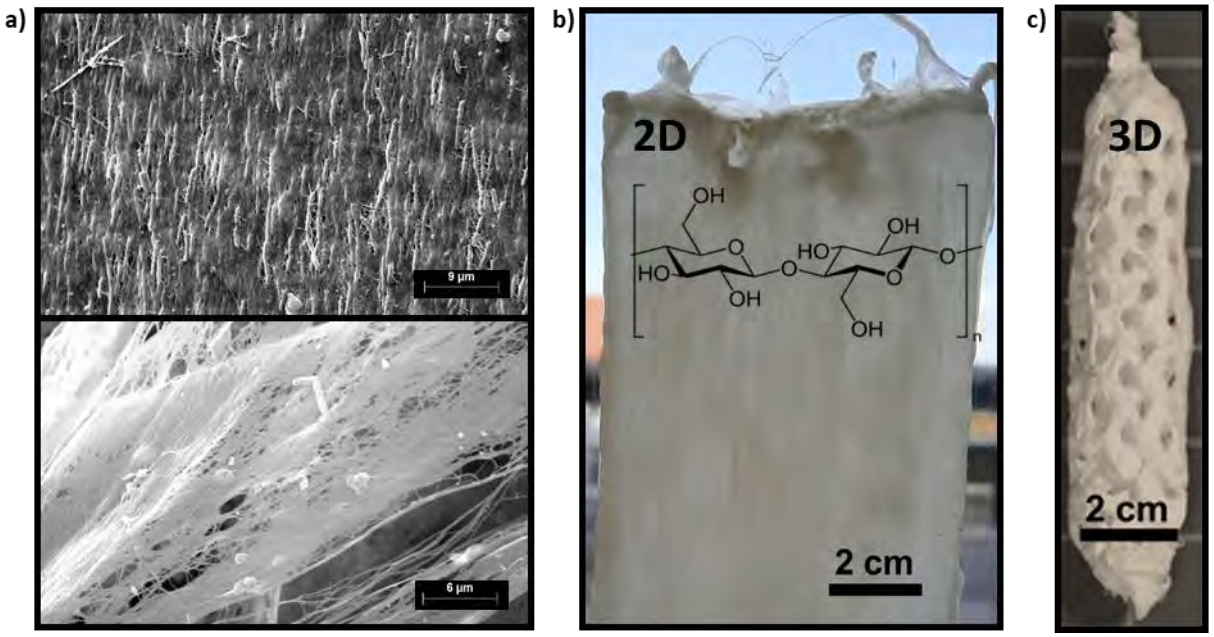

Figure 1: Aligned BC were produced in 2D and 3D shapes inspired by biofilms under water flow. a) Scanning electron microscope image of aligned BC. b) Two dimensional (2D) body of oriented BC. c) Three dimensional (3D) body of oriented BC.

We present a new biomediated approach to the preparation of structured and porous materials. By adjusting the manufacturing parameters it allows to tailor the material texture from the nanometre to the millimetre scale. We have fabricated 2D and 3D anisotropic materials by a combination of structured growth surfaces and continuous media flow [3, 4].
These materials are currently being tested for their use in the in-vitro immobilisation of co-cultures by means of symbiotic growth for the production of exopolysaccharides, the degradation of pollutants in aqueous systems and the development of biosensors as living materials.

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Translating the natural hierarchical composite design into inorganic-organic composites through direct-ink-write 3D printing

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Natural inorganic-organic composites exhibit an exceptional combination of stiffness, strength, and toughness, owing to their intricate hierarchical composite structures. Firstly, as natural materials form through bottom-up growth, they are assembled from the most basic building blocks in a hierarchical manner. Secondly, their compositions feature (1) aligned stiff anisotropic fibres or particles with a thickness ranging from one to a few hundred nanometres, (2) connected by a soft matrix, and (3) a tight interface in between. This hierarchical organization, from molecular to macro scales, achieves a strong coupling between the inorganic and organic constituents, fostering synergistic interactions across multiple length scales. Inspired by the hierarchical composite design in nature, this presentation introduces a hierarchical design strategy via direct-ink-write (DIW) 3D printing, which integrates shear-induced alignment of ceramic platelets within a polymer matrix at the unit strut level, and further 3D prints the unit struts into macro-structures for desired properties.

