



Bionicomposites

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Incorporating nanomaterials in living systems could force the latter to produce “bionicomposites”. We report a review of the first attempts with such bionicomposites, e.g. showing how the control of the eating and dormant states of microorganisms can provide nano-architectures with novel mechanical and functional properties, and how introducing nanomaterials in the diets of animals producing silks (spiders or silkworms) leads to intrinsically reinforced fibers with strengths higher than those of their natural counterparts, as well as those of synthetic polymer fibers or carbon fiber-reinforced polymeric composites.

1. Introduction

We defined as “bionicomposites”^{1–3} a new class of composites produced by living organisms after assimilating synthetic nanomaterials from the surrounding environment. Considering the complexity and performance of such materials, their design and production is an interesting area of exploration.

The metabolic activity of microorganisms stimulates the formation of a wide range of nanomaterials that have unique physical properties as well as nanostructures that cannot be reproduced in laboratory by abiotic synthesis. It is well known that the natural presence of biominerals in the protein matrix and hard tissues of insects,⁴ worms⁵ and snails⁶ enables high strength and hardness (>500 MPa) materials. Such biogenic materials are often in the nanometer scale as a by product of cellular or extracellular reactions. From this point of view, the artificial incorporation of nanomaterials in a biological environment to obtain novel nanoarchitectures with smart functions should, in principle, be possible.

The elastic properties of honeybee combs, for example, are due to the hierarchical structure of the comb walls that are continuously strengthened and stiffened by the addition of silk produced by the bee larvae during their pupation period.⁷ This ancient and natural example is reminiscent of modern fiber-reinforced composite laminates.⁸ Artificial engineering can achieve the microstructural sophistication of their natural

counterparts by a bionic approach. Feeding spiders or *B. mori* silkworms with diets containing carbon nanomaterials could result in intrinsically reinforced silkworm silk fibers.^{1,9}

Fermentation of bacteria in the presence of nanomaterials could also create a biogenic composite. Starting with a small jar, it is possible to transfer large cultures until enough material is obtained to create composites with unexpected properties. This approach offers new possibilities to biogenic reinforced composite manufacturing. Yeast is a cellular factory and reproduces asexually through a process called “budding” in which a “mother” cell grows a “daughter” cell that separates to become fully independent. Yeast fermentation could be exploited to transfer (carbon) nanomaterials from one yeast cell to another by internalization process or to incorporate them on the cell wall.^{2,3} These techniques used commercially available bread yeast but can be generalized to any living cells without a special surface modification of the materials. In turn, this process could lead to the development of hierarchical and interactive structures programmed to self-assemble into specific patterns, such as those on strain sensors, or on self-healing materials able to sense and repair mechanical damage. Artificial living surfaces have been prepared from porous polymer layers and inoculated fungi; such composites can provide a novel form of functional or smart materials with the capability to be both active (eating) and waiting (dormant, hibernation) states with additional recovery for the reinitiation of a new active state by observing the metabolic activity over two full nutrition cycles of the living material (active, hibernation, reactivation).¹⁰

Here, we provide a review of this emerging materials engineering area that developed from living materials. We define as bionicomposite as a composite produced by living organisms that assemble artificial nanomaterials, modulating the functional properties in some manner. Although there are many overlaps with biohybrid engineering materials, we restrict our exploration to processes that adhere to the most fundamental aspect: the living organisms must fabricate nanomaterial and/or nanocomposites. The engineered aspect of a bionic material

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will be restricted to engineering of the material components and mechanical aspects.

2. Bionic silk

Silks produced by spiders are the toughest materials and there are many attempts to emulate the properties of their natural counterparts.^{11–13} Natural silk fiber is a hierarchical material in which hydrogen bonded β -strands, β -sheet nanocrystals, and a hetero-nanocomposite of stiff nanocrystals embedded in a softer semi-amorphous phase assembles into macroscopic silk fibers. It follows that the large breaking stress of spider silk fibers can be explained according to the hierarchical structures of the crystal domain and network.¹⁴ In view of their high strength, the utilization of spider silk fibers as a reinforcement for polymer composites is gaining attention in different fields of applications. Several attempts have been made with silkworms due to their large availability even if their silk is weaker than that of spiders. Hu *et al.*,¹⁵ for example, were able to regulate the crystallinity of regenerated silk fibroin by exposing silk to hot water vapor; Zhang *et al.*¹⁶ showed the preparation of silk directly dissolving fibers in CaCl_2 – formic acid, preserving the nano-fibril structure and allowing high-quality silk materials, while recently Buehler *et al.*¹⁴ reported a bioinspired spinning method to obtain regenerated silk fibers by pulling a silk microfibril solution. However, these methods exploit dissolution and post-treatment processes that are time-consuming, require solvents that are difficult to remove and in some cases are toxic and in general the achievement of the natural β -sheet crystal structure is challenging.^{17–20} Carbon nanotubes (CNTs), which possess superior mechanical properties, are widely applied as reinforcement in preparing high-performance materials.^{21–24} A number of groups introduced such nanoparticles with a non-bionic approach²⁵ on the surface of spider silk fibers, achieving an enhancement of toughness²⁶ or electrical conduc-

tivity.²⁷ Recently, the *in vivo* incorporation of CNTs and graphene into spider silks has been explored (Fig. 1).¹

