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Review article

Artificial inorganic biohybrids: The functional combination of microorganisms and cells with inorganic materials

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ABSTRACT

Biohybrids can be defined as the functional combination of proteins, viable cells or microorganisms with non-biological materials. This article reviews recent findings on the encapsulation of microorganisms and eukaryotic cells in inorganic matrices such as silica gels or cements. The entrapment of biological entities into a support material is of great benefit for processing since the encapsulation matrix protects sensitive cells from shear forces, unfavourable pH changes, or cytotoxic solvents, avoids culture-washout, and simplifies the separation of formed products. After reflecting general aspects of such an immobilization as well as the chemistry of the inorganic matrices, we focused on manufacturing aspects and the application of such biohybrids in biotechnology, medicine as well as in environmental science and for civil engineering purpose.

Statement of significance

The encapsulation of living cells and microorganisms became an intensively studied and rapidly expanding research field with manifold applications in medicine, bio- and environmental technology, or civil engineering. Here, the use of silica or cements as encapsulation matrices have the advantage of a higher chemical and mechanical resistance towards harsh environmental conditions during processing compared to their polymeric counterparts. In this perspective, the article gives an overview about the inorganic material systems used for cell encapsulation, followed by reviewing the most important applications. The future may lay in a combination of the currently achieved biohybrid systems with additive manufacturing techniques. In a longer perspective, this would enable the direct printing of cell loaded bioreactor components.

Contents

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1. Introduction

The self-sustaining mechanism of living (micro-)organisms or cells makes them the epitome of efficient working engines driven by their immanent genetic information. This circumstance arouses ever since the interest of men, who begun very early to utilize living microorganisms for the production of food, e.g. in fermentation processes, or for the synthesis of pharmaceutical products. The technical application of living biological entities was finally manifested in an independent scientific discipline known as *Biotechnology* in the 19th century. In the last 40 years the encapsulation of living cells and microorganisms became an intensively studied and rapidly expanding research field, aiming at a sustained or temporal utilization of metabolically active cells for manifold applications in medicine, bio- and environmental technology, or even for civil engineering.

The integration of viable microorganisms into industrial biotechnological processes is challenging and requires a well-engineered and durable delivery system to address control, stability, cost, and longevity concerns [1]. Biotechnological applications cover for example the remediation of heavy metal ions or aromatic hydrocarbons such as phenol or naphtene. The encapsulation of microorganism into a support material is of great benefit for processing, since the embedding into a matrix material protects the cells from shear forces, unfavourable pH changes, or cytotoxic solvents, prevents culture-washout and simplifies the separation of formed products [2]. The encapsulation can be performed in either organically cross-linked hydrogels (e.g. alginate or synthetic polymers) or into inorganic matrices such as silica or cements. The latter have the general advantage of a much higher chemical resistance towards harsh environmental conditions during processing compared to their polymeric counterparts.

Mineral encapsulation is a biomimetic concept, characterizing it as a natural occurring state in which life is possible. Diatoms for example are the archetype of living biohybrids, being capable of performing self-encapsulation, also known as biomineralization [3]. This process is typically occurring under humid conditions, in which microorganisms assimilate environmental minerals and build porous but extremely stable exoskeletons by an intercellular mechanism. But also fungi, bacteria, cyanobacteria, and algae are capable of performing precipitation of minerals like calcium carbonate, calcium phosphate, calcium oxalate, magnetite or greigite, and cadmium sulfide, mercury sulfide, and lead sulfide through autotrophic or heterotrophic pathways [4,5]. Besides biomineralization, several microorganisms including cyanobacteria, fungi, algae, yeasts, diatoms or lichens temporally or permanently colonize lithic habitats as another successful strategy to protect themselves against harsh environmental conditions or predators [6]. Such bioinorganic materials are also naturally found worldwide in form of endoliths, in which bacteria or fungi are encapsulated in rocks or soils even in the oceanic crust, that is considered to be the largest fungal habitat on the planet [7].

This article aims to present a review on the encapsulation of microorganisms and eukaryotic cells in inorganic matrices such as silica gels or cements. After reflecting general aspects of such an immobilization as well as the chemistry of the inorganic matrices, we focused on manufacturing aspects and the application of such biohybrids in biotechnology, medicine as well as in environmental science and for civil engineering purpose.

2. General aspects of cell encapsulation in inorganic matrices

Biohybids can be defined as the functional combination of proteins, viable cells, and microorganisms with non-biological materials [8] and was first mentioned in terms of engineering an artificial pancreas in 1970^{ths} by Chick and co-workers [9]. Since then, a variety of mammalian cells [10,11], plant cells [12–14], and microorganisms like yeasts [15,16], fungi [17], bacteria [18,19], protozoa [20], algae [21,22] or cyanobacteria [23,24] have been immobilized within organic or inorganic materials in order to use these biohybridal structures as artificial organ [10,11,25], biosensor [21,26,27], biosorbent [17], for biosynthesis [12,22] or as biocatalyst [19,28].

Generally, the fixation of biological entities provides several benefits compared to their free or adsorbed counter parts, because it results in an increased process economy during their industrial utilization (Fig. 1) [14]. This includes in particular an easy separation and recycling of the bioactive species from the broth with drastically reduced cell loss, which also facilitating the recovery of end-products. Moreover, the use of immobilized cells allows a uniform supply of nutrition without influencing the cell density, a reusability of the culture or the ability of running continuous processes or extending the reaction time at least [13]. Additionally, encapsulation was proved to be a suitable strategy to improve cell stability and storage time as well as photosynthesis efficiency and enzymatic activity, as a result of an efficient protection against harsh environmental conditions like heat, UV-irradiation, enzymatic attacks or shear forces [13,29]. The limitation of cell division is also being discussed as a possible reason for an enhanced productivity of immobilized cells, since energy usually being consumed during cell proliferation will be transferred to metabolic activity [30]. Especially worthy to mention is the possibility to protect transplanted mammalian cells against the immune system of patient treated with biohybrids as artificial organs [11].

The main challenges for the creation of a biohybrid pose the maintenance of the organisms viability and activity during the encapsulation procedure as well as during the state of encapsulation for a prolonged time [31]. Essential requirements are related to the simulation of a native environment specifically for the fixed organisms. Most microorganisms and particularly eukaryotic cells only tolerate minor deviation of their natural living conditions. This demands a precise control over temperature, pH, ionic strength as well as the absence of cytotoxic substances like

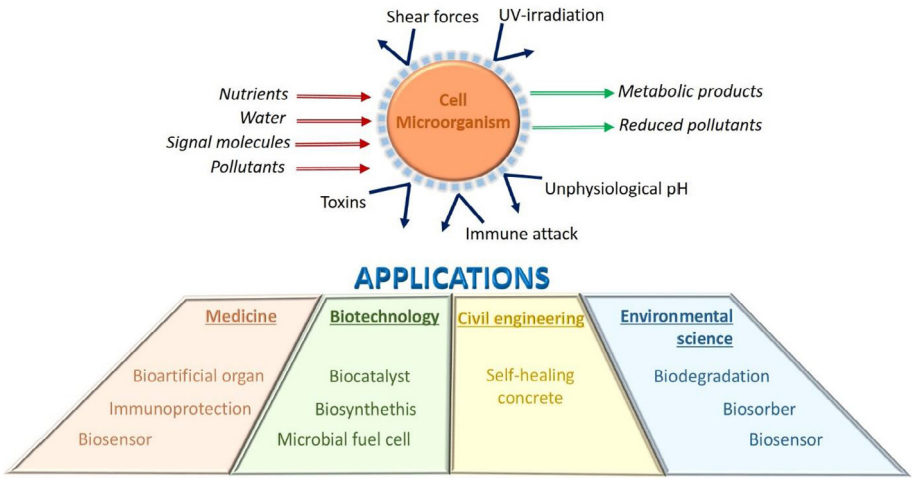

Fig. 1. Benefits and applications of cell encapsulation in inorganic matrices.

alcohol or residual alkoxisilane monomers during the whole encapsulation process or within the final biohybrid construct. Additionally, a gentle solidification and the prevention of shrinkage or dehydration of the host matrix are essential to avoid cell lysis [30,32]. Furthermore, the possibility to precisely tune the physical properties of the host matrix by synthesis parameters is a crucial factor for the functionality of the biohybrid. Particularly, in inorganic host materials the inevitably required diffusion processes like exchange of metabolic excretes and nutrients are allowed due to their small molecular sizes and weights, whilst cell leaching or the permeation of larger harming cells can be permitted by regulating the pore size and overall porosity [33,34]. While immunoprotection demands extremely small pore sizes (<30 nm) [35] and a narrow pore size distribution in order to prevent antibodies (e.g. IgG diameter = 11.8 nm) to enter the material, biohybrids with pore sizes below the diameter of encapsulated or competing cells from the medium are sufficient, if permission of cell diffusion is aimed only [10]. For a deeper understanding of the physical and chemical requirements of host matrices in general and characterization protocols, the interested reader is referred to the review of de Vos [36].

Although first attempts of cell encapsulation were made by using organic host matrices in form of synthetic [37] and natural [11,12,25,38] polymers, polysaccharide [39] or silk [40], which are usually fulfilling the precondition for a cytocompatible processing route, these systems show limitations in terms of reliability and durability of the immobilization due to their gelular structure [13,41]. Inorganic materials were investigated as alternatives with a higher chemical and mechanical stability, making these biohybrids suitable for a long-term application. Successful fixation of living biological entities was performed with inorganic host materials composed of silica [13,42], alumina [41], titanium dioxide [43], zirconia [28], graphene [16], calcium carbonate [44], calcium phosphates [15,45] or magnesium phosphate [19,23]. Predestined characteristics of these materials are the biological and chemical inertness, thermal and mechanical stability, resistance against fouling and absence of swelling in organic or inorganic solvents, which prevents leaching of the cells as well as altering of the porosity or the mechanical stability of the biohybrid over time. Transparent or translucent inorganic materials attain special interest for the fabrication of biohybrids, because these matrices are suitable for embedding photoactive microorganisms like algae or cyanobacteria aiming at an improved environmental friendly production of energy [13].

3. Material systems for inorganic bioencapsulation

3.1. Sol-Gel process derived materials

As mentioned in the previous chapter, diatoms use silicate as an exoskeleton. This silicate skeleton is specially structured as it serves not only for stability, but also as a protective cover against other physicochemical influences [46]. It is not surprising that organisms use silica for this purpose, because this material has many excellent properties, e.g. it is mechanically robust, chemically inert, optically transparent, thermally stable and insulating, and biocompatible [46,47]. Due to all of these advantages and to the possibility of low temperature synthesis, silicates are predominantly investigated for the immobilization of bacteria and cells in inorganic matrices. Here, the silica matrix can be either silica microspheres [48], thin films [49] or a mesoporous 3D matrix [50]. SiO_2 networks are commonly produced via a sol–gel process, which results in different application forms, depending on temperature, pressure, solvent, precursor, pH or the amount of water used for hydrolysis and gelation. In addition to dense ceramics and highly porous aerogels, it is also possible to produce thin layers or fibers as well as non-agglomerated nanoparticles in the size range of 20–500 nm [51,52]. A major advantage of sol–gel processing is that in addition to pure SiO_2 ceramics also hybrid materials (e.g. sol–gel polymer composites) can be fabricated by using precursors with additional functionalities. The following section describes briefly the chemical principles of such sol–gel-derived silica ceramics, whereas besides the "classical" use of alkoxide precursors also aqueous synthesis routes are described. The latter has the advantage, that no cytotoxic alcohol is generated during matrix synthesis and hence the survival and activity of immobilized microorganism is less influenced than by the alkoxide pathway.

