

## BIOMIMETICS CLUES FOR TISSUE ENGINEERING MATERIALS

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### Abstract

Tissue engineering involves creation of three-dimensional tissue structures, also referred to as *scaffolds*, on which cells or other bio-molecules may be incorporated. Scaffolds are aimed at guiding the organisation, growth and differentiation of cells in the process of forming functional tissue, providing physico-chemical cues and therefore need incorporating both native anatomy and necessary tissue culture criteria. The successful incorporation of any scaffold within a host body is dependent on appropriate communication between cells, tissues, and the host system as a whole.

This paper aims to review the biologically synthesised materials that have found interest for biomimetics applications, discussing their present or possible application for tissue scaffolding. Besides, indications on biomimetics material design are also provided, aimed at suggesting how in practice a successful extraction of clues from nature is obtained.

### 1. Introduction

Scaffolding systems need primarily to be biocompatible and are therefore built using biomaterials. In practice, the concept of biocompatibility can result in a number of criteria, including lack of cytotoxicity, minimal immune responses, and inflammation and chemical compatibility with aqueous solutions and physiological conditions. Moreover, it is important to dispose of basic units amenable to design and modification, so to allow as far as possible the self-templating of the scaffold. From the manufacturers point of view, it is also essential that material production, purification and processing are easy and scalable, whilst the users would require a controlled rate of biodegradation of the materials [1]. A further specific set of properties would guarantee the physico-chemical success of implantation: in this respect, critical parameters include macroporosity, cell attachment properties, localised growth factor release, degradation, and mechanical properties [2]. It is noteworthy that different properties are required for different types of tissue e.g., a cartilage region would have reduced need for transport, hence a lower porosity and reduced access to transport channels or a diffusion network. By contrast, osteoblasts would require greater mass transport and greater space for migration [3].

The most common scaffolds used in tissue engineering are still adapted from materials utilised in the current medical devices industry, because this offers certain regulatory advantages. However, these materials were never designed for an application as such. In contrast, materials able to support three-dimensional tissue growth have been designed

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in Nature thanks to evolution. For example, materials ranging from spider silks to extracellular matrix proteins provide excellent examples of biomaterial scaffolds. In addition, naturally occurring biomaterial scaffolds, such as collagen, can be chemically modified to confer desirable properties. In other words, the study of biological tissues based on biomimetics concepts, hence from "the abstraction of good design from nature" [4], can provide useful clues for tissue engineering. As a result of this experience, biomimetics is gradually entering the cellular level, hence the molecular scale [5]. The high level of organisation, present in a hierarchical way from the nanoscale to the macroscale in biological tissues, which are synthesised in aqueous environment under mild physiological conditions, is ideal to control tissue formation, biological functions or physical performance. This suggests that biological proteins will be at the basis of future biomimetic systems, hence including scaffolds for cell growth.

## **2. Characteristics of biologically synthesised materials**

A number of characteristics presented by biological materials, are not always available, at least in principle, in engineered materials. In practice, biologically synthesised materials are capable of:

### *Responding dynamically to applied forces (smart design-for-function)[6]*

In good engineering practice, it is required to manufacture something that does what it is supposed to do (e.g., a material beam which is able to withstand a given mechanical stress for a given time). Far too often, however, ability to design for function is limited when unexpected changes are introduced in the environment and/or when for some reason the geometry needs to be modified e.g., by integration in a larger structure. In other words, adaptation to change is limited for engineered materials. In contrast, biological structures are able to develop, hence changing geometry and possibly shape, adapting meanwhile to significant environmental changes and different degrees of mechanical stress. This is because they recognise, as is typical of most biological tissues, that the functions they have to perform in successive stages of their development are different.

### *Building themselves in a hierarchical and optimised way (self-assembly)[7]*

Biologically synthesised materials are built in a hierarchical way i.e., using assemblages of molecular units or their aggregates embedded within other particles or aggregates that may, in turn, be part of larger units of increasing levels of organisation. Good examples of hierarchical structures are bones, hair and muscles, built at different levels from the cell up to the whole structure. The advantage of hierarchical structures is that the individual building blocks carry not only the information to interact with their neighbours, but also the information for assembling into materials of hierarchical structure. This implies the ability for self-assembly in micro- and nano-scale components. In addition, the hierarchical structure has been also optimised for function at different stages of development and is therefore integrated with a smart design for function, as discussed above. Insect sensors, such as campaniform sensilla in locust skin, represented in Figure 1, do characterise themselves for an optimised self-assembly coupled with the attitude to respond to environmental changes. In addition, they are embedded in a system, the skin, which is aimed at fulfilling other functions, such as protection and defence.

