Chapter 7
Bionanocomposites Assembled by "From Bottom to Top" Method

Particles, which take part in different biological processes, have a special place in nanometer composites. Rich and variable biochemistry of proteins displays key importance of a nanometer phenomenon for a working mechanism of living organisms [1, 2], and this provides a wide range of possibilities for development of new types of hybrid nanomaterials with constructible structures, functions and shapes [3–5].

Integration of nanoparticles and biomolecules, each having unique properties, on the one hand, and being in the same nanometer scale (enzymes, antigens, and antibodies of typical sizes 2–20 nm like nanoparticles), on the other hand, as of two structurally compatible classes of materials, gives resultant new hybrid nanomaterials with synergetic properties and functions (Fig. 7.1).

Interaction of nanoparticles with biopolymers (proteins, nucleic acids, polysaccharides) plays very important role in enzyme catalysis, biosorption, biohydrometallurgy, geobiotechnology, etc.). There is a great interest to nanomaterials, which can be used in biomedical and pharmaceutical applications due to their biocompatibility and biodegradation. At the same time bionanocomposites should meet some demands to plasticity of a matrix, they should have improved barrier, antimicrobial properties, ability to controlled release of bioactive substances such as antimicrobial agents, antioxidants, drugs, calcium compounds in biologically available form and their mixtures, etc. Biopolymers, such as natural and synthetic proteins, obtained by chemical methods or by genetic modification of microorganisms or plants, nucleic acids (including synthetic ones), biodegraded complex polyethers, such as polylactic acid, and its derivatives, oligo-hydroxyalkanoate, most often, polyhydroxybutyrate, their copolymers, biomedical materials, such as hydroxyapatites, are used. There is an interesting group of materials for this purpose including synthetic and natural (vegetable and animal) polysaccharides, such as cellulose and its derivatives, alginates, dextranes, acacia gum, chitosan, and any of its natural and synthetic derivatives, especially chitosan acetate, and proteins obtained from raw animal materials, as well as proteins from maize (especially zein) or soya, gluten derivatives, gelatin, casein, etc. Relatively widely used in

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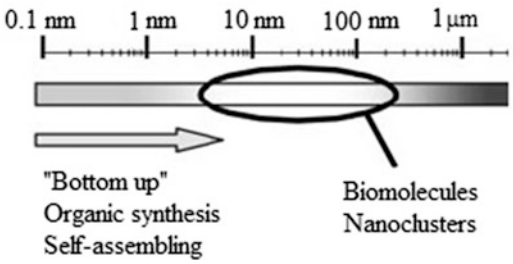


Fig. 7.1 Integration nanometer scale of nanoparticles, biomolecules and bioobjects

biomedical applications are intercalated bionanocomposites based on organically modified materials with layered structures (layered phyllosilicates, montmorillonite, etc.), including their usage for release of active ingredients, such as volatile odoriferous substances and their components (for example, lynalol, polar ether oil with anti-microbial properties).

Biocomposites also include products of concentration and biomineralization with participation of natural, also inorganic, polymers. Perfection of bioprocesses, principles of their realization and self-regulation of biosystems are still not only stunning, but permanently impel researchers to their modeling under laboratory conditions, to development of bio-imitating concepts, to designing of artificial analogues. Following, perception and copying methods of Nature, a desire to unravel its mystery bring to a new research direction, biomimetics, whose purpose is transportation of its laws into inanimate nature.

Almost all approaches and problems of nanomaterials science considered in this book more or less concern development of biocomposites. Their applications are especially important in medicine: it is a basis for progress in diagnostics and therapy on cellular and genetic levels. While modifying surfaces of drug carriers with biocompatible polymers, it is necessary to optimize functions of polymeric component, which can play a role of a binding material for therapeutic or diagnostic drugs, to provide some characteristics of drugs (solubility, bio-availability, prolongation of their action due to slow desorption of drugs from polymer matrix, their shelf life, etc.), etc.

At last, we shall note that quite many biological materials are known, such as tissues [6], fungi [7], bacteria [8], viruses [9–11], and biomolecules [12, 13], which by their spatial configuration are very close to organic-inorganic nanostructures obtained by functioning of organic fibers with nanoparticles [14].

First of all, we shall consider variants, when there is a bio-object in a system, which can initiate reduction of metal ions and thus form nanoparticles to produce nanobiocomposites.

7.1 Bioreducing Agents in the Synthesis of Nanocomposites

During the recent years biosynthesis methods have become widely used, because they are relatively simple and efficient ways of synthesis of nanoparticles in reduction processes as compared to physical or chemical methods. Fungous or

mold microorganisms [15–18], bacteria [19], plant extracts [20, 21] are used as biological objects. In the base of many applications of microorganisms including bioleaching, bioremediation, bacterial corrosion, in particular, in biosynthesis of nanoparticles, there is their ability under extreme external conditions to induce specific protective mechanism of stress suppression, for example, toxicity of metal ions or metals due to a change in redox-state of ions or intercellular deposition of a metal [22].

Among the abovementioned approaches there is biochemical synthesis based on usage of biologically active components, which is in the group of the most efficient and environmentally appropriate methods.

7.1.1 Vegetable Biomasses and Extracts as Reduction Agents of Metal Ions

Production of nanoparticles with the use of various plants, plant-mediated synthesis, is a widely used technique of synthesis of nanobiocomposites. There are especially numerous examples of this type of composites based on gold and silver (Table 7.1) [23], extracellularly produced in leaf extracts of *Cinnamomum camphora* [24],

Table 7.1 Plants used in biogenic synthesis of silver and gold nanoparticles [23 and references there]

Plants	Silver and (or) gold nanoparticles	Shape (morphology)	Particle size
Medicago sativa	Au	fcc twinned, crystal and icosahedral	4–10 nm
Medicago sativa	Au	fcc tetrahedral, hexagonal platelets, icosahedral multiple twinned, decahedral multiple twined and irregular shaped.	15–200 nm
Chilopsis linearis	Au	–	1.1 nm
Pelargonium graveolens	Au	Spherical, rods, flat, sheets and triangular	21–70 nm
Cymbopogon flexuosus	Au	Triangular, hexagonal	–
Cymbopogon flexuosus	Au	Triangular	–
Avena sativa	Au	fcc tetrahedral, decahedral, hexagonal, icosahedralmultiple twinned, irregular shaped, rod shaped	–
Cicer arietinum	Au	Triangular	–
Tamarindus indica	Au	Triangular	–

(continued)

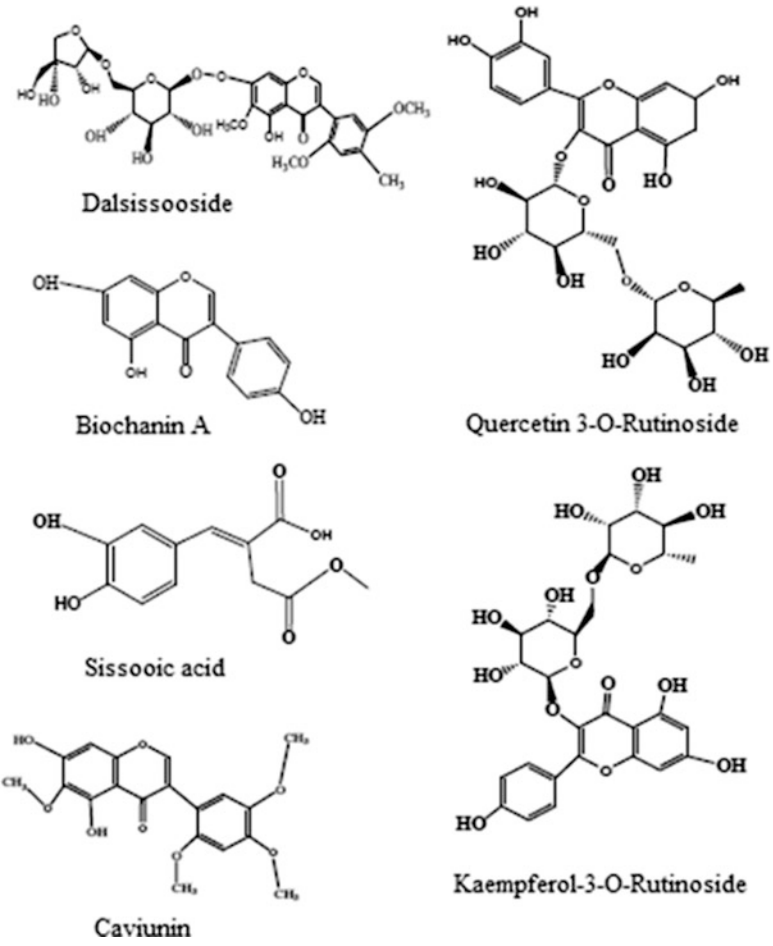
Table 7.1 (continued)

Plants	Silver and (or) gold nanoparticles	Shape (morphology)	Particle size
Triticum aestivum	Au	fcc tetrahedral, hexagonal platelets, irregular shaped, rod shaped, decahedral multiple twined, icosahedralmultiple twined	10–30 nm
Sesbania	Au	Spherical	6–20 nm
Medicago sativa	Ag	Spherical	2–20 nm
Quercetin	Ag	–	Radius 1–1.5 mm
Tetrapanax	Ag	–	< 100 nm
Capsicum annuum	Ag	–	–
Pelargonium graveolens	Ag	–	–
Aloe vera	Au, Ag	Triangular, spherical	–
Brassica juncea	Au, Ag, Cu	–	–
Emblica officinalis	Au, Ag	–	10–20 nm, 15–25 nm
Azadirachta indica	Au, Ag and Ag core –Au shell	Polydisperse, flat, plate-like, spherical, peculiar core–shell structure	5–35 nm, 50–100 nm
Cinnamommum camphora	Au, Ag	Triangular, spherical	55–80 nm

gooseberry *Emblica officinalis* [25], *Aloe vera* [21], poon *Dalbergia sissoo* [26], geraniume *Pelargonium graveolens* [27], black tea [28], etc. Biosynthesis can be conducted directly in biomass, i.e. intracellularly. This way was used to produce gold nanoparticles in alfalfa *Medicago sativa* [20] biomass, desert willow C*hilopsis linearis* [29], *Sesbania* grains [30], etc. Moreover, formation of nanoparticles can proceed in vivo. Alfalfa roots can absorb Ag(0) from agar medium and transport them into saplings, where nucleation and growth take place [31]. Similarly, the desert willow *(Chilopsis linearis)* absorbs Au (160 mg in Au/L in agar) and synthesizes gold nanoparticles in roots, stems and leafs with average size 8, 35, and 18 Å, respectively [29]. Probably, following the same general scheme: reduction and absorption of atomic Au(0) by roots of plants, transportation to some parts of a plant, growth and coalescence of nanoparticles, *Brassica juncea* biomass contained nanoparticles of Au, Ag, and Cu alloys sized from 5 to 50 nm after 14 days of growth in soil enriched with gold chloride, silver nitrate, and copper chloride [30].

It is supposed that [26], combined components of biological extracts show synergetic reduction effect during formation of nanoparticles from metal ions. Proteins, polyphenol, carbohydrates can be involved in reduction processes in these systems.

Chemical structures of the most important representatives of these substances extracted from the poon leaves *Dalbergia sissoo* are shown in the Scheme 7.1.



Scheme 7.1 Chemical structures of important constituents in green leaves of *Dalbergia sissoo*

It has been shown that naphthoquinones [32] and anthraquinones [33] from *F. oxysporum* can be efficient carriers of electrons during reduction of metals. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) (see Scheme 7.1) is one of the components of the plant extracts which was obtained in pure state and for which reducing properties with respect to $AgNO_3$ or $Cu(NO_3)_2$ in AOT-n-alkane system were confirmed experimentally [34, 35]. It is assumed that citronellol and geraniol molecules, which are the basic components of terpenoids contained in high concentrations in the extract of *Pelargonium graveolens* [27, 36] leaves, are simultaneously stabilizers and reducing agents. Chemosorption of proteins from

P. Graveolens on the surface of Ag nanoparticles is confirmed by widening of peaks of amide line I (1,640 cm^{-1}) and of ether group C═O (1,748 cm^{-1}) in FTIR spectra of synthesized biocomposite [36]. Usually, phyto proteins similarly to many synthetic polymers (see the Sect. 2.2.4) show dual function in formation of nanobiocomposites. On the one hand, they have bioreducing ability due to amino acid residuals, such as L-tryptophan, L-tyrosine, L-arginine, L-lysine, L-asparagine acid, etc, whose reducing properties, in particular, are demonstrated with respect to gold ions [37, 38]. On the other hand, presence of various functional groups in content of proteins (hydroxyl group, carboxyl group, etc.) advances their chemosorption on the surface of nanoparticles, an important role in this case has molecular mass of a macromolecule. For example, highly molecular fraction ($M > 5$ kDa) of water soluble proteins of soy reduces $NaAuCl_4$ in water medium and stabilizes the formed nanoparticles, while low molecular proteins with molecular mass <5 kDa, though initiate formation of nanoparticles, are unable to prevent their aggregation [39], in this aspect, their behavior is similar to that of synthetic polymers, considered above.