Unfortunately, due to spiders' territorial and cannibalistic nature, their silk has been impossible to mass produce, so practical applications have yet to materialize. To overcome this challenge, Zhang *et al.*²⁸ have engineered bacteria that produce spider silk with a performance similar to its natural counterpart. Assembling a DNA part containing 192 repeat motifs of the *Nephila clavipes* dragline spidroin, they spun fibers that replicate the mechanical performance of their natural counterparts, *i.e.*, tensile strength (1.03 ± 0.11 GPa), modulus (13.7 ± 3.0 GPa), extensibility ($18 \pm 6\%$), and toughness (114 ± 51 MJ m^{-3}). Another approach to overcome the impossibility to cultivate spider farms is the utilization of silkworms that are available on a large scale and can be integrated in bioengineering structures. Feeding *B. mori* silkworms with diets containing CNTs led to intrinsically reinforced silkworm silk fibers (Fig. 2).⁹ The silk fibers produced by such natural processes showed superior tensile strength and toughness thanks to a spontaneous silk-spinning process that converts helical and random coils into β -sheet structures, with a strength comparable to that of steel (1 GPa) but 5 times smaller than the best bionic spider silk. Moreover, given concerns about the toxicity of CNTs in whole organisms, Weisman *et al.*²⁹ investigated the effects on the overall viability and growth of feeding CNTs to *Drosophila* larvae. They found that CNT feeding did not affect the survival to either stage; survival to the pupal stage and adulthood was somewhat higher for the CNT-fed group ($83.5 \pm 3.8\%$ versus $78.0 \pm 3.9\%$ to the pupal stage, and $79.5 \pm 3.4\%$ versus $69.4 \pm 3.9\%$ to the adult stage).

3. Yeasts and bacteria as microfactories for engineering nanomaterials

Living microorganisms have long been used in food preservation; such microorganisms form living surfaces that provide

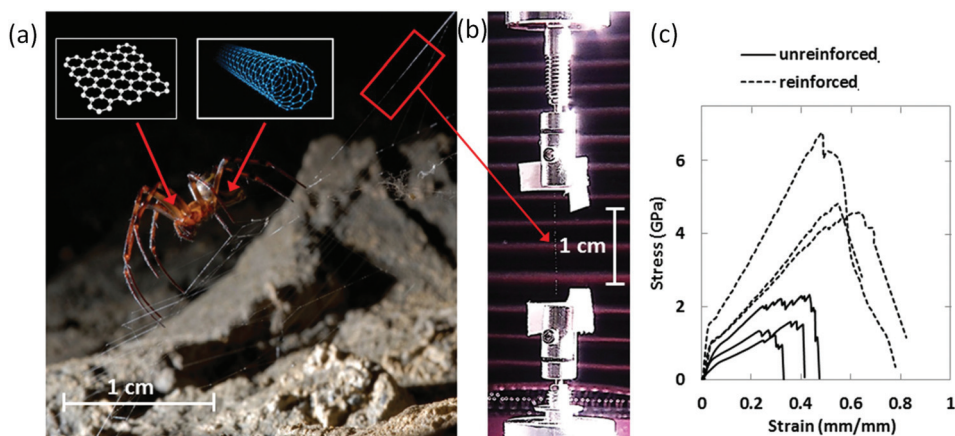


Fig. 1 Schematic diagram of the experimental procedure proposed by Pugno *et al.*¹ (a) Spiders are fed with dispersions containing graphene or CNTs; (b) the corresponding spun silk is collected and tested in a nanotensile system; (c) stress–strain curves show improved mechanical properties when compared to pristine samples and are, statistically, superior to any other material available today.¹

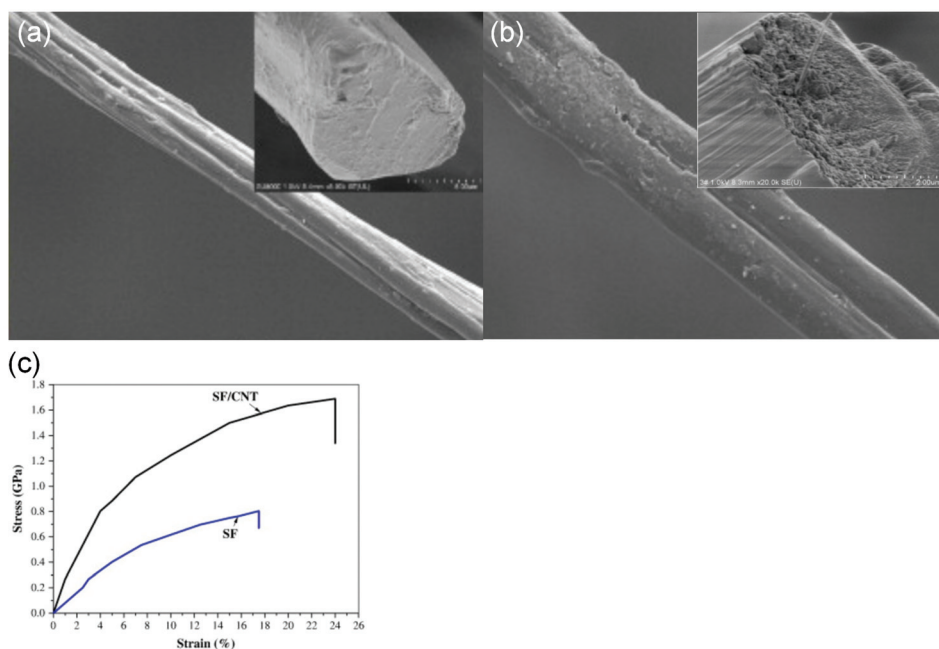


Fig. 2 A comparison of the morphology of (a) silk fiber (SF) and (b) CNT-embedded silk fiber (SF/CNT). The inset of panel (b) shows a higher resolution image. (c) A comparison of the stress–strain curve of SF and SF/CNT.⁹

an attractive platform for the development of functional materials. At present, biotech companies use fungi to produce valuable products; thus, combining the fermentation mechanism of some microorganisms with nanomaterials could give rise to bionic composites with novel properties.³⁰