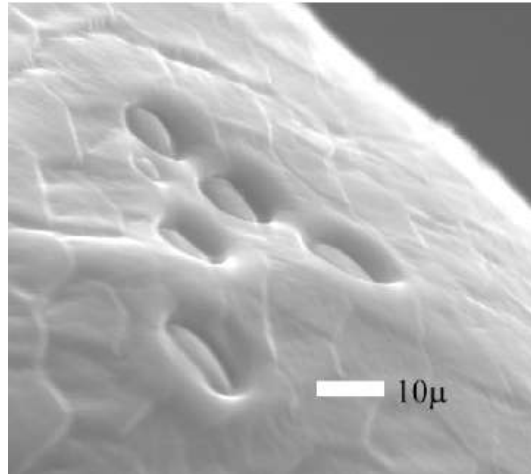


Figure 1 Electron micrograph of a region in locust skin with campaniform sensilla

*Performing different functions when required (multi-functionality)[8]*

One of the most critical problems, for example in polymeric composite materials, is the necessity of measuring material strain locally. This can be obtained via embedding optical fibres in the composites structure, but the two functions remain separated and somehow conflicting, at the point that each of them can interfere with the other one. An example of this interference is that embedded optical fibres can act as delamination sites for the composite structure, hence possibly producing damage in the laminate [9]. In contrast, biological structures, such as arthropods cuticles, respond in the same time the function of protection, sensing and locomotion [10]. In the same way, dentin, enamel, and bone are multifunctional, serving as load-bearing systems with piezoelectric properties [11].

*Responding in an active way by sealing cracks before they become catastrophic (self-healing)[12]*

In most materials, when stress is applied to a system in man-made materials failure of the material occurs shortly after the initial defect forms. There are several applications where the ability to prevent or postpone failure after the initial defect would be extremely useful: the abalone shell, a micro-laminate composite of calcium carbonate crystals and proteins, provides this ability. Cobble-like polygonal nanograins are basic building blocks used to construct individual aragonite platelets into a mother-of-pearl configuration, known as nacre. Consequently, abalone shell has fracture toughness 3,000 times greater than that of the crystals alone [13]. Although the proteins comprise only a few percent of the mass of the composite, they are responsible for the tremendous enhancement of strength of the material and the precise control of its unique nanostructure, which allow the defects to be compensated for [14]. In addition, biological tissues are able to provide self-healing in Nature, because they are conceived with some form of engineering redundancy, which means that the tissue is still able to perform its function, whilst a part of it is “out of order”. This function is particularly interesting for what a scaffold is supposed to be able to do. It should provide a sufficiently rigid framework to fill the defect, degrade after the new tissue forms, and leave a product fully integrated with surrounding tissue. Recently, the concept of biomimetic self-healing has also been applied in aircraft materials including hollow

fibres, often referred to as *bleeding composites* [15]. These allow the two effects typical of abalone shells to be performed, namely *self-knowledge of damage* through crack penetrating ultra-violet fluorescent dye and *restoration of mechanical properties* by a self-activating process with a stored healing agent.

### 3. Materials biomimetics and tissue engineering

Natural composites exhibit outstanding combinations of properties over a temperature and relative humidity range. This can represent a limitation for other applications: in tissue engineering operation in physiological conditions, where temperature and humidity range are hardly variable is required nonetheless.

The fact that natural materials are hierarchically designed means also that control has to take place at any level, from the nanoscale up to the macroscale. This implies that in many cases design according to simple approximation, such as e.g., the “rule of mixtures” for synthetic composites, would have reduced significance for hierarchically built materials. Also, for practical reasons, the above control cannot always be carried out in physiological conditions, while it would be important to do so, because properties of natural material dramatically change with humidity. For example horn keratin matrix has Young's modulus of 0.9 GPa with 40% water and 6.1 GPa in the dry state [16].

Mechanisms for toughness improvement are considerably specific in natural materials: in nacre (mother-of-pearl) the fracture path shows evidence of crack blunting and diversion [17]. The work of fracture is highly directional and is governed by crack stopping at interfaces, followed by crack diversion through delamination. It has been demonstrated that providing weak interfaces in laminated ceramics can increase the work required to propagate a crack by over two orders of magnitude. This is the function of the thin matrix material between the calcium carbonate platelets, which constitute mollusc shells, such as abalone [18].

