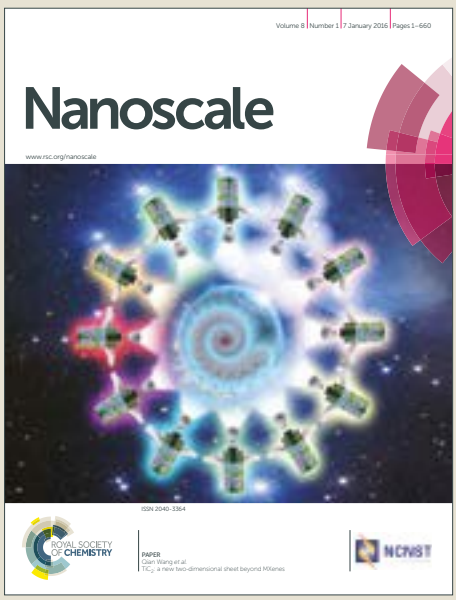
Nanoscale

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: N. Pugno and L. Valentini, *Nanoscale*, 2019, DOI: 10.1039/C8NR08569B.



ROYAL SOCIETY OF CHEMISTRY

rsc.li/nanoscale

Bionicomposites

View Article Online
DOI: 10.1039/C8NR08569B

Nicola Pugno*[1,2,3], Luca Valentini**[4]

1 Laboratory of Bio-Inspired and Graphene Nanomechanics, Department of Civil, Environmental and Mechanical Engineering, University of Trento, Trento, Italy. E-mail: nicola.pugno@unitn.it

2 School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, United Kingdom

3 Ket-Lab, Edoardo Amaldi Foundation, via del Politecnico snc, I-00133 Roma, Italy

4 Dipartimento di Ingegneria Civile e Ambientale, Università di Perugia, UdR INSTM, Strada di Pentima 4, 05100 Terni - Italy. E-mail: luca.valentini@unipg.it

Abstract

Incorporating nanomaterials in living systems could force the last ones to produce "bionicomposites". We report a review on the first attempts on such bionicomposites, e. g. showing how the control of eating and dormant states of microorganisms can provide nano-architectures with novel mechanical and functional properties, and how introducing nanomaterials on the diet of animal producing silks (spiders or silkworms) led to intrinsically reinforced fibers with a strength higher than their natural counterpart as well as than synthetic polymer fibres or carbon fiber-reinforced polymeric composites.

1. Introduction

Bionicomposites are a new class of composites produced by living organisms, like microorganisms, cells and bacteria, that reshape in new more complex structures synthetic nanomaterials through their capacity to metabolize them from the environment. Considering the complexity and performance of such materials, their design and production is an interesting area of exploration.

The metabolic activity of microorganisms stimulate 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,[1] worms[2] and snails[3] enables high strength and hardness (>500 MPa) materials. Such biogenic materials are often in the nanometer scale as by product of cellular or extracellular reactions. From this point of view, the artificial incorporation of nanomaterials in 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.[4] This ancient and natural example is reminiscent of modern fiber-reinforced composite laminates.[5] Artificial engineering can achieve the microstructural sophistication of their natural counterparts by bionic approach. Feeding spiders or *B. mori* silkworms with diets containing carbon nanomaterials could result in intrinsically reinforced silkworm silk fibers.[6,7]

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 to obtain material enough to create composites with unexpected properties. This approach offer new possibilities to biogenic reinforced composite manufacturing. Yeast is a cellular factory and reproduces asexually through a

View Article Online
DOI: 10.1039/C8NR08569B

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 replicate 2D materials on the cells walls.[8,9] These techniques used commercially available bread yeast but can be generalized to any living cells without special surface modification of nanostructured carbon 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, and of self-healing materials capable to sense and repair mechanical damage. Artificial living surfaces have been prepared from porous polymer layers and inoculated fungi; such composite can provide a novel form of functional or smart materials with capability to be both active (eating) and waiting (dormant, hibernation) states with additional recovery for 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 develops from living materials. We define as bionicomposite a composite produced by living oorganisms that assemble artificial nanomaterials, modulating the functional properties in some manner. Although there are many overlapping 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 nancomposites. The engineered aspect of a bionic material will be restricted to engineering of the material components and mechanical aspects.

2. Bionic silk

Silks produced by spiders are the most tough materials with many attempts to emulate the properties of their natural counterparts.[11-13] Natural silk fiber is a hierarchical material where 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 surprising strength, the utilization of spider silk fibers as reinforcement for polymer composites is gaining attention in different fields of applications. Several attempts have been done with silkworms due to their large availability even if their silk is weaker than that of spider. 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 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 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 case 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 approach[25] on the surface of spider silk fibers, achieving an enhancement of toughness[26] or electrical conductivity.[27] Recently, the in vivo incorporation of CNTs and graphene into spider silks has been explored (Figure 1).[7]

View Article Online
DOI: 10.1039/C8NR08569B

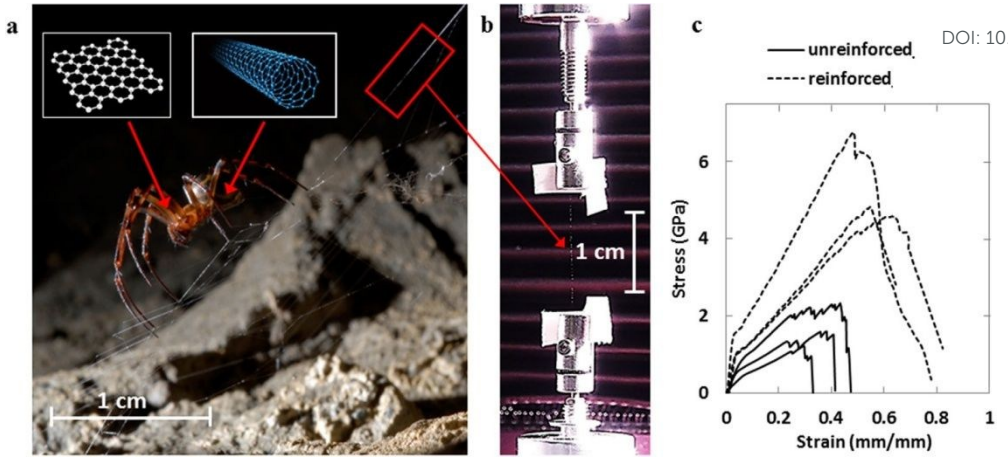


Fig. 1. Schematic of the experimental procedure proposed by Pugno et al.[7] (a) Spiders are fed with dispersions containing graphene or CNTs; (b) the corresponding spun silk is collected and tested in a nanotensile system; (c) stress – strain curves show improved mechanical properties when compared to pristine samples and in general superior than any other material available today.[7]