3.2. Alkoxide pathway

For the alkoxide pathway, precursors of the form $Si(OR)_4$ are used, wherein R is preferably an alkyl group. The gold standard of this synthetic route is to use tetraethoxyorthosilicate (TEOS, $R = C_2H_5$) and tetramethoxyorthosilicate (TMOS, $R = CH_3$) as precursors [53–55]. The mechanism (Fig. 2A) for initiation of the sol–gel process depends on the prevailing pH, whereby both an acidic or basic pH are favoring hydrolysis of the alkoxide groups to form silanols (Si-OH) in the first step. Silanols are unstable and tend to form Si–O–Si bonds in a condensation reaction

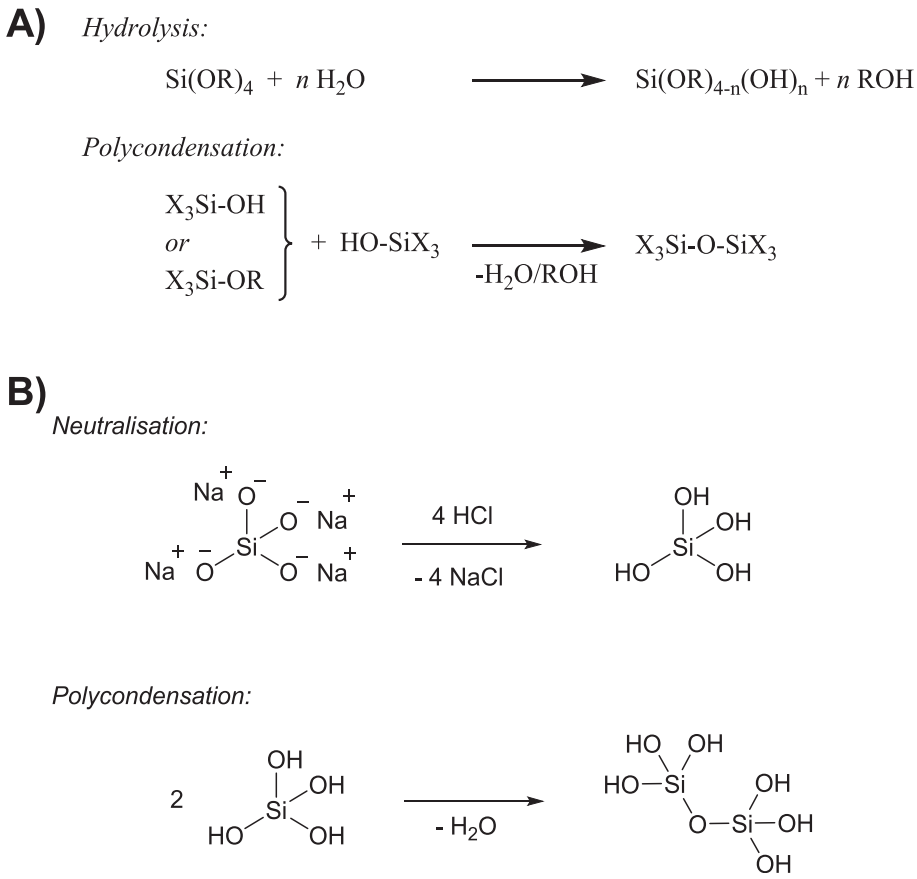

Fig. 2. A) Reaction equations of the formation of silicone bonds from alkoxysilane precursors (X = OR/OH.). First, hydrolysis produces Si-OH bonds, which subsequently form into a 3D network through (alcohol-) condensation. B) Non-alcoholic reaction equations for the formation of silicone bonds. First, neutralization causes Si-OH bonds, which subsequently develop into an oxo-bridged SiO_2 network through condensation.

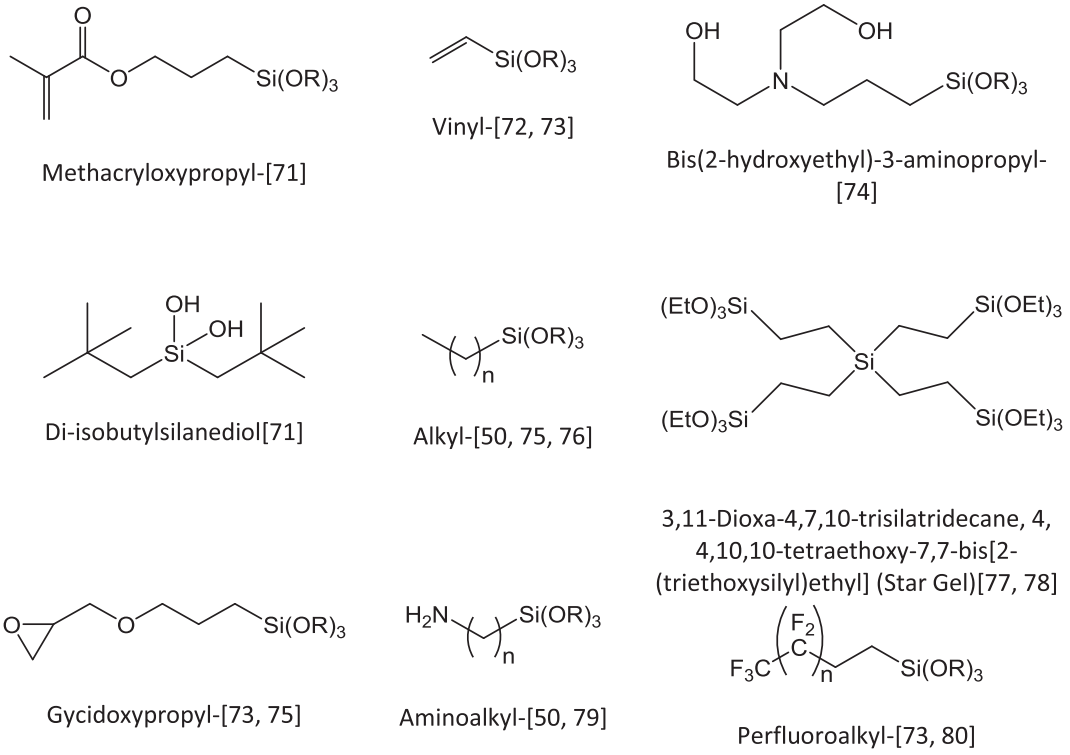

Fig. 3. A selection of modified siloxane precursors with R = alky groups. Dialkoxysilanes having two functional groups are (See above-mentioned references for further information) also possible.

[55–57]. This condensation can take place by either the reaction of two silanol groups (dehydration) or by the reaction of the silanol with an alkoxide group (dealcoholation). As the reaction / condensation progresses, nanometer sized silica particles are initially formed, which further grow and finally interlink until a 3D network is formed [47]. For immobilization of microorganisms via such a system, the biological systems can be mixed into the prehydrolyzed precursor solution [50,55]. The external conditions in this step are mild regarding pressure and temperature [47], however, great care must be taken to have control over pH and alcohol development, as this can have cytotoxic effects and proteins can be denatured [58,59].

3.3. Aqueous pathway

Although the cytotoxic alcohol can be removed from the above described system by evaporation, this will alter the gelation kinetics and hence processability of the silica matrices. As an alternative to the alkoxide pathway, there is also the option of using an aqueous route to produce silica matrices, which directly avoids the release of toxic alcohols by using sodium silicate as a precursor [47]. This route also takes place in two steps, of which in the first one silanols are produced by acidifying a solution of sodium silicate (Fig. 2B). A suitable acid is hydrochloric acid, whereby the resulting by-product is NaCl and a buffer can be used to control the pH [56]. Optionally, a cation exchanger resin may be applied (which replaces Na^+ ions from the solution by H^+ ions) to initiate the first reaction step [60–62]. In a second step, the neutralized silanols react in a polycondensation reaction to form a 3D network analogous to the alkoxide synthesis route (Fig. 2B).

3.4. Variation of precursors

The sol–gel process with TEOS or TMOS results in the formation of a SiO_2 network, in which ideally all silicon atoms are fourfold linked to other Si-atoms. However, it is also possible to use modified precursors, which contain at least one covalently bound spacer with functional moieties such as amines, carboxylic acids, thiols or oxiranes (Fig. 3). In this way, properties of the 3D structures can be specifically changed and adjusted depending on the intended use. Siloxane derivatives with functional groups such as aminoalkyl and carboxyalkyl have great influence on ionic interactions by forming networks with either brittle, transparent or soft hydrogel-like properties, which can be used to immobilize biological materials, improve wettability, and extend biocompatibility [50,63].

Alkyl and aryl siloxanes are capable of forming meso- and microporous glasses with the ability to adjust mechanical and optical properties over a broad range by varying the precursor composition. Since hydrophobic properties and porosity are well controllable in such glasses, these materials are suitable as surface biosensors [64–66], stimulating matrixes for enzymes like lipases [67], and porous foam scaffolds for cells [50]. In contrast, hydroxyalkyl siloxanes are used for the synthesis of hydrophilic materials. These may have properties similar to soft hydrogels, which are particularly well suited for the immobilization of labile proteins and cells.

Modified siloxanes used in combination with the standard silica and silicate precursors can act as catalyst or redox systems. The resulting networks are transparent glasses, which have either semi-hard or a brittle fracture behavior. In particular, they are used in conjunction with biosensors and enzymes. Further research in this field could have much impact on the used sol–gel matrices, and it remains interesting how siloxanes could be differently modified and used [50,68,69], e.g. by using star-gel-like precursors [70]. Surprisingly, most of the described precursor modifications are not yet applied for the encapsulation of microorganisms, although these would offer the possibility to adjust crucial properties (mechanics, optics, chemical resistance, surface properties) in a much broader range than pure TEOS.

3.5. Cements as encapsulation matrices

Currently, the main research topic of cement encapsulated microorganisms is the creation of self-healing properties in Portland cements for civil engineering [5,81]. Such cements are based on a mixture of different calcium silicate (Ca_2SiO_4 and Ca_3SiO_5) and calcium aluminate ($CaAl_2O_4$) phases, which form gels at a highly alkaline pH value during setting [82]. Here, the microorganisms are thought to produce calcite mineral after exposure to air (in a crack), such that the precipitated mineral will close the crack and inhibit further crack propagation. However, due to the alkaline pH, such cements are likely unsuitable for most of the other applications described in this review.