These examples highlight the difficulties in *controlling the material behaviour* and *optimising its performance*, when materials are mimicked on natural ones. This can suggest the viability of a biomimetic approach for tissue engineering, which can be to come as close as possible to a completely regenerating material, mimicking what happens in complex organisms, such as vertebrate tissues. In practice, the future aims of tissue engineers would include growing large, vascularised solid organs such as the liver. Such structures require very complex scaffolds filled with channels that would allow liquid to flow and blood vessels to develop inside the tissue. This suggests the interest of looking at the regenerating capabilities in vertebrates.

There are different forms of regeneration, of which the simplest one is the *axonal outgrowth* of the nervous system, whilst *regeneration by simple proliferation*, as seen in organs, such as intestines, liver, or adrenal gland, appears to be more complex. Simple proliferation and differentiation of the stem cells can then channel the regeneration of other organs and tissues. In other cases, such as in amphibia, damaged site cells first dedifferentiate then differentiate again into their type. The maximum degree of vertebrate regeneration takes place in the urodele amphibians (salamanders and newts). After a newt limb is amputated, the wound is sealed over by the rapid migration of epithelial cells from surrounding tissue. Mesodermal cells beneath the epidermis lose their differentiated character and reproduce to form a conical mound of cells called the *blastema*. The blastemal cells eventually differentiate again into the cartilage,

connective tissue, and muscle of the new limb. This allows regeneration of a new forearm or hindleg in approximately six weeks [19].

In mammals, true regeneration is limited to a very few tissues, such as liver, bone, retina and skeletal muscle [20]. The only example of complete regeneration of mammalian appendages is the annual replacement of antlers in deer, with other notable examples include bats being able to plug holes in their wing membranes, rabbits, domestic cats and bats filling in ear holes [21]. Liver cells come closest to mimicking the urodele regeneration phenomenon, in which differentiated cells revert to an embryonic dividing state and then differentiate once more into specialized cells. For example, a rat can replace two-thirds of its liver in 5 to 7 days. Mature liver cells only partially dedifferentiate, however. In response to tissue destruction, liver cells, which normally do not reproduce, undergo rapid cell division. A baboon liver transplanted into a human will double in size to reach that of a human liver within a week. In general, it is still not clear how much the blastema formation process is diffused and can be duplicated in mammals. They lack the ability to grow new limbs through blastema formation: this is not completely true for limb parts, and has been observed for fingertips, for example in the case of re-growth of the tips of mouse toes [22]. Recently, tissue regeneration has been linked to by the action of the so-called knockout proteins, such as thrombospondin, whose elimination from the wound site might promote improved healing. This may explain the good limb regeneration properties of animals, such as some salamanders, in particular the Mexican Mole Salamander. As an example, to avoid as much as possible the foreign body reaction, “stealth” materials are being designed that can enter the body and remain free of a layer of adsorbed protein, aiming at getting a Teflon-like substance that strongly resists the attachment of proteins [23].

#### 4. Case studies

Given the above considerations, it is not surprising that a number of biological materials, coming from a variety of animals and plants, are investigated to provide scaffolds for tissue engineering. Since the research in this field is particularly thriving, it would be difficult to supply information on all the tissue-engineering projects aimed at development of bio-inspired scaffolds. For this reason, only a limited number of case studies are presented and the rationale for their application from the point of view of materials science is discussed. In Table 1 four types of biological materials are reported, which particularly attracted the attention of biomimeticists.

Material	Characteristics	Structure
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Molluscs shells (abalone)	Strength Toughness Impact resistance	Layers of calcium carbonate with organic binder
Rat tooth enamel	Hardness Damage tolerance	Cylindrical rods of hydroxyapatite (95%) with proteins + water
Sponges skeletons	Elasticity Toughness Porosity	Formed by a structure made of spongine, a material not very different from horn
Diatoms	Hardness Malleability Biosynthesis	Silica in various shapes