Another group of plant components, biologically active in synthesis of nanoparticles are polyphenols (catechin gallate, epicatechin gallate, epi-gallocatechin, gallocatechin gallate, etc.). For example, water extracts of leaves and seeds of *Syzygium cumini* contain 21 and 36 mg g^{-1} of polyphenols, respectively. It is shown that sizes of formed nanoparticles depend on concentration of these components [40]. And an increase in concentration of phenol compounds in the sorghum *Sorghum spp* extract (2,010, 2,375 and 2,520 mg/L GAE[1] at the temperatures of extraction 25, 50, and 80 °C) caused respective increase in intensity of the absorption band of the obtained Ag particles at 390 nm, which directly confirmed that particularly phenol compounds were the main reducing agents in this system [41]. According to the data from Table 7.1, the produced nanoparticles are characterized by great variety of shapes. The most widely appeared is triangular morphology, including truncated (prismatic) anisotropic structures. These nanotriangles can serve building blocks for electrically conducting thin films [42]. Bioreduction of $HAuCl_4$ by extract of tamarind leaves brings to formation of highly anisotropic flat triangular structures, which are interesting for optoelectronics, photonics, and sensor devices [43]. It should be noted that precision control over the shape of formed nanoparticles and their size under conditions of biogenic synthesis is often difficult, because the mechanisms of reduction and absorption of protective agents are not quite clear. However, like in chemical reduction reactions, morphology and size of formed nanoparticles can be varied efficiently by a change in composition of biocomponents. For example, as amount of lemongrass *Cymbopogon flexuosus* extract added to $HAuCl_4$ solution increased, average size of triangular and hexagonal particles decreased, while a fraction of spherical particles with respect to them increased [44]. Average size of gold nanoparticles

[1] GAE – equivalent of gallic acid used as a standard phenol compound in spectrophotometric analysis of total concentration of phenols.

synthesized in presence of native biomass of hop was 17.3 Å, and when etherified bioreagent was used, 9.2 Å particles were produced, while hydrolyzed biomass produced nanoparticles of ~25 Å [45]. For silver nanoparticles made by treatment of silver ions by capsicum extract *Capsicum annuum* L, nanoparticle size was a time function of the reaction [46]. During 5 h spherical particles with diameter 10 ± 2 nm formed, increase in duration of the process from 9 to 13 h caused formation of nanoparticles with sizes 25 ± 3 nm and 40 ± 5 nm, respectively. It should be noted that optimization of synthesis conditions in presence of tetrapanax (rice-paper plant) biomass allowed production of silver nanocomposites with 1.8 wt % concentration and antimicrobial activity (minimal concentration of inhibition) of biogenic composite was 14.1 mg 14.1 mg (Ag) L^{-1} and 28.1 mg (Ag) L^{-1} for *Escherichia coli* and *Candida albicans*, respectively [47], which was comparable with action of colloid nanosilver.

On the whole, rates of biosynthesis are lower than in reactions of chemical reduction, and time of reaction may reach several hours, however, in some cases kinetic parameters can be compatible [48]. It is interesting to note that reduction of Au(III) ions in presence of vegetable surfactants from coconut and castor oils goes during several minutes without adding of special reducing or stabilizing agents [49]. At the same time if the chemical surfactant butyl ammonium bromide is used, Au nanostructures are formed only the next day.

The advantage of using of plant raw materials or their extracts with respect not only to chemical or physical methods of synthesis of nanocomposites, but in comparison with other biogenic processes are simplicity and minimization of a number of technological cycles, almost entire implementation of "green chemistry" at all stages of synthesis, including formation of non-toxic biocompatible materials applicable for biomedical purposes. Soft condition of synthesis, aqua media, quite high product yield make plant-mediated processes attractive for scaling, i.e. for producing a great amount of nanobiocomposites as, for example, is shown in the case of reduction of silver ions by extract of solid biomass of oil-palm (*Elaeis guineensis*), though the produced nanoparticles have wide size distribution from 5 to 50 nm [50]. Volume production can be favored by the fact that many species of raw plant materials are used for industrial purposes. A good example is *Sorghum spp*, which are used in alcohol production and other industrial products. It was recently found that *Sorghum* extracts have efficient reducing properties with respect to silver and iron ions and can provide substantial stability of colloids [41].

7.1.2 Microbiological Synthesis of Metal Nanoparticles

Microbiological synthesis of metal nanoparticles, though requires a special stage of cellular cultivation, oppositely to the abovementioned biogenic plant-mediated processes, is characterized by selectivity and high level of molecular control over metabolic processes, providing reproducible synthesis of nanoparticles of certain

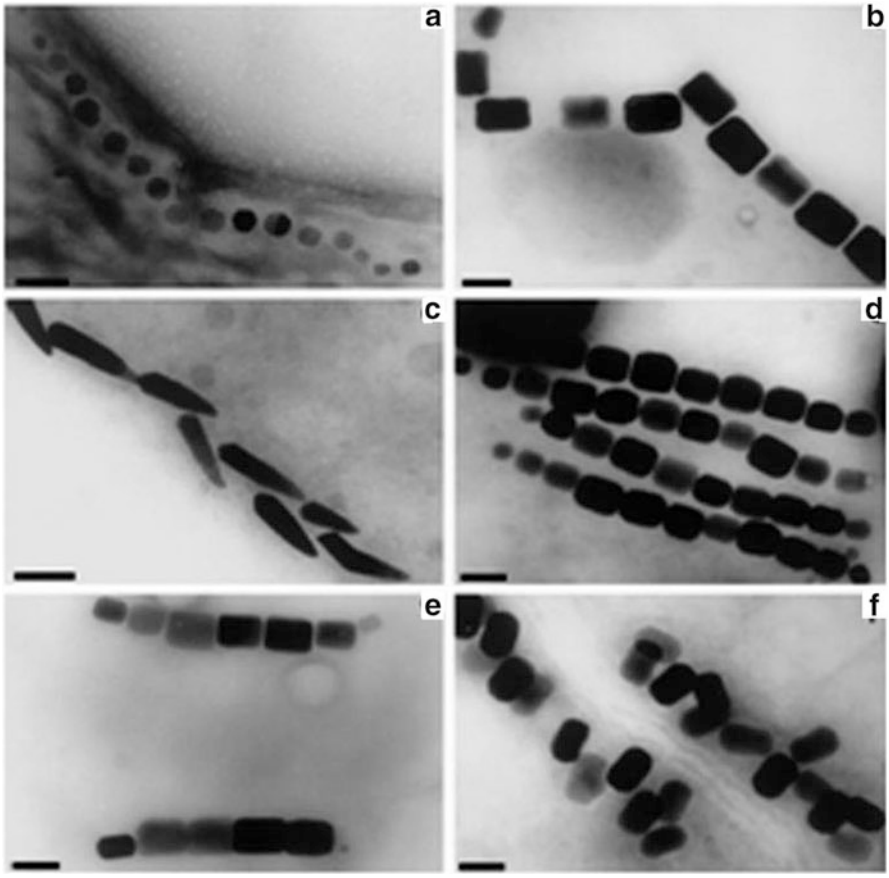


Fig. 7.2 Electron micrographs of crystal morphologies and intracellular organization of magnetosomes found in various magnetotactic bacteria. Shapes of magnetic crystals include cubo-octahedral (**a**), elongated hexagonal prismatic (**b**, **d**, **e**, **f**) and bullet-shaped morphologies (**c**). The particles are arranged in one (**a**, **b**, **c**), two (**e**) or multiple chains (**d**), or irregularly (**f**) (bar equivalent to 100 nm) [51]

sizes and structure. Evolution of microorganisms create the necessary prerequisites for development of their ability to produce spatially organized nanomaterials, among which the focus is on chemical lithography for reproducing of energy, usage of highly dispersed particles for special functions, detoxing functions for surviving in a toxic medium [51]. For example, sulfate-reducing bacteria reduce sulfates, thiosulfates, sulfites, and other sulfur containing compounds in oxidized to sulfides form. Some species of microorganisms synthesize inorganic materials at nanometer scale and integer them in functional components. Thus, magnetotactic bacteria contain intercellular chains of magnetite nanocrystals, magnetosomes (Fig. 7.2), via which they orient in geomagnetic field of Earth [52].

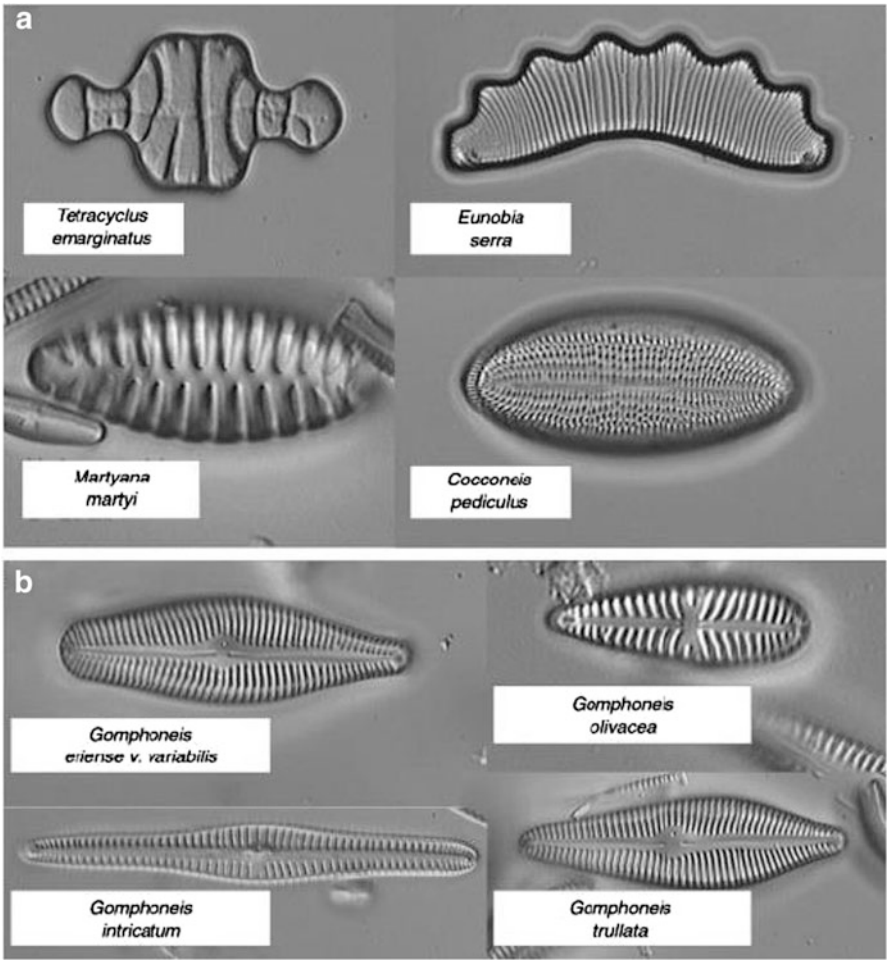


Fig. 7.3 Genera (**a**) and species (**b**) of diatoms

Depending on a type of bacteria, size of magnetosomes can vary from 35 to 120 nm. Biological mechanism of formation of magnetosomes controls accumulation of iron and biomineralization of magnetic crystals with a typical size and morphology inside membrane vesicles consisting of protein-containing lipid biolayers. Their combination with bioactive materials such as nucleic acids, enzymes, antibodies, etc. make it possible to fabricate materials with a possibility of magnetic manipulation with their biocomponents.

A probability of ordered disposition of nanoblocks of mineral crystals in intercellular or extra-cellular matrix of living organisms is clearly demonstrated by different kinds of diatoms (Fig. 7.3) [53].

Diatoms belong to a large group called the heteroconts or multi-flagella algae of *Bacillariophyta* class. They are composed of a cell and range in size from 2 μm to

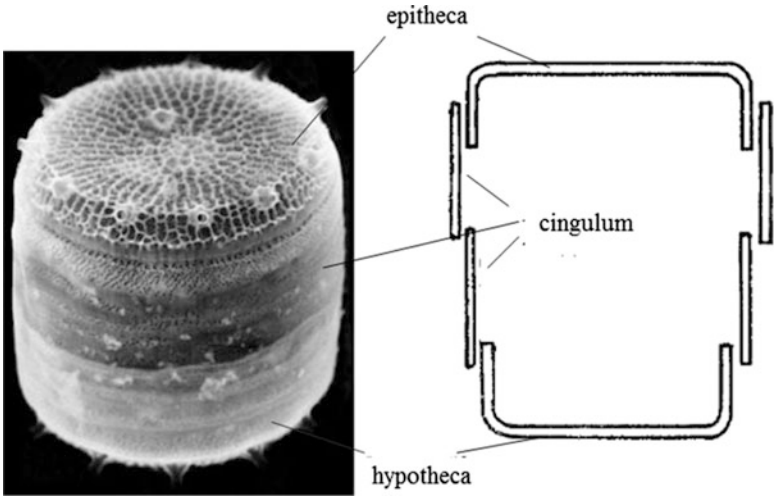


Fig. 7.4 Silica components of cell walls of *Thalassiosira tumida*

2 mm, some cells can unite into colonies [54–56]. Diatom cells are contained within a unique silica cell wall (a frustule) comprising two separate valves typically overlap one over the other like the two halves of a petri dish. One half, the hypotheca is smaller than the other half, the epitheca [57, 58]. Each of the valves contains additional ring structures called cingulum (Fig. 7.4). The life cycle of diatoms consists of three stages to compose the cell wall. The biogenic silica is synthesized intracellulary by polymerization of silicic acid taking from the environment. This material is then extruded to the cell exterior and added to the wall.