Suitable cement alternatives are mineral cements used for bone replacement in medicine and dentistry. The chemistry of such cements is usually based on calcium or magnesium phosphates [83,84], in which raw materials like tricalcium phosphate or trimagnesium phosphate react in an aqueous phase to form a hardened cement matrix by a dissolution – precipitation reaction:

$$3\alpha - Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(PO_4)_5HPO_4OH$$

$$Mg_3(PO_4)_2 + (NH_4)_2HPO_4 + 15H_2O \rightarrow 2MgNH_4PO_4 \cdot 6H_2O + MgHPO_4 \cdot 3H_2O$$

The major advantage compared to Portland cement are setting conditions within a physiological pH range and short setting times of usually less than 10 min. This is beneficial for both, processing (rapid hardening) as well as cell survival. In addition, those biocements provide high strength of up to ~80–100 MPa in compression [83], whereas especially magnesium phosphate cements develop such strengths within a short time of <1 h. The cements are usually microporous with pore sizes in the sub-μm range, but also nanometer-sized pores can be introduced by combining cements with a silica sol [85]. This is likely beneficial to avoid an outspreading of the encapsulated microorganisms from the intrinsically microporous cement matrices, while maintaining nutrition supply by the nanoporous silica gel. A similar effect might be achieved by applying "dual-setting" biocement matrices, in which a further polymeric hydrogel phase is simultaneously build up during cement setting [86].

4. Processing approaches for biohybrids

Beside properties like chemical composition and mechanical strength, the design of materials such as porous scaffolds, spheres or films becomes more and more essential for their application due to the fact of a growing understanding of interaction between the material and its surrounding. The encapsulation of living cells in silica biohybrids follows in most cases the following procedure. The precursor is prehydrolyzed, the alcohol, which is released by the hydrolysis, is removed and a neutral pH is adjusted prior to a gently admixture with the suspension of living cells. Thereafter, the gel suspension can be processed to reach its final shape. Here, the simplest way is casting into a mold for bulk gelation [87]. Although the appeal of this method lies in its easy operability, it sometimes lacks in its unfeasible diffusion parameters due to undersized pores and long diffusion paths [88] causing metabolism reduction or even cell death. Life-sustaining molecules like nutrients and proteins must be transported to the cells through the matrix, while excreted waste or therapeutical products should be

released [46]. On the other hand, the cell migration must be prevented and the invasion of adverse molecules like antibodies must be blocked in order to suppress an immune reaction in the case of medical applications [89,90]. Since these processes are diffusion controlled, both the total porosity as well as the pore diameter and their length must be adjusted for a sufficient supply. Furthermore, the compartment for the living cells must be adapted to their task. Entrapped cells behave different to "free" planktonic cells due to their new environment with limited available space, reduced diffusion rates, and interactions with the surrounding matrix. Prolonged life time is often a result of encapsulation, which may be attributed to the metabolic shift from proliferation to metabolic production [91]. This fact could be important in a bioreactor or in case of biosensors. For other applications, the "normal" behavior of the cells is of interest. By using a low density silica sol–gel (~0.5 M silica content), Eleftheriou *et al.* could erase this effect and indicated, that silica encapsulated *E. coli* showed a similar cell growth and cellular regulation like cells in suspension [92]. Therefore, not only the chemical composition of a material influences the encapsulation, but also the design and hence the processing approach.

The diffusion coefficient is proportional to the porosity and has to be adapted to guarantee an adequate network for transit. The easiest way to increase the porosity of the encapsulating matrix is to reduce its concentration. Therefore, the ratio of water to precursor is enlarged typically in the range between 25 and 200 (molar ratio water: precursor) in the case of silica gel [93,94]. For cementous biohybrids the liquid to powder ratio has to be increased for a higher porosity [95]. By drying the matrices after setting the evaporating water leaves micro pores for a better nutrient supply. Additionally, the incorporation of leachable porogenes or foaming agents could create macro pores. Nieto *et al.* successfully entrapped mouse fibroblasts and ephithelia cells in a TEOS based silica sol, where the polysaccharide D-glucose acted as a porogen and additional as nutrient for the encapsulated cells [93]. After dissolving the glucose in water it was added to TEOS to create a prehydrolyzed sol. The arising alcohol was removed from the sol by evaporation at 80 °C for 10 min with simultaneous water addition in the same ratio to avoid gelation. Thereafter, the prehydrolyzed sol was gently mixed with the cell suspension prior to gelation. An adequate permeability for nutrient and oxygen supply was created by leaching of the glucose. Another strategy is to work with water-soluble polymers, which act as place holders to produce a suitable porosity. An additional benefit is here the reduced shrinkage of the xerogel during drying by the polymeric phase. By its supplementary volume and its implicated elastic properties, the polymer network works as a supporting frame and thereby prohibits embrittlement as well as cracking [96]. Sometimes the cells are directly encapsulated in the polymeric phase e.g. as microbeads, which protect the cells during setting and build a living space in the closed matrix after leaching [97,98]. The use of foaming agents can result in further porosity increase. Typically, CO_2-bubbles from an acid-base reaction act as the expanding agent like it is the case for commercially available baking-powder ($NaHCO_3$ and $Ca(H_2PO_4)_2$). Chen *et al.* encapsulated human umbilical cord mesenchymal stem cells in a mineral biocement by using alginate-fibrin microbeads as cell friendly compartment within a calcium phosphate cement during setting and a sodium hydrogen carbonate/citric acid as foaming agent [99]. Here, the goal was no long-term entrapment of the cells, but their release and ingrowth after implantation. The authors observed an increasing cell viability from 49% (without porogen) to 86% with 15% porogens.

A different approach to integrate pores within a bulk matrix is the freeze-gelation technique (Fig. 4). Here, the sol together with cells and potentially necessary additives like nutrients or protectives are frozen in a mold. By the formation of ice crystals water is extracted from the sol, resulting in a localized sol-concentration and thereby a sol–gel-transition. After drying by evaporation or lyophilization a highly porous monolith is built with pores copying the ice crystals geometry. The cells tend to dehydrate during the freezing process and the formation of intercellular ice crystals limits cell survival [100]. Nevertheless, freeze-gelation is a simple and promising encapsulation method. Soltmann *et al.* noted merely a survival of 0.3‰ of *Pseudomonas fluorescence.* After the addition of 15% lactose as cryoprotective the survival rate rises about 58-times [101]. Furthermore, the proliferation ability within the porous matrix seems to compensate the loss of viability during freezing.

Since the diffusion pathway is minimized in thin films, they are typically used for the purpose of biosensors, providing an excellent response time within seconds [103–106]. The fabrication of such films is usually performed by dip coating [107,108], spin coating [109,110] or spray coating [111] to obtain films with high crack resistance, sufficient adhesion to the substrate and uniform film thickness. Thereby, the typical film thickness is usually in the submicron range, which limits the application to enzyme encapsulation, since whole cells exceed the film dimension and are not completely coated and entrapped [91,112]. However, the formation of thin films is used to encapsulate whole living cells by the so called "biosil" method. In this method vaporized alkoxide precursors are used to immobilize living microorganisms previously deposited on scaffolding materials like silk, polyester or glass fibers or sponges [54,113,114] within a sol–gel silica film. The solidification of the silica film takes place directly on the cell surface by reaction of the gaseous precursors with water sorbed on the cell surface. Toxic alcohol generated as sol–gel reaction by-product will be eliminated simultaneously to the silica gel deposition via gas flux [54]. A precise control of membrane thickness is given by the exposure period of the cells to the gas, while membrane porosity and stability can be adjusted by the choice of the precursor. This process is suitable for yeast and bacteria as well as for large cells, like animal or plant cells, since the size of the cells is not constrained by the pore size of the sol–gel silica network and the film thickness [115]. Plant cells [54] and mammalian cells including hepatocytes [113,116] or pancreatic islets [115] were successfully encapsulated by the biosil method with a viability of the encapsulated cells in the same range or even higher as for free planktonic cells. For example, Li et al. immobilized *Sphingomonas* sp. *GY2B* in sawdust as a model scaffold and improved the cell viability by 30% by an additional silica vapor deposition step for a better protection of the bacteria [117]. The encapsulated cells benefit from the protection of the SiO_2-shell against environmental conditions like shear forces, whereby their survival is prolonged [115]. The comparison of different immobilization methods is however difficult, since most studies use only free cells as a reference for their analyzed encapsulation strategy. A detailed comparison of different encapsulation method would be desirable, since it has not only an effect on the obvious parameters like protection or permeability, but also on the bio-silica interface, which has a high influence on the cell's behavior as demonstrated by Fazal *et al.* [91]. They analyzed the metabolic state of *Saccharomyces cerevisiae* within three different designed matrices (thin films by cell-direct assembly, particles by spray drying and glycerol-doped gel monoliths) and found extreme differences in their biological processes like stress-related gene ontology categories.

5. Medical application of biohybrids

In the last decades cell and microorganism encapsulation attains the interest of researchers as appropriate tool for the delivery of bio-

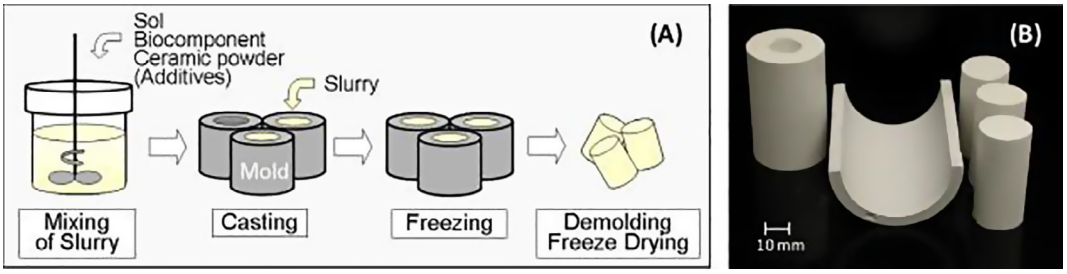

Fig. 4. A) Preparation of biohybrids from microorganism loaded preceramic slurries by casting and freeze drying and B) examples of finally processed biohybrids. Reproduced from [102] with permission from Elsevier (2010).