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 performance similar to its natural counterpart. Assembling 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 ± 6%), and toughness (114 ± 51 MJ/m^3). Another approach to overcome the impossibility to cultivate siper farms is the utilization of silkworms that are available on large scale and can be integrated in bioengineering structures. Feeding *B. mori* silkworms with diets containing CNTs led to intrinsically reinforced silkworm silk fibers (Figure 2).[6] The silk fibers produced by such natural process showed superior tensile strength and toughness thanks to a spontaneous silk-spinning process that converts helical and random coils to β-sheet structures, with a strength comparable to that of steel (1GPa) 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 overall

View Article Online
DOI: 10.1039/C8NR08569B

viability and growth of feeding CNTs to Drosophila larvae. They found that CNT feeding did not affect survival to either stage; survival to pupal stage and adulthood was somewhat higher for the CNT-fed group (83.5±3.8% versus 78.0±3.9% to the pupal stage, and 79.5±3.4% versus 69.4±3.9% to the adult stage).

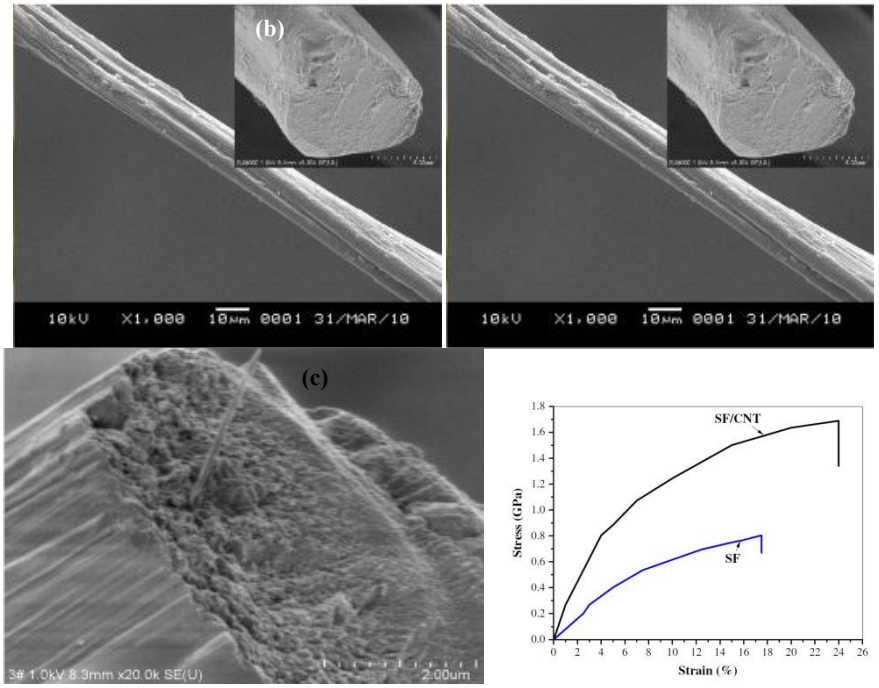
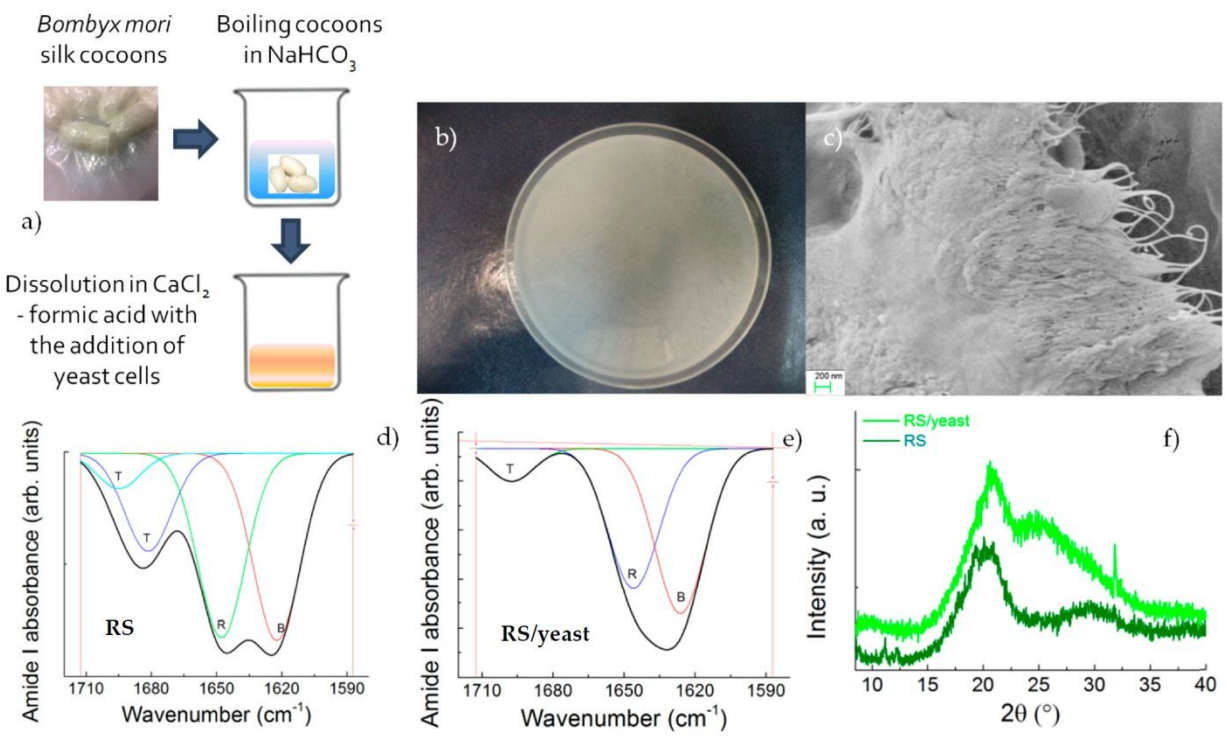


Fig. 2. A comparison of the morphology of silk fiber (SF) (top) and CNT-embedded silk fiber (SF/CNT) (bottom). (c) A comparison of the stress-strain curve of SF and SF/CNT.[6]

Living microorganisms have long been used in food preservation; such microorganisms form living surfaces that provide an attractive platform for the development of functional materials. At present, biotech companies uses 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]