Inspired by our previous work in which the metabolic activity of living microorganisms was used as an engineered platform for the fabrication of advanced carbon-based materials,² Valentini *et al.*³¹ extended this approach, with emphasis on the fermentation process used for centuries in wine- and bread-making, to produce bionic composites which integrate regenerated silk nanofibrils that, from the geometrical point of view and in terms of mechanical properties, are similar to carbon nanotubes. The resulting reduced volume fraction of nanofibrils within the film could make the fermented hybrid composite more resistant to fracture, with self-repairing properties exploiting the microorganisms' growth process that allows for the intracellular transport of nanomaterials. The CO₂ bubbles produced during fermentation could be used to realize porous architectures ideal for biomedical applications. As conceptual proof, they demonstrated that the deposition of such a living coating on fruits helps the preservation of their shelf-life and that bionic film layers can be laminated onto a soft substrate for the realization of a temperature-responsive bilayer system (Fig. 3).^{31,32}

Yeast is a cellular factory which is capable of taking simple molecules from their environment, such as sugars, and synthesize new elements needed for its growth at mild temperatures.^{33–36} Water is an important factor in this regard as dry yeasts are fully impermeable to molecules whereas wet yeasts promote the transfer of molecules from outside to

inside the cell. Thus, a yeast cell is ideal for realizing composite materials that combine advantages of both the living and nonliving worlds. The mechanism of nutrition and growth of microorganism represents an unexplored field to design interface between microorganism and such nanomaterials. Several examples of bionic carbon-based composites, involving graphene and nanotube materials, have been recently proposed as a method to produce bionic composites with novel and unexpected properties.

Valentini *et al.*,³⁷ exploited the bubbles formed during yeast fermentation to assemble nanomaterials at the water–oil interface of a mixture, and used this process to fabricate nanocomposites with intractable polymers.³⁷ Although the resulting composites were porous, they had improved mechanical properties. Valentini *et al.*² adopted a similar strategy based on *Saccharomyces cerevisiae* yeast cells that multiply with a process where a daughter cell is initiated as growth from the mother cell. To feed yeast the highest possible doses of CNTs, dry Baker's yeast (the normal food) was mixed with concentrated suspensions of water soluble CNTs. Firstly, a solution of raw functionalized CNTs in water was prepared, followed by the addition of sucrose.² The resulting dispersions (which differed somewhat in nanotube content) were used as the sole food source for yeast. The average concentration, expressed as nanotubes per mm³, was 3100 ± 550. They also exploited such a mechanism to obtain the intracellular transport of CNTs during this growth process (Fig. 4a); the resulting reduced volume fraction of CNTs within the film made the fermented composite more resistant to fracture, and the CNT bridges between yeast cells contributed to enhancing electron transfer. In the case of graphene sheets, the bionic composite showed

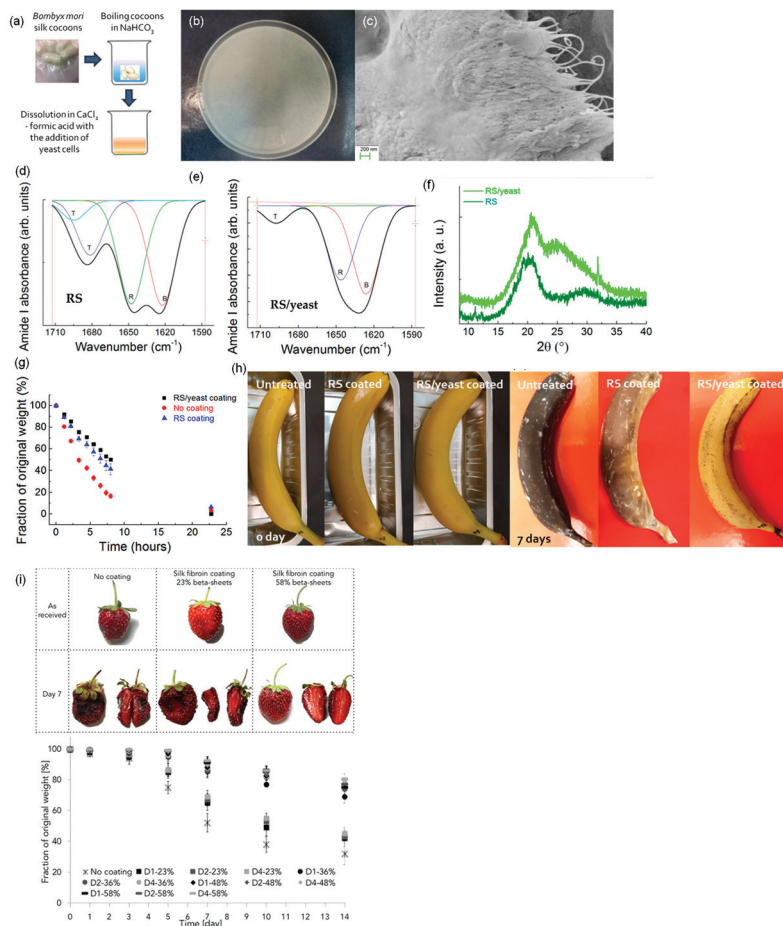


Fig. 3 (a) Schematic diagram of silk fibroin production using regenerated silk (RS); (b) visual appearance of the RS/yeast film and (c) FESEM image of silk nanofibrils; (d) and (e) FTIR spectra of regenerated silk and RS/yeast films, respectively. The colored lines represent the components of the amide I band and are indicated as β -sheets (B), random coils (R), and turns (T); (f) XRD results of regenerated silk and RS/yeast films.³¹ (g) Weight loss of sponges soaked in water and stored for up to 25 h at 23 °C and 32% relative humidity (RH). (h) Time lapse photography of banana degradation, indicating that the silk/yeast coating reduces the degradation rate. (i) Time-lapse of strawberries ripening when stored at 22 °C and 38% RH (no coating) or dip coated in silk fibroin suspension (23% β -sheet coating). Weight loss of strawberries stored for up to 14 days at 22 °C and 38% RH. Strawberries were stored as picked (*i.e.* no coating) or after coating with a silk fibroin suspension (Dx–xx%). Dx stands for 'x' dip coating steps. xx% stands for the relative amount of beta-sheet content.³²