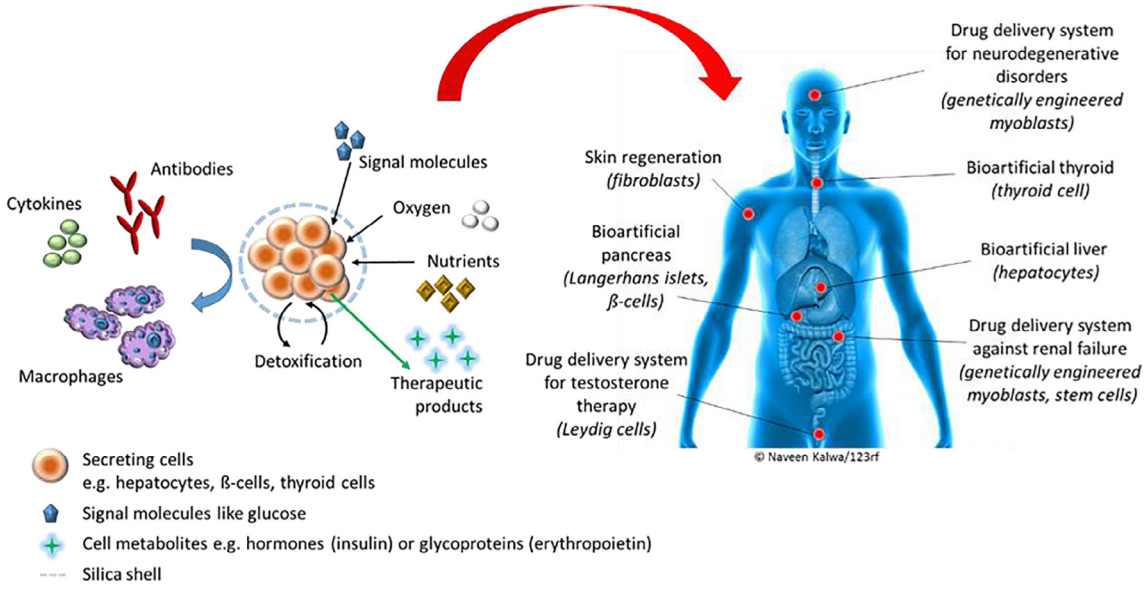

Fig. 5. The concept of bioartificial organs is based on the implantation of living and metabolically active cells, which are intend to assist the function of diseased organs by the delivery of required metabolites. Other cell therapies use engineered cells as implantable cellular drug delivery system in order to treat cancer or degenerative diseases like Alzheimer. Here, silica encapsulation of viable cells provides a successful strategy for their immunoprotection as macrophages, antibodies, and cytokines are not able to diffuse through the nanoporous silica shell. At the same time, small molecules like oxygen and nutrients can pass the membrane and guarantee the metabolic activity and viability of cells. The cell metabolites act as therapeutic agents, which will be produced and delivered either in response to extern signal molecules for example glucose (*principle of bioartificial pancreas*) or in a continuous manner. Moreover, a detoxification system according to the liver can be realized by the encapsulation and implantation of hepatocytes. Up to now, various cell types were investigated with regard to their ability to substitute physiological functions of diseased organs or to treat cancer or organic malfunctions [128].

logically active substances like hormones [10], cytostatics for cancer treatment [39], pain reducing agents [118], or probiotics [38] to specific sides in human body, while protecting the implanted biological entities against the atypical biological environment or deleterious cells at the same time. These implantable biohybrids are intended either for a prolonged or even permanent use in order to avoid systemic administration of released agents and helping to enhance the patient compliance in particular in the case of lifelong drug administration like in diabetes. Thereby, the encapsulated biological entities either show a continuous [119] or a physiologically controlled release behavior triggered by extern stimuli [115]. The biocompatibility of the biohybrid carrier material can be considered as a fundamental criterion, since this determines whether or not a malfunction of the implanted biohybrids occurs, caused by an inflammatory response and fibrotic overgrowth *in vivo* [10,120]. Beside this, encapsulated cells are investigated as suitable medical sensor system for the fast and precise detection of diseases, toxins, or specific blood values like glucose levels [121].

5.1. Bioartificial organs

The transplantation of encapsulated and metabolically active cells is considered as outmost promising strategy for the therapy of chronic endocrine diseases like diabetes mellitus [10,120,122], hypothyroidism [119], hypogonadism [123], neurodegenerative diseases [124,125], or hepatic [37,116,126] and renal [127,128] failure. Thereby, the implanted cells either substitute the physiological functions of diseased tissue with the release of required metabolites or treat organic disorders or cancer by the delivery of therapeutic agents like cytokines or glycoproteins (Fig. 5). Although the durability of the biohybrid system is an essential aspect, only few carrier matrices consist of inorganic materials such as silica spheres [129] or silica coatings, which are deposited directly on the cell surface [42,115] or can be coated as additional matrix on initially polymer encapsulated cells to improve the stability of hydrogels [122,130]. In the last decades silica encapsulation of various mammalian cell-types were investigated concerning their applicability as bioartificial organ, among them Langerhans islets or isolated ß-cells for the use as bioartificial pancreas [10,130], hepatocytes as bioartificial liver [37], or thyroid cells as a thyroid hormone replacement approach [119]. These studies utilized the selective permeability of nanoporous silica matrices for an immunoisolation of implanted cells. While cells or molecules with a larger molecular structure like antibodies or immune cells are unable to pass the silica membrane, the diffusivity for lower molecular weight substances is high [42]. This provides an effective immunoshielding of encapsulated cells and inhibit cell leaching on the one hand, while on the other hand

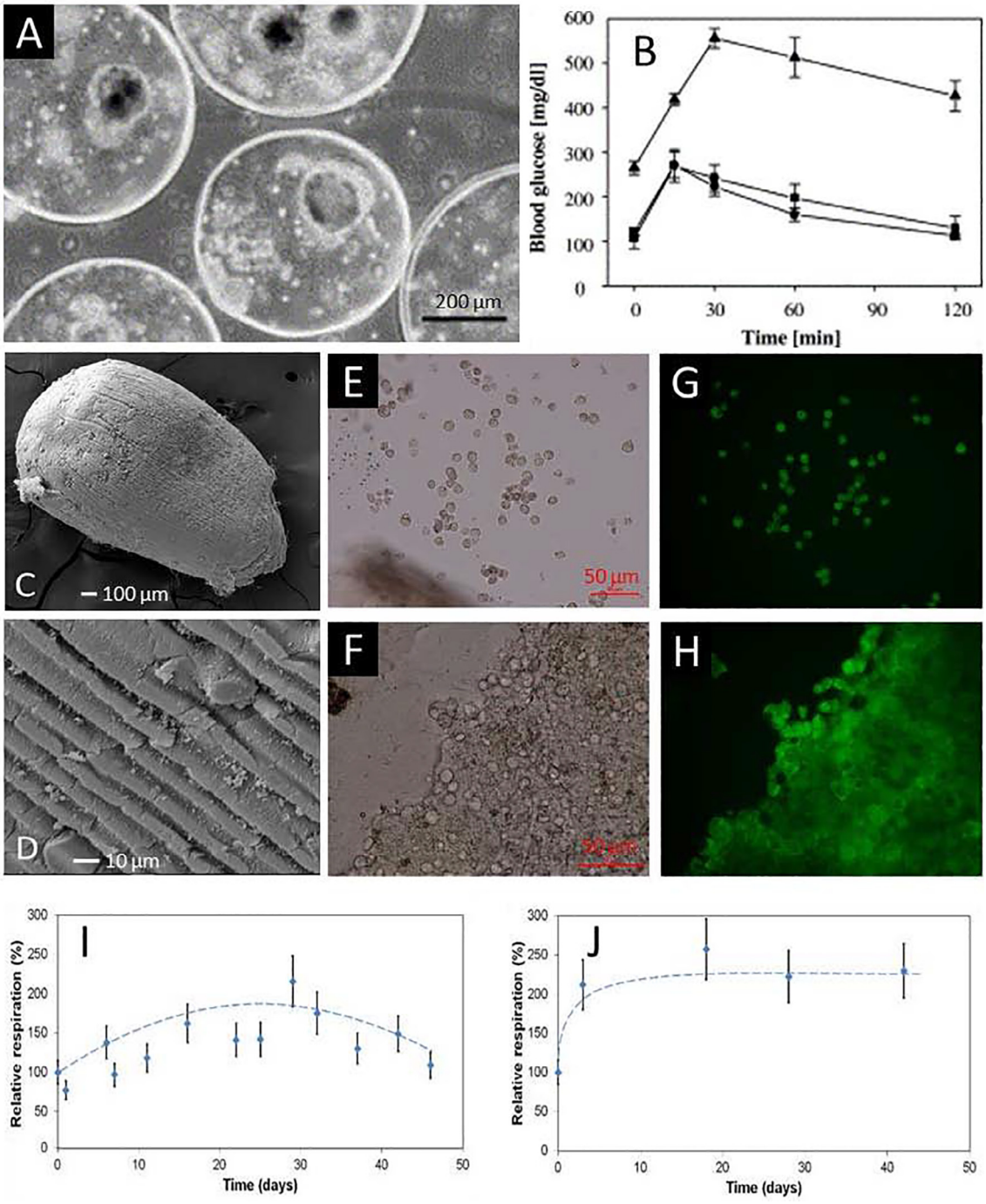

Fig. 6. A) Microscopic image of alginate/aminopropyl-silicate/alginate coated alginate beads loaded with rat islets, after 2 months implantation in a peritoneal cavity of a diabetic mouse. B) Blood glucose levels of non-diabetic mice (•), streptozotocin-induced diabetic mice (▲), and mice treated with encapsulated islets (■). Mice with transplanted islets could establish normoglycemia after 1 day and showed this constant glucose level for up to 1 month. This clearly indicates the efficiency and suitability of silica entrapped islets for the application as bioartificial pancreas (Reproduced from from [130] with permission from Elsevier (2002)). C-J) Biocompatible mineralized beads composed of an alginate-silica composite core coated with a Ca-alginate layer used for cell encapsulation for medical purpose. Human hepatocellular carcinoma cells (HepG2) were entrapped within these beads as cell model for other therapeutic cell lines like ß-cells. The study verifies the biocompatibility, the improved mechanical stability, the reliability of long-term cell encapsulation, and the immunoprotection ability of the ceramic-like, silica reinforced hydrogel beads. C/D) Scanning electron microscopy image of mineralised beads. HepG2 cells survive more than 42 days after encapsulation within the mineralized beads. This was shown by microscopic investigation of the biohybrids after viability staining with fluorescein diacetate. (E/F) Brightfield and (G/H) fluorescent microscopy images of silica/alginate encapsulated HepG2 after 1 day (E/G) and after 42 days (F/H). I/J) The metabolic activity was also proven by the measurement of the oxygen consumption of HepG2 cells encapsulated within pure alginate (I) or within mineralized beads (J). While respiration activity of alginate entrapped cells decreased 30 days post-encapsulation, the oxygen consumption of cells entrapped within mineralised beads increased sharply in the first hours and stayed constant afterwards. The authors explained this by an early cell proliferation followed by a constant viability of silica encapsulated cells. Reproduced from [35] under the Creative Commons Attribution License (2011).

diffusion processes for essential nutrients and oxygen are maintained. Moreover, the exchange of small molecule enables the cells to detect rapidly extern stimuli like glucose levels or pH-changes, which results in a prompt responsive release of therapeutically desired metabolic products.