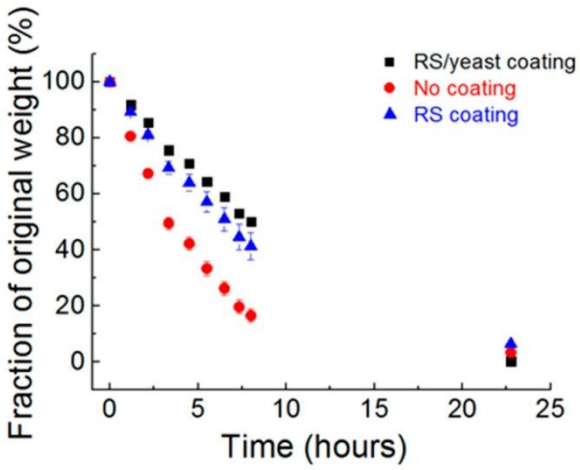
Inspired by previous work where the metabolic activity of living microorganisms was used as an engineered platform for the fabrication of advanced carbon-based materials,[8] Valentini et al.[31] extended this approach, with emphasis on the fermentation process used for centuries in wine- and

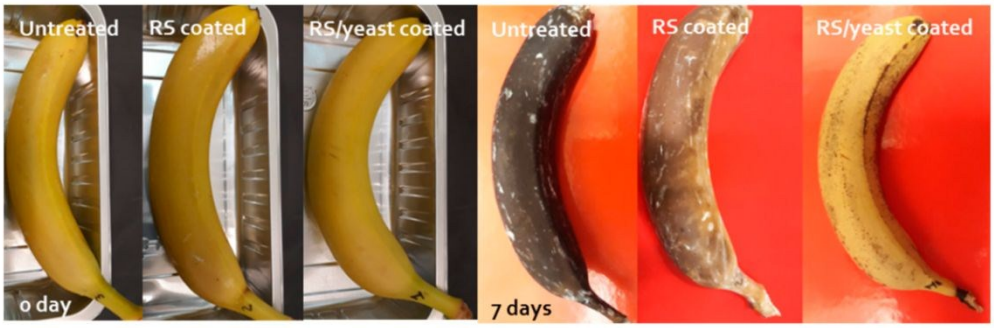
bread-making, to produce bionic composites which integrate regenerated silk nanofibrils that from the geometrical point of view and in terms of mechanical properties are similar to carbon nanotubes. The resulting reduced volume fraction of nanofibrils within the film could make the fermented hybrid composite more resistant to fracture with self-repairing properties exploiting the microorganisms' growth process that allows for the intracellular transport of nanomaterials. The CO_2 bubbles produced during fermentation could be used to produce porous architectures 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 temperature-responsive bilayer system.[31,32]



View Article Online
DOI: 10.1039/C8NR08569B

f)





g)

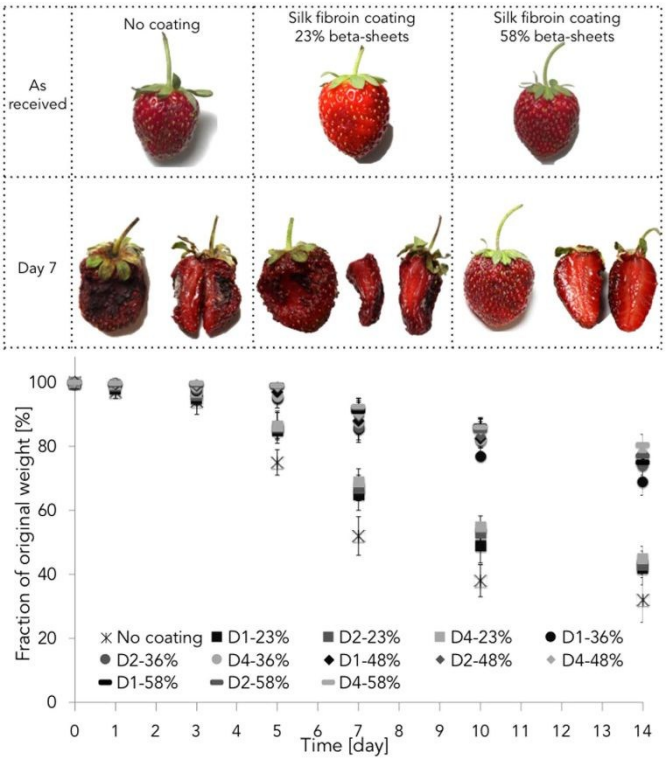


Fig. 3. a) Scheme of silk fibroin production using regenerated silk (RS); b) Visual appearance of the RS/yeast film and c) FESEM image of silk nanofibrils; d) and e) FTIR spectra of regenerated silk

View Article Online
DOI: 10.1039/C8NR08569B

and RS/yeast films, respectively. The coloured 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.[32] g) Weight loss of sponges soaked in water and stored for up to 25 h at 23 °C and 32% relative humidity (RH). Time lapse photography of banana degradation, indicating that the silk/yeast coating reduces the degradation rate. h) Time-lapse of strawberries ripening stored at 22 °C and 38% RH (no coating) or dip coated in silk fibroin suspension (23% β-sheets 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 silk fibroin suspension (Dx-xx%). Dx stands for 'x' dip coating steps. xx% stands for relative amount of beta-sheet content.[32]

3. Yeasts and bacteria as microfactories for engineering nanomaterials

Yeast is a cellular factory which is able of taking simple molecules from its environment, such as sugars, and synthesize new elements needed for its growth at mild temperature.[33-36] Water is an important factor in this regard being dry yeasts fully impermeable to molecules whereas wet yeasts promoted transfer of molecules from outside to inside the cell. Thus 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 bionicomposites 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.[8] adopted a similar strategy based on *Saccharomyces cerevisiae* yest 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.[8] The resulting dispersion (which differed somewhat in nanotube content) were used as the sole food source for yeast. The average concentration, expressed as nanotubes per mm^3 was 3100±550. They also exploited such mechanism to obtain the intracellular transport of CNTs during this growth process (Figure 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 again a higher failure strength, and was able to self-repair after placing the composite material containing living yeast cells back into growth medium.[9] Finally, a recent example of a graphene, CNT, and liquid rubber composite produced through yeast fermentation was reported:[38] CO_2 bubbles produced during fermentation along with collapsed yeast cells resulted in the transformation of conventional silicone rubber composites to auxetic robust rubber. Rubber-like materials when compressed along an axis expand in directions orthogonal to the applied load (e.g., positive Poisson's ratio) being the Poisson's ratio values for the majority of them about 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 in 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 high temperature (≈150°C). Moreover, from a practical perspective, rubber-based materials with such reversible auxetic architectures can find applications in broad fields 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-

View Article Online
DOI: 10.1039/C8NR08569B

assembly. For instance, a biofilm of *Shewanella oneidensis* was used to bind and reduce graphene oxide in solution, forming an electroactive thin film (Figure 4b).[41] Synergistic effects were observed between the living cells and the graphene, resulting in improved charge storage and properties.