Table 1 Case studies for innovative scaffolding materials

#### 4.1 Sponge skeletons

The sponge skeleton is an aggregation of spicules, which give the animal its shape and will persist as a structure after the sponge has died. Sponges are classified according to the composition of these spicules, and therefore their skeleton. About 80% of sponges belong to the class Demospongiae and have a skeleton made of a nitrogenous, hornlike substance, called *spongine*. These are the soft elastic sponges, which can be harvested for household and commercial use. The other 20% belong to the class Calcispongiae, having calcium carbonate spicules, and Hyalospongiae, having silicic acid spicules. The latter are also called glass sponges because their skeletons have a glass texture and can show an absolutely constant refractive index, which means they can act as light propagating devices, like fibre optics, with mechanical properties much higher than commercial glass [24]. In addition, experiments carried out on a marine sponge skeleton demonstrated that it was able to act as a potential scaffold, supporting growth of human osteo-progenitor cells. This was attributed to the hydration potential of the fibre, the presence of open interconnected channels created by the fibre network, the collagenous composition of the fibre, and the structural diversity of fibre architecture [25].

#### 4.2 Diatoms

Peptide-based biomaterials can be fabricated to form two- and three-dimensional structures. Recent studies show that biomaterial promotion of multi-dimensional cell, cell interactions and cell density are crucial for cellular differentiation and subsequent tissue formation. Other refinements in tissue engineering include the use of stem cells, cell pre-selection and growth factor pre-treatment of cells that are used for seeding scaffolds. These cell-culture technologies, combined with improved processes for defining the dimensions of peptide-based scaffolds, might lead to further improvements in tissue engineering [26]. Novel peptide-based biomaterial scaffolds seeded with cells show promise for tissue repair and for other medical applications.

Diatoms offer a suggestive example of peptide-based materials: these silica precipitated peptides, micro-algae about 10 microns long. They exist in countless numbers throughout the world, helping to make up the base of the food web, are photosynthetic and are responsible for approximately 25% of the world's total carbon sequestration [27]. In fossilized form, they are the source of diatomaceous earth and the ingredients in all-natural insecticides, concrete and animal feed. Diatoms extract calcium, magnesium

and silicate ions from ocean water to form hard tissues to protect the living organisms, therefore making their shells out of silica dissolved in seawater. The optimisation of mechanical properties means in practice a control over the orientations and morphologies the silica structure assumes. Diatoms act as lithographers, building nanostructures with high precision and repeatability. This is significant also because tissue engineering increasingly uses techniques such as soft lithography, alternative to the traditional moulding and rapid prototyping techniques to model scaffolds [28]. As a matter of fact, programmed molecular assembly using diatoms has been proposed, so that in practice, tissue engineering can potentially exploit the fact that diatoms produce a scaffold of magnesium silicate that is constant across generations, i.e., is genetically determined and is programmed in the diatom DNA. Biomolecular chemistry techniques can easily decode the genetic sequence associated with a specific shape, and then reprogram it, therefore the diatom can be used as a model organism to test the feasibility of these ideas, i.e., that of a prototype nanofabricator [29].

### 4.3 Abalone

Other future goals for better synthetic biomaterial scaffold design include constructing mixed materials that contain inorganic molecules or metal-binding groups, which could lead to *biomaterials with greater tensile strength*. In nature, as mentioned above abalone shell, a natural material with high tensile strength and hardness, contains a mixture of organic and inorganic materials. Abalones are members of a large class (Gastropoda) of molluscs having one-piece shells. They belong to the family Haliotidae and the genus *Haliotis*, which means “sea ear”, referring to the flattened shape of the shell.

Abalone shells are rounded or oval with a large dome towards one end. The shell has a row of respiratory pores. The muscular foot has strong suction power permitting the abalone to clamp tightly to rocky surfaces. An epipodium, a sensory structure and extension of the foot that bears tentacles, circles the foot and projects beyond the shell edge in the living abalone. A number of species of abalone exist, including black (*H. cracherodii*), flat (*H. walallensis*), green (*H. fulgens*), pink (*H. corrugata*), pinto (*H. kamtschatkana*), red (*H. rufescens*), threaded (*H. assimilis*), Western Atlantic (*H. pourtalesii*), and white (*H. sorenseni*) abalone.

Abalone shells include microlaminates with outstanding impact resistance i.e., layered, columnar, and foliated structures of crystalline units, allotropic forms of calcium carbonate: this architecture results from an evolutionary design for an ideal impact-resistant material providing armour to the mollusc [30]. The structure of abalone suggests that a possibility to have an effective bone graft would be in a composite of these two materials, with the ceramic armour providing the hardness and resistance to impact, whilst a softer, probably polymeric, protein material needs to provide the templating function. Other properties, such as the pore size, could be optimised so to provide the best conditions for cell growth [31].