In the first study, ceramic nanocomposites with a high inorganic content (95 wt.%) and a minimal organic phase are prepared, which reveal excellent flexibility for shape morphing owing to their bioinspired concentric lamellar microstructure. These flexible ceramic nanocomposites can be used in 4D printing through a programmable prestrain approach to deform into complex structures hardly achievable with conventional 3D printing. With a high inorganic content, the materials also exhibit excellent mechanical properties, anisotropic thermal properties, and resistance to corrosion and high temperatures.[1]

In another study, the hierarchical strategy is leveraged to fabricate strong and tough ceramic-reinforced organo-hydrogels consisting of (1) 5 wt.% ceramic platelets, (2) a highly crystalline poly(vinyl alcohol) (PVA) organo-hydrogel matrix, and (3) silane-treated ceramic-polymer interfaces. By 3D printing unit struts with the above compositions into a selection of bioinspired macro-architectures, this strategy is capable of translating mechanical mechanisms from natural materials into composite organo-hydrogels with a combination of high stiffness, strength, and toughness, through multi-scale energy dissipation mechanisms. Also, the composite organo-hydrogels can be simultaneously endowed with excellent operation tolerance and electrical conductivity for potential applications in flexible electronics under mechanically demanding conditions.[2] Hence, these studies showcase a model strategy that extracts the hierarchical composite design principles from nature and applies them to harness the benefits of both inorganic and organic constituents in composite materials. Being versatile and applicable in diverse material compositions, this strategy shall also inspire future research on the design and fabrication of bioinspired materials.

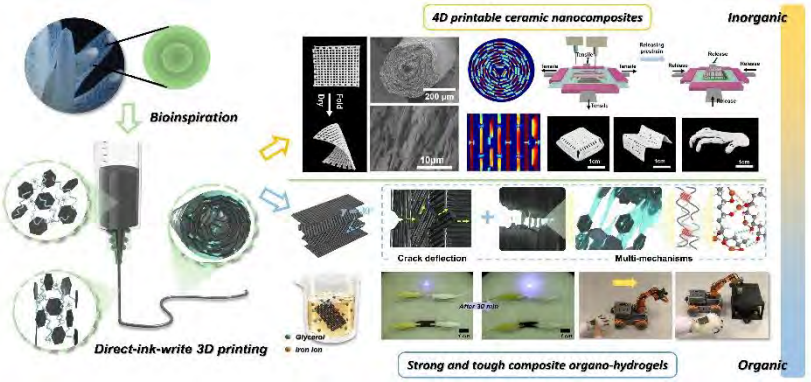

Figure 1. Hierarchical design strategy for inorganic-organic composites through direct-ink-write 3D printing.

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Ultra-tough and Multi-functional Hydrogels with Bioinspired Hierarchical Structures and Multi-mechanisms

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Flexible and biomimicking hydrogels are highly attractive engineering materials of biosensors, soft robotics and flexible electronics. However, the practical application of hydrogels is currently limited by a series of challenges, including inherently fragile networks, trade-offs between mechanical and electrical properties, and limited environmental adaptability. In contrast, natural materials such as tendons and skins, are renowned for their excellent combination of high strength and toughness, owing to their hierarchical structures with synergistic reinforcement mechanisms ranging from nano to macro scales. Moreover, due to their nature as biological hydrogels, these bio-tissues have high sensitivity and exceptional environmental adaptability that are difficult to achieve in artificial materials.

To replicate the hierarchical structures and excellent properties of natural materials, we devised an intricate and versatile processing route for preparing strong and tough hydrogels by a freeze casting-assisted solution substitution strategy (FASS). FASS enables the formation of organo-hydrogels in one step with tissue-like hierarchical structures including anisotropic honeycomb microstructures, entangled and aggregated fiber bundles and fibers, high-functional nanocrystalline domains, and strong molecular bonding. This optimized hierarchical structure endows the organo-hydrogel with outstanding tensile strength (20.8 MPa), stretchability (2227%) and crack insensitivity with a record-high fracture toughness (260 MJ/m3). In addition, the organo-hydrogel is endowed with broad-spectrum multi-functions, including strain and stress sensitive, fatigue- and fracture-resistant, as well as even long-term stability, tolerant to both high temperature and freezing that are difficult to achieve with conventional hydrogels, making it an all-purpose material that can overcome the challenges in real-life applications of flexible electronics. More importantly, combining multi-scale simulation and experimental modelling, our work establishes a systematic roadmap for strong and tough functional hydrogels by unraveling the processing-structure-property relationships in our organo-hydrogel through its multiple levels of structure and functionality. The roadmap also offers an all-around approach that can inspire the development of other advanced functional materials for a multitude of applications.