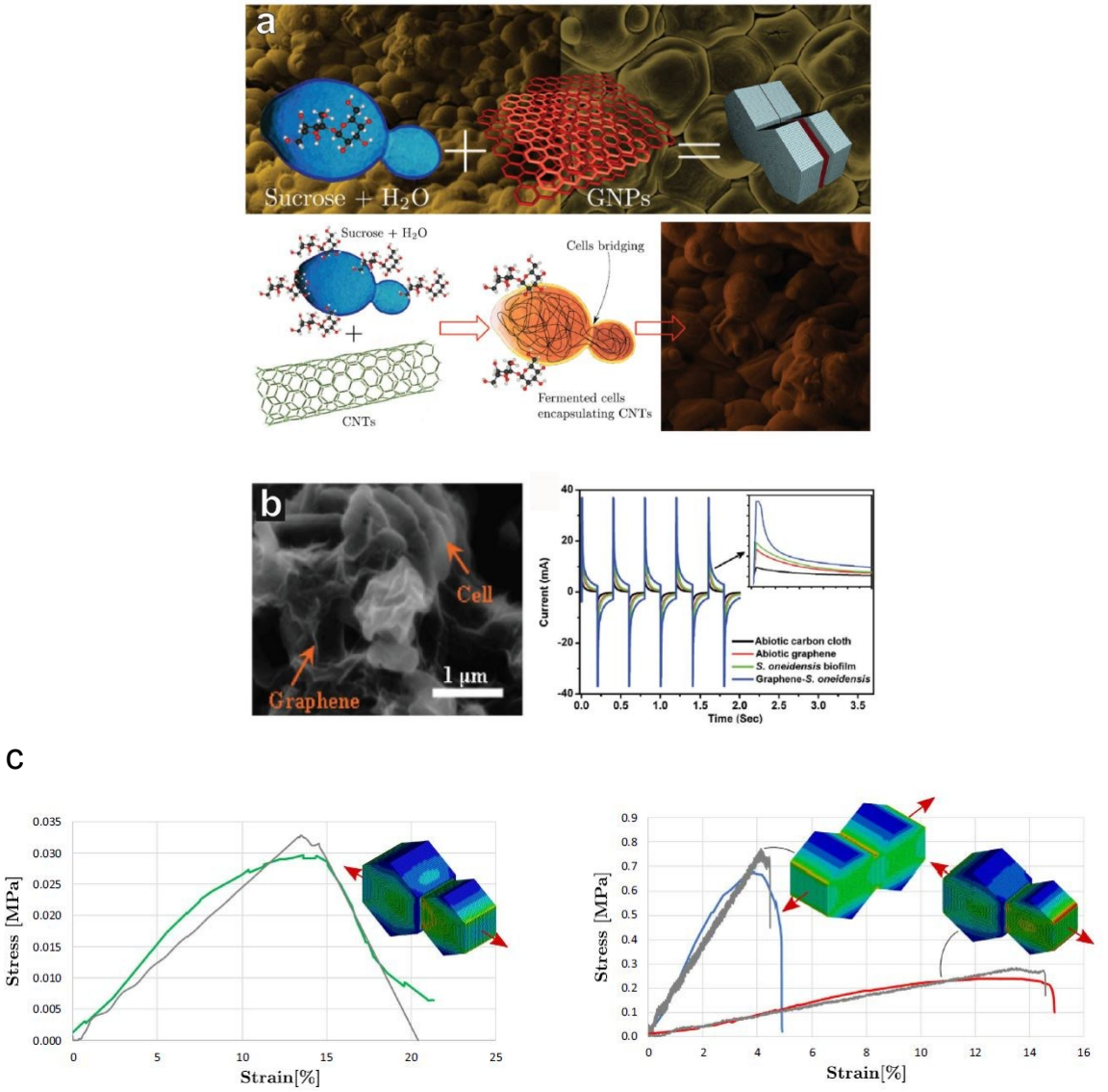


Fig. 4. Living composites with carbon materials. Integrating living microorganisms with inorganic materials serve for the fabrication of various devices. (a) Bionic composites made of yeast cells fermented with graphene nanoplatelets (GNPs) (top) or carbon nanotubes (CNTs) (bottom).[8,9] (b) Biomemory 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

View Article Online
DOI: 10.1039/C8NR08569B

device).[41] (c) Stress-strain curves obtained from tensile tests on fermented yeast sample (left panel) and yeast/CNT composites (right panel) prepared before fermentation (blue curve) and after fermentation (red curve), respectively.[8]

Based on the fermentation method proposed above a novel microorganism inspired macro/super-porous hydrogel composed of specific polymers and yeast was prepared. The authors found that the integration of polymeric materials and fungi has 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 property (Figure 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 Figure 5a, indicates that actually the compressive strength and modulus may have a little difference for samples with almost the same density (Figures 5a-c). This mainly attributes to the tiny difference of microstructure, which cannot be controlled so precisely by this method (Figure 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.