Other potential biomedical applications of abalone include improved biosensors, such as a DNA chip used for robotic diagnostic screening for HIV, other viral and microbial infectious agents and genetically inherited diseases [32]. A “genetic switch” controls the abrupt transition from calcite to aragonite synthesis in the abalone shell and flat pearl. Here, proteins purified from the calcite and aragonite crystals are shown to control the “polymorph selection” and atomic lattice orientation of calcite and aragonite crystals produced in vitro, matching with perfect fidelity the control exhibited in vivo. These

results then served as the basis for the use of the crystal-controlling proteins to produce polymetallic crystalline thin-films with useful semiconductor and magnetic properties [33].

#### **4.4 Rat tooth enamel**

The tooth is a cranio-facial structure that is the focus of intensive tissue engineering studies. The outer enamel layer is almost 95% mineral, the hardest structure in the body. During its formative stages enamel consists of a protein matrix that forms the framework for mineral deposition [34]. The matrix proteins have been identified and cloned and now scientists hope to use this knowledge to replicate the natural enamel-forming process. Amelogenin, produced by specialized cells called ameloblasts, is the major enamel protein, constituting about 90% of the matrix material. Amelogenin is believed to play a role in developing enamel by stabilizing newly formed enamel crystals and in allowing their subsequent growth. In addition to amelogenin, there are other proteins such as tuftelin and ameloblastin that play an undetermined role in enamel formation. There is compelling evidence that enamel formation begins at the outer edge of the dentinal layer, at the dentino-enamel junction. Crystallite ribbons rise up from the dentin and are separated by globules, or nanospheres of amelogenin. The nanospheres appear to spiral upward around the growing crystallites, eventually degrading and ultimately disappearing as the crystallite ribbons coalesce into solid enamel.

In evolution, there appears to be a significant correlation between evolutionary changes in amelogenin protein sequence and enamel complexity [35]. A theory suggests a negative correlation between amelogenin evolution and enamel complexity, explained with complex occlusal and masticatory patterns, which place strong selective pressure on enamel structure and, therefore, amelogenins. This may slow the rate of evolution in the proteins by increasing selection against new variants. There can be also a positive correlation between the number of different amelogenin proteins in a species and the complexity of enamel microstructure: the more complex the enamel, the more amelogenin variants are present, because amelogenins are differentially removed during enamel maturation [36].

Therefore, the structure of enamel appears to be possibly optimised to achieve in the best way its function. Once again, this can be suggestive for scaffolding, in that the differentiation between proteins is achieved with great success in rat enamel. Rat tooth bud cells were used as a host for cell-seeded biodegradable scaffolds with the objective of leading to regeneration of mammalian dental tissues [37].

### **5. Discussion**

In the practical use, a tissue has to fulfil a number of different functions: however, not all materials and materials combinations are adapted to achieve multifunctionality. Therefore, the aspect of materials selection becomes crucial once again: in that respect the hierarchical structure of biological materials allows to minimise the influence of defects on materials properties, in that at a nanoscale materials have a notch sensitivity reduced to practically nil [38]. This has the important consequence that pre-existing damage, which is not allowed to develop, has the indirect function of enabling the

sensors to start the biological remodelling of the material [39]. In the case of a typical structure formed by perfectly staggered mineral inclusions embedded in a protein matrix, for example the abalone shell, a high level of impact resistance is offered by the high shear zones of protein between the long sides of mineral platelets. The protein not only offer a possible higher resistance to strain by unfolding their domain structure [40], but a different proportion of protein and mineral allow to modify the compliance and the viscoelastic properties of the biocomposite. As a consequence, multifunctionality could be achieved e.g., via a different degree of folding of the proteic structure. In practice, biomimetic multifunctional structures obtained by tanning of a protein matrix within a helicoidal fibrous structure, such as most commonly found in insect cuticle, were developed, using peptide-impregnated paper cellulose in place of chitin, resulting in a waterproof material with very high wet strength [41]. Some polypeptides, presenting secondary protein-like structures, such as the  $\alpha$ -helix, have also been found particularly promising in this regard, for the perceived link between the presence of two levels of helices and the possibility of tuning template-template interactions [42].