Highly specific structures such as enzymes or proteins included in bacterial membranes provide the specific interactions with reactive components during biosynthesis of nanocomposites and favor high product yield. Bacterial cells can serve as reducing agent or matrix carrier for nanoparticles. Thus, extract of bacterial culture *Rhodopseudomonas capsulata*, containing 65 % of protein, 20 % of soluble polysaccharides, and 7 % of lipids show bioreducing activity in the reaction Au (III) → Au(0) [59, 60]. At this, change in concentration of $HAuCl_4$ in the range from $2.5 \cdot 10^{-4}$ to $4.0 \cdot 10^{-4}$ M can be used to control shape of nanoparticles from spherical (10–20 nm) to nanowires (50–60 nm), respectively [60]. On the whole, the main factors of regulation of size and shape of nanoparticles in biosynthetic methods are similar to the considered above for chemical reduction: concentration of metal ions and proteins in extract, pH of a medium, time of reaction, etc. Certainly, it is necessary to take into account that additionally to specific absorption of proteins also non-specific interactions take place, which bring, for example, to isotropic growth of nanoparticles. And oppositely, by varying the ratio [$HAuCl_4$]/[extract] in the fungous strain *Rhizopus oryzae*, various shapes of gold nanoparticles were obtained (triangle, hexagonal, nanorods) [61]. Yield of the

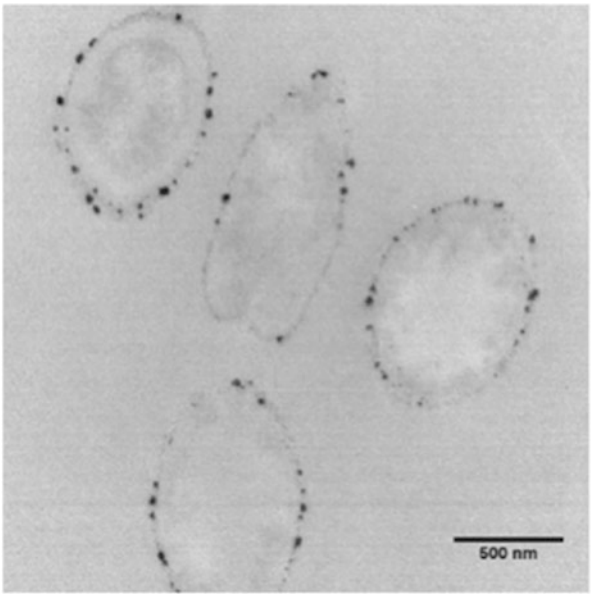


Fig. 7.5 Nanoparticles of Pd(0) in and on the outer cell parts of *Shewanella oneidensis* [64]

latter, for example, was 70–80 %. The amount of the metal adsorbed may come to 25 % of the dry substance of microbial cells [62].

It is interesting that in the case of bacterium *Shewanella oneidensis* at low concentrations of precursor there was exclusively intercellular formation of nanoparticles, and at high, predominantly deposition on walls of *S. Oneidensis* cells was observed, nanoparticles were rather coarse to 100 nm and more [63]. It is reported [64] also about deposition of Pd(0) in periplasmic space of *S. Oneidensis* (Fig. 7.5).

It should be noted that while biosorption of metal ions by microorganisms is characterized by quite high rates, bioreduction processes go far slower, and in some cases to increase their efficiency it is necessary to use donors of electrons, for example, H_2, formate, lactate, pyruvate, etc. [63, 64]. Addition of external donor of electrons was also required for synthesis of Pd nanoparticles with usage of *Desulfovibrio desulfuricans* [65] bacterium. Rather high reduction rates of Au(III) by *Pyrobaculum islandicum* in presence of H_2 is associated with hydrogenase [66]. An interesting approach is when fermentation of *Clostridium pasterianum* under anaerobic conditions causes generation of H_2, which then participates in reduction of Pd(II) to Pd(0) and the following deposits on walls of cytoplasm of a bacterium [67]. Moreover, thus produced biohydrogen can additionally serve as donor of hydrogen in catalytic activity of Pd(0). Thus, efficient catalyst can be obtained at one step.

Usually, the processes of reduction by bacteria are conducted under anaerobic conditions, and, on the whole, they are more efficient and fast than aerobic processes [68]. Though examples of the latter are also known, they are realized and important from practical point of view, for example bioreduction of $HAuCl_4$ by cyanobacteria

[69, 70] or by *Bacillus subtilis* [71]. It has been shown that intermediate product obtained during formation of intercellular Au(0) is Au(I) sulfide complex [69]. Nanoparticles of Pd(0) are obtained under aerobic and anaerobic conditions [64] under action of *S. Oneidensis*. Polyethyleneimine (PEI) or amino-enriched cationic polyelectrolytes behave both as binding molecules and reduction agents for synthesis of Au-bacterial nanocomposites similarly that when Au-composites were obtained in different ways [72, 73]. During reduction of the $HAuCl_4$ using PEI in solution the bacterial fibers become from colorless to purple that indicates the formation of Au nanoparticles on the fibers [74].

Protein environment facilities producing the complicated products both on the composition and form. Thus, when ionic silver enters in the organism it is bonded by affinic biopolymers and is reduced by biological substrates up to high dispersed metallic state [75]. Special attention is paid to soluble metal-polymer nanocomposites containing silver or other metal nanoparticles as potential antibacterial and antivirus agents.

Often in the synthesis of complex bionanocomposites individual biological molecules are preferred to whole bacterial cells. Biotemplates in addition to wide variety of shape and sizes show reproducible template structures, which make them ideal matrices for deposition of nanoparticles. DNA [76, 77] and viruses [78] biomolecules are used for production of metal nanorods, nanowires. Among proteins bacterial S-layers [79] and flagella[2] [80, 81] are successfully used. In particular, S-layers included in content of cellular wall of most bacteria are regular protein subunits, and are most widely spread structures in prokaryotic organisms. They consist of numerous copies of individual polypeptides, which spontaneously form highly organized nanoporous superlattices of different symmetry (Fig. 7.6).

Bacillus sphaericus were used to produce 405-nm gold nanoparticles spaced at 13 nm at the surface of S-layer with square periodicity [82]. Hexagonally packed S-layers of *D. Radiourans* [83] allow fabrication of ordered structures of micron sizes from regularly arranged gold nanoparticles, so that they completely follow a biotemplate structure. It is interesting to note that from the equal mixture of citrate-stabilized nanoparticles of 5 and 10 nm sizes only 5-nm colloid is bound by S-layer, while during absorption of 20-nm particles long-ordered structure is broken. Depending of shape and electrostatic properties of a biotemplate, nanobicomposite materials of various spatial arrangements are formed [53]. It is important that for modification of topographic or chemical properties of the S-layer just construction of one gene is required.

[2] Flagella – is a surface structure present in many prokaryotic and eukaryotic cells and serving for their motion in liquid medium or on the surface of solid media. Bacterial flagella thickness is 10–20 nm and length 3–5 μm. Basal body in a cellular wall drives exterior semi-rigid protein helix fiber via the body rotation, thus generating hydrodynamic force driving the cell directionally. Basal body of a flagella is a miniature electromotor, due to which bacterial cell is able to develop very high speed, 100 μm/s, i.e. more than 50 lengths of the cell body per second. The driving force takes energy from ionic gradient on the inner membrane of the bacterial cell – transmembrane potential of hydrogen or sodium ions.

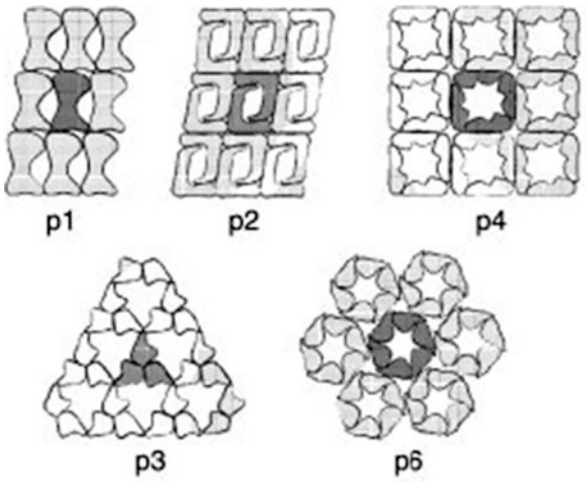


Fig. 7.6 Different types of bacterial surface layers (S layers) with a diagonal (p1, p2), square (p4), or hexagonal (p3, p6) symmetry [53]

Method of molecular engineering of biological molecules can be applied to fabricate templates having a great affinity to a certain metal ions, for example, to Au(III) [84, 85]. Introduction of a histidine fragment into flagellate protein caused a controlled deposition of Au(0) nanoparticles of 5 nm in diameter due to formation of Au(I) complex with structured amino and imidazole groups. Native flagella *Desulfovibrio desulfuricans* shows high affinity to Pd(II) complexes due to nitrogen-containing ligands, and protein fibers are completely coated by Pd (0) nanoparticles, whereas attempts to synthesize Au(0) under the same conditions failed [81]. Genetic manipulation with peptide sequence of RP437CysFliC coli allows production of RP437CysFliC line with addition of up to 12 added cysteine residuals per flagella monomer. Presence of cysteine thiol groups causes immobilizing of Au(0) nanoparticles on the surface of bacterial flagella fibers. Flagella-bound nanoparticles are in the range 20–50 nm, most suitable for catalytic applications of gold nanocomposites.

In this aspect it is also important that formation of Au-S complexes, as is known, increases catalytic activity of these systems [86].

One of the important properties of bacteria is their ability to reduce or oxidize trace elements, including toxic metals and radionuclides, which can be efficiently applied for in situ bioremediation of metal-polluted soils and water and for extraction of precious metals from dilute spent solutions [87]. To the early works in this field belong bioutilization of Pd(II) from solutions in form of Pd(0) [88] with usage of sulfate-reducing bacteria. *D. Desulfuricans* demonstrates bioreducing activity with respect to ions of the considered metals in the medium of liquid wastes and in sewage in spent car catalysts [65, 89]. Processes of reduction are implemented in electrobioreactors, containing bacterial cells immobilized on external surface of Pd-Ag electrode. Hydrogen is formed electrochemically and its transport goes through membrane of the electrode. The similar scheme was used for bioselective

utilization of Au(0), Pd(0), and Cu(II): firstly Au(III) was reduced by native biomass *D. Desulfuricans,* then Pd was extracted by pre- palladinized mass (see below), and at last, Cu(II) was deposited in hydroxide and sulfates compounds using gas emission from bacterial culture [90].

Living cells show high ability to bioreduction. It follows from this that some enzymes remain active at low pH (2–3). At the same time, bioextraction of Pd from wastes (scrap) of electronic devices was ineffective, because they, as a rule, contain a great amount of Cu^{2+} ions (25 wt% or more), which inhibit hydrogenase (see below) [90]. But if bacterial cells, which were subjected to pre-palladinizing are used, Pd(0) seeds can be catalysts for further chemical reduction of Pd(II) from Cu^{2+} containing solutions. In this case extraction is not enzymatic, but autocatalytic growth of Pd(0) clusters proceeds on cells. The same is true for *E. coli* [91], however, bacterial cultures *C. necator* and *Cupriavidus metalliduran* reduced Pd without pre- palladinizing [92].

Biomineralization can be caused by dissimilating reduction of metals. For example, for oxygenless respiration Fe(III) as electron acceptor exudates Fe (II) which, in turn, inspires formation of mineral phases such as magnetite, vivianite or siderite [93]. Other products of microbiological activity can include manganese oxide, silicates, phosphates, etc. Direct transformation of impurities in heavy metals and radionuclides in nanometer mineral phases are enzymatic reduction of U (VI) to U(IV) [94, 95], and also Au(III), Ag(I), Tc(VII), Cr(VI), Se(VI)/(IV) and Pd(II) ions [96].

7.1.3 *Fermentative Synthesis in the Formation of Nanoparticles*

The main problems in usage of biological systems are caused by difficulties in control over fineness of particles and their morphological characteristics, difficulties with obtaining biological material, etc. Therefore, approaches of fermentative methods of synthesis of metal nanoparticles are developed. For example, water solution of $AuCl_4$ was subjected to reduction with purified sulfite reductase ferment, extracted from *Fusarium oxysporum* fungous [97].