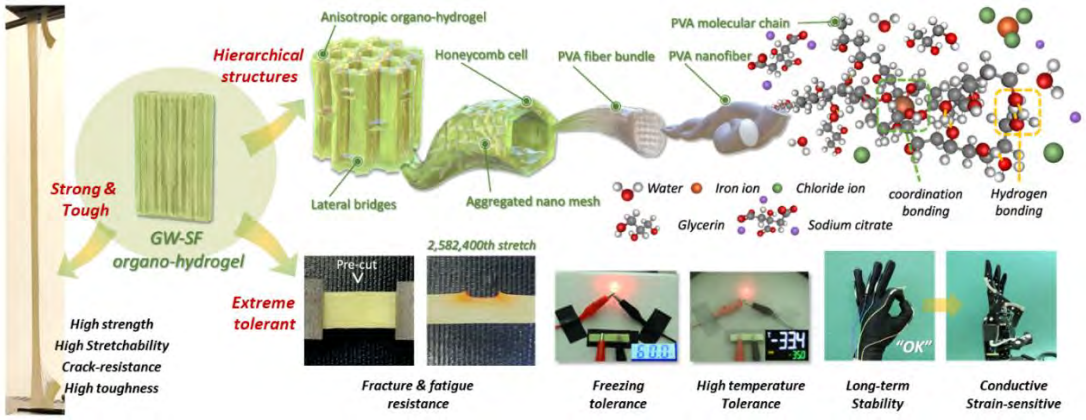

Figure 1. Strong and tough organo-hydrogels with tissue-like hierarchical structures and multi-functions.

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C: Complex-shaped biological materials

Fomes fomentarius fruiting body: How hierarchical structuring and chemical composition influence the compressive mechanical properties

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Fungal composites are attracting growing interest in the development of sustainable, degradable structures, but little is known about the structure-property relationship of their natural counterparts. The fruiting bodies of *Fomes fomentarius* are surprisingly resilient and lightweight materials. We investigate the interplay between hierarchical structure and mechanical resistance in different segments (crust, trama, hymenium and mycelium core) of the fruiting body. We found considerable differences in the chemical composition and arrangement of hyphae in these different segments, with the crust and hymenium in particular being characterized by a sophisticated structuring that leads to high strength.

Our analysis encompasses the structural, chemical, and mechanical characterization of the fungal structure, with a specific focus on the composition of the cell wall, including chitin and glucan content, degree of deacetylation, and trace element distribution. Notably, the hymenium exhibited superior mechanical properties alongside the lowest porosity. Our findings suggest that this exceptional strength can be attributed to the abundance of skeletal hyphae, the highest chitin content in the cell wall, and the honeycomb structure. Additionally, an elevated calcium content was observed in the hymenium and crust, with SEM-EDX confirming the presence of calcium oxalate crystals.

Interestingly, the crust displayed layers with varying densities and different calcium and potassium content, along with the presence of α-glucan, while β-glucan was identified in the other segments. These results underscore the significance of considering the diverse structural and compositional characteristics of different segments when designing materials and products inspired by fungi. Moreover, the porous yet sturdy structure of the hymenium holds promise as a blueprint for the development of modern smart materials.

Relationship between structure, mechanical properties and function in locust cuticle

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Insect cuticle is a multifunctional biological material. It is the exoskeleton of insect, forming various functional surface structures. One of its striking characteristics is the wide range of its mechanical properties. The elastic modulus of insect cuticle, for example, covers a range of more than eight orders of magnitude [1]. Why do cuticle properties vary so dramatically? With the aim to address this question, researchers have used a set of different testing methods to measure properties of cuticle specimens, which were selected from various body parts, across a variety of insect species and often preserved/prepared in different ways [2,3]. Almost all these factors can influence cuticle properties. Hence, the literature data cannot be simply compared with each other, and no solid conclusion can be drawn regarding the mechanisms that underlie the property variations in the cuticle. To fill this literature gap, our studies are focused on two key questions. First, how do the mechanical properties of insect cuticle vary in a single species, when all testing conditions are kept constant? Second, what are the mechanisms behind the variations of the cuticle properties?

Using a combination of scanning electron microscopy (SEM), micro-computed tomography (micro-CT), confocal laser scanning microscopy (CLSM) and nanoindentation, we performed one of the most comprehensive studies to date, where we simultaneously investigated the microstructure, sclerotization and the elastic modulus of locust cuticle. We have shown that, in the desert locust *Schistocerca gregaria*, the elastic moduli of tibiae, femora and compound eyes range from 0.5 GPa to 8 GPa [4-7]. This property change can be explained almost fully by the differences in the microstructure and sclerotization of the investigated specimens. Our results suggest that, in most cases, sclerotization determines the difference between the elastic moduli of different body parts, whereas anisotropic properties of each body part are controlled by their microstructure, in particular by fiber orientation. However, it is still possible that a cuticle specimen that is not as sclerotized as others shows a stiffness as high as more sclerotized specimens. This is in contradiction to the common understanding and is particularly observed in the hind femur, which consists of a cuticle that is less sclerotized than those of the fore and mid femora but can still reach a stiffness higher than others in certain directions. This, therefore, suggests that the interaction between the microstructure and sclerotization may not be as simple as previously thought. We expect that our results help to better understand the complex structure-material function relationship in insect cuticle. In addition, the obtained detailed data might be potentially interesting for biomimetic development of lightweight structures for various applications.