again a higher failure strength and was able to self-repair after placing the composite material containing living yeast cells back into growth medium.³ Finally, a recent example of a graphene, CNT, and liquid rubber composite produced through yeast fermentation was reported:³⁸ CO₂ bubbles produced during fermentation along with collapsed yeast cells resulted in the transformation of conventional silicone rubber composites into auxetic robust rubber. When compressed along an axis, rubber-like materials expand in directions orthogonal to the applied load (*e.g.*, positive Poisson's ratio), the Poisson's ratio values for the majority of them approaching 0.5. Designed mechanical instabilities on soft materials have been used to realize materials with a negative Poisson's ratio (*i.e.*, the so called auxetic behavior) that will contract (expand) in the transverse direction when compressed (stretched).³⁹ Such materials may find perspective practical applications; for example, once infiltrated in a cavity with a smaller dimension,

auxetic materials can be used as swell tools that expand by means of tensile stress to conform to the wellbore and to remain anchored to it.⁴⁰ For such applications it is required to maintain an expansion above 300% at a high temperature (≈ 150 °C). Moreover, from a practical perspective, rubber-based materials with such reversible auxetic architectures can find applications in a broad field, from energy absorbing materials to tunable membrane filters. Other examples of living composites rely on the self-organization of bacterial biofilms to induce particular morphologies on carbon materials *via* self-assembly. For instance, a biofilm of *Shewanella oneidensis* was used to bind and reduce graphene oxide in solution, forming an electroactive thin film (Fig. 4b).⁴¹ Synergistic effects were observed between the living cells and the graphene, resulting in improved charge storage and properties.

Based on the fermentation method proposed above, a novel microorganism inspired macro/super-porous hydrogel com-