Formation and stabilizing of nanoparticles was performed in situ in presence of phytochelatin peptide.[3] Similarly nitroreductase was extracted from *Fusarium oxysporum*, which catalyzed silver nitrate reduction to silver nanoparticles [98]. Active center of this ferment, as in the case of the abovementioned sulfite

[3] The general structure of phytochelatin is (γ-Glu-Cys)n-Gly, n = 2–11. In Greek "phyto" means it presents in plants and "chelatin" is its ability to form chelate complexes with many metals including Cd^{2+}, Pb^{2+}, Zn^{2+}, Sb^{3+}, Ag^{+}, Ni^{2+}, Hg^{2+}, $HAsO_4^{2-}$, Cu^{2+}, Sn^{2+}, SeO_3^{2-}, Au^{+}, Bi^{3+}, Te^{4+}, W^{6+} ions.

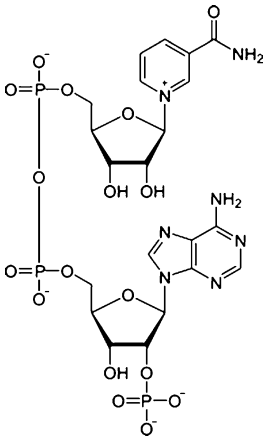
reductase is NADPH.[4] As is assumed, for catalytic formation of nanoparticles reduction of α-NADPH до α-$NADP^+$ is needed to Au(II) in intermediate processes and then of metal gold [97].

Hydrogenase activity of *D. desulfuricans* and *Escherichia coli* is found in biosynthesis of Pd(0) [88, 99] and Au(0) [68] nanoparticles. Though reduction of Au(III) is partly suppressed by Cu(II) ions, which are known as inhibitors of periplasmic, not cytoplasmic hydrogenases, finally a mechanism of biochemical ways of formation and growth of Au(0) nanoparticles stays unknown. Role of various hydrogenases in reduction of Pd(II) is studied in detail also in the case of *Desulfovibrio fructosivorans* [100]. It is assumed that enzymes serve sources of electrons for the reduction process and nucleation and growth centers of particles (see Table 7.2 [16, 27, 101–111]).

7.2 Sol-Gel Process as a Way of Production of Template-Synthesized Bionanocomposites

Bioglass® glass ceramics was first used in biopractice in early 1970s [112]. For the first time encapsulation of active ferments in sol-gel matrix was fulfilled in 1990 by mixing of biomolecules with sol-gel precursors [113], and just in several years many different hybrid bioceramic materials of this type were designed. Presently incorporation of bioactive substances into ceramic gel is widely used technique for

[4] Nicotinamide adenine dinucleotide phosphate is frequently naturally occurred co-ferment of some dehydrogenases, ferments, which catalyze oxidizing-reducing reactions in living cells. NADP accepts hydrogen and electrons of oxidized compound and serves a carrier of them.



In chloroplasts of vegetable cells NADP reduces in light reactions of photosynthesis and then supplies with hydrogen synthesis of carbohydrates in dark reactions.

Table 7.2 Biosynthesis of nanocomposites

Nanoparticles	Organism	Size (nm) and shape of nanoparticles	Reaction conditions	References
Bacteria				
Ag(0)	*Pseudomonas stutzeri*	35–46, >200	$AgNO_3$	[101]
Ag(0)	*Lactobacillus sp.*	20–50, >100, 15 and 500	$AgNO_3$	[102]
Au(0)	*Rhodopseudomonas capsulata*	10–20	$HAuCl_4$, extracellular reduction	[60]
Au(0)	*Escherichia coli*, *Desulfovibrio desulfuricans*	20–50	2 мМ $HAuCl_4$, periplasmic space, cell surface, extracellular reduction	[68]
Au(0)	*Shewanella oneidensis*	<10	$HAuCl_4$ $3H_2O$, Intracellular reduction	[63]
Au(0)	*Bacillus subtilis*	5–25	AuCl	[106]
Au (0)	*Shewanella algae*	10–20	Au(III), anaerobic conditions	[107]
Au(0)	*Lactobacillus* strains (buttermilk)	25–50, >100	$HAuCl_4$	[102]
Au(0)	*Thermomonospora, actinomycetes*	7–12	Extracellular reduction	[16]
Au(0)	*Rodococcus actinomycetes*		Intracellular reduction	[110]
Yeast				
Ag(0)	*MKY3 strain*	2–3	Ag^+, Extracellular reduction	[103]
Fungus				
Ag(0)	*Verticillium* sp.	2–20	Extracellular reduction on the cell wall	[104]
Ag(0)	*Fusarium oxysporum*	2–50	Extracellular reduction	[105]
Au(0)	*Colletotrichum* sp.	8–20	$HAuCl_4$	[27]
Au(0)	*Verticillium* sp.	2–20, 25	Au(III), intracellularly	[108]
Au(0)	*Fusarium oxysporum*	20–40	Extracellular reduction	[109]
Au(0)	*Rhizopus oryzae*	50–70, triangular, hexagonal, pentagonal, spherical, nanowires, nanorods	$HAuCl_4$ $3H_20$, Extracellular reduction	[61]
Au(0)	*Fusarium oxysporum*	7–20	$HAuCl_4$, Sulfite reductase *in vitro*	[97]

(continued)

Table 7.2 (continued)

Nanoparticles	Organism	Size (nm) and shape of nanoparticles	Reaction conditions	References
Au-Ag сплав	*Fusarium oxysporum*	8–14	Extracellular reduction	[111]
Plants				
Ag(0)	*Pelargonium graveolens* (экстракт листьев)	16–40	10^{-3} M aqua solution, $AgNO_3$	[36]
Ag(0)	*Dalbergia sissoo*	5–55, spherical	$AgNO_3$	[26]
Au(0)	*Dalbergia sissoo*	50–80, spherical, triangular, hexagonal	$HAuCl_4$ $3H_2O$	[26]

formation of bionanocomposites. Bioceramics is ideal material, because it has high rigidity, mechanical strength for fracture and impact strength. Inorganic matrices include silicon, titanium, zirconium oxides, TiO_2–cellulose composites, etc. These composites can be obtained also by dry method, like xerogels and ground powders. Based on these materials biosensors and ferment electrodes can be developed, encapsulating agents for drug delivery, adsorbents for pharmacy and cosmetic industry, photocatalysts for air and water cleaning, etc. Bioinorganic and hybrid nanostructures combine optical, electronic, and mechanical properties of inorganic materials and low cost of natural biomaterials, and are already used potentially not only in biomedical applications, but also as optical [114–118], magnetic [119, 120], catalytic and other materials [121].

Organic polymers are widely used materials for transplantation of soft tissues, though they do not have biological activity, they are most often bio-tolerant. Moderate temperatures and soft conditions of hydrolysis and condensation-polymerization of monomer alkoxides of metals and metalloids allow trapping of protein molecules without their denaturation. High stability of thus incorporated biomolecules, inertia and high S_{sp} of the matrix, its porosity facilitate heterogenization procedure, without need of covalent bonding of the matrix, and make attractive sol-gel variants for immobilizing proteins, including whole cells [122].

One more widely used among considered approaches in biomedical applications is coating of a surface of metal implants (more often Ti_6Al_4V) alloys with organic polymer, which has a great importance for integration into a bone and binding with it. Finding a mechanism of biocompatibility and effect of dynamics of physiological processes has an important place in structuring of the implant/host interface. Though titanium alloys show perfect corrosion resistant properties, metal ions can be released in physiological medium, and this can have an adverse effect on organism, especially in the case of vanadium ions.

Here were confine to analysis of the main approaches and listing of the obtained materials.

7.2.1 *Biomedical Nanocomposites*

Bionanocomposites consisting of ceramics and resolved polymeric implant are promising for successful regeneration of bone tissue [123]. One of typical examples is nanocomposite material based on glass ceramics and nanofiber degraded polymer of poly(lactic acid) (PLA) [124, 125].

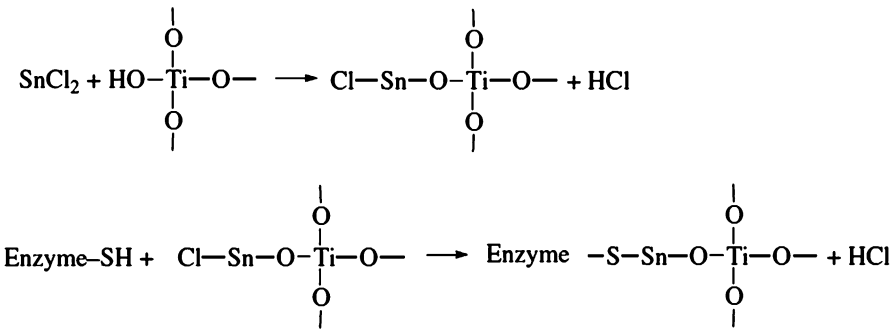
This method can be used for encapsulation into mesostructured microspheres and cells [126]. Examples of post-synthetic modifying of ceramics (after aerosol drying and surfactant removal) are rare. This way is mainly used for development of multifunctioning vector for diagnostics and therapy, producing of incorporated drugs such as ibuprofen [127], triclosan, doxorubicin [128], alendronate, zoledronate [123], etc. and biocomponents (phospholipidsliposomes, transmembrane proteins) [129, 130].

Proteins, such as copper-zinc superoxide dismutase, myoglobin, hemoglobin, and bacterio-rhodopsin are encapsulated in porous silica gel matrix prepared by sol-gel synthesis, which strongly holds these molecules without loss of their fermentative activity and without change in their spectral properties [131]. The matrices provide transport of small molecules to reaction center and transport of reaction products, strongly hold protein molecules in pores. The same way was applied for heterogenization of glucose oxidase and peroxidase used as active solid phase elements in glucose sensor.

The same way is applied to bind antibodies for potential use in medicine, immune chromatography, immune sensing, etc. For example, sol-gel captured immunoglobulins preserve their ability to bind external antigens (2,4-dinitrophenylhydrazine) from solution. Atrazine binding properties of a sol-gel matrix doped with 10 % PEO and including monoclonal anti-atrazine antibodies are studied in detail [132]. This matrix "recognized" in solution and bound widely used atrazine herbicides. It is important that there was neither leaching of antibodies, nor non-specific physical sorption of atrazine by ceramic matrix. At that, there was no decrease in activity, at least, during 2 months, while activity in a solution decreased under these conditions to 40 %. Antibodies encapsulated in these particles can be used as sensors for recognizing of specific antigens [131]. There are examples [133] of the first successful attempts to capture catalyst antibodies by sol-gel matrix and their usage: antibodies 14D9 contained in these matrices catalyze different reactions including hydrolysis of cyclic acetals, ketals, epoxides, etc. Peroxidase trapped by silica particles shows high stability as compared to non-immobilized ferments at change in temperature and pH. Encapsulation of ferments in silica gel nanoparticles allows compensation of ferment deficiency in living systems, and their usage in medicine without hazard of allergy or proteolytic reaction with almost negligible leaching. It should be stressed that there are some more advantages of these materials: high thermal and pH stability, prevention of leaching of captured proteins, fermentative reaction, which is suitable for spectral control (both in pores and in matrix), convenient storage, a possibility of re-usage, etc. Moreover, these systems provide control over morphology and sizes of particles.

Lipidic bilayered vesicles with interior water cells are widely used as models of biomembranes in supramolecular chemistry. They are often used as nanocapsules for drugs delivery or for transfection of genetic structures of nucleic acids, ferments, they are candidates for designing of supramolecular devices.

Sol-gel technique is realized for immobilising of ferments acting as bioreactors [134], for this chemically active end groups of ferments and active bonds of ceramic dopants, for example, -Sn-Cl are used. The process of immobilizing and synthesis of these materials can be presented by the following Scheme 7.2:



Scheme 7.2 Covalent binding of enzyme molecule with the TiO_2 surface

By this mechanism, for example, alkoholdehydrogenase is immobilised inside nanotubes of template synthesized TiO_2, which (cofactor NAD^+, phosphate buffer, pH8) is active in ethanol oxidization. Since that nanotubes are open from both ends, this configuration makes it possible to use them as a flow nanoreactor. There are numerous similar examples including covalent binding of antibodies for functioning of sol-gel films (Fig. 7.7) [135–141].

At the same time this causes formation of particles with uncontrolled sizes, because distribution of molecules captured by ceramic matrix is far from uniform, and kinetics of the catalyzed reactions does not obey to Michaelis-Menten laws [142].