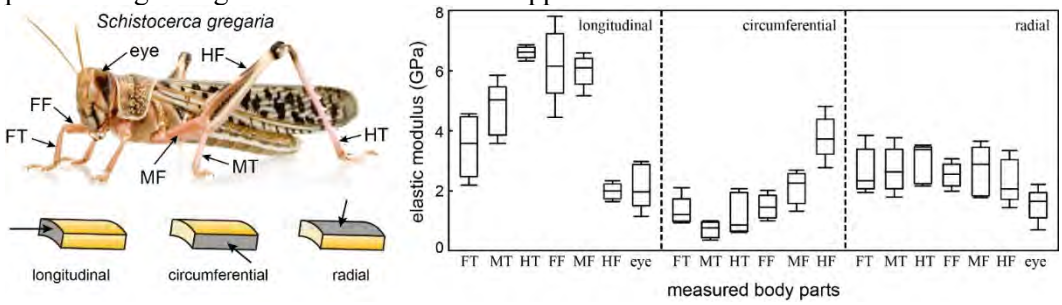

Fig. 1. Mechanical properties of locust cuticle in different body parts.

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D: Biomineralisation inspired materials and systems

Fetuin A functionalization of biodegradable nonwovens for improved osteoblastic growth behaviour

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Especially in the context of hard tissue defects, electrospun nonwovens are a versatile material to be applied as biodegradable implantable scaffolds. Owing to fiber diameters in the micro- and nanometer range and a high surface-to-volume ratio with high porosity, they can mimic the natural structure of the extracellular matrix (ECM) [1] and are replaced within remodelling and regeneration processes in the long term. [2] Yet, the treatment of bone matrix defects with porous synthetic materials for guided bone regeneration often fails due to missing mechanical and biological cues. The blood plasma binding protein Fetuin A offers a high calcium affinity, thus enhancing the formation of initial CaP crystallization nuclei and promoting calcium phosphate mineralization. [3]

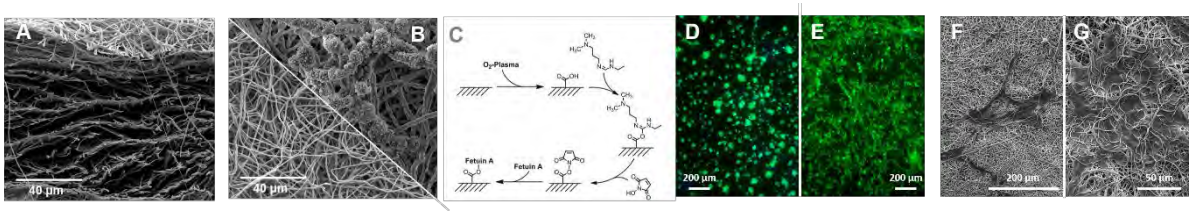

Figure 1. SEM images of A) PLLA-co-PEG nonwoven (cross-sectional) and B) PLLA-co-PEG nonwoven compared to a calcified Fetuin A functionalized one. C) Schematic of the Fetuin A functionalization by EDC/NHS coupling. D/E) Fluorescence and F/G) SEM images of MG63 osteoblasts on unmodified (D/F) and Fetuin A functionalized (E/G) nonwovens after 48h cultivation.

PLLA-co-PEG electrospun nonwovens have been pre-treated with O_2-plasma, functionalized by covalent bonding with Fetuin A and subsequently calcified in vitro. CaP affinity of the functionalized nonwovens has been investigated with a tailored in vitro-calcification protocol. CaP load was quantified using a photochemical assay and morphological characterization of the mineralization was performed using SEM. Cell seeding behaviour over 48h was investigated using human MG-63 osteoblasts, followed by the determination of metabolic activity as well as cell morphology and migration via fluorescence staining and SEM. The type I collagen and cytokine and chemokine levels were verified via ELISA.