View Article Online
DOI: 10.1039/C8NR08569B

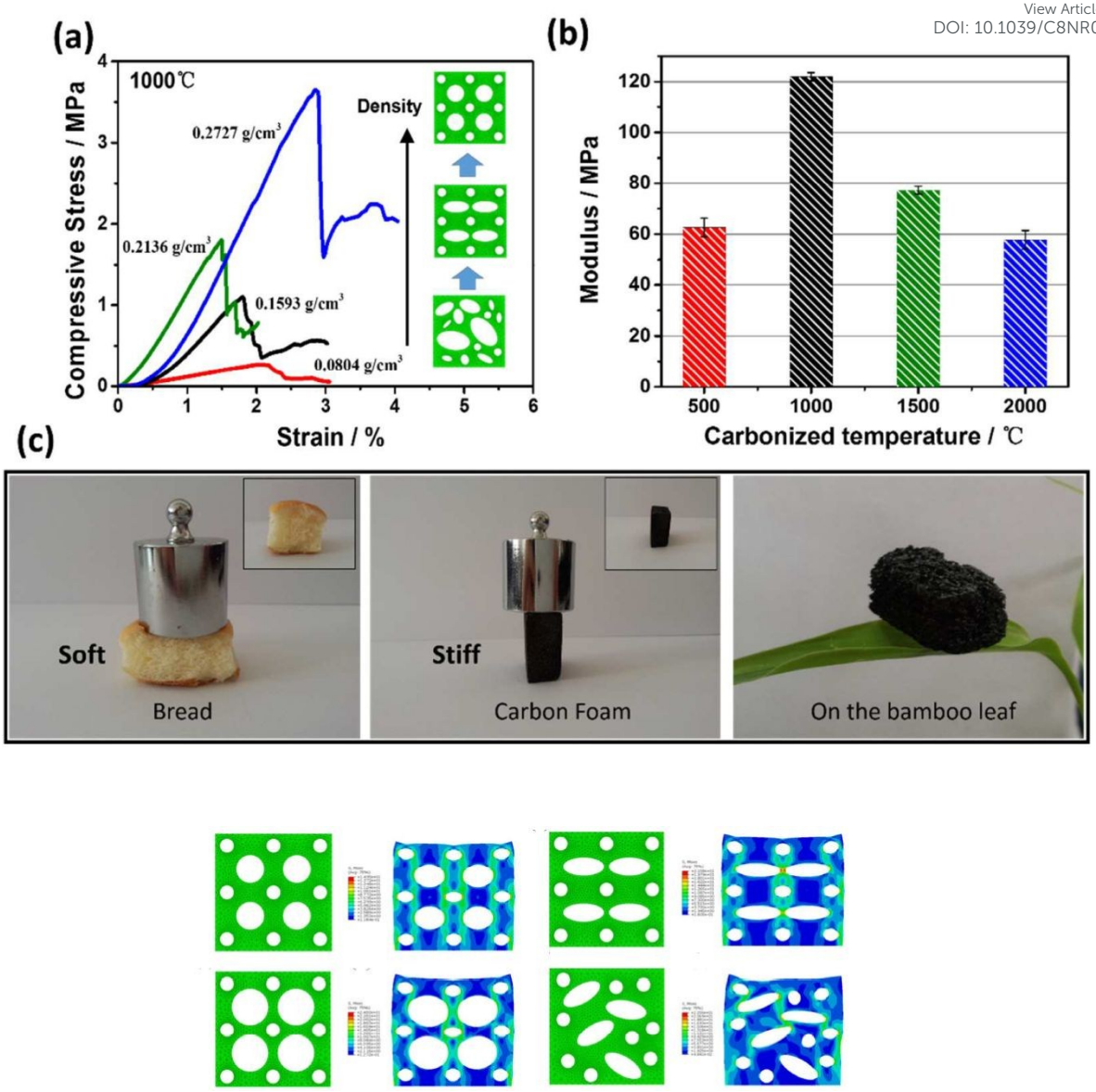


Fig. 5. (a) Stress–strain measurements of carbon foam with different bulk density carbonized at 1000℃, (b) Compressive moduli E of carbon foam carbonized at different temperature, bulk densities of measured samples are all between 0.21~ 0.22 g/cm3, (c) Left: a 100g weight standing on a piece of bread, middle: a 100g weight standing on a carbon foam; right: the carbon foam standing on a bamboo leaf.[43] (d) The 2D simulation geometry of compression test and projection of stress on the x-y plane of the microstructure.[43]

High porous bionic hybrid materials made of cardiac cells and CNTs might also provide muscle for robots made of living tissues.[44] Khademhosseini 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 gelatin framework was strengthened with a reduced electrical impedance. The fractal-like CNT networks were found also to alter tissue organization (Figure 6) which are particularly suitable as cell delivery system for cell therapy.

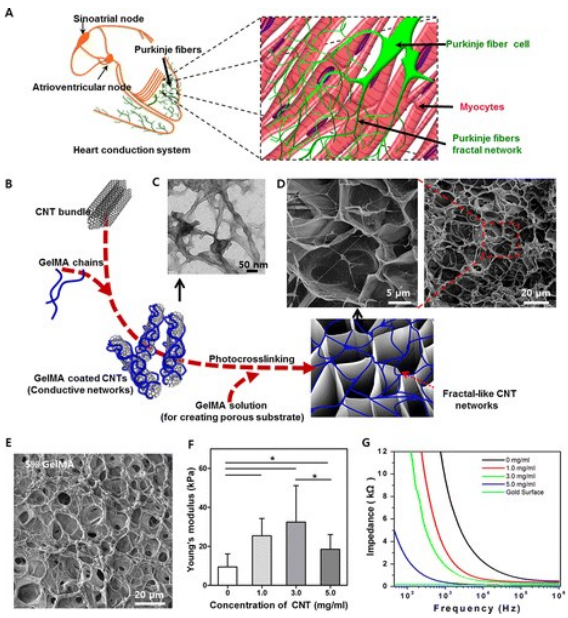


Fig. 6. (a) Schematic diagram illustrating the isolated heart conduction systems showing the purkinje fibers, which are located in the inner ventricular walls of the heart. (b) Preparation procedure of fractal-like CNT networks embedded in hydrogel. (c) TEM image of hydrogel-coated CNTs. (d) SEM images show porous surfaces of 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]

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] there exists examples in attaining both strength

View Article Online
DOI: 10.1039/C8NR09569B

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 challenging. 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 μm to 11 nm), revealing an highly desirable scaling laws of the mechanical properties of cellulose nanopaper: the smaller, the tougher (uncommon trend) and the stronger (common trend) (Figure 7).