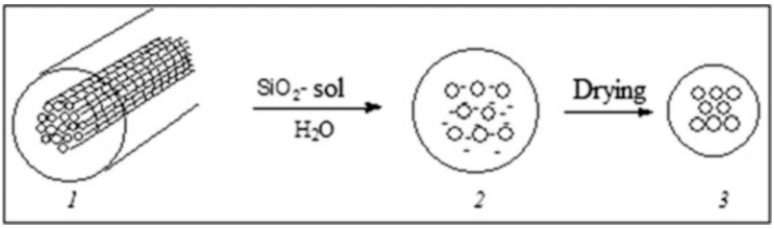


Fig. 7.7 A scheme of an organized macroporous SiO_2 structure, formed by the bacterial templates: bacterial filaments with multicellular fiber structure) (*1*); a mineralization of the inter lament space (*2*) and a macroporous replica formation by drying at 873 K (*3*)

Usage of polysaccharides as templates in sol-gel processes allows control over organized hybrid nanocomposites [143–145]. Formation of 3D fiber net [143] of a composite is due to hydrogen bonds formed between hydroxyl of macromolecules and products of TEOS hydrolysis. These materials are used in many fields as protective coatings, food package or structural composites as is shown in some examples in the Table 7.3. Biopolymers from marine mollusks can be fixed on silica gel obtained from different sources (silicic acid, sodium silicate, silicon alkoxide) forming hybrid materials. Carbohydrate polysaccharides (arabinogalactane, cellulose derivatives, etc.) containing active hydro- and carboxyl groups are capable to bind magnetite nanoparticles. Magnetitodextranes, dextranferrites, carboxymethyldextraneferrit obtained are used as biocompatible magnetic carriers, immune-magnetic sorbents, preparations for immune-magnetic separations of antigens. Moreover, ferroarabinogalactane possesses membrane-acting properties and is immune-response modulating agent [146, 147]. Although carbohydrates reveal weaker stabilizing effect than proteins but they have quite a number advantages. Thus, they do not denaturate at high temperatures and pH as well as in organic solvents.

Nanocomposite materials based on TiO_2 and poly-ε- caprolactone (PCL), containing 6, 12 and 24 wt% TiO_2, obtained in sol-gel process [148] have bioactive properties. Polymer is incorporated in the net due to hydrogen bonds between carboxyl groups and functional groups of inorganic matrix (see also Sect. 6.3). Kinetics of ampicillin desorption shows that the studied material releases high doses of antibiotic during first hours, and then slower release of the drug provides supplying dose to the end of experiment. When adding poly-N,N- dimethylaminoethyl methacrylate, soluble structures and insoluble composites form. In the insoluble ones there are about 70–75 % links of polymer amine absorbed on the surface of sol particles, while for the soluble ones the main fraction of the links is in loops [149, 150]. Thus, a net of polymer amine is formed bound to silica particles. This is confirmed by the examples of oligomer amines [151], synthetic oligopropylamines similar to those found in diatoms.

As in the case of traditional nanocomposites, hybrid thin films obtained by aerosol technique [152] by integration of (bio)organic component in microspheres at one stage form organic-inorganic materials of two types: class I (weak interaction between components) and class II are materials with strong interaction between components.

There are interesting biocomposites based on mixed oxides, for example SiO_2 -ZrO_2 or SiO_2-CaO-P_2O_5 in one matrix [153]. Bioactive materials based on calcium phosphates (hydroxyapatite and tri-calcium phosphate, see Sect. 7.3) and glass/ceramic glasses are widely used in dentistry and orthopaedics. The first of them, silica gel glasses considered as promising substitutes for bones and regenerated tissue matrix, because they have high compatibility with tissue (rigid and soft), osteoconductivity (they are carriers for regenerating material, which in-grows into implant), osteo-induction (ability to stimulate regeneration of bone tissue).

Among different types of bioactive glasses, sol-gel derivatives have been developed relatively recently [154, 155]. Comparison of sol-gel glasses with their

Table 7.3 Hybrid biocomposites based on silicon containing materials and polysaccharides for different applications [259, 379–385]

Molecular precursor or building block	Polysaccharide, cell, microorganism	Properties	Application	References
TEOS	Vinyl-modified guar gum	Absorbed properties, mechanical stability	Decontamination	[379]
Sodium silicate	Alginate	Nanometric scale, nontoxic for cells	Drug delivery	[259]
TMOS	Chitosan	Nontoxic for cells, proliferation	Repair of bone	[259]
Sodium silicate	Collagen	Bioactivity, nontoxic for cells	Tissue engineering	[259]
3-glycidoxypropyltrimethyloxysilane	Gelatin	Hydrophobic, biocompatibility, transparency	Antiwetting coatings	[259]
Sodium silicate	*Horseradish peroxidase* and glucose-6-phosphate dehydrogenase	High catalytic activity, Michaelis-Menten kinetics, nonleaching of enzyme	Materials for biosensors, immobilization ability, fermentative reactor	[380]
Silicone alkoxides, organoalkoxysilanes	Bovine Serum Albumin	High stability, optical isomer separation of D- and L-tryptophan	Monolithic columns for chromatography	[381]
Silicone alkoxides, organoalkoxysilanes	*Horseradish peroxidase*	Templates obtained by sol-gel method for solid phase lithography, stability, reusing	Optical waveguide of biosensors	[382]
MCM-41 (Mesopore silicate)	Cytochrome C	Nontoxic for cells, light internalization with living human cells	Transmembrane protein delivery	[259]
Organoalkoxysilanes	Microphytic alga *Chlorella vulgaris*	Long-term stability, heavy metal ion's affinity	Amperometric sensors	[259]
Silicone alkoxides	Langerhans insula	High long-term stability, immune isolation of transplant tissue with a minimal resection and fibrosis	Bioartificial organs	[383]
Sodium silicate and colloid silica	Cyanobacteria,	Long-term stability, photoactivity protection	Photobioreactor	[384]
Zeolite	Xylanolytic bacteria	Stable accumulation, easy-to-use	Biogas production	[385]

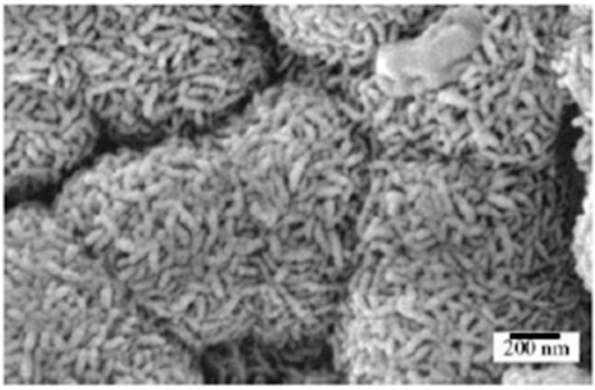


Fig. 7.8 Morphology of bioactive collagen-glassfibrillar nanocomposite [162]

metal-derivatives analogues in a wide range of concentrations (up to high content of SiO_2) makes it possible to optimize solubility and bioactivity. Sol-gel glasses can be used as powders, coatings, porous foams, and they can show good ability to formation of bone tissue in vivo [156–159]. In this view sol-gel approach has some technological advantages [160]. This method followed by electrospinning was used to prepare bioactive glasses shaped as nanofibers [161]. Diameter of these fibers is extremely small (10–100 nm) as compared with the fibers produced by spinning form melt (usually 10–100 μm). For rehabilitation medicine a new composite biomaterial is developed [162], which consists of nanofibers and reconstructed collagen matrices (Fig. 7.8). The process is carried out as follows. Sol-gel derivative of bioactive composite ($58SiO_238CaO4P_2O_5$) was transformed by electrospinning into nanometer fibers with average fiber diameter 320 nm, which were consequently treated with hydrazine and collagen, the main organic component of bone matrix, and as a result a stitched nanocomposite was produced shaped as a thin membrane.

Bioactivity of nanocomposite *in vitro* was estimated by incubation period in SBF medium[5] [163]. There are a lot of similar examples (see, for example, [164–173]).

For biomedical application also hybrid material is used based on poly (2-hydroxyethylmethacrylate) (pHEMA) and silica gel, which is obtained by sol-gel synthesis and shows some bioactivity, for example, in proliferation [174–177]. Amount of inorganic precursor (TEOS) is taken so that in a mixture with organic monomer fraction of silica gel would be 30 wt%. There is strong interaction between phases, which inhibits interphase separation: thermal stability of pHEMA improves, silica gel content has effect on decomposition temperature and has no effect on T_g of pHEMA; swelling decreases as silica gel content decreases (10–30 wt%), while increase in the latter content causes increase in *in vitro* bioactivity, which advances formation of apatite on a surface of modified

[5] SBF is artificial surrounding tissues liquid which contains ions (pH = 7.4, 142 mM Na^+, 5 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 125 mM Cl^-, 27 mM HCO_3^-, 1 mM HPO_4^{2-}, 0.5 mM SO_4^{2-}) in concentrations close to human plasma (in a typical experiment 50 mg of microporous composite was placed in biological activity at 37 °C up to 7 without renewing) [163].

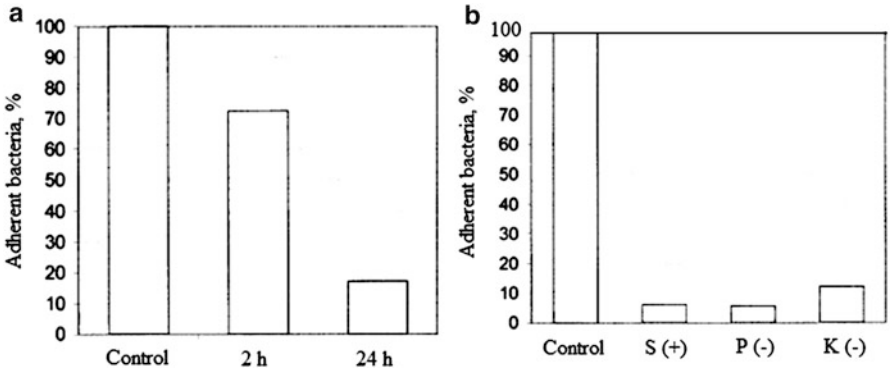


Fig. 7.9 Survival rate of gram positive (**a**, **b**) and gram negative (**b**) adherent bacteria on SiO_2 nanosol coatings with embedded colloidal silver and chlorohexidine after 2 (**a**) and 24 h (**a**, **b**) *S Staphylococcus*, *P Pseudomonas aeruginosa*, *K Klebsiella pneumonia* [171]

hydro gel soaked with biological liquid SBF. As it was noted, bioactive nanocomposites can be used for frames structuring in engineering of bone tissue [170, 172, 174, 175, 178–180].

The similar example is Ampicillin-β-lactam antibiotic active against gram-positive and gram-negative bacteria, including the form of bound at the stage of sol-gel synthesis, which is widely used for treatment of infections [181, 182] (Fig. 7.9).

The main part of application of hybrid mesoporous particles relates to multifunctional vectors. Thus, aerosol particles are important as biomedical carriers [130] in pharmacy, preparation of ointments, etc.

7.2.2 Biocomposites as Means for Drug Transfer

Reconstruction of damaged tissue, artificial substrate for cell growth, locally acting system for drug transfer are the most widely spread application fields of bionanocomposites. Nanoparticles used for multifunctional vectors of drugs (MVD) should have hydrophilic surface. As a rule, their size should not exceed 60 nm. Loading of drugs is a simple thermodynamic absorption process: precise amount of mesoporous microspheres is added to a drug solution and stirred from 5 to 20 min to 24–28 h. Amount of bound solution depends on a carrier structure and on force of interphase interaction between a matrix and drug molecules. In order to increase circulation time of these particles in blood flow, they are coated by hydrophilic elements, often PEGs. This, on one side, prevents their coagulation, and on the other hand, this eliminates or minimizes absorption of proteins on them, and makes a particle "invisible" for cells of immune system. For example, local transport of antimicrobial drugs in periodontal pocket has advantages caused by delivery of more drugs to a target with minimal damage for the organ [183]. Supplying of desirable

constant drug concentration, a decreases in systematic level of the drug, and decrease in potential harm are also reached by MVD means. Oxides used for MVD should form strong ion-covalent interaction between oxide matrix and phosphonated form of a drug or they should form biocompatible apatite-like phase.

There is increasing interest to mesoporous silica gel materials (such as MCM-41 and SBA-15) for their usage as MVD and controlled release of a drug. Their advantages are non-toxic originality, suitable diameters of pores and high specific surfaces, abundant with Si-OH bonds. Moreover, these systems show prolonged action though their capacity to drugs is relatively low, and they typically have irregular volume morphology, which is not ideal for MVD. In this connection there is an interest in hollow silica gel spheres formed on interface boundary in oil/water emulsion. To form particles with stable lamellar mesostructure, close to spherical, silica can be integrated in interlayer areas of multilamellar vesicle. Linear polysiloxane colloids such as templates formed *in situ* during emulsion polymerization are coated with cross-linked polysilsesquioxane shell at gel formation of trimethoxymethylsilane, hollow particles form by extraction of soluble core by a solvent. Colors as functional molecules were encapsulated by a silicon net and hollow particles. Hybrid particles with core-shell structure were obtained by coating of surfaces of monodisperse polystyrene beads by homogeneous coating made of silica.

In other variant, loading of a drug is done by mesoporous microspheres. This way provides release of a bound drug up to 50–70 %. Two-stage release of a drug is determined by absorption of the drug by two mechanisms (centers): molecules of the drug are absorbed on exterior surface of microspheres or in pores, the first are released fast, the second ones more slowly. Mesoporous silica gel material (powder or thin films) under biologic conditions can be degraded during several hours, destruction rate depends on content, porosity, calcination temperature and causes loss in activity of a drug [184, 185].