Superficially immobilized Fetuin A complexes calcium ions from the surrounding medium, which act as crystallization nuclei initializing biomimetic hydroxyapatite crystallites mineralization (Fig. 1 B). CaP formation takes place homogeneously distributed over the entire surface of the functionalized material. Against the use of seed crystals, the entire fibers are thus surrounded by a CaP layer, maintaining the fibrous ECM-like morphology with its high surface-to-volume ratio and porous fiber interstices. This morphology supports both cells and hydroxyapatite to replace the scaffold structure from within. Initial results indicate a highly elevated metabolic activity on Fetuin A functionalized nonwoven, which led to increased cell ingrowth with spread cell morphology and dense cell layers. ELISA showed decreased collagen levels in the supernatant of Fetuin A surfaces, maybe indicating binding of collagen propeptides to Ca^{2+} growth nuclei. [4]
Fetuin A modification of biodegradable nonwovens enables a distinct CaP crystal growth on the material surface being a feasible way towards enhanced biomineralization and osteoblastic growth behaviour.

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Stabilization of Amorphous Calcium Phosphate by Barium: A Novel Approach Inspired by a Marine Worm

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Amorphous calcium phosphate (ACP) is a transient precursor phase in bone and teeth, transforming into crystalline carbonated hydroxyapatite. Some rare organisms like the Nemertean worm *Amphiporus lactifloreus* produce the biomineral ACP that is stable throughout the organism's lifetime in its stylet [1]. A microscopy image can be found in Figure 1A. The stylet is a 100-micron long nail-shaped "tooth" at the end of the proboscis and is used to capture and pierce prey. The mechanism of proboscis extraction is depicted in Figure 1B. ACP, although transient under aqueous conditions, could be a valuable mineral phase for tissue-engineering of bone due to its similar elemental composition to carbonated apatite in bone. We aim to study the worm's ACP stability mechanisms to develop methods to stabilize synthetic ACP. We study the 3D structure using x-ray nano computer tomography and focused ion beam electron microscopy (FIB/SEM), as well as the elemental composition and distribution of stylets extracted from *A. lactifloreus.*

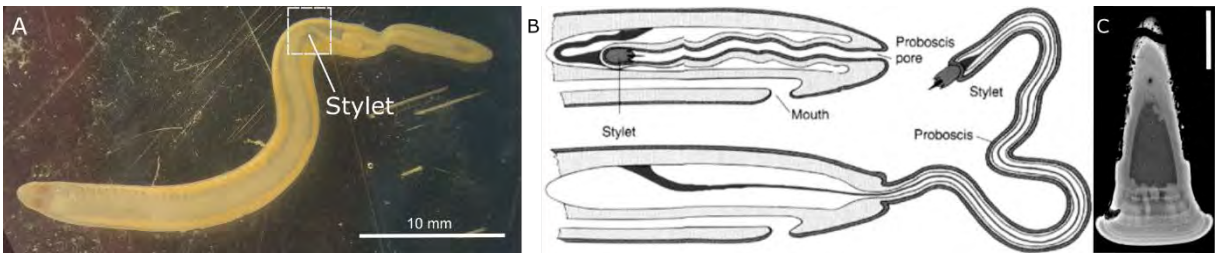

Figure 1. Microscopic image of *A. lactifloreus* (A), schematic drawing of stylet extension (B, adapted from [1]) and SEM image of stylet with a scalebar of 10 µm.

The stylet consists of an inner core region, surrounded by an outer lamellar structured layer. This can be seen in the SEM image in Figure 1C. In both regions, Ca and P are the main elements. Surprisingly, the concentrations of Ba and Sr are high with Ca/Ba 5:1 by atoms and Ca/Sr 10:1. The outermost layer, only a few tens of nanometre thick, exhibits elemental contrast of heavier elements in nano-CT and is enriched in S and Ba according to EDS. Raman detected SO_4^{2-} . Nanoindentation results showed an elastic modulus of 10–28 GPa and a Vickers hardness of 30-160.

We carry out syntheses with different elemental compositions and monitor the ACP stability by FTIR. The addition of Ba at atomic ratio Ca/Ba = 9:10 is capable of stabilizing ACP and with Ca/Ba = 3:1 and 19:1 can delay crystallization. By substituting Ca in the ACP synthesis with different ratios of Ba and Sr, the crystalline transformation of ACP can be slowed down for weeks. This result shows that Ba can be used to modulate ACP stability. In the future this might lead to ACP that can be tailored to various industrial and medical applications in the field of bone repair and replacements.