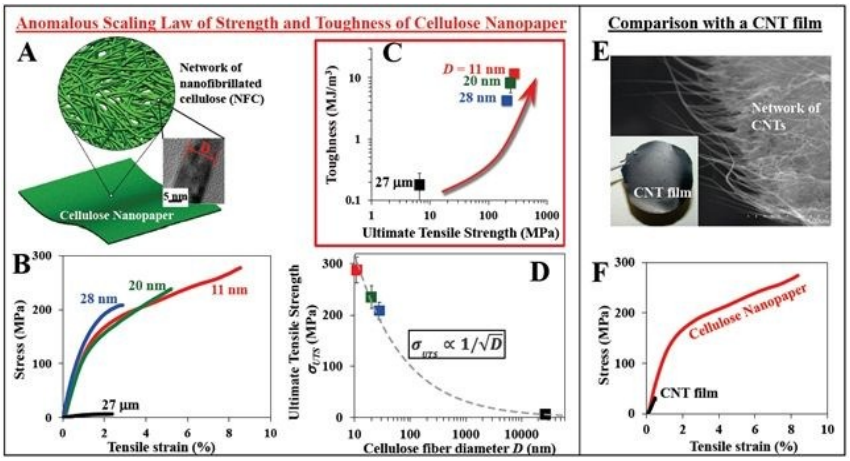


Fig. 7. An "anomalous" scaling law of strength and toughness of cellulose nanopaper: The smaller, the tougher (anomalous) and the stronger.[47]

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 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

View Article Online
DOI: 10.1039/C8NR08569B

bionic fibers of BC when EPS are involved in bacterial nutrition or interaction of bacteria with CNTs was reported by Park et al. (Figure 8).[50]

The mechanical properties of such bionic fibers were also investigated[51] and the results are reported in Figure 9 where the Young's modulus of the pristine BC, BC fiber, and BC/CNTs fibers was 7.3, 29.2, and 38.9 GPa, respectively. 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.

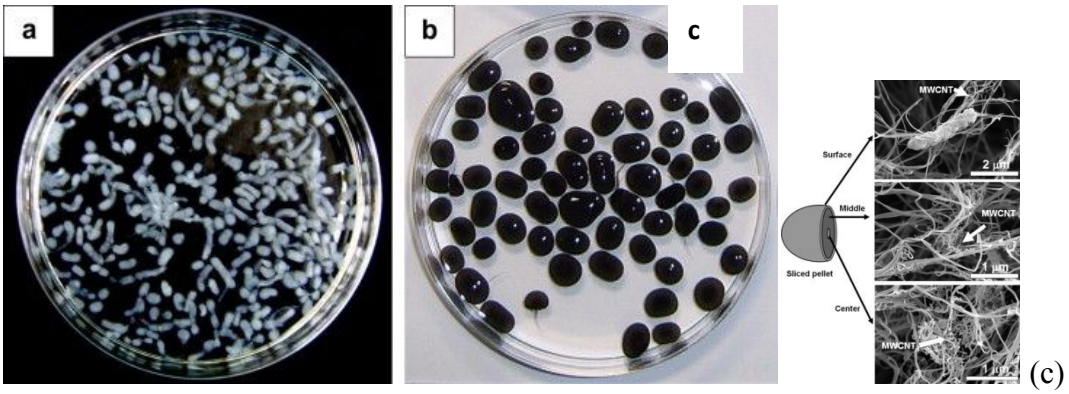


Fig. 8. Photographs of bacterial cellulose pellets synthesized in (a) Hestrin and Schramm medium and (b) MWCNT-dispersed Hestrin and Schramm. (c) FESEM images of bacterial cellulose synthesized in MWCNT-dispersed Hestrin and Schramm medium.[51]

Further, molecular dynamics simulations[52] 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 resistant force in cellulose fiber case is substantially higher than that in the CNT case, shedding light on molecular scale understanding of the huge difference in tensile strength between

View Article Online
DOI: 10.1039/C8NR08569B

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 intrinsical three-dimensional (3D) structure which distinguishes it from other natural polymers. Biogenic incorporation of graphene or CNTs is thus challenging because of the small pores in pristine BC.[53]

To overcome such problem Yoon et al.[54] reported a post-processing immersion method consisting in 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 grapheme 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 showed 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 (Figure 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 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.

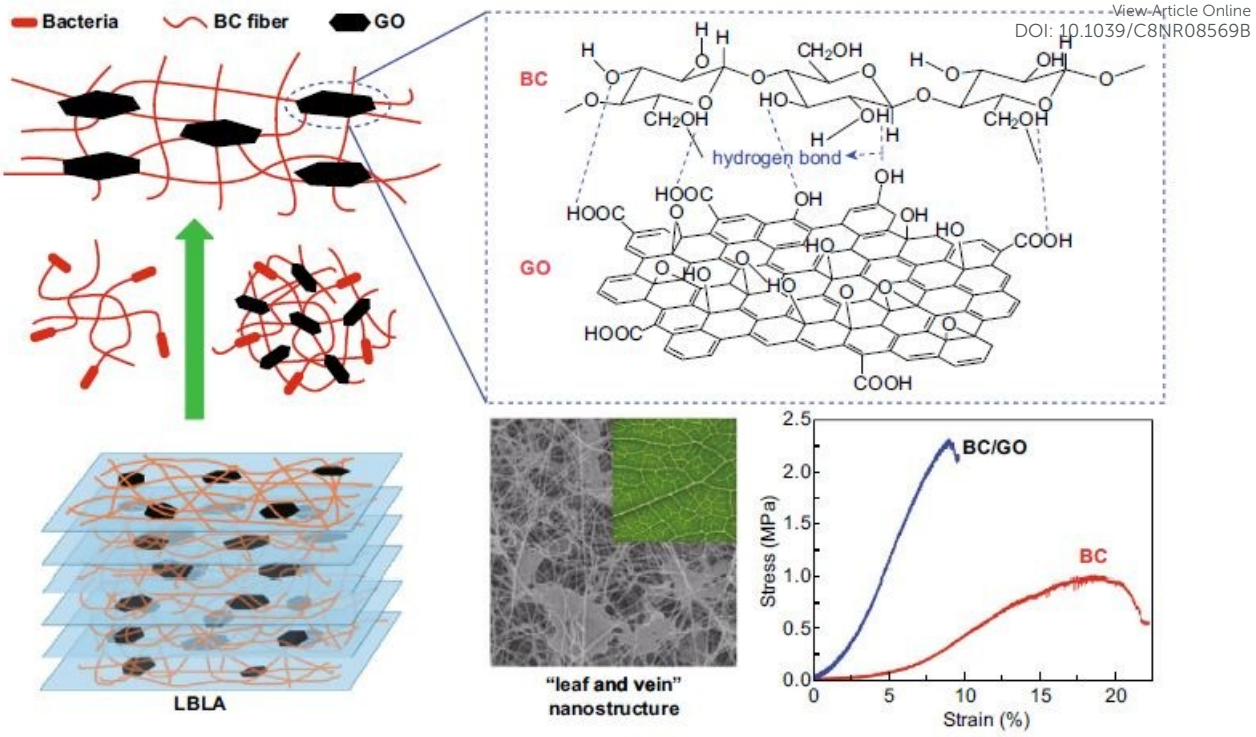


Fig. 9. Schematic diagram of ‘‘leaf and vein’’ nanostructure of BC/GO prepared by LBLA strategy. The layer-by-layer culture mode facilitates the bundling of 2D GO nanosheets by 1D BC nanofibers and promotes distribution of 2D GO nanosheets in 3D BC matrix.[56]

5. Conclusion

This article reviews the recent attempts in the emerging field of bionicomposites 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 as self-assembling, self-healing, and responsiveness to external stimuli. From the examples reviewed here, we discussed two main different efforts that lead to bionic materials. One attempt to use microorganisms and their metabolic activities for materials production. All approaches related to this item, have focused on the relative simplicity and ease of growth of such living organisms. We believe that this is the future as more scientists develop engineered cellular systems to secrete synthetic materials mimicking the natural counterpart.