New type of organic-inorganic hybrids includes organic part of biological origin bound to inorganic nano-object [186–191]. In biomedical applications [192–196] intercalated systems are widely used (Sect. 6.3). Simplicity of synthesis, versatility, biodegrading and biocompatibility of layered dihydroxides (LDHs) are especially interesting for production of bionanocomposites [197, 198]. For example, combination of alginate and maize protein (zein) provides new matrices for MVD [199], which can directly encapsulate a drug, for example, ibuprofen (IBU). These MVDs were tested for controlled extraction of ibuprofen used as a model drug under conditions of transport through gastrointestinal tract. Velocity of transfer of encapsulated drug decreases with increase in zein content, probably, due to hydrophobic character of this polymer. Ibuprofen is intercalated in $[Mg_2Al]Cl$ by ion-exchange reaction. The procedure of obtaining of alginate-zein drugs is shown schematically in Fig. 7.10.

Controlled kinetics of ibuprofen extraction from alginate-zein biocomposite beads can be useful for different kinds of therapy. Simplicity of zein production as a film and a possibility of usage of small-sized LDHs provide broadening of this approach for immobilizing of different drugs.

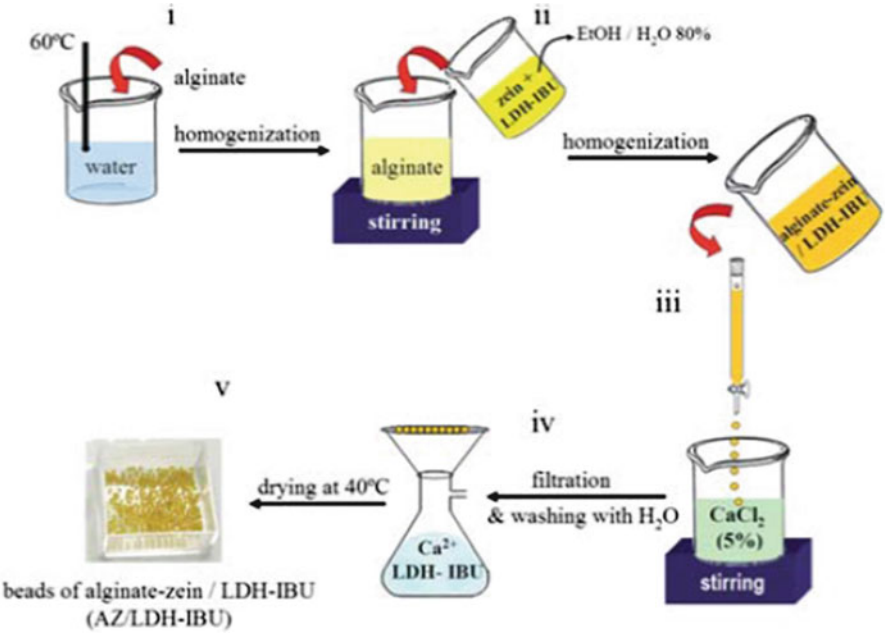


Fig. 7.10 Scheme of the general procedure employed for the preparation of the alginate–zein beads entrapping the LDH-IBU hybrid [199]

From the chemical point of view, all multifunctional vectors based on silicon dioxide (pure SiO_2, organically modified SiO_2, SiO_2-ZrO_2, SiO_2-CaO-P_2O_5 mixed oxides) are often synthesized in presence of template agents [200–209]. The SiO_2 – ZrO_2 mesoporous microparticles containing ZrO_2 between 0 and 20 mol % were far more stable in relevant biological conditions, than pure silica gel. Moreover, interesting properties were found in presence of zirconyl caused by complex formation of drugs with zirconium and by hydrophilic/hydrophobic character of the drugs (using for example hydrophobic zoledronate, containing imidazole ring or hydrophilic alendronate with propyl chain and terminal amino-group).

As regards loading of a drug, there is a common tendency: independently of a type of absorbed drug its absorption is increased as content of zirconium oxide in silica gel matrix increases. However, in composites with the highest zirconium oxide concentration (20 mol %) there are lower textural characteristics (surface area, volume and size of pores). Loading of a drug is more efficient (by 3.5 times) in the case of hydrophilic forms (alendronate), than for hydrophobic drugs (zolendronate). This result can be explained by hydrophilic neighboring of microparticle surface provided by Si-OH and Zr-OH groups. On the other hand, hydrophilic/hydrophobic character of drugs can impact the process of its release. At the same time the process of alendronate release is its fast release followed by slower more controlled process, profile of alendronate release is more sigmoid and can be factorized into three phases: delay phase, explosion phase and saturation phase.

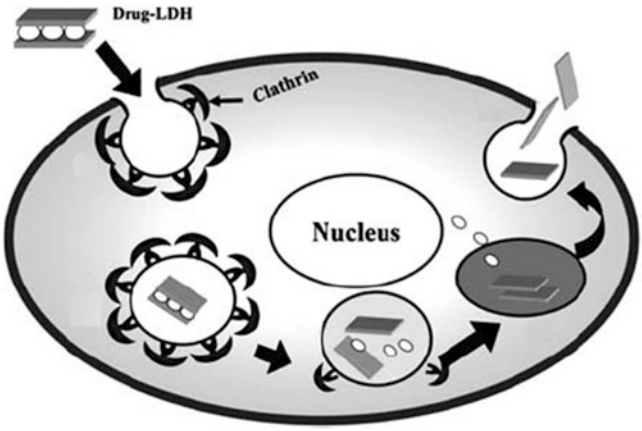


Fig. 7.11 The proposed cellular uptake mechanism of drug-LDH nanohybrids: internalization of the nanohybrid via clathrin-mediated endocytosis, transport and release of the drug in the lysosome and LDH externalization via exocytosis

This profile is caused by hydrophobic property of zolendronate, which impedes diffusion of water physiological medium into pores causing the delay phase. Immediately after release of a part of zolendronate hydrophilic/hydrophobic balance of a surface is distorted thus providing diffusion of a solvent into pores, and normal character of a drug release is accompanied by the explosion phase and saturation. It is interesting to note that amount of released drug is far lower (especially in the case of zolendronate) in the cases when drugs are strongly bound to the surface (mixed silicon and zirconium oxides), than in the case of a simple absorption (pure silica gel). Another way of control over drug release is by aggregation of liposomes with mesoporous microparticles (Fig. 7.11).

This biomimetric approach bringing to cell-like structure or "protcells" is one of elegant ways of development of contemporary multiplatform for biocompatible drug delivery. It is based on ability of membranes in a cell to control metabolism and, in particular, hinder diffusion of ions and charged hydrophilic molecules. These studies are based on electrostatic interactions under conditions of pH neutral medium between microparticles based on silicon oxide, which can be anionic in the case of non-modified silica gel or cationic in the case of silica gel modified with amines.

7.2.3 Biomineralization and Bioconcentration

Sol-gel processes is a widely spread way of formation of bionanocomposite materials by the methods similar to polymer sol-gel synthesis [210]. The main synthetic approaches developed for production of hybrid nanocomposites and discussed in detail above, are the common methods for sol-gel mineralization,

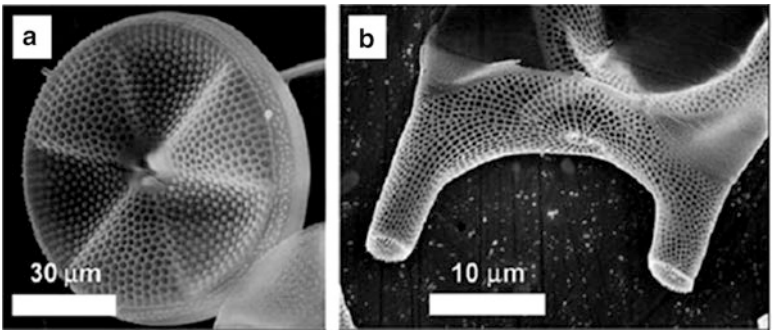


Fig. 7.12 Diatom valve view for *Actinoptychus spec*. (**a**) and *Eucampia zodiacus* (**b**) [226]

including mechanism of switching of biologically active macromolecules at the stage of formation of ceramics, glasses, and other inorganic composites (see, for example [211–214]). Many of these methods are taken from living nature and are used for realization of different bioprocesses, biosorption, biomineralization, during formation of natural composite materials with hybrid structure, for example, bone, boron-silicate glasses and diatoms, etc. [215]. Almost all metals take part in biomineralization, however, the best studied are the processes with participation of Si, Mg, Ca, Sr, Ba, and also Mn, Fe ions [216–220].

Laws of biomineralization processes are best of all studied for condensation of silicic acid in presence of water soluble polymers [149, 221–223]. Condensation of monomer silicic acid proceeds via intermediate formations of organic-silicon nanoparticles in presence of poly(allylamine) [224], poly-(1-vinylimidazol) [225]. In nature these processes happen under impact of biosiliconized organisms, diatoms and sponges, which produce more than 20 % of photo-synthetic oxygen. Diatoms are one-cell organisms with silicon exoframe, they can accumulate a great amount of silicon and use it as silica for structuring important elements of their organisms (Fig. 7.12) [226]. However, the main stages of structuring of silicon claps: capturing of silicic acid from environment, its storage in cytoplasm and transport of silica deposit to vesicle, formation of micro- and nanostructured video-specific claps are not understood. It is known that important role in biosynthesis of the claps play polymer ampholytes, silaffins, which are proteins with polyamine (3–20 nitrogen atoms) links and phosphate groups [227, 228].

Polyamines have been found in diatoms in free form, they were also extracted from silicon sponges, which stimulated studies of synthesis of new silica and composite materials and biomimetric modeling of processes in living nature.

Depending on initial configuration of polylysine (PLys) (α-spiral or β-sheet), silica nets are formed with different pore sizes consisting of aggregated spherical nanoparticles with diameter 200 nm. In the poly(allylaminehydrochloride)/phosphate – TMOS system silica also forms as a result of extraction of polymer phase in water solution [229–231].

In-cell concentration of soluble forms of silica in diatoms is from 19 to 340 mM, which exceeds significantly equilibrium concentration in solution (2–3 mM). Silica is bound with organic substances, most probably, in form of spherical particles in special silicon transport vesicles (STV), their size is about 30–40 nm [232, 233, 229]. However, it is not excluded that silicic acid presents in cytoplasm in form of soluble complexes of oligo-silicates with polymers, though it is unknown how high can be concentrations of silica in cytoplasm to do no harm to cells.

An organic matrix (template) controls nucleation processes, growth and formation of inorganic materials with perfect morphology during *bioconcentration – biomineralization*; on this basis a complex hierarchical structure of composites is developed with unusual chemical and physical properties [234]. Consequently, study of biomineralization includes solution (or, at first approach, at least understanding) of two problems: how strictly organized inorganic materials form (morphogenesis) and how these processes can be reproduced in biomimetic systems (morphosynthesis or mineralization *in situ*). There are five main stages of morphogenesis of diatoms [235–238]: formation of silica spheres of 30–50 nm in diameter (in STV); delivery of silica in STV to silicaleme and release of silica to SDV; beginning of silica deposition [239]; pore formation [240].

Understanding of morphogenesis of diatoms brought to discovery of new proteins, silaffins, which can take part in biomineralization process in SDV. Most probably, morphogenesis of porous silica structures with silaffines and polyamines has a mechanism similar to synthesis of mesoporous structures with usage of surface-active substances and block-copolymers [237, 241, 242]. Therefore, organic-silicon nanoparticles are a synthetic model of vesicles responsible for storage and transport of silica precursors in cytoplasm of diatoms, variations of composition of a stabilizing polymer and of conditions for further condensation provide a possibility for production of silica close to biogenic by composition.

Molecular recognition and molecular tectonics are very important aspects of biomineralization, however, genetic grounds of evolution of biomineralogic picture remain unknown, as is an answer to the principal question: how morphogenetic compatibility is implemented on the *biology-inorganic chemistry* interface. Most often this form of biominerals is predeterminated by spatial structure, as a result of some conformation or location of a cell. Rubber-like ormosil with dispersed Ca^{2+} ions can be obtained in sol-gel process, this composite can replace soft tissues. This relates to SiO_2-PMMA composites with incorporated Ca^{2+} ions, which show biological activity [243]. It is shown that silanol groups formed in ormosils are dominating as a factor, which controls biological activity, while the effect of dissolved Ca^{2+} ions is secondary. Moreover, in bioaggregation processes can be also included [244] such relatively complex particles as $BaTiO_3$, $SrTiO_3$, $NaNbO_3$, perovskites with ABO_3-type, synthetic analogues of which were considered in - Chapter 5. It is assumed [245] that fine mono-disperse precursors of high temperature superconducting ceramics, etc. can be obtained with the help of bacteria.

General problems of sol-gel synthesis applied to formation of organized matter include four approaches [246]: formation of self-aggregated organic matrices (transcription syntheses); cooperative assembling of ensembles, template and

building blocks (synergetic syntheses); morphosynthesis, in which organized non-linear chemical neighboring, reaction fields (static, reconstructing, transiting), and their combination (integrated synthesis) are used for generation of models. This strategy (reaction ensemble → replication → metamorphism) is similar to general scheme of mineralization. It can be illustrated on examples of template-directed syntheses of ordered mesoforms and organic clays, micro-framework structures, also with usage of bacterial templates. Especially clear this appears in reproduction of hierarchic macro-structured ordered silicates, which can be shown on example of *Bacillus subtilis* multicell fibers as scaled organic templates [246].