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The role of extracellular matrix in mineralized *E. coli* biofilms

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Abstract

Biofilms are biological living tissues that occur after bacteria colonize a surface and synthesize an extracellular matrix, as a survival strategy in challenging environments. In addition to the organic matrix, some biofilms precipitate mineral particles such as calcium phosphates and calcium carbonates. Previously, we investigated the role of alkaline phosphatase (ALP) in the precipitation of calcium phosphates in the form of hydroxyapatite in biofilms produced by the model strain *E. coli* K-12 W3110 (*1*). From cryo-focused ion beam-scanning electron microscopy, we could identify both mineralized bacteria and mineralized portions of the extracellular matrix. With few exceptions, microbial mineralization usually results from adventitious precipitation of inorganic compounds led by their interactions with different metabolic processes (*2*). Such biologically induced mineralization results directly from microbial activity, tends to accumulate minerals on the surface of the bacteria, and eventually embed them in the growing crystals (*2*). Moreover, biominerals can also be influenced by interactions of their precursors with the environment, e.g. the extracellular matrix (*3*). In this context, we investigated the influence of different macromolecules in the biofilm on the formation of calcium phosphate crystals. On nutritive agar substrates inducing mineralization, we cultivated diverse *E. coli* strains, which produce as extracellular matrix only curli fibers, only phosphoethanolamine-cellulose fibers, both or none of them (*4*). We estimated crystalline lattice parameters using wide-angle x-ray scattering and assessed crystal strain at the nanoscale. Microindentation was exploited to study the influence of the extracellular matrix on the mechanical properties of the mineralized and unmineralized biofilms. Uncovering the influence of the biomolecules present in the extracellular matrix is an important piece of knowledge to engineer living composites.

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F: Engineered biohybrid living materials

Directed Evolution of Microorganisms for Engineered Living Materials

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Engineered living materials (ELMs) represent an enticing new class of materials whose structure and properties are governed by metabolically active microorganisms.[1-4] Besides their adaptive capabilities, engineered living materials are usually grown at ambient temperatures and pressures and rely on abundant and environmentally friendly chemistries, thus offering a more sustainable alternative to their synthetic counterparts. The rich biochemical diversity in microorganisms provides an immense design space for engineered materials with exquisite structures and living functionalities.

To achieve functionalities of relevance, wild-type microorganisms must have evolved traits to fulfill a biological function that matches an engineering need. Another approach is to re-program microbial genomes to achieve desired functionalities. Although synthetic biology tools continue to expand, the bottom-up rational design of engineered living materials still relies on prior knowledge of genotype-phenotype links for the function of interest. Even if a function-encoding gene is known, the fitness of the whole microorganism to an engineering context will likely involve more than one specific trait. These challenges call for the development of other strategies for the selection of genetically programmable microorganisms for engineered living materials.

Here, we utilize a high-throughput directed evolution platform to enhance the fitness of whole microorganisms under selection pressure and integrate them into complex engineered living materials.[5] Given the broad interest in cellulose fibers as a sustainable natural resource, the model high-yield cellulose producer *Komagataeibacter sucrofermentans* was chosen to illustrate our platform. We show that our droplet-based microfluidic approach enables the directed evolution of these bacteria towards a small number of cellulose overproducers from an initial pool of 40'000 random mutants. Sequencing of the evolved strains reveals an unexpected link between the cellulose-forming ability of the bacteria and a gene encoding a protease complex responsible for protein turnover in the cell.

The ability to enhance the fitness of microorganisms towards specific phenotypes and to discover new genotype-phenotype links makes this high-throughput directed evolution platform a promising tool for the development of the next generation of engineered living materials.

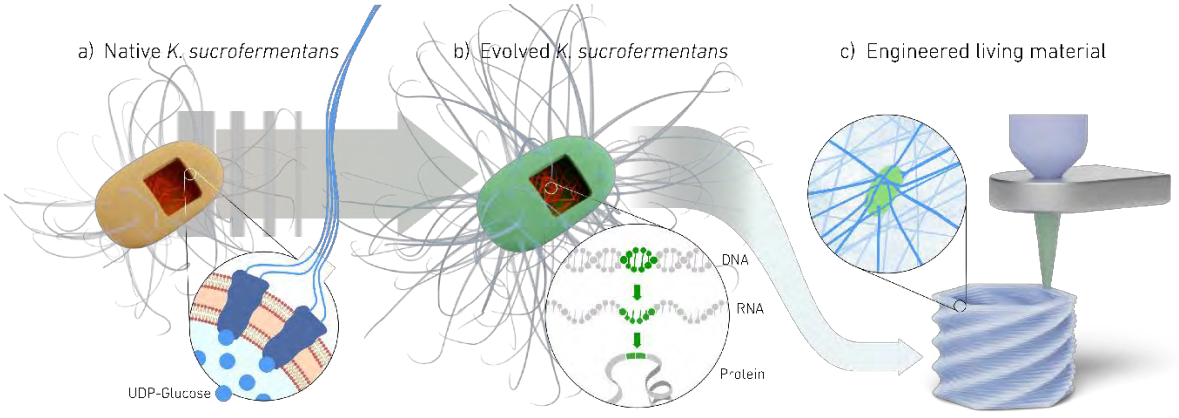

Figure 1. Direct evolution of cellulose-producing microorganisms for engineered living materials. a. Native *K. sucrofermentans* metabolizes sugars to UDP-glucose, which is polymerized to glucan chains by the cellulose synthase transmembrane complex. The glucan chains are exported through the cell wall and self-assembled into bundles to form cellulose nanofibers. **b.** Evolved microorganisms produce more cellulose, partly due to mutations in their genome, translated into the corresponding proteins. **c.** Evolved cellulose overproducers can be shaped into living objects by combining the bottom-up self-assembly of cellulose fibers with the top-down shaping freedom of 3D printing.