Based on several studies about silica encapsulated pancreatic islets Leoni *et al.* reported, that pores between 7 and 66 nm offer a sufficient diffusivity in order to maintain metabolism and viability of cells, whereas antibody permeability will be efficiently inhibited with pores sizes <18 nm resulting in a save immunoprotection [33]. In another study Dandoy *et al.* verified these pores size values. They successfully entrapped cells of a hepatocellular carcinoma cell line within silica modified alginate beads. These mineralized beads had pore size diameters between 22 and 30 nm, which provided an efficient immuno-isolation and at the same time allows diffusion of nutrients and metabolites [35]. The most important clinical advantages of an immune isolated cell-therapy are the possibility of xenogenous or allogenous cell transplantation and the prevention of a permanent systemic immunosuppression, which is recognized to increase the risk for malignancies or recurrent infections [131]. Moreover, easy and minimal invasive implantation and retrievability in the case of failure or complications are predefined characteristics of those bioartificial organs [10]. For example Sakai *et al.* showed that pancreatic islets maintain metabolically active after silica encapsulation and responded to glucose stimulation with insulin secretion [122]. In a subsequent *in-vivo* study, Sakai *et al.* demonstrated, that diabetic mice treated with silica encapsulated pancreatic islets recovered normal glycemia levels for a period of 15 weeks, without the need of the administration of immunosuppressive drugs [130] (Fig. 6).

Boninsegna *et al.* used the biosil technique for the encapsulation of Langerhans islets from rats with promising *in vivo* and *in vitro* results [115]. In this study a minimum of 90% of encapsulated islets remained viable and could be verified with substantial maintenance of islet function. Furthermore, the nanoporous silica membrane allowed an unhindered mass transfer for glucose stimulation and subsequent insulin release. The allogenic transplantation of these biohybrids into diabetic rats resulted into a rapid decrease of glycaemia levels and the restoration of normal glycaemia values for more than 2 months suggesting a constant viable cell number. Beside silica, porous macro capsules composed of alumina [41], titanium dioxide [43] or calcium phosphate [45] were investigated as suitable compartment for cells. La Flamme *et al.* investigated nanoporous alumina macro capsules as alternative biocompatible carrier material for insulin secreting MIN6 cells [41]. These capsules were produced by an anodization process, whereas pore size and thickness were controlled by voltage and time. With this method the authors produced membranes with spherical pores in the range of 44.4–79.4 nm. A collagen matrix doped with MIN6 insulinoma cells was encapsulated within the alumina membrane system, resulting in a superior immunoprotection of cells verified *in vitro*. Although the cells retained their viability and functionality over a period of 24 h, the insulin release was almost inhibited due to the limited diffusivity of glucose through the alumina membrane. Further studies showed promising results regarding the application of immobilized cells for wound dressing and skin regeneration. For this medical indication, Zhu *et al.* and Desimone *et al.* encapsulated fibroblasts within silica nanoparticles reinforced hydrogels resulting in a favoured cell viability and improved skin regeneration *in vivo* [132,133] (Fig. 7).

5.2. Biosensors

Cell encapsulation for health care applications is not restricted to artificial organs; this concept is also of high interest for a simple to operate diagnosis device known as biosensor. This type of sensor is used as clinical analytical tool for a fast and definitive diagnosis of infections with pathogens, organ failure, tumors, toxins, abuse of drugs, ascertain pregnancy, or elevated glucose levels by detecting a particular molecule in a biological medium and converting this information into an easy accessible physical signal [26,121,134]. For this purpose, biological sensing elements with a binding affinity for specific molecules are fixed on the biosensor's surface. Typically, these sensing elements are enzymes, antibodies, whole cells or microorganisms [135]. The recognition event between those biological entities and the analyte will be converted by an optical, electrochemical, piezoelectrical, thermometrical, or colorimetrical transducing mechanism into a quantifiable physical signal [136]. A special kind of biosensors known as immunosensors uses antibodies or antibody-related reagents and the formation of highly specific antigen–antibody reactions for the diagnosis of diseases [137,138]. Although antibodies are the most utilized biological component in immunosensors, Copello *et al.* showed that encapsulated pathogenic protozoa *Leishmania guyanensis* and *Trypanozoma cruzi* can be used as a direct parasitological test for Leishmaniasis infection [20]. For this purpose, thin sol–gel silica films on glass slides were incubated in the protozoa containing solution, resulting in a covalent attachment of the parasites. In contact with the patient's serum the specific antibody-antigen reaction between the protozoon and the specific antibodies can be detected via a conjugated enzyme colour reaction as an unambiguous sign of Leishmaniasis positive sera. The sensitivity and specificity of this biohybrid was comparable to immunoassays based on non-covalently heat fixed cells. However, the latter demands rigorous storage conditions and suffers from increased cell loss during the involved washing steps, resulting in a reduced reliability. The silica based immunosensor maintained its performance up to 2 months of storage at 4 °C, which makes this method suitable for the direct detection of pathogens with minimal equipment or under restricted storage conditions.

6. Biotechnological application of biohybrids

Plant cells as well as several microorganisms like yeasts, fungi, algae and bacteria are used as whole-cell based bioreactors, biosensors or biocatalysts and represent a promising and cost-effective alternative to isolated enzymes often used for these biotechnological applications. The major benefit of using whole cells originates from the self-repairing mechanisms and the robustness of intact organisms. Beside this, cell-type specific advantages like the synthesis of intra- and extracellular enzyme production in the case of fungi, the eukaryotic expression mechanisms and the bacteria-like growth and handling of yeasts as well as the ability to proliferate, growth in high cell densities, and the accessibility to genetic engineering are well-founded arguments for the utilization of complete organisms or cells instead of single components.

Following the biomimetic concept of biomineralization, encapsulation of viable plant cells, yeasts, algae, and cyanobacteria into porous inorganic carrier matrices is a recent approach, which was successfully applied in order to simplify separation procedures of broth and culture associated with minimal cell loss, to permit or even increase the reusability of cultures or to enhance processing time for continuous running processes as well as the productivity and efficiency of the culture [14,89]. Dickson and Ely summarized these advantages in their review and also emphasized, that due to the constraining growth of embedded cells more energy is directed toward the production of secondary metabolites [89]. In the last years, photosynthetically active organisms such as algae and cyanobacteria, attained special interest in the biotechnology community, since these organisms are capable of assimilating carbon

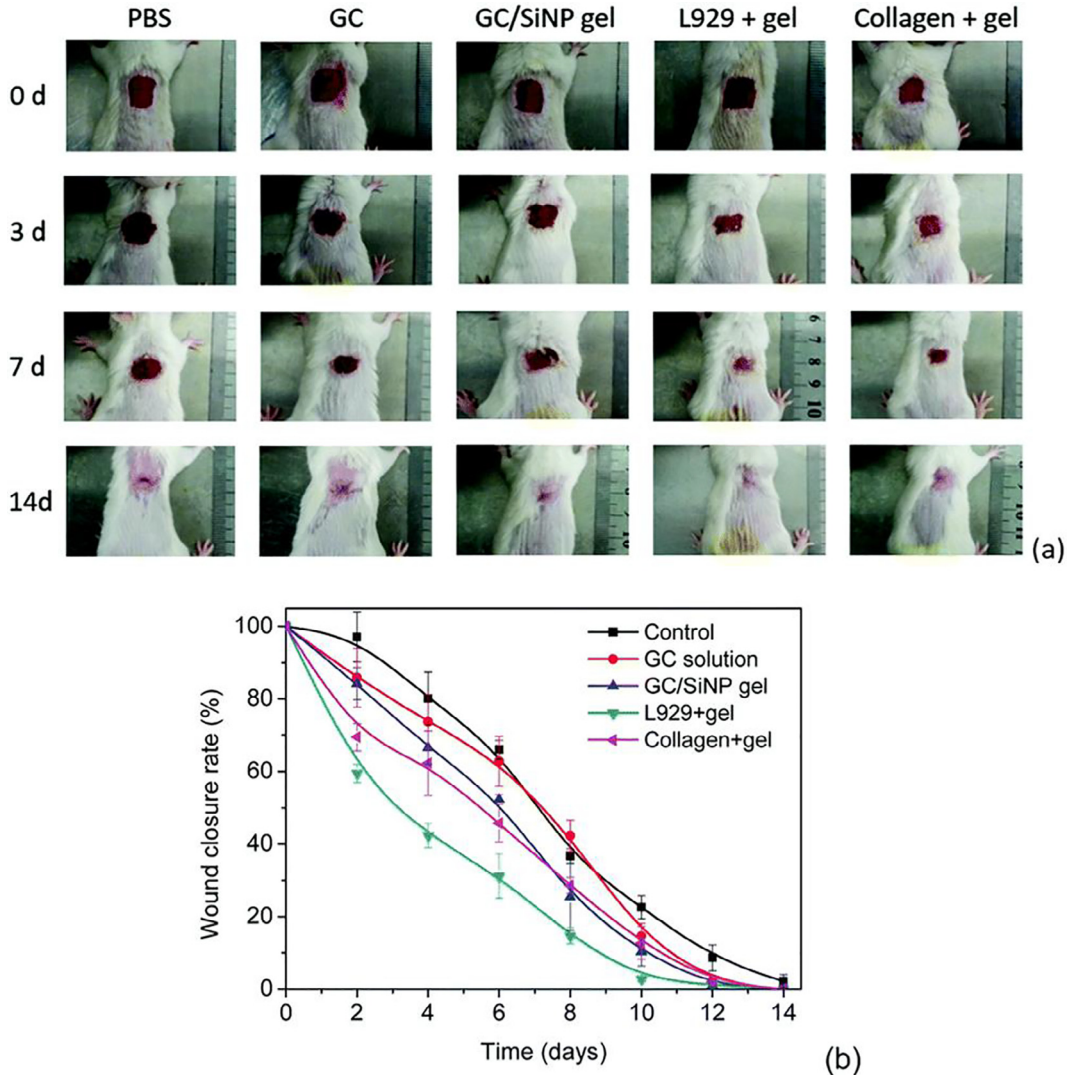

Fig. 7. Skin healing ability of different hydrogels. (a) Mices with skin wounds (10×10 mm^2) were treated with injectable hydrogels consisting of glycol chitosan (GC), silica nanoparticles modified glycol chitosan (GC/SiNP gel), silica nanoparticles modified glycol chitosan loaded with fibroblasts (L929 + gel), or silica nanoparticles modified glycol chitosan loaded with collagen (collagen + gel). A treatment with phosphate buffered solution (PBS) served as control. A complete closure of the wound with new epidermis was found for each formulation except for the control group. (b) The quantification of the wound closure rate showed, that fibroblast loading resulted into an accelerated wound healing and improved the quality of skin regeneration. The modification of glycol chitosan with silica nanoparticles improved the adhesive properties toward tissue and the gelation as well, making this modified hydrogel version a suitable carrier system for fibroblasts. Reproduced from [132] with permission from the Royal Society for Chemistry (2017).

dioxide and solar energy and convert them into valuable products based on hydrocarbons, oils and sugars. For this, translucency or transparency of the matrix is a special requirement, which has to be fulfilled by the host matrix for the encapsulation of photoactive organisms. This special optical feature is only met by few inorganic materials, which satisfy the requirement of a biocompatible encapsulation process at the same time, namely silica [89] and magnesium phosphate cement [23].