7.3 Intercalation Processes in Development of "Green" Nanobiocomposites

Principle of action of many biosensors and transportation means is based on surface recognition of biosystems. Nanoparticles present a variety of places for selective binding with biomolecules. By development of this surface nanoparticles can be organized for surface recognition of biomolecules and cellular structures. The recent review [247] reports on progress in the field, which deals with interaction between nanoparticle and bimacromolecule (on the example of interaction with proteins, DNA) (Fig. 7.13).

Efficient and selective interactions with biomacromolecules depend on area of receptor contacts and dynamically organized structures with high binding

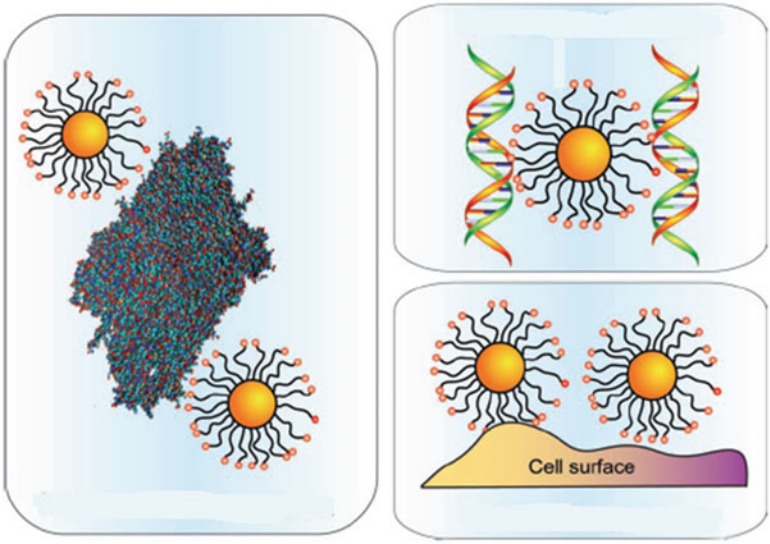


Fig. 7.13 Schematic presentation of nanoparticle interactions with proteins, DNA, and cells [247]

ability [248]. However, recognition of biomolecular structures is very difficult because of their large dimensions and complexity of the surface topology [249].

Among potential nanofillers, MMT nanoclays are widely used in producing of bionanocomposites due to their availability and deeply studied intercalation chemistry (see Chapter 5) [250]. A vast variety of modern hybrid and biohybrid materials based on clays, including those that contain living entities or their fragments, are produced. Bionanocomposites show not only improved structural characteristics, but also act as useful functional materials for ecological and biomedical purposes and other application areas. Thus, bio-objects and their components easily broaden interlayer distance of, for example, MMT (0.98 nm) to 1.10 nm (alcohols), polyethylene glycol ($M_w = 1{,}000$) to 1.11 nm, cellulose acetate butyrate to 1.13 nm, starch to 1.21 nm, glucose to 1.25 nm, etc.

In saponite L-DOPA zwitterions are vertical in interlayer distance as a monolayer of partially superimposed formations [251]. Besides, clays are ecologic and their antimicrobial properties are widely accepted in pharmaceutics, cosmetics, and food industry [252, 253]. Layered silicates from smectite family [254–258] or fiber clays, such as sepiolites are used as inorganic reinforcing components in new materials like native bone and tissues [259]. The ways of formation of biohybrid materials based on layered silicates are similar to synthetic polymers and can be presented on the example of hemoglobin intercalation in organically modified magadiite (Fig. 7.14). Preliminary intercalation of ammonium tetra-butyl widens interlayer gallery of clay (to 2.56 nm), thus facilitating access of a bulk protein molecule [140].

The most widespread types of intercalated polysaccharides, cells, microorganisms, proteins, and ferments are shown in Table 7.4.

Magnesium silicate modified by aminopropyl with intercalated DNA is exfoliated in water solutions due to protonation of amino groups with formation of

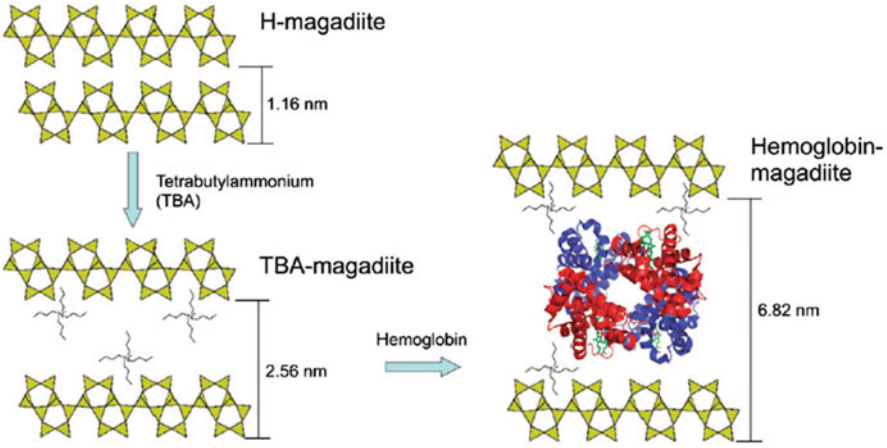


Fig. 7.14 Haemoglobin intercalated in organically modified magadiite [140]

Table 7.4 Intercallated biohybrids (polysaccharides, cells, microorganisms) for various applications [386–394]

Precursors	Polysaccharides, proteins, cells, microorganisms	Properties	Application	References
MMT, cloisite, caolin, hectorite	Starch, its derivatives	Barrier properties (for gases, water vapors), mechanical stability	Food packing	[386]
MMT	Chitosan	Adsorption and anion exchange properties	Anionic decontamination	[387]
Sepiolite	Chitosan	Anion exchange properties, mechanical stability	Potentiometric sensors	[388]
Wollastonite	Silk fibroin	Bioactivity, nontoxic for cells, enhanced mechanical properties, barrier properties (for gases, water vapors)	Tissue engineering	[389]
Cloisite-Na	Gelatin	Barrier properties (for gases, water vapors)	Food packing	[390]
Laponite	Polyphenol oxidase	Sensitivity towards citrus flavonoid, prolonged catalytic activity	Amperometric biosensors	[391]
Sepiolite	Lipasa	High stability, recycling	Fermentative reactor for biodiesel fuel	[392]
Bentonite	Algae *Ulva* sp.	High biomass loading, easy regeneration, reuse	Biosorbent for uranium (VI) recovery from water	[393]
Sepiolite	Influenza virus	Protection against antigen activity, increase the immune response	Intranasal or intramuscular vaccines	[394]

ordered meso-lamellar (a) and individual DNA molecules coated by super-thin disperse nanosheets formed due to exfoliation of the silicate by the scheme (Fig. 7.15) [260]:

However, intercalation of bio-structures is associated with some specific features. Thus, topography of light sensitive protein of bacteriorhodopsin in a purple membrane lied in 2D crystal and forming a layered composite during intercalation polymerization of methacrylate is analyzed *in situ* (Fig. 7.16) [261]. At that a polymer film can be subjected to additional cross-linking. This approach can be used for production of optically transparent biocomposites, because molecules of the purple membrane captured into the polymerized system are characterized by stability of structure and chemical composition and preserve their photochromic functionality.

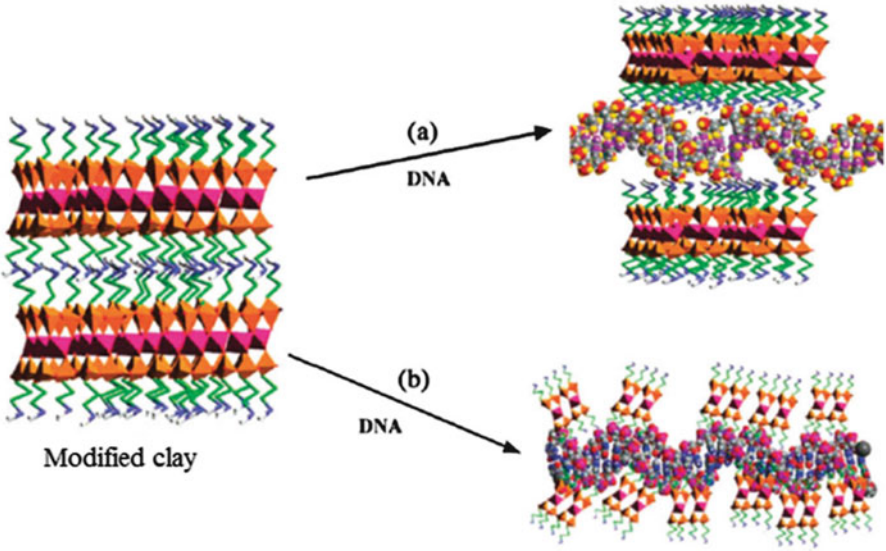


Fig. 7.15 Reassembly of aminopropyl-modified magnesium silicate organoclay layers by association with DNA leading to an ordered mesolamellar nanocomposite (**a**) or to an ultrathin organoclay covering on individual DNA molecules with dispersed nanosheets formed during exfoliation (**b**) [260]

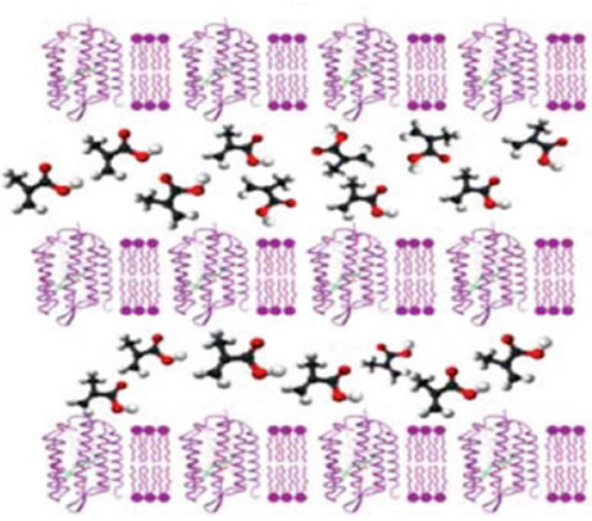


Fig. 7.16 A thin film consisting of an ordered lamellar stack of purple membrane sheets containing transmembrane bacteriorhodopsin protein molecules and intercalated monomer [261]

7.3.1 *Biodegrading "Green" Bionanocomposites*

One of the interesting research directions is production of biodegrading nanocomposites for preparing a frame for a tissue construction, therapy of periodontal bone defects and ridge augmentation, drug delivery (Fig. 7.17) [262].

Commercial starch, chitosan, cellulose, polylactide, poly(hydroxybutyrate) are used as biopolymers for thermoplastic matrices. The most often used biodegraded polymer for biomedical applications is poly(lactic) acid (PLA), because it can be obtained under controlled conditions, consequently, with predictable properties, such as elasticity modulus, ultimate strength, and rate of biodegradation [192, 195, 263–269]. Besides, PLA has high biocompatibility *in vivo* and ability to bone tissue regeneration (osteoconductivity) [270–272]. Stereoisomers and most interesting transformations of lactic acid including polymerization are shown in the scheme (Fig. 7.18).

Though PLA has good mechanical and physical properties, including biocompatibility and biodegradation, it does not have some fundamental characteristics of biomaterials. For example, in tissue engineering PLA not only should stimulate and support tissue growth, but in the process the growth and degradation rates of the tissue should be correlated. To some degree these properties can be controlled by OMMT introduction (Fig. 7.19) [262].

Nanobiocomposites of the PLA containing 3–5 % of organically modified MMT (OMMT) are obtained by mixing in a melt in two different mixers, mini-twin-rotor extruder, and in a mixer with inner dispensers bringing to different degree of dispersion, characterizing rheology of the melt. The obtained PLA/OMMT samples had percolation nets in the melt [273].