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In vitro plant cell encapsulation via high-resolution laser lithography

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The development of plant tissue is strongly controlled by mechanical signals, which can originate from either the growing tissue itself or its environment. While biochemical factors involved in plant development have been extensively studied, techniques for investigating geometric effects on single cell level have been limited. This project aims to mechanically control plant tissue development by establishing a new method focusing on the encapsulation of plant cells in a defined geometry at the micrometer scale. Therefore, a plant-compatible hydrogel ink was developed with tuneable stiffness suitable for high-resolution laser printing. This material will be used to encapsulate protoplasts from *Arabidopsis thaliana* in defined geometrical scaffolds in vitro, creating distinct geometries that mimic native plant shapes (e.g. shoot). For the encapsulation, a high-resolution laser lithography system is used (Nanoscribe GT2). The growth of the plant cells will be monitored over time using confocal imaging. Within this project, we show a novel plant-compatible hydrogel in combination with high-resolution laser lithography. Furthermore, we provide preliminary results of plant cells growing inside the hydrogel, the viability of the cells in this artificial environment and their development.
The method can be used to investigate the influence of shape and force on plant cell fate and plant tissue organization. This project seeks to shed light on how the physical environment influences multicellular plant tissue, with potential implications for advancements in agricultural and biomaterial sciences.

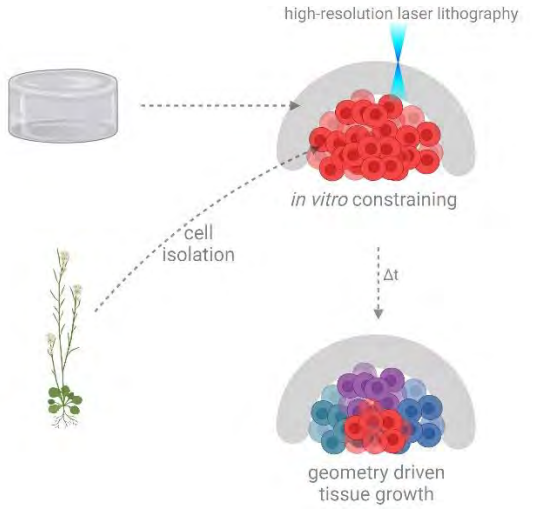

Figure 1. Project description created by biorender.com

H: Artificial intelligence and bioinspired materials

Vi-Fi – AI-Based Secure Health Monitoring System Using Wi-Fi Signals

K.A. Qassem[1], S.M. Seyam[1], Y.S. Al-Hamadi[1], A.H. Al-Dubaisi[1], M. Al-Rajab[1]

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For Millennials, individuals often encounter discomfort with traditional vital signs monitoring technologies, relying on invasive methods that leave them vulnerable unless under hospital supervision. Delayed detection outside hospitals poses significant risks, especially for vulnerable groups like the elderly and those with chronic conditions, contributing to 10-13% of deaths [1]. Current monitoring solutions face challenges including discomfort, false alarms, and financial constraints, compounded by limited access to advanced equipment in remote areas. Rising healthcare costs, with ICU expenses in the US growing from $56.6 billion in 2000 to $108 billion in 2010 [2], and a 3.1% increase in medical device spending from 2009 to 2019 [3], further complicate the issue. Additionally, the susceptibility of monitoring devices to security breaches compromises patient data privacy and integrity.

To address these challenges, we propose Vi-Fi, a smart and secure contactless system for real-time vital signs monitoring using Wi-Fi signals. Vi-Fi aims to prevent heart attack-related deaths and detect sleep apnea. The system utilizes two routers to extract channel state information (CSI) data, which is then encrypted and sent to a secure server for analysis using AI-based deep learning algorithms. These algorithms extract information such as breathing and heart rates. The results are presented on a web dashboard for healthcare professionals, while an intuitive mobile application allows remote patients and caregivers to track vital signs and communicate with the medical team from home. Vi-Fi enables real-time monitoring of vital signs within the ICU and offers convenience for elderly or chronic condition patients at home. It also addresses challenges associated with monitoring patients during a pandemic, alleviating pressure on both patients and healthcare professionals. Additionally, Vi-Fi employs AI models to analyze complex patterns in vital signs, identifying anomalies and predicting chronic diseases, sleep apnea, and changes in respiration rate for early prevention and improved patient healthcare.