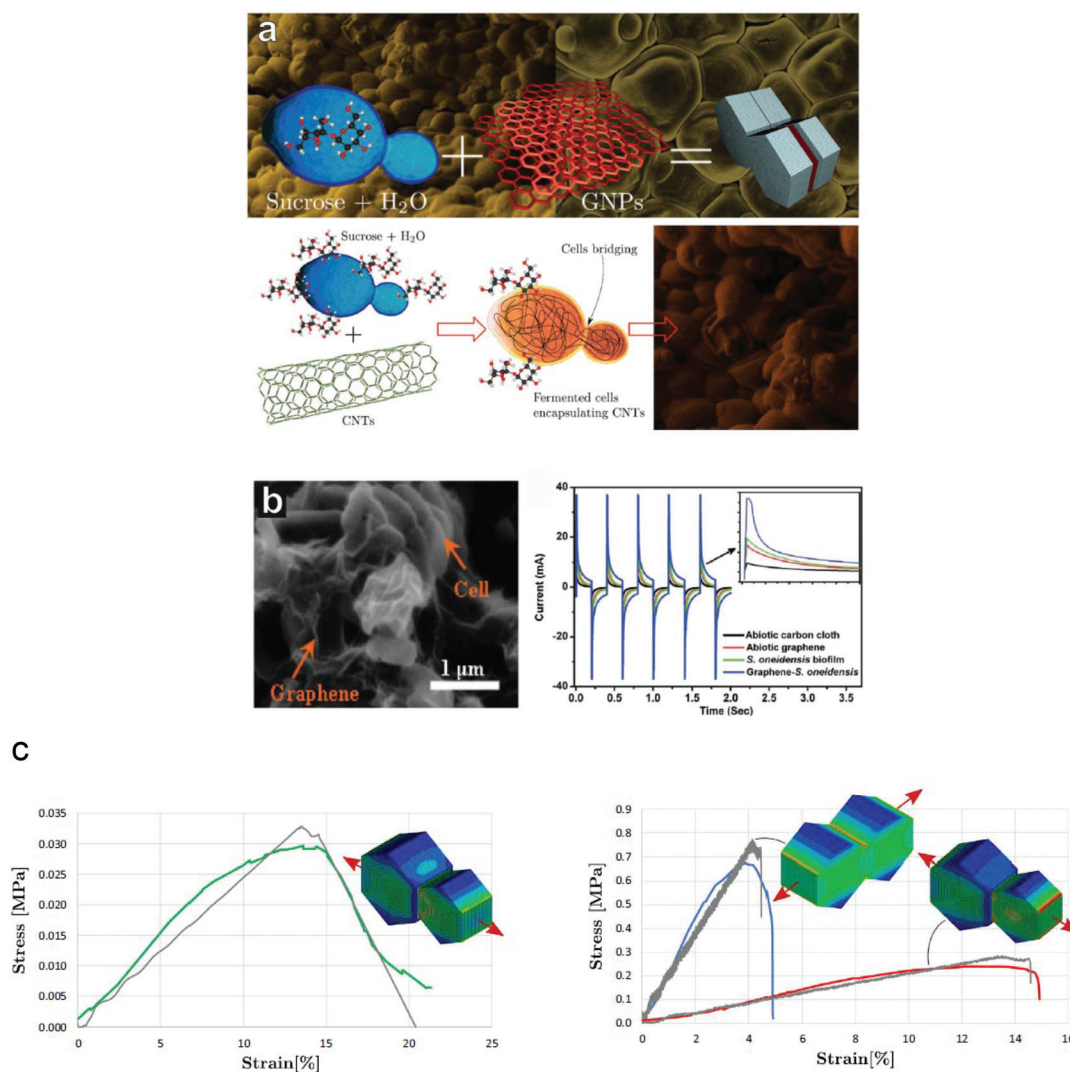


Fig. 4 Living composites with carbon materials. Integrating living microorganisms with inorganic materials serves for the fabrication of various devices. (a) Bionic composites made of yeast cells fermented with graphene nanoplatelets (GNPs) (top) or carbon nanotubes (CNTs) (bottom).^{2,3} (b) Biomemory device fabricated through the self-assembly of *Shewanella oneidensis* bacteria with graphene oxide (left: SEM image of the composite, right: write/erase function of the biomemory device).⁴¹ (c) Stress–strain curves obtained from tensile tests on a fermented yeast sample (left panel) and yeast/CNT composites (right panel) prepared before fermentation (blue curve) and after fermentation (red curve), respectively.²

posed of specific polymers and yeast was prepared. The authors found that the integration of polymeric materials and fungi significantly improved the pore shape/size, swelling and adsorption properties of the hydrogels.⁴² More recently a low-cost, green and template-free carbonization method was adopted to form a tunable hierarchical morphology carbon foam with good mechanical stability, high electromagnetic interference (EMI) shielding efficiency and thermal properties (Fig. 5).⁴³ The carbon foam is mechanically stiff and able to sustain a considerable load without significant deformation. However, the presence of secondary peaks after the main drop in Fig. 5a indicates that actually the compressive strength and modulus may have a little difference for samples with almost the same density (Fig. 5a–c). This mainly attributes to the tiny

difference of microstructure, which cannot be controlled so precisely by this method (Fig. 5d). They also demonstrated potential applications of such carbon foams as high performance electromagnetic interference shielding and thermal insulating porous carbon materials with high fire resistance.

High porous bionic hybrid materials made of cardiac cells and CNTs might also provide muscle for robots made of living tissues.⁴⁴ Shin *et al.*⁴⁴ realized a hybrid scaffold, with CNTs homogeneously incorporated into a gelatin derivative, and addressed simultaneously the three principal requirements for tissue engineering: high porosity for cell adhesion, biodegradability and high elastic tensile/compressive modulus. Due to the fiber-like structure, high electrical conductivity, and high mechanical strength of CNTs, the porous

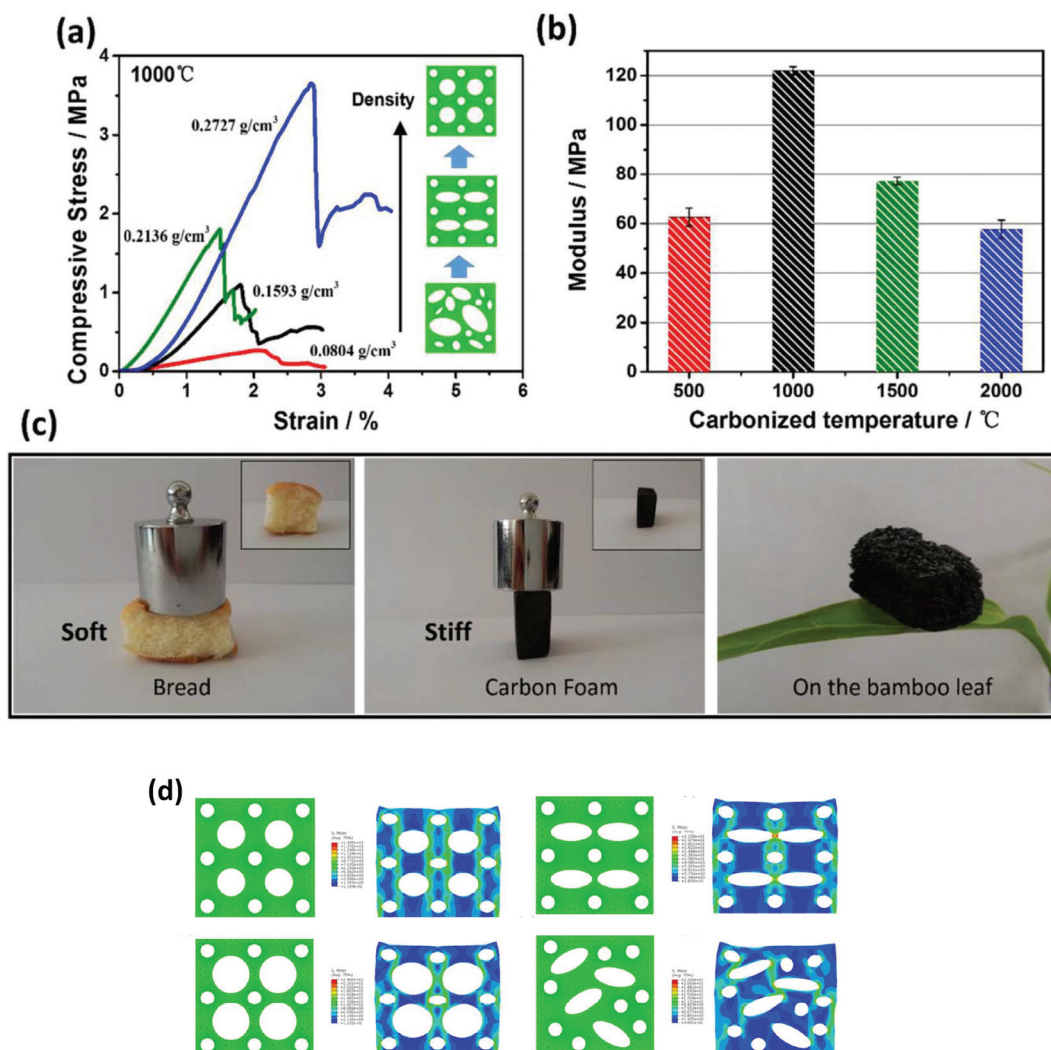


Fig. 5 (a) Stress–strain measurements of carbon foam with different bulk densities carbonized at 1000 °C, (b) compressive moduli E of carbon foam carbonized at different temperatures; bulk densities of measured samples are all between 0.21–0.22 g cm⁻³, (c) left: a 100 g weight standing on a piece of bread, middle: a 100 g weight standing on a carbon foam; right: the carbon foam standing on a bamboo leaf.⁴³ (d) The 2D simulation geometry of the compression test and projection of the stress on the x – y plane of the microstructure.⁴³