The other approach in developing bionic composites where the living component are used to modify the materials giving to the final product novel and unexpected functions. This method allow the realization of complex functional materials that are not possible to obtain with the bottom-up approach reported above.

As these two engineering technologies combine, the emerging field of Bionics (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, Bionics is the natural evolution of Biomimetics.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

N.M.P. is supported by the European Commission under the Graphene Flagship Core 2 grant No. 785219 (WP14 "Composites") and FET Proactive "Neurofibres" grant No. 732344 as well as by the Italian Ministry of Education, University and Research (MIUR) under the "Departments of Excellence" grant L.232/2016. L.V. is supported by the European Commission under the Graphene Flagship Core 2 grant No. 785219 (WP14 "Composites") as well as by the Italian Ministry of Education, University and Research (MIUR) under the "Departments of Excellence" grant L.232/2016.

References

View Article Online
DOI: 10.1039/C8NR08569B

1 A. George and S. Ravindran, *Nano Today* 2010, **5**, 254 – 66.

2 H. C. Lichtenegger, T. Schoberl, M. H. Bartl, H. Waite and G. D. Stucky, *Science* 2002, **298**, 389-392.

3 Y. Politi, R. A. Metzler, M. Abrecht, B. Gilbert, F. H. Wilt, I. Sagi, L. Addadi, S. Weiner and P. Gilbert, *Proc. Natl Acad. Sci.* 2008, **105**, 17362 – 17366.

4 H. R. Hepburn and S. P. Kurstjens, *Apidologie* 1998, **19**, 25–36.

5 K. Zhang, H. Duan, B. L. Karihaloo and J. Wang, *Proc. Natl Acad. Sci.* 2010, **107**, 9502–9506.

6 J.-T. Wang, L.-L. Li, M.-Y. Zhang, S.-L. Liu, L.-H. Jiang and Q. Shen, *Materials Science and Engineering C* 2014, **34**, 417–421.

7 E. Lepore, F. Bosia, F. Bonaccorso, M. Bruna, S. Taioli, G. Garberoglio, A. C Ferrari and N. Pugno, *2D Mater.* 2017, **4**, 031013. (Available from 2015 at https://arxiv.org/ftp/arxiv/papers/1504/1504.06751.pdf)

8 L. Valentini, S. Bittolo Bon, S. Signetti, M. Tripathi, E. Iacob and N. M. Pugno, *Scientific Reports* 2016, **6**, 27031.

9 L. Valentini, S. Bittolo Bon, S. Signetti and N. M. Pugno, *ACS Applied Materials & Interfaces* 2016, **8**, 7607.

10 L. C. Gerber, F. M. Koehler, R. N. Grass and W. J. Stark, *Proc. Natl Acad. Sci.* 2012, **109**, 90–94.

11 F. Vollrath and D. P. Knight, *Nature* 2001, **410**, 541–548.

12 C. J. Fu, Z. Z. Shao and V. Fritz, *Chem. Commun.* 2009, **43**, 6515–6529.

13 A. Koeppel and C. Holland, *ACS Biomater. Sci. Eng.* 2017, **3**, 226–237.

View Article Online
DOI: 10.1039/C8NR08569B

14 S. Keten, Z. Xu, B. Ihle and M. J. Buehler, *Nat. Mater.* 2010, **9**, 359-367.

15 X. Hu, K. Shmelev, L. Sun, E.-L. Gil, S.-H. Park, P. Cebe and D. L. Kaplan, *Biomacromolecules* 2011, **125**, 1686-1696.

16 F. Zhang, X. You, H. Dou, Z. Liu, B. Zuo and X. Zhang, *ACS Appl. Mater. Interfaces* 2015, **7**, 3352-3361.

17 S. Ling, Z. Qin, C. Li, W. Huang and D. L. Kaplan, *Nature Communications* 2017, **8**, 1387.

18 X. Q. Wang, J. A. Kluge, G. G. Leisk and D. L. Kaplan, Biomaterials 2008, **29**, 1054–1064.

19 S. Xu, Y. Lin, J. Huang, Z. Li, X. Xu and L. Zhang, *J. Mater. Chem. A* 2013, **1**, 4198 − 4206.

20 Q. Wang, Q. Chen, Y. Yang and Z. Shao, *Biomacromolecules* 2013, **14**, 285 − 289.

21 S. Kim, J. Byun, S. Choi, D. Kim, T. Kim, S. Chung and Y. Hong, *Adv. Mater.* 2014, **26**, 3094 − 3099.

22 C. J. Shearer, A. Cherevan and D. Eder, *Adv. Mater.* 2014, **26**, 2295 − 2318.

23 C. Wang, Y. Zhang, L. Lin, L. Ding, J. Li, R. Lu, M. He, M. Xie and R. Cheng, *Eur. J. Lipid Sci. Technol.* 2015, **117**, 1940 − 1946.

24 J.-T. Wang, L.-L. Li, M.-Y. Zhang, S.-L. Liu, L.-H. Jiang and Q. Shen, *Mater. Sci. Eng., C* 2014, **34**, 417 − 421.

25 E. Steven, W. R. Saleh, V. Lebedev, S. F. A. Acquah, V. Laukhin, R. G. Alamo and J. S. Brooks, *Nat. Commun.* 2013, **4**, 2435.

26 S.-M. Lee, E. Pippel, U. Gösele, C. Dresbach, Y. Qin, C. V. Chandran, T. Bräuniger, G. Hause and M. Knez, *Science* 2009, **324**, 488 – 92.

View Article Online
DOI: 10.1039/C8NR08569B

27 E. Steven, J. G. Park, A. Paravastu, E. B. Lopes, J. S. Brooks, O. Englander, T. Siegrist, P. Kaner and R. G. Alamo, *Sci. Technol. Adv. Mater.* 2011, **12**, 055002.