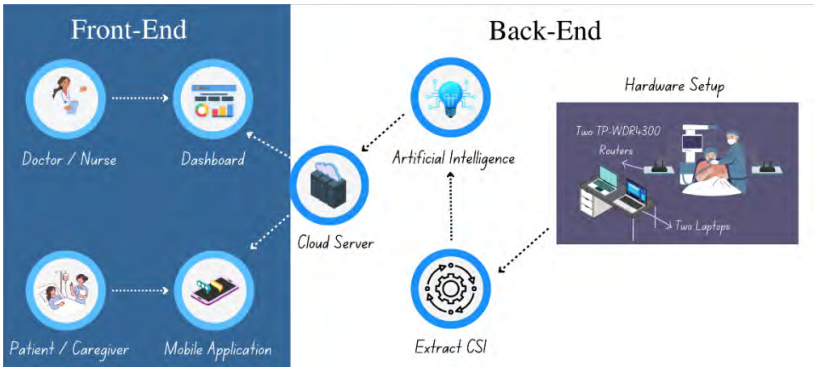

Figure 1. System Architecture

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J: Synthesis of bioinspired materials

Beyond Traditional Boundaries: Harnessing Cellulose Synthase Catalytic Promiscuity To Design Cellulose-Chitin Co-Polymers In Vivo And In Vitro

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Glycosyltransferases (GTs) synthesize a wide range of glycoconjugates essential for organisms' adaptation and survival. Cellulose and chitin synthases, belonging to the GTs family 2, are enzymes responsible for polymerizing cellulose and chitin fibers, respectively. Each exhibits organism-specific functions without cross-functionality in biopolymer synthesis. In this presentation, I will present our findings that bacterial cellulose synthase from *Rhodobacter sphaeroides* (rBscA-B) cleaves uridine diphosphate N-acetylglucosamine (UDP-α-D-GlcNAc) with slightly lower substrate affinity and less efficiently comparable to the synthase natural substrate, uridine diphosphate with the formation of chitin fibrils. *R. sphaeroides* grown with UDP-α-D-GlcNAc results in the production of chitin oligomers. These findings challenge the traditional view of organism-specific biopolymer synthesis and advocate new possibilities for manipulating their composition in cellulose-producing organisms, modulating their interaction with their environment and/or influencing their evolutionary trajectories.[1]

References

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Suitability of Ti-PMMA sandwich materials for biomedical applications

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The need for implant materials for hard tissue replacements have been increasing with a fast pace. Keeping this in mind, the main goal of this work was to develop craniomaxillofacial implants for patient specific implants (PSI) applications, with a minimal stress shielding effect, being a major problem for metallic implants such as Ti [1]. Combinations of sheet-like metal-polymer-metal sandwich materials (SMs) were chosen as the ideal material structure. The idea of SMs is nature based, as structures such as a human bone, beak of birds are generally comprised of SMs to maximize its performance with the minimum use of material and weight [2]. These composites provide several benefits for biomedical applications, such as tuneable mechanical properties, along with great vibrational and thermal insulation properties. To make the implants biocompatible Ti was used as skin material along with PMMA as core material. As the bonding between them also needs to be biocompatible, a "*grafting from"* process was applied to graft PMMA on Ti [3]. This grafted PMMA was used as an adhesive to achieve a good bonding between the metal and the polymer. The bonding was attained via fusion bonding.

The thermal, vibrational and shaping possibilities of these SMs was investigated. SMs showed significantly better thermal and vibrational properties than that of Ti. The shaping possibilities of these SMs for PSI applications were investigated via V-bending and deep drawing tests. When tests were performed at a temperature range where these polymers have sufficient ductility, the SMs were able to be shaped without failure. The results illustrate the promising possibility of applying nature-based SMs for CMF application.

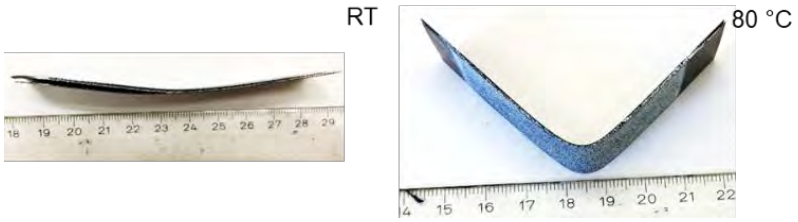

Figure 1. V-bending of Ti-PMMA SMs. Where at room temperatura (RT) it had early delamination, at 80 °C it was able to bent without problems.

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