In general, as reported above, outstanding mechanical properties are obtained in biological materials through their hierarchical structures. However, their resolution i.e., the basic level of hierarchy, strongly depends on the structure, passing from the length scale of mineral platelets in sea-shells which is around 100-500 nm to more complex hierarchies, such as in bone and dentin, where the lowest dimension of the mineral crystals is in the order of a few nanometers [43]. For as regards the morphology of the optimal scaffold, honeycomb-like structures have been widely investigated, in particular trying to model them with rapid prototyping techniques, such as solid freeform fabrication technology [44] or fused deposition modelling [45]. The cellular structure with a honeycomb pattern, largely applied in nature, results in 3D in an open-pore solid with pores interconnecting through adjacent faces [46]. This type of structure allows controlling the porosity, in that its mechanical properties are simply dependent on the level of porosity, regardless of the lay-down patterns and channels size, as is the rule for biological porous materials [47]. In this regard, it is also worthy to note that porosity modulation is critical to biocompatibility, since an insufficient porosity can lead to a material that when implanted, does not have a sufficient vascularity, possibly resulting in foreign body reaction to it [48].

## **6. Conclusions: biomimetic design for materials**

Once the specific characteristics of biological materials have been discussed, and some cases of promising natural materials have been presented, it is important to move back and try and understand what biomimetic design is about. In other words, it would be essential to clarify in what a biomimetic clue represents and how it is really possible to extract a clue from nature, especially in the case of tissue engineering.

A definition such as "biomimetic design" can appear incoherent, since design normally denotes a practice that is both intentional and teleological (aimed at an objective), whereas the ordering principles of nature appear to be proactive rather than active, therefore learning from experience. However, the dichotomy between the teleology of design and the causality of evolution is only apparent: the human brain operates by variation and selection, so that thoughts and ideas are evolutionary selected rather than deliberately created [49].

In this sense, the notion of biomimetics can be still helpful to optimise materials design, hence in heuristic terms. In practice, the biomimetic approach, based on exploiting the knowledge obtained in nature during evolution, is not confined to biological aspects, which would seem to be reductive in materials terms. In contrast, it includes three main ideas, which can all be included in the concept of biomimetics [50]. All of the following three ideas can be useful in designing tissue-engineering materials.

These are:

- Artificial synthesis of naturally occurring materials, substances or other structural configurations
- Mimicking biological processes in creating life-like products
- Mimicking of complex self-organizing natural processes to obtain dynamic artefacts

The above definitions encompass a horizontal process, based on the different functions that biological structures can present. However, there is also a vertical approach, based on different levels of “smartness” achievable, when passing from engineered to natural materials, as it is described in Figure 2. This justifies on the point of view of improving the “state of the art” the adoption of a biomimetic approach to tailor research projects.