6.1. Biosynthesis

The utilization of microorganisms for the synthesis of valuable products like pharmaceutics or flavorings and fragrances is well known since decades, but recently attracting great interest again in the scientific community in order to use photoactive organisms as cell factories for the production of "green" nanomaterials or biofuels [14,139]. However, various microbial cells, among them yeasts, bacteria or fungi, are used as key components in biotechnological processes to produce chemicals like pesticides, food additives, recombinant proteins or biofuels such as ethanol [14,140]. In the last ten years, the creation of biohybrids has extensively been studied as suitable tool for the prolonged or continuous production and excretion of those products, whilst circumventing expensive and highly energy consuming harvesting and extraction steps [89]. Although, first achievements in this area were made, the preservation of the cell viability and enzymatic function, the permanent entrapment of the organisms, and the maximization of the process yield remains a challenge and especially demands sophisticated encapsulation protocols and material systems [49,89,141]. Due to the diversity of encapsulation techniques, silica is the most common and successful applied host matrix for the creation of biohybrids, capable of preserving the viability and metabolic activity of the biological additives. Pioneer work was performed by Carturan *et al.*, who developed the biosil technique, which enables the biocompatible coating of viable cells with a thin silica layer [42]. In an early study the authors demonstrated, that the productivity of secondary metabolites by *Catharantus roseus* plant cells was increased by a factor of 100 after encapsulation

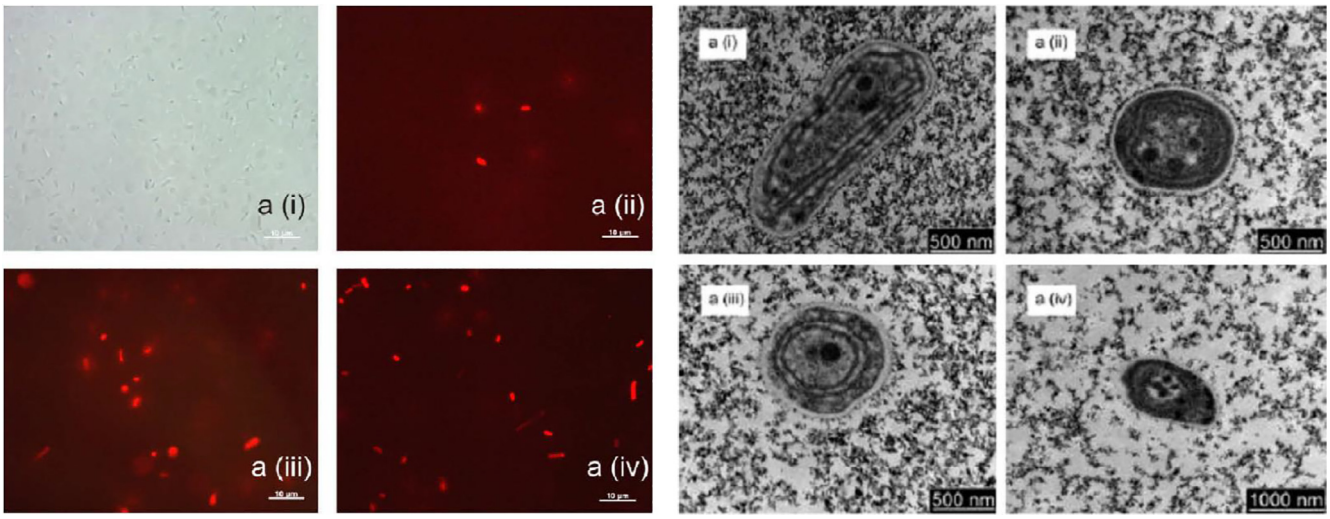

Fig. 8. Left: Optical light microscopy images of cyanobacteria *Synechococcus* encapsulated within silica gel after (i) 1 week and epifluorescence microscopy images of cyanobacteria encapsulated within silica gel after (ii) 1 week, (iii) 4 weeks, (iv) 12 weeks. Right: Transmission electron micrograph of cyanobacteria entrapped into a glycerol-containing gel after (i) 1 h, (ii) 1 day, (iii) 1 week and (iv) 1 month. Reproduced from [149] with permission from Royal Society for Chemistry (2008).

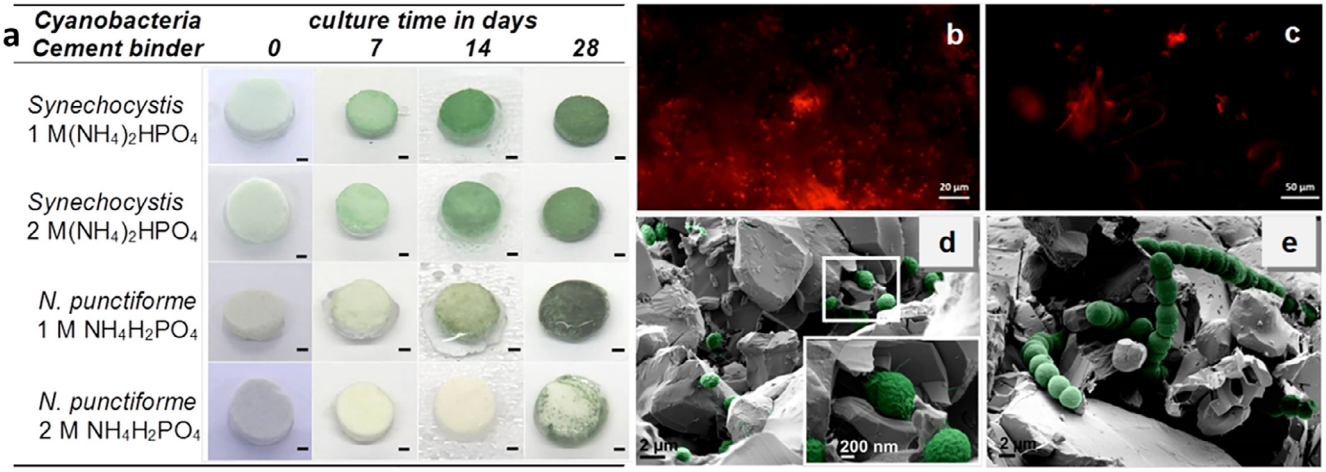

Fig. 9. a) Cyanobacteria of the strain *Synechocystis* or *Nostoc punctiforme* immobilized within magnesium phosphate cements. The cement powder was combined with a 1 M or 2 M binder solution of either $(NH_4)_2HPO_4$ or $NH_4H_2PO_4$. Scale bar = 2 mm. The chlorophyll-a-autofluorescence (b/c) and the scanning electron microscope pictures (d/e) showed that the cyanobacteria *Synechocystis* (b/d) or *N. punctiforme* (c/e) are viable and photosynthetically active even after 4 weeks of immobilization. Reproduced from [23] with permission from Elsevier (2016).

with this method [142]. In contrast, an aqueous pathway of silica encapsulation was used by Chen *et al.* [143]. The authors encapsulated whole bacteria cells of the species *Methylomonas* within a gel derived from sodium-silicate. *Methylomonas* is a methanotrophic bacterium, which contains methane monooxygenase and enables these bacteria to catalyse the epoxidation of propene to epoxypropane. Entrapped cells could be repeatedly used for more than 25 times without significant loss of activity. In a recent study, Lotfabad *et al.* showed that biohybrids consisting of TEOS derived silica and encapsulated bacteria *Pseudonomas aeruginosa* are suitable for the economic production of biosurfactants [18]. 84% of the encapsulated cells retained their viability for up to 1 year and more than 90% of their production efficiency after 5 cylces within 35 days, whereas the silica stability was the limiting factor for the application time. Moreover, under economic and environmental considerations soyabean waste oil was used as carbon containing growth medium. Besides this, various other biohybrids were used for biosynthesis of antibiotics from bacteria or fungi [49], food supplements from algae [140], gold-nanoparticles [139], bioethanol [144] or even energy sources like hydrogen [145].

The utilization of algae and cyanobacteria as biocatalyst, for the production of valuable organic products such as biofuels or pharmaceutically active metabolites can be considered as a further improvement of an environmental friendly production route, because it is not only based on solar energy transformation, but also involves the sequestering of CO_2. Many excellent reviews address the benefits and challenges of encapsulating photoactive microorganisms, among them reviews authored by the workgroup of B.-L. Su [14,30,89,146,147]. Su *et al.* describe silica encapsulation as suitable method to induce the release of intracellularly produced metabolites instead of the destruction derived extraction, which is the most common biotechnological followed strategy [148]. The same workgroup could proof the sustained viability and photoactivity of cyanobacteria after encapsulation within a silica-gel matrix and by this provide evidence for the production of biofuel or biogas by fixed phototrophs [30] (Fig. 8). A concrete

example for continuously working biophotoreactors based on living biohybrids is given by Fiedler *et al.*, who encapsulated the microalga *Haematococcus pluvialis* within sol–gel silica layers [140]. Under the influence of stressors these algae produce astaxanthin, a carotenoid used as supplement in fish food and an additive in food and cosmetic products. In this study the viability of the algae could be maintained over 40 days. Moreover, the metabolic activity was optimized by the addition of iron ions, NaCl and hydrogen peroxide, which are acting as stressors and result in an increased intracellular formation of astaxanthin. By using glycol ethers as extracting agent, a yield of 22% was obtained and only moderate cell damage of 70–80% was detected, allowing the encapsulated microalgae to be recultivated. This reusability offers a more efficient process, as compared to the commercial production of astaxanthin, where the extraction process is associated with the complete biomass destruction.

As alternative to silica-biohybrid working bioreactors, Soltmann *et al.* and Vorndran and Lindberg used hydraulically setting magnesium phosphate cements for the gentle encapsulation of viable microorganisms [19,23] (Fig. 9). While fixed yeast cells and bacteria *Saccharomyces cerevisiae* respectively *Rhodococcus ruber* showed a reduced metabolic activity after encapsulation, the photoactivity of the two model cyanobacteria strains, namely *Synechocystis* and *Nostoc punctiforme,* was comparable to planktonic cells and could be maintained over the complete investigation period of 4 weeks. In both studies a favored cell growth was detectable on the surface of the cements.

In a study performed by Taylor *et al.* the superficial overgrowth of the host matrix was inhibited by a daily UV-irradiation of the biohybrid and the growth medium for up to 20 days [49]. The local sterilization enabled the authors to differentiate between the metabolic activity of encapsulated or surface-immobilized fungi and bacteria cells. The model hybrid structures consisted of a layer-by-layer casted silica gel, inoculated with cells of the penicillin producing fungus *Penicillium chrysogenum* or the antibiotic producing bacterium *Streptomyces rimosus.* Although the colloidal silica gel used in this study was more porous compared to that one produced by the acid-catalyzed alkoxide pathway, the yields were still poor compared with suspended or superficially fixed cells. The authors explained this by the slow oxygen and nutrient diffusion, but without determining the cell number of viable cells or the porosity of the matrix.