gelatin framework was strengthened with a reduced electrical impedance. The fractal-like CNT networks were found also to alter tissue organization (Fig. 6), and so are particularly suitable as a cell delivery system for cell therapy.

4. Hierarchical bionicomposites

Composites consisting of a material with a high strength, reinforced with a ductile and tough phase, are very important in advanced material design;⁴⁵ and there exist examples in attaining both strength and toughness, which often involve complicated geometries (*e.g.*, carbon nanotubes connected by Y-junctions forming a super-graphene)⁴⁶ that are hardly applicable to other materials. A general approach to address the conflict between strength and toughness still remains challen-

ging. Recently, Zhu *et al.* have reported a study on the mechanical properties of cellulose-fiber-based paper,⁴⁷ where they observed that both the strength and toughness of cellulose nanopaper increase simultaneously (40 and 130 times, respectively) as the size of the constituent cellulose fibers decreases (from a mean diameter of 27 μ m to 11 nm), revealing a highly desirable scaling law of the mechanical properties of cellulose nanopaper: the smaller, the tougher and the stronger (Fig. 7).

Many different types of cellulose can be found in plants, or microorganisms. Among these, bacterial cellulose (BC) harvests a specific interest in the field of biogenic materials because it is a specific product of the primary metabolism of bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina*.⁴⁸ Because of its unique properties resulting from the ultrafine structure, BC can be transformed into

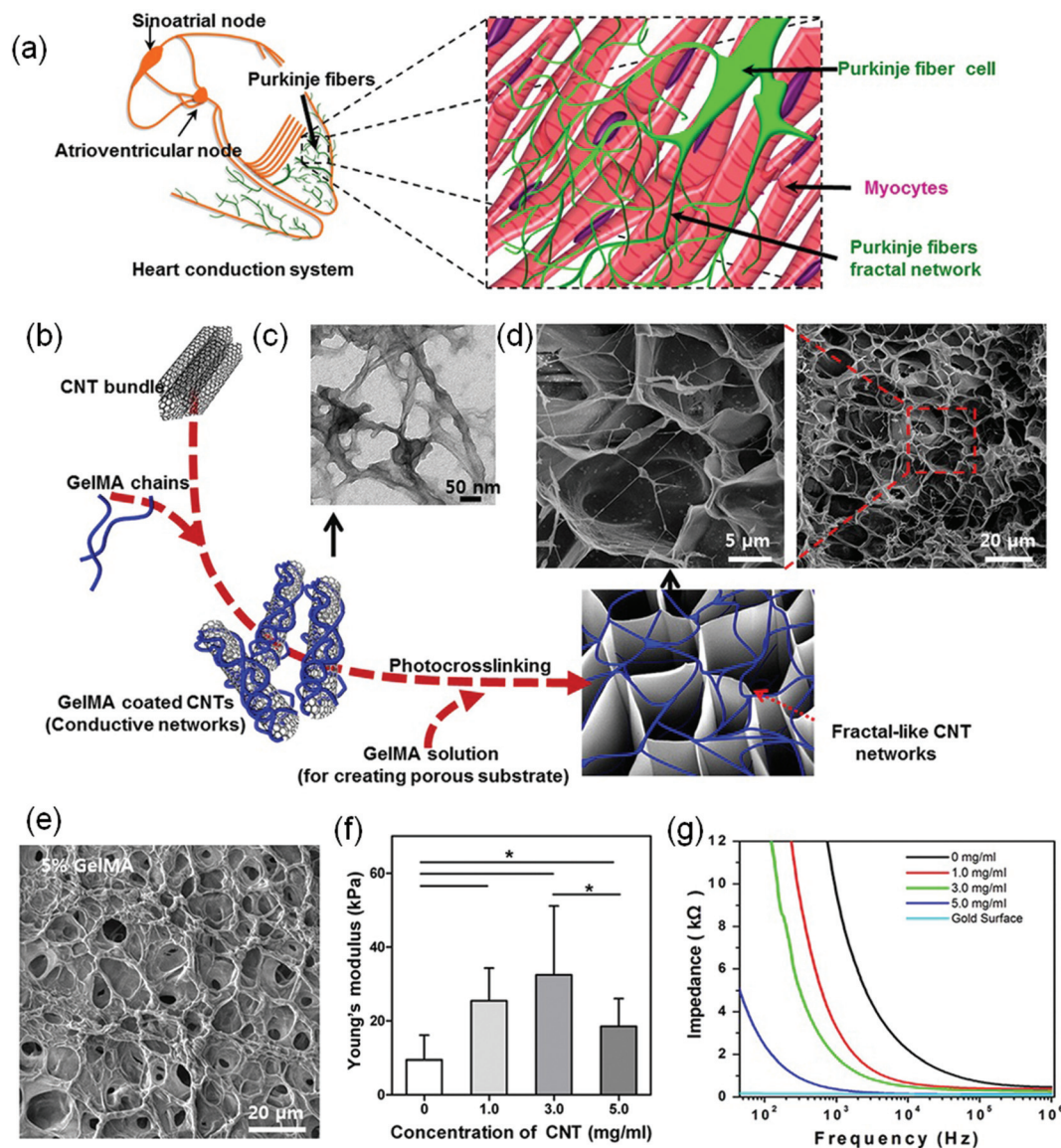


Fig. 6 (a) A schematic diagram illustrating the isolated heart conduction systems showing the Purkinje fibers, which are located in the inner ventricular walls of the heart. (b) Preparation procedure of fractal-like CNT networks embedded in hydrogel. (c) TEM image of hydrogel-coated CNTs. (d) SEM images show the porous surfaces of a CNT-hydrogel thin film. (e) SEM images of pristine hydrogel. (f) Young's moduli of CNT-hydrogels. (g) Decreasing of the overall impedance of a 50 μm thick hydrogel thin film with increased CNT concentrations.⁴⁴