28 C. H. Bowen, B. Dai, C. J. Sargent, W. Bai, P. Ladiwala, H. Feng, W. Huang, D. L. Kaplan, J. M. Galazka and F. Zhang, *Biomacromolecules* 2018, **19**, 3853–3860.

29 T. K. Leeuw, R. M. Reith, R. A. Simonette, M. E. Harden, P. Cherukuri, D. A. Tsyboulski, K. M. Beckingham and R. B. Weisman, *Nano Lett.* 2007, **7**, 2650-2654

30 R. P. Ross, S. Morgan and C. Hill, *Int. J. Food Microbiol.* 2002, **79**, 3–16.

31 L. Valentini, S. Bittolo Bon and N. M. Pugno, *Nanomaterials* 2018, **8**, 518.

32 B. Marelli, M. A. Brenckle, D. L. Kaplan and F. G. Omenetto, *Sci. Rep.* 2016, **6**, 25263

33 Y. Chen, L. Daviet, M. Schalk, V. Siewers and J. Nielsen, *Metab. Eng.* 2013, **15**, 48–54.

34 K. K. Hong and J. Nielsen, *Cell. Mol. Life Sci.* 2012, **69**, 2671–2690.

35 W. Runguphan and J. D. Keasling, *Metab. Eng.* 2014, **21**, 103–113.

36 E. P. J. M. Everaert, H. C. van der Mei and H. J. Busscher, *Colloids and Surfaces B: Biointerfaces* 1998, **10**, 179–190.

37 L. Valentini, S. Bittolo Bon, S. Signetti and N. Pugno, *ACS Applied Materials & Interfaces* 2016, **8**, 22714.

38 L. Valentini, S. Bittolo Bon and N. M. Pugno, *Adv. Funct. Mater.* 2017, **27**, 1606526.

39 K. Bertoldi, P. M. Reis, S. Willshaw and T. Mullin, *Adv. Mater.* 2010, **22**, 361–366.

40 R. Mody, D. Gerrard and J. Goodson, *Rubber Chem. Technol.* 2013, **86**, 449–469.

41 Y. Yuan, S. Zhou, G. Yang and Z. Yu, *RSC Adv.* 2013, **3**, 18844.

42 Q. Zhang, B. Chen, L. Tao, M. Yan, L. Chen and Y. Wei, *RSC Adv.* 2014, **4**, 32475–32481.

43 Y. Yuan, Y. Ding, C. Wang, F. Xu, Z. Lin, Y Qin, Y. Li, M. Yang, X. He, Q. Peng and Y. Li, *ACS Appl. Mater. Interfaces* 2016, **8**, 16852–16861.

44 S. R. Shin, S. M. Jung, M. Zalabany, K. Kim, P. Zorlutuna, S. B. Kim, M. Nikkhah, M. Khabiry, M. Azize, J. Kong, K. T. Wan, T. Palacios, M. R. Dokmeci, H. Bae and X. S. Tang, *ACS Nano* 2013, *7*, 2369–2380.

45 A. Carpinteri and N. M. Pugno, *Nat. Mater.* 2005, **4**, 421-423.

46 G. Meng, Y. J. Jung, A. Cao, R. Vajtai and P. M. Ajayan, *Proc. Nat. Acad. Sci.* 2005, **102**, 7074-7078.

47 H. Zhu, S. Zhu, Z. Jia, S. Parvinian, Y. Li, O. Vaaland, L. Hu and T. Li, *Proceedings of the National Academy of Sciences* 2015, **112**, 8971-8976.

48 R. Jonas and L. F. Farah *Polym. Degrad. Stabil.* 1998, **59**, 101-106.

49 H. P. Fink, P. Weigel, H. J. Purz and J. Ganster, *Prog. Polym. Sci.* 2001, **26**, 1473-1524.

50 W.-I. Park, H.-S. Kim, S.-M. Kwon, Y.-H. Hong and H.-J. Jin, *Carbohydrate Polymers* 2009, **77**, 457–463.

51 W.-I. Park, H.-S. Kim, S.-M. Kwon, Y.-H. Hong and H.-J. Jin, *Carbohydr. Polym.* 2009, **77**, 457–463

52 Y. Li, H. Zhu, S. Zhu, J. Wan, Z. Liu, O. Vaaland, S. Lacey, Z. Fang, H. Dai, T. Li and L. Hu, *NPG Asia Materials* 2015, **7**, e150.

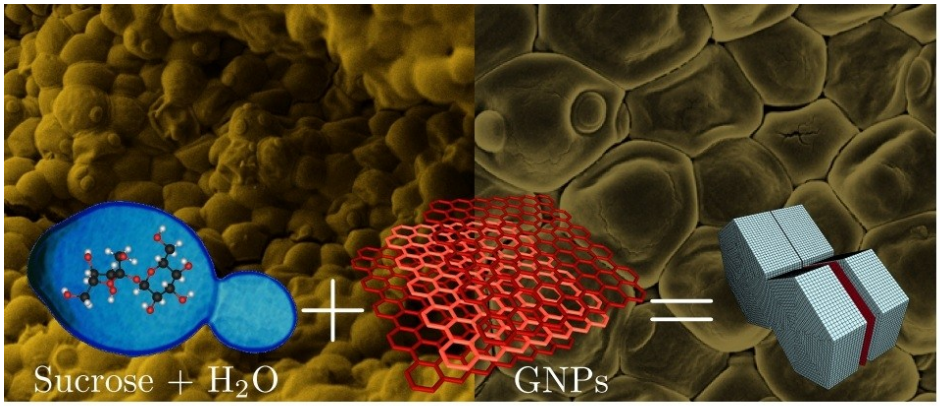
53 C. Gao, Y. Wan, C. Yang, K. Dai, T. Tang, H. Luo and J. Wang, *J. Porous Mater.* 2011, **18**, 139–145.

54 S.H. Yoon, H.J. Jin, M.C. Kook and Y.R. Pyun, *Biomacromolecules* 2006, **7**, 1280–1284. View Article Online
DOI: 10.1039/C8NR08569B

55 H. Si, H. Luo, G. Xiong, Z. Yang, S.R. Raman, R. Guo and Y. Wan, *Macromol. Rapid Commun.* 2014, **35**, 1706–1711.

56 H. Luo, J. Dong, F. Yao, Z. Yang, W. Li, J. Wang, X. Xu, J. Hu, *Nano-Micro Lett.* 2018, **10**, 42.

View Article Online
DOI: 10.1039/C8NR08569B



134x85mm (192 x 192 DPI)