Beside using cell encapsulation for biosynthesis, bioreactors can be supported by the addition and co-immobilization of microorganisms, which have a high sensitivity and a high selectivity for the detection of environmental changes and can be used to stage the fitness of microorganisms [150] or to detect water pollution with drugs, toxins or chemicals [151,152].

6.2. Microbial fuel cells

Another way of generating energy is given by microbial fuel cells, which utilize microbes as biocatalyst or the direct electron transfer of electrochemically active bacteria like *Shewanella, Geobacter,* or *Rhodoferax* to convert chemical energy into electricity [153,154]. Since usually organic compounds in wastewaters function as nutrition source for those microorganisms, microbial fuel cells are simultaneously working as wastewater treatment, resulting in the generation of electricity without a net carbon emission [153]. Various interesting reviews are addressing the general working principles of microbial fuel cells and different designs of electrodes [153,155–157]. In an alternative approach to most bio-electrodes, which are based either on microorganisms immobilized on the surface of an electrode by biofilm formation, or on a chamber electrode, where microorganisms are suspended or encapsulated within hydrogels [158], few studies describe the encapsulation of microorganisms within inorganic [16,159] or organic/inorganic composite materials [160] as a suitable concept for microbial fuel cell electrodes. Szöllösi *et al.* investigated the electrical performance of a novel bio-anode by the encapsulation of the marine bacterium *Shewanella* as biocatalyst in an organic/inorganic composite [154]. The electrode consisted of alginate and a titanium dioxide doped conductive polymer polyaniline network, which was improved by the modification with graphene. This modification resulted in a 105-fold higher conductivity and 7-fold higher power-density output of the microbial fuel cells. Another study performed by Bahratan *et al.* verified the suitability of graphene oxide encapsulated and genetically engineered cells of *Saccharomyces cerevisiae* or *exo*-electrogenetic bacteria *Shewanella oneidensis* for biofuel cell applications [159]. Here, the microorganisms were entrapped within a conductive graphene oxide hydrogel coating, which enabled the direct communication between the non *exo*-electrogenic yeast cells and the surface of an electrode. Yeast viability and bio-catalytic activity remained stable for a period of 15 days. Although minor reports on inorganic encapsulation for microbial fuel cell application are available up to now, this research could offer great potential, if scientific efforts in the combination of conductive materials and microorganisms will be done in future.

7. Applications of biohybrids in environmental science and for civil engineering

The colonization of bacteria, algae, cyanobacteria, and fungi is known to cause mineral dissolution and alteration, which presents a particular problem in the preservation and restauration of monuments and frescoes [161,162] or building materials [163]. As an initial step for a successful attachment on minerals the microorganisms secrete an extracellular polymer inducing the formation of a biofilm. This self-generated microenvironment provides essential conditions for further proliferation of the microorganisms. In particular, the biofilm serves as mechanical protection and as a hydrophilic environment, which prevents microbes from dehydration, and enables diffusion controlled nutrition with water soluble readily available elements [6]. Another pathway of nutrition is given by the release of microbial metabolites, including the formation of organic acids, metal binding compounds like siderophores and cyanide, which cause leaching of minerals and metal ions from solids and are responsible for the acquisition of essential nutrients [6]. The ability of several microorganisms to bind or to degrade specific metals is a highly versatile tool in environmental science and in civil engineering, where they are applied in biosensors [164], biosorbers [165] or as active component in self-healing concretes [166].

7.1. Environmental sensors

Environmental pollution with carcinogenic and neurotoxic substances and their risk on human health and harmful effects on ecosystem is worldwide a topic of great concern and has led to legislative interventions aiming at the avoidance and the reduction of environmental contamination by the establishment of preventive measures and early action plans [167]. In this context, biosensors are indispensable instruments for a continuous *in situ* environmental monitoring of air, soil or water quality to guarantee a prompt intervention. As described earlier, a biosensor is composed of a biological component, the bioreceptor, and a transducer, which converts a specific biological event into a detectable physical signal. This signal can be an electric current, fluorescence, luminescence or color [168]. As the signal strength is proportional to the analyte concentration, a quantitative measurement is possible. The main

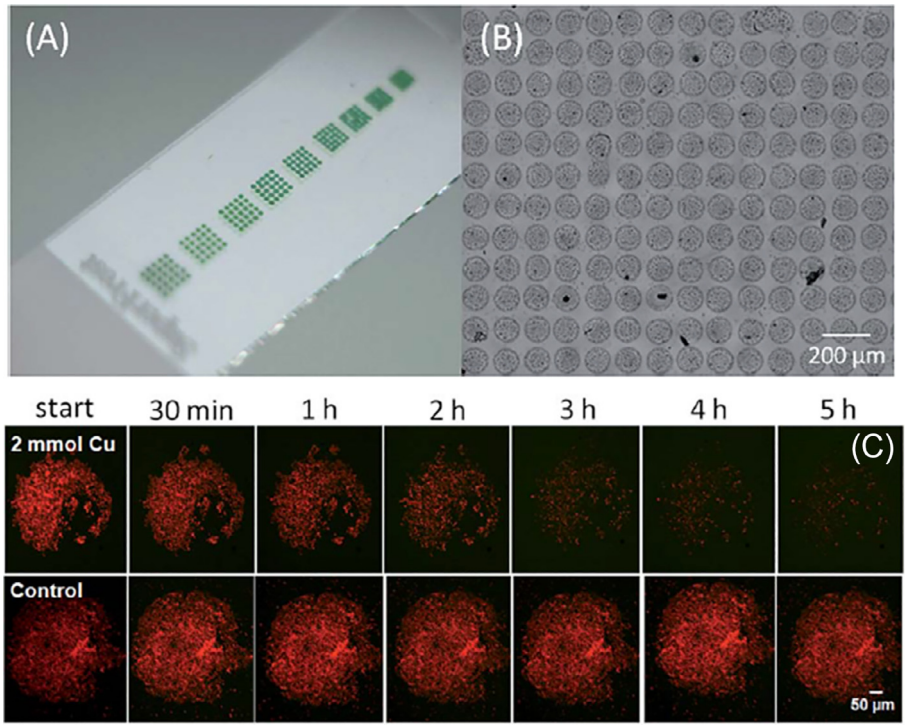

Fig. 10. (A) Alginate/silica arrays containing *C. vulgaris* cells printed onto Catiofast 159-modified glass slide with varying dot distance (ranging from 300 µm to 900 µm) and a water contact angle about 15. (B) Alginate array spotted onto a hydrophobic surface with a water contact angle of about 90 (dot distance 10 µm). (C) Fluorescence images of a single dot of immobilized *C. vulgaris* cells after different periods of exposure time; (above) in the presence of 2 mmol copper-ions and (below) control without Cu. Reproduced from [21] with permission from Royal Society for Chemistry (2014).

environmental application of biosensors relates to a specific and rapid detection of toxins like pesticides notably atrazine, herbicides, hormones, metal ions or the registration of oxygen depletion in aquatic habitats as indirect result of the microbial degradation of organic pollutants [168,169]. Compared to traditionally used spectroscopic or chromatographic analytical methods, biosensors are characterized by their high specificity, reliability, cost-effectiveness, comfortable handling, and easy sample preparation [170]. Besides enzymes, antibodies, nuclide acids, specific proteins, or organelles, whole cells or microorganisms are used as biological recognition elements in biosensors, among them native or genetically modified yeasts [171], fungi [172], bacteria [173] or photosynthetic microorganisms like cyanobacteria [174] or algae [21,168]. In particular, the use of vital microorganisms or whole plant or mammalian cells is the only method for a direct evaluation of bioavailability, toxicity, and genotoxicity of pollutants [169]. In this case, toxic effects caused by environmental pollutants can be easily quantified due to either the inhibition of cellular activity, in particular cell growth, motility depletion, respiration, and inhibition of photoactivity, or to the expression of a reporter gene or molecule [169]. The detection limit for hazardous compounds of microbial biosensors ranges between a few nM to some mM [169]. Apart from electrochemical, thermometric or piezoelectric biosensors, optical biosensors are used for environmental monitoring. The latter utilize reporter genes of enzymes or fluorescence proteins, which convert the biological event based on colorimetric, fluorometric or luminometric reaction in response to the molecular recognition of a biological event. Here, eukaryotic luciferase, ß-galactosidase or green fluorescence protein isolated from the jellyfish *Aequoria Victoria* are the most common used reporter genes in optical biosensors [169]. For a deeper understanding the interested reader will be referred to several excellent reviews on this topic [121,148,170,173,175]. Generally, cell immobilization or encapsulation is not only necessary for the creation of a sensor it also provides a vital microenvironment for cells or microorganisms. Consequently, most carrier systems consist of polymers such as alginate, gelatin, agar, pectin or polyvinyl alcohol satisfying the demand of minimal humid conditions [168]. Although bioreceptors encapsulated within inorganic materials show a higher stability, only the minority of toxicity testing systems are using inorganic materials such as pure silica gels or silica in combination with hydrogels as cell carrier [21,167]. Liu *et al.* created an electrochemical biosensor for the detection of sulfide by using an engineered *Escherichia coli* BL21 (DE3) strain, which overexpressed sulfide:quinone oxidoreductase and immobilized these cells within porous gold nanoparticles. With this microbial sensor system, sulfur concentrations in a wide range between 50 µM and 5 mM could be detected [176]. In another study, Pannier *et al.* fabricated an optical biosensor by micro-contact printing for the detection of the herbicide atrazine and copper ions [21]. For this, algae cells of the species *Chlorella vulgaris* were immersed in an alginate/silica gel and the biological doped gel was deposited onto a glass plate (Fig. 10). The authors could prove that the microorganisms maintained their vitality and responded sensitively to atrazine for up to 8 weeks. Also a repeated use of up to 8 cycles was proven to be possible. Another optical biosensor design based on algal cells encapsulated in a translucent alginate/silica hydrogel was applied by Durrieu *et al.* [167]. For this sensor system algal cells were encapsulated within a protecting calcium alginate matrix followed by silica gel immobilization. With this sensor an atrazine concentrations ranging from 0.001 µM to 10 µM were detectable after 40 min exposure time.