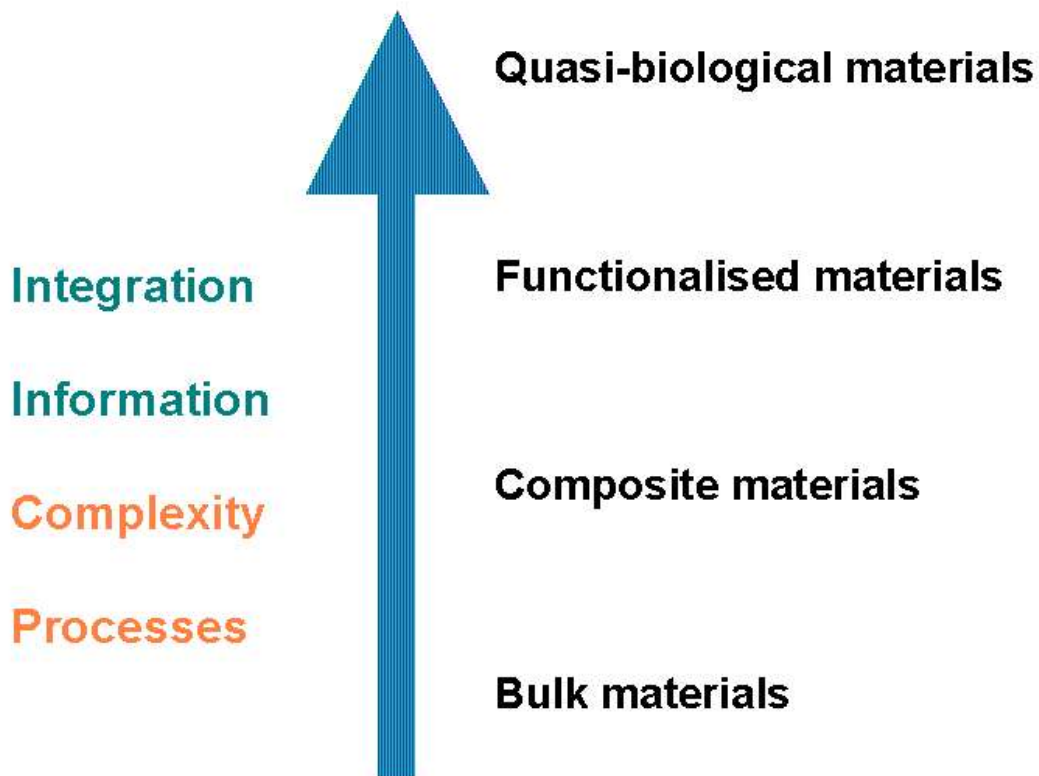


Figure 2 Vertical approach to materials, with aspects of “smartness” introduced (elaborated from [51])

In practice, biomimetics projects should possibly and hopefully include all of these aspects. As an example, CICADA (*Cricket Inspired perCeption and Autonomous Decision Automata*), a European project involving a number of researchers with different expertise, as detailed in Figure 3, involves all of the aforementioned aspects. The aspect of **artificial synthesis** is implicated in trying to produce microchips based on

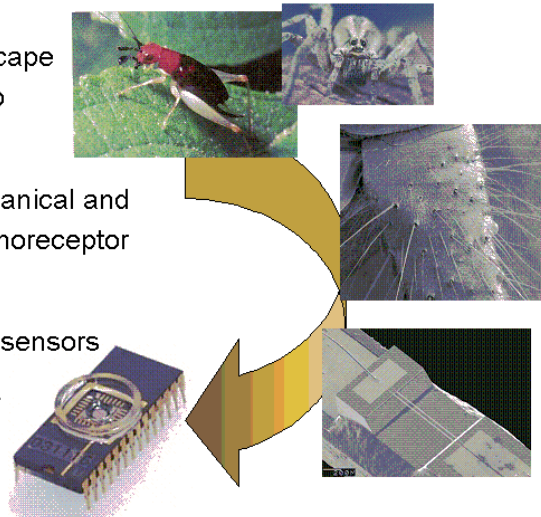
the crickets' sensory principle. The **investigation of biological and behavioural processes** in their escape strategies allows the reflection and the study of how biological sensors work and how their redundancy principle can be usefully introduced in micro-sensing devices. The **level of self-organisation** reached by the insect in pulse transmission is mimicked by the use of cricket neurones in a hybrid system, partly biological partly engineered (bio-MEMS). These three ideas should also be ideally at the origin of tissue engineering projects aimed at obtaining clues for self-assembly and tissue regeneration from nature.

## CICADA

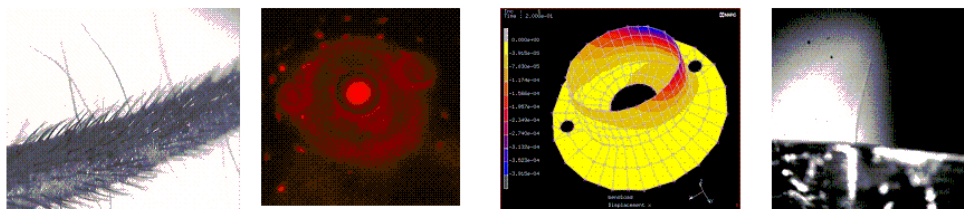
### Cricket Inspired PerCepTION and Autonomous Decision Automata

#### APPROACH

- ❑ Investigate perception and escape action of crickets responding to attacking predators (spiders)
- ❑ Characterize and model mechanical and functional properties of mechanoreceptor hairs and hair canopy
- ❑ Design large arrays of MEMS sensors
- ❑ Build a miniature demonstrator using living computers (cricket neurones)



#### INTEGRATION OF INFORMATION



Biological System
Reconstruction
Modelling
System Response

#### EXPERTISE

- ✓ Université Francois-Rabelais - FRANCE – *Sensory ecology*
- ✓ The University of Reading - UNITED KINGDOM - *Materials science*
- ✓ Universiteit Twente - NETHERLANDS - *Nanosensors*
- ✓ Forschungszentrum Juelich - GERMANY – *Hybrid systems*

Figure 3 Operational scheme of the CICADA project

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