regenerated materials (fibers, films, food casings, membranes, sponges, etc.).⁴⁹ Extracellular polymeric secretions (EPS) of such bacteria are multipurpose polymers that are important for applications in several fields. The preparation of bionic fibers of BC when EPS are involved in bacterial nutrition or interaction of bacteria with multi-walled CNTs was reported by Park *et al.* (Fig. 8).⁵⁰

The mechanical properties of such bionic fibers were also investigated⁵¹ and the results are reported in Fig. 9 where the Young's moduli of the pristine BC, BC fiber, and BC/CNTs fibers were 7.3, 29.2, and 38.9 GPa, respectively. The Young's modulus of the bionic composite fibers was approximately 430% higher than that of the pristine BC. The major finding of this study lies in that the well-aligned hybrid microfibers are much stronger

and tougher than the microfibers composed of nano-fibrillated cellulose or CNT alone.

Further, molecular dynamics simulations⁵² revealed that the synergetic interaction of hydrogen bonds between the nanocarbons and cellulose nanofibrils is the key to the enhanced mechanical performance. The variation of total hydrogen bonding energy, which matches well with that of the total potential energy in terms of both peak location and amplitude, offers strong evidence for the cascade of events of hydrogen bond breaking and re-forming during the sliding process and the dominant role of hydrogen bonding in the toughening mechanism of cellulose nanopaper. The magnitude of the resistant force in the cellulose fiber case is substan-

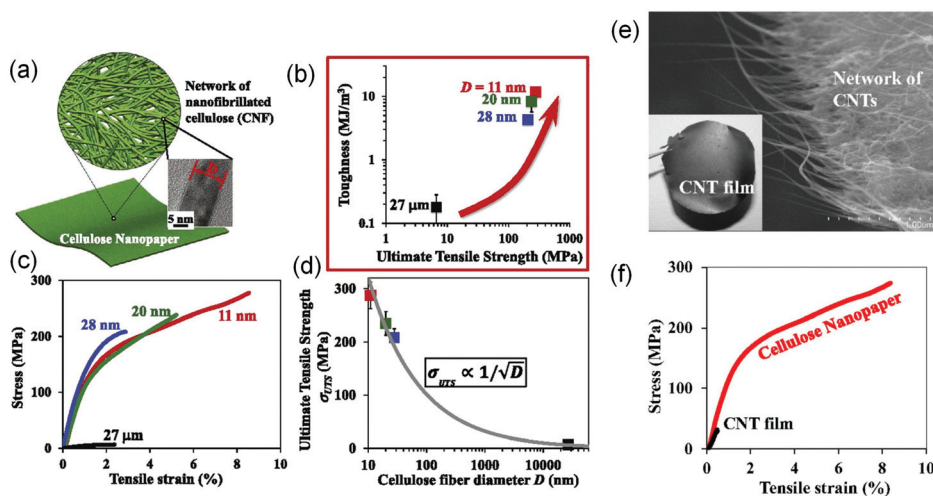


Fig. 7 Scaling law for the strength and toughness of cellulose nanopaper.⁴⁷ (a) Schematic of cellulose nanopaper, made of a random network of CNF fibers. (b) Stress–strain curves of cellulose paper made of cellulose fibers of various mean diameters. As the cellulose fiber diameter decreases from micrometer scale to nanometer scale, both tensile strength and ductility of the cellulose paper increases significantly (c). (d) Plot of the ultimate tensile strength that scales inversely with the square root of cellulose fiber diameter. (e) and (f) Comparison with morphology and stress–strain curves of CNTs network, respectively.

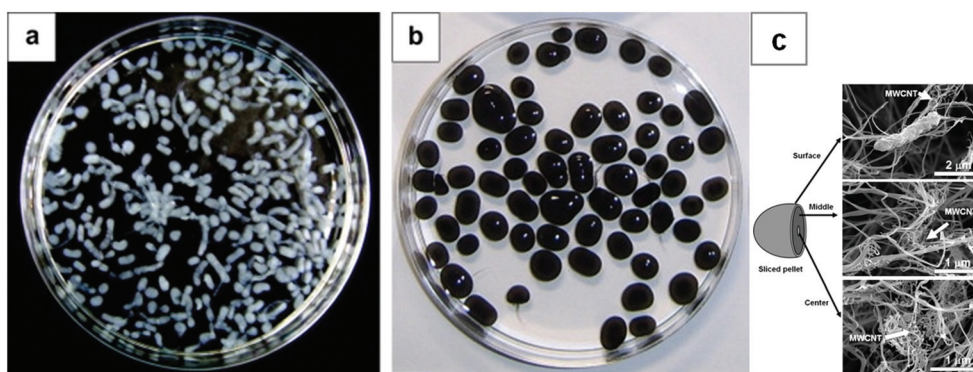


Fig. 8 Photographs of bacterial cellulose pellets synthesized in (a) Hestrin and Schramm medium and (b) CNT-dispersed Hestrin and Schramm. (c) FESEM images of bacterial cellulose synthesized in CNT-dispersed Hestrin and Schramm medium.⁵¹

tially higher than that in the CNT case, shedding light on the molecular scale understanding of the huge difference in tensile strength between cellulose nanopaper and CNT films. The synergistic interaction between cellulose fibrils and CNT is thus applicable to other material building blocks, facilitating a new design strategy to create a wide range of mechanically strong bionic microfibers.