7.2. Bioremediation and biorecovery

The ability of several microorganisms to degrade metal containing materials or toxic molecules or to bind metal ions in solution is a versatile tool for environmental bioremediation, biorecovery and biorecycling of scares elements or metals. Therefore, embedding these bioadsorbing species is a suitable strategy to minimize the

pollution caused by the release and accumulation of those metal loaded microorganisms in the environment [6]. Moreover, the encapsulation and immobilization of the sorbing microbial components in physically and chemically stable matrices facilitates an easy separation of the sorbing phase or a save removal of adsorbed compounds from the purified water. This fact also increases the efficiency of immobilized microbes for bioremediation by allowing a repeated or continuous use [177]. Another outstanding feature of immobilized microbial cells compared to free cells is their enhanced degradation ability, due to the protection against biotic (e.g. completion, predation) and abiotic (e.g. pH, temperature) stress factors in the area of application [178,179]. To summarize, the inorganic encapsulation increases the lifetime and the reusability of purifying biological doped systems, which are essential factors for an economical process. Generally, the purification of contaminated water sources by biological species is based on bioaccumulation, biotransformation, biomineralization or biosorption, and does not require viable cells in all cases. While accumulation and degradation are intracellular processes requiring metabolic activity, biosorption is a passive binding process on the cell surface and is even possible for dead cells [17]. In the case of biodegradation, the transformation of pollutants such as metal ions [180], phenol [181,182], methanol [183], herbicides [184] and pesticides [179] or dyes [177] into non-toxic components is a metabolic and hence an active process. A concrete example of bioremediation with immobilized bacteria could be shown by the work of Alvarez *et al.* [185]. In this study silica encapsulated bacteria *Burkholderia* sp. were used to reduce toxic metal ions like Cr (VI) into the non-reactive version Cr (III). Here, the silica matrix acted as protector against toxic chromium ions, since the porosity of the silica network limited the diffusion of these ions and with this the contact to the microorganisms. This additionally resulted in a higher viability of immobilized cells compared to free cells, and in turn prolonged the bioremediation process. Immobilized bacteria were able to completely reduce 100 μg / ml Cr(VI) after 4 days of incubation in contaminated water and to transform 200 μg / ml Cr(VI) after 7 days in soil.

The binding capacity of microorganisms is also used for biorecovery of valuable and rare metals from contaminated waters, biomining leachates or e-waste extracts. This topic was intensively reviewed by Nancharaiah and co-workers, whereas the urgent need to promote biorecovery strategies was emphasized [186]. The authors predict an upcoming interest of highly cost-efficient biological recovery, since the demand for metals like Pd, Ru or rare earth elements such as yttrium increases with increasing technological progress, while the availability is continuously decreasing as result of limited resources. Although several microorganisms such as algae, bacteria and fungi are able to perform metal recovery by either bioaccumulation, biosorption, bioreduction or bioprecipitation [186,187], to the best of our knowledge the immobilization of sorbing phases was not addressed in literature up to now.

7.3. Microbial concretes

Several strategies have been developed to combat the unavoidable problem in civil engineering, namely crack formation in cementitious building materials. Therefore, reinforcement of clay or concrete by the addition of fibers was one of the first man-made solutions developed to bridge or redirect cracks, and is also instinctively used by animals like swallows to build nests. Nowadays, beside steel reinforcement, also the addition of polymer phases to concrete or the superficial treatment with repairing agents are some of the current strategies to increase mechanical strength and durability of concrete. An innovative approach to improve cementitious building materials is given by microbial self-healing concretes. The active repair mechanism is induced by the addition of microbes and mineral precursor compounds to the concrete matrix [188]. Although almost all kinds of microorganisms are known to cause biodeterioration of building materials, it is just the ability to metabolically transform minerals, which makes microorganisms extensively studied objects as promising crack healing agents in building materials [189]. Excellent overviews about this topic can be found in several review articles [5,189,190].

Due to the high alkalinity of concrete only alkaliphilic or alkali-tolerant bacteria survive the encapsulation process within concrete. Moreover, the addition of endospore-forming bacteria is beneficial, since spores survive harmful environmental conditions and form the germ for a new bacteria generation. In general, three different bacteria species are utilized for crack healing applications. One of them are ureolytic bacteria strains, among them *Bacillus sphaericus, Sporosarcina pasteurii*, and *Bacillus megaterium* [189]. These strains are able to degrade enzymatically urea into ammonium and carbonate ions, which creates optimal conditions for the calcium carbonate precipitation:

$$H_2N - CO - NH_2 + 2OH^- \rightarrow 2NH_3 + {CO_3}^{2-}$$

The biomineralization of ureolytic bacteria is a very effective way for carbonate precipitation, because these strains are able to produce carbonate in a high precipitation rate of up to 10–26 g carbonate / L / day, resulting in a fast crack filling [5]. Other bacterial strains, e.g. *Bacillus cohnii*, *Bacillus pseudofirmus* and *Bacillus alkalinitrilicus* use a different mineralization pathway. These bacteria are able to oxidize organic compounds like lactate or acetate to water and CO_2. In combination with the highly alkaline conditions in concrete, the formation of CO_3^{2-} is induced, which than results in the precipitation of calcium carbonate in the presence of a calcium source. Another way of carbonate precipitation within construction materials is given by nitrate reducing bacteria such as *Pseudomonas denitrificans, Diaphorobacter nitroreducens, Pseudomonas aeruginosa*. An alternative technology was followed by Zhu *et al.*, who investigated the suitability of autophototrophic cyanobacteria, *Synechococcus PCC8806* for the restauration of concrete by mineral precipitation [191]. They could show that more than 80% of the cyanobacteria population survived under mimicked concrete conditions and were able to precipitate silicate and calcite.

The incorporation of bacteria within cement matrices was proven to result in increased strength, durability and crack-healing of up to 0.97 mm-wide cracks [81]. Usually bacteria and mineral precursors, mainly calcium sources and nutrients, are mixed to the concrete during the preparation process via the liquid phase [5]. Thereby, the preservation of the metabolic activity is the most challenging aspect, which can be drastically diminished by the harsh conditions within the concrete matrix. Limiting and utmost influencing factors for the choice of the bacterial strain are given by the dense matrix structure with pores <0.5 μm and porosities about 1%, the reduced content of oxygen and water as well as the highly alkaline pH of 12–13 [189,192]. In order to avoid a drastic impact on bacterial vitality, the encapsulation of bacteria in protective capsules prior to the admixture is a preferred strategy. Typical encapsulation materials are diatomaceous earth, expanded clay, zeolites, silica gel, hydrogels or porous glass [81,192,193], whereas capsules with increasing porosity in course of an aging process are favored as the activation of the healing system during crack formation is more reliable [5]. Wang *et al.* could show, that hydrogel encapsulation of spore-forming bacteria within a modified copolymer of poly (ethylene oxide) and poly (propylene oxide) resulted in a complete healing of 0.5 mm wide cracks after 7 days, which was 20–60% higher than for bacteria-free concrete [194]. In general, the activation of the microbial healing system is directly

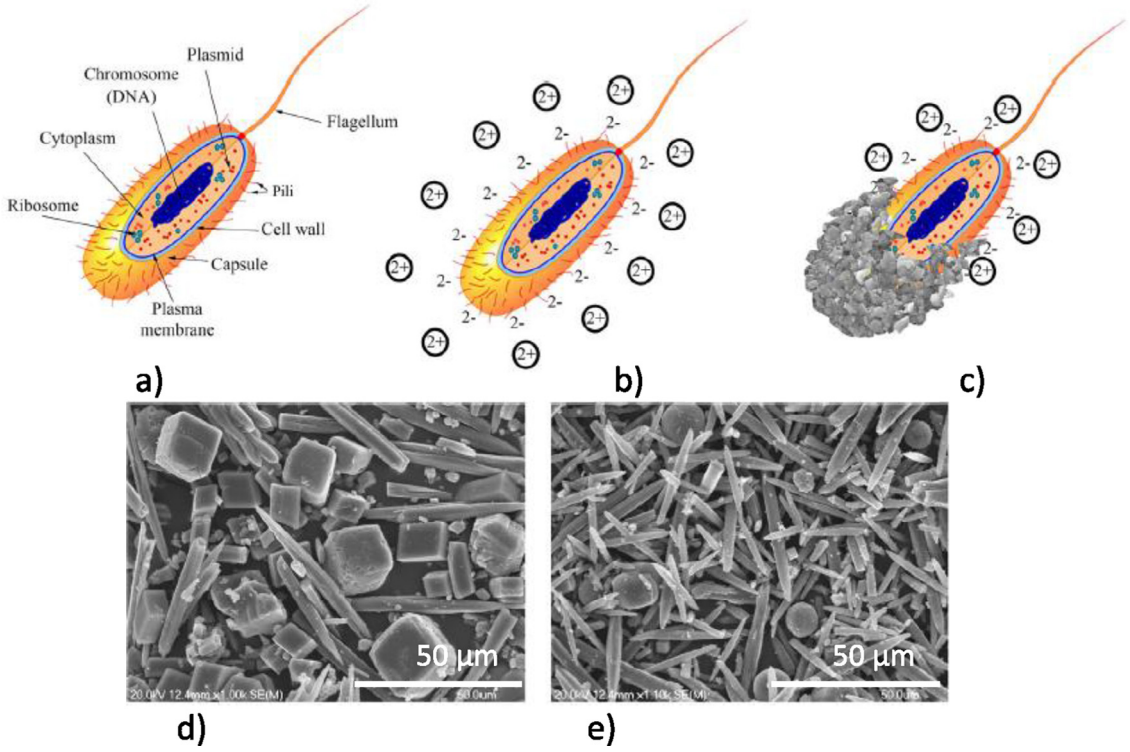

Fig. 11. a) Bacteria structure with b) negatively charged cell wall with the presence of positively charged ions. c) Biomineral formation by means of binding ions to the cell wall. Scanning electron micrographs show calcite precipitation by d) *B. sphaericus* and e) *B. subtilis*. Reproduced from [5] with permission from Springer-Nature (2016).

connected to the incidence of cracks and the following penetration of water, working even after days or months after concrete preparation. The healing process, namely the biomineralization, is starting immediately after the dissolution of the incorporated salts and their enzymatic driven decomposition. The extracellular precipitation of calcium carbonate occurs then by the attachment of cations, e.g. calcium ions to the negatively charged microbial cell walls (Fig. 11). This closes the crack and hence prevents further crack propagation and structural failure of the concrete matrix.

8. Outlook and future perspectives

The encapsulation of microorganisms and cells in silica or cement matrices has made great progress in the past years as demonstrated by many research approaches in the field of biotechnology, biomedicine or environmental science. However, there are still some underdeveloped fields regarding application and material processing. One example are microbial fuel cells: although minor reports on inorganic encapsulation for microbial fuel cell application are available, this research could offer great potential, if scientific efforts in the combination of conductive materials and microorganisms will be achieved in future. Another aspect is related to the processing of biohybrids into macroscopic forms. Currently, this is mainly performed by "classical" techniques, such as slip casting, while macro pores are introduced by porogen dissolution. Here, the application of additive manufacturing methods will likely help to generate more defined biohybrid matrices in terms of their outer and inner shape to control mass transfer and diffusion paths by variation of macro pore dimensions. This aspect was recently addressed by a work of Seidel *et al.* [12], who used an extrusion based plotting method to create macro porous alginate / agarose / methylcellulose scaffolds loaded with plant cells ("green bioprinting"). This technique should be also adaptable to the inorganic matrices described in this review as printable biocement pastes are already established in the fabrication of macro porous calcium phosphate scaffolds [195].

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