The most important feature of BC is the intrinsic three-dimensional (3D) structure, which distinguishes it from other natural polymers. The biogenic incorporation of graphene or CNTs is thus challenging because of the small pores in pristine BC.⁵³

To overcome such problems, Yoon *et al.*⁵⁴ reported a post-processing immersion method consisting of the immersion of BC pellicles in CNT solutions. However, this method cannot work when the BC pellicles are thick. Moreover, this method is not applicable for graphene oxide (GO) because GO is much larger than CNT and thus cannot enter the 3D structure of BC

pellicles. A one-pot *in situ* biosynthesis approach was developed, and a BC/GO nanocomposite with homogeneous GO nanosheets in a BC matrix was successfully fabricated.⁵⁵ The authors showed that the BC/GO nanocomposite had high mechanical properties and improved electrical conductivity, compared to those of the pristine BC.

The same authors very recently reported a novel *in situ* layer-by-layer assembly (LBLA) method for fabricating thick (≥ 5 mm) BC/GO nanocomposite hydrogels with highly dispersed GO nanosheets bundled by 3D interconnected BC nanofibers (Fig. 9).⁵⁶ The BC/GO hydrogels showed improved mechanical properties over that of bare BC. The bioinspired nanostructure with strong hydrogen bonding, close mechanical bundling, and an even distribution of 2D GO nanosheets throughout the 3D BC network, are the main reasons why the LBLA-derived BC/GO hydrogels are ultra-strong.

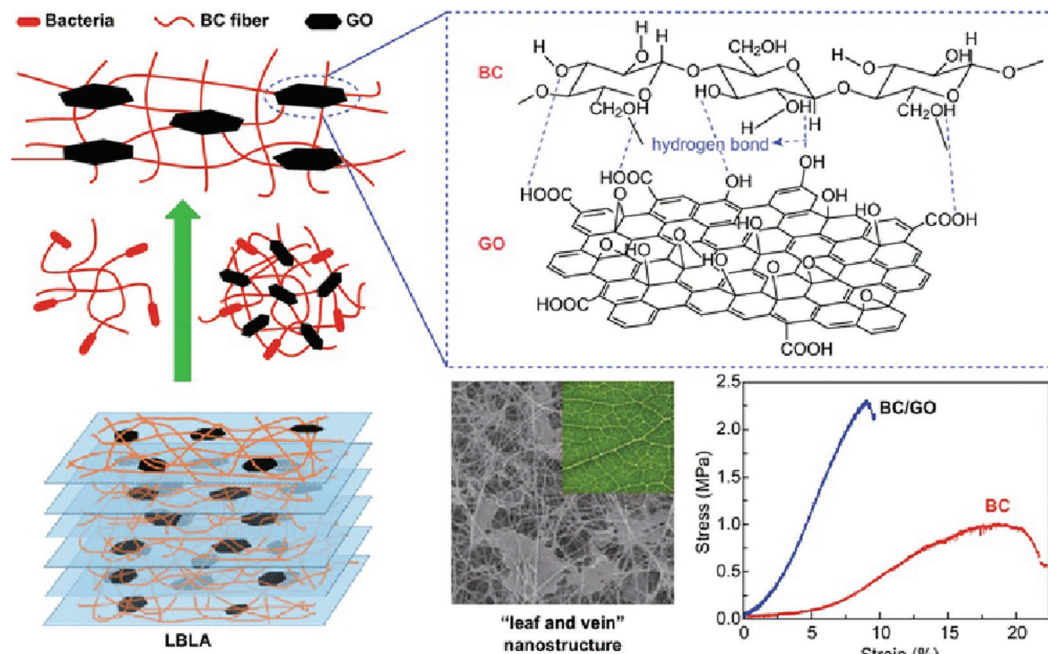


Fig. 9 Schematic diagram of the "leaf and vein" nanostructure of BC/GO prepared by the LBLA strategy. The layer-by-layer culture mode facilitates the bundling of 2D GO nanosheets by 1D BC nanofibers and promotes the distribution of 2D GO nanosheets in 3D BC matrix.⁵⁶

5. Conclusions

This article reviews recent attempts in the emerging field of bioncomposites that utilize living organisms and artificial nanomaterials to fabricate novel composites. The living organisms act as factories and thus the bionic composites can have the typical properties of living systems, being self-assembling, self-healing, and responsive to external stimuli. From the examples reviewed here, we discussed two main different efforts that lead to bionic materials. One attempt is to use microorganisms and their metabolic activities for materials production. All the approaches related to this point focused on the relative simplicity and ease of growth of such living organisms. We believe that more scientists will develop engineered cellular systems to secrete synthetic materials mimicking natural counterparts.

The other approach in developing bionic composites is where the living components are used to modify the materials and give to the final product novel and unexpected functions. This method allows the realization of complex functional materials, such as the bionic silk, that are not possible to be obtained with the bottom-up approach reported above.

As these two engineering technologies combine, the emerging field of bioncomposites (according to our strict definition) could lead to a revolution in material science thanks to the production of superior materials directly by evolved organisms (such as spiders or silkworms) when in the presence of artificial nanomaterials.

In this sense, bioncomposites/bionics is the natural evolution of bioinspired materials/biomimetics.

Conflicts of interest

There are no conflicts to declare.

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