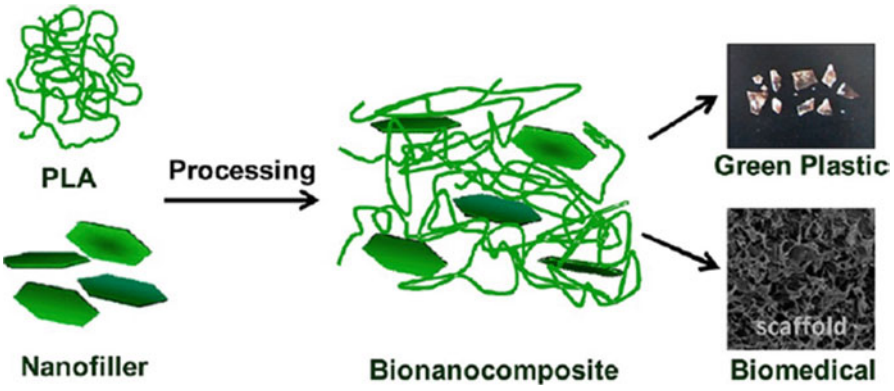


Fig. 7.17 A general scheme of synthesis of bionanocomposites based on biodegradable polymers [262]

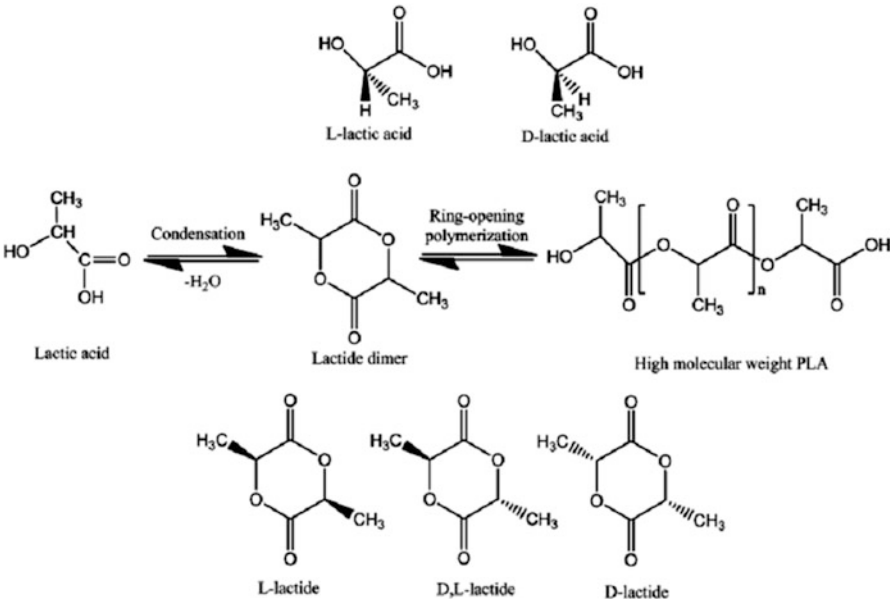


Fig. 7.18 Stereoisomers of lactic acid, and the schemes of lactic dimer, high-molecular-weight PLA and diastereoisometric forms of lactides

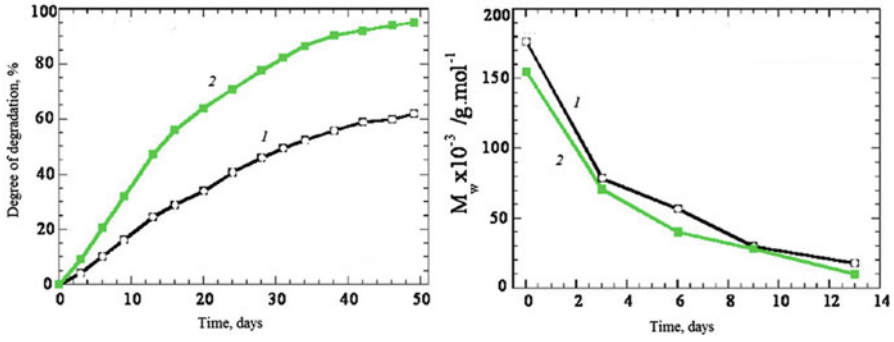
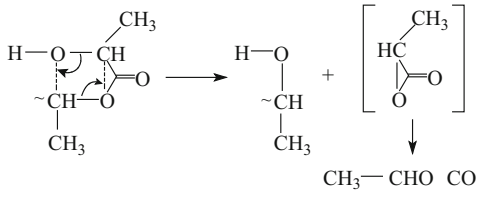


Fig. 7.19 Biodegradation ability of neat PLA (*1*) and biocomposite modified with 4 % OMMT (*2*) [262]

The main mechanism of PLA degradation includes alcoholysis/acidolysis inside, and molecular ether bond of polylactide ether groups in a chain [274]:



Traces of metals, which can present in a polymer (for example, like impurities or remnants of catalysts), such as Sn, Zn, Al, and Fe catalyze reactions of in/intermolecular alcoholysis/acidolysis and depolymerization at high temperatures: the more selective is a catalyst, the less efficient is its depolymerization effect at high temperatures. Though this concerns, mostly PLA, the similar behavior can be expected for other biopolymers [275–277]. Decomposition of plastified PLA filled with Na-MMT in nitrogen shows that an increase in a filler content causes a significant decrease of thermal stability: temperature of the maximal decomposition rate (according to TGA data) decreases continuously from 370 to 325 °C with increase in MMT concentration from 1 to 10 wt% [278, 279]. Biocomposites based on PLA also have flame-retardant properties [280, 281]. Modifying impact of organically modified clays on different physical and biological properties appears also in other "green" nanobiocomposites obtained from renewable sources, for example, polyethers: polyhydroxyalkanoates (PHAs), poly(3-hydroxobutyrate), copolymer poly(3-hydroxobutyrate-co-70 % 4-hydroxobutyrate [P(3HB-co-70 % 4HB)], etc. [253]. These materials are future candidates to biodegrading composites, they attract keen attention for medical applications, because they have properties which are not typical for known synthetic polymers, such as biocompatibility and biodegradation [282]. Mostly, lactides were used for studying of flame-protective properties of plastics from renewable sources: clay/polyether bionanocomposites [283, 284].

For qualitative measurements of antimicrobial activity of the nanocomposites gram-positive (*Staphylococcus aureus*) and gram-negative (*E. coli*) bacteria were used and antimicrobial properties of different PLA composites were studied using the disc-diffusion method by diameter of composite-inhibited zone against these bacteria. The poly(3-hydroxybutyrate) copolymer (P(3HB-*co*-4HB)) has shown a discernible microbial-inhibiting zone related to incorporated nano-clays. However, other poly(hydroxyalkanoate) composites did not show any activity even being incorporated in clays, maybe due to their special morphology. Most probably, antimicrobial properties of biocomposites reinforced by modified clay can be caused by antimicrobial activity of their substitutes, quaternary ammonium groups. These groups carrying alkyl chains can destroy bacterial cell membranes suppressing their metabolic activity and causing lysis of a bacterial cell with time [252]. Increase in clay concentration appears in stronger antimicrobial activity of the composites. According to [285], higher specific surface can be reached by increase in clay concentration on which more bacteria can be adsorbed. Stability of gram-negative bacteria against the composites can be explained by special features of the bacterial cellular structures. Biocomposites based on P(3HB-*co*-4HB) will be applied in medicine and pharmaceutics due to their biodegradation and biocompatibility. Thus, it seems promising usage of P(3HB-co-4HB) nanocomposites in view of *in vitro* decomposition for biomedical applications.

Laminar clays, such as kaolin contain 2D lamellas bound via intercalation (or intermediary) layer. Lamellas should, first of all, exfoliate or chemically separate; they work as nanofillers and should disperse in a polymer matrix. Organic clays based on smectites and less on sepiolites were intensely studied for production

of pesticide composites, which diminish loss of bioactivity caused by volatility or photodegradation of insecticides and herbicides bound with organic clays. Another important application is removal of pollutions, in particular, those having organophilic properties.

New tends in syntheses based on organic clays are related to usage of non-toxic modifiers of biological origin, for example, lecithin or various biopolymers instead of traditional quaternary ammonium surfactants. This approach is applied not only for environmental protection, but also for biomedical purposes. Usage of these nanostructural hybrids can be practically important for development of frames for tissue engineering or as adjuvants for vaccines. The future abilities of natural two-layer aluminum silicate halloysite (with clay nanotubes) mean a possibility of filling halloysite nanotubes with active ingredients, which will provide their usage in cosmetics, scent disguise, agriculture, medicine and other areas.

As is known, synthetic apatites have high affinity to host tissues and biological activity, they bring to increase in compatibility of materials due to their chemical and structural similarity to the natural apatite crystals. Nanometer apatite crystals in mineral basis of bone tissue provide higher metabolic activity than synthetic apatites. Synthesis of apatite crystals is well described with usage of vast variety of methods, including solid phase chemical reactions, for example, mechanical-chemical from the mixture $Ca(OH)_2$-P_2O_5 and CaO-$Ca(OH)_2$-P_2O_5 CaO and $CaHPO_4$ [286–290].

We shall consider a composite consisting of gelatin-poly-D, L-lactide-hydroxy-apatite nanofiber made *in situ* by hydrothermal mineralization [291, 292]. Nanofibers of hydroxyapatite (HA) are uniformly distributed in gel in a polymer matrix. There are ion interactions between Ca^{2+} HA ions and negatively charged gel functional groups in the nanocomposite. Besides, HA plays important role of a bridge binding the polymer gel and poly-, D,L-lactide in the nanocomposite, due to which dense net forms. When the polymer is removed from the composite, pure HA crystals remain. The Fig. 7.20 shows three-phase model linking two polymer chains with inorganic

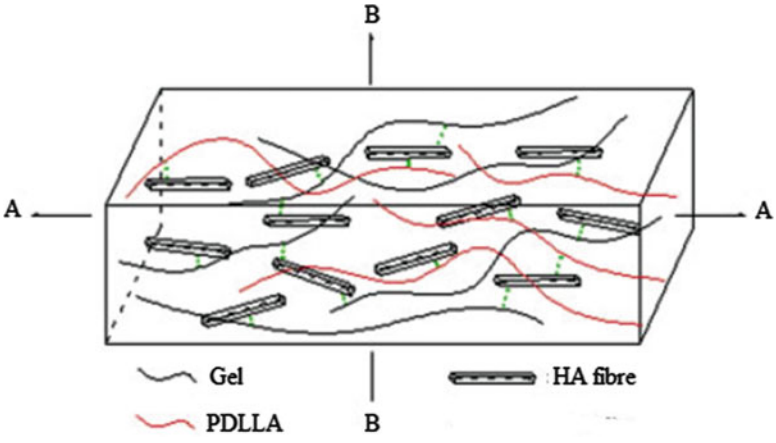


Fig. 7.20 The schematic model of interactional bonding between Ca^{2+} in HA nanofibers and negative charged functional groups in gel and PDLLA molecular chains [292]

nanofiber based on experimental results and explaining improvement of mechanical properties of the composite [293, 294].

Colloid apatite nanospheres of 2–5 nm in diameter are obtained in presence of PAA polyelectrolyte used as structurally directing agent for synthesis of calcium-deficient apatite. PAA-superpressant is a substance supressing nucleation and growth of apatite crystals during *in situ* wet chemical synthesis, widely used for production of micro and nanocrystalline apatite [295, 296] and biometric formation of its particles [297, 298].

We shall also highlight glass filler with bioactive composition with average size 320 nm generated by electrospinning process. Nanofibers with different concentrations (to 35 %) are uniformly distributed in PLA solution. The following drying and thermal pressing were used to produce dense nanocomposites, which induce fast formation of artificial hydroxycarbonate apatite layer from physiological solution. As nanofiber concentration increased from 5 to 25 %, bioactivity *in vitro* improved under artificial conditions (osteoblast response was estimated in terms of cell growth, differentiation (fitness) and mineralization).

The purpose of the future investigations in this direction is development of nanostructured, multifunctional and bio-induced hybrid materials including usage of new synthesis methods, giving of predetermined physical and mechanical properties in combination with calculated and other theoretical studies.

7.3.2 Nanocomposites Based on Polysaccharides

Polysaccharides are polymer hydrocarbon structures consisting of repeated links bound by glycoside bond, which present one of the most widely spread group of natural polymers (cellulose, starch, dextran, and chitin). Their usage in development of nanostructured hybrid materials has recently increased, because natural polysaccharides can be promising and available substitutes for non-degrading polymers due to their biodegradation, biocompatibility and availability. Hybrid nanocomposites based on polysaccharides are used for immobilizing of ferments without loss of their activity and increase in stability [233, 299, 300].

Nanocomposite based on natural montmorillonite (MMT – Cloisite) and pullulan (exopolysaccharide of microbial origin, Mn @200,000) is approbated as a suitable candidate for replacement of synthetic polymers and oxygen-barrier coatings and for production of highly wetted surfaces of new coatings [301]. In particular, oxygen barrier and wettability of US sounded pullulan composite, as well as of bionanocomposite coatings based on it, depends on volume concentration of a filler and is compatible with theoretically predicted (Table 7.5).

Cellulose consisting of repeated links of *d*-glucose structural blocks is a highly functioning, linear, rigid-linked homopolymer characterized by its hydrophility, chirality, biodegradation, wide possibilities of chemical modification, and formation of various semi-crystalline fiber morphology. According to the data of review [302], nanometer cellulose fiber materials (i.e. microfiber and bacterial cellulose) are

Table 7.5 Volume fraction of filler and oxygen permeability coefficients of coated PET and bionanocomposite coatings [301]

	$P'O_2$ [mL · μm · m^{-2} · (24 h)$^{-1}$ · atm^{-1}]	
Filler volume fraction	Total	Coating
0.017	883.24	142.32
0.046	659.76	83.25
0.073	476.06	50.98
0.098	332.15	31.79
0.123	228.01	20.27
0.145	163.66	13.93
0.167	139.08	11.65
0.188	154.29	13.06
0.207	209.28	18.37

promising for production of bionanocomposites, because cellulose is abundant, has high strength and hardness, light weight and is characterized by biodegradation. High-strength nanocomposites are produced [303] using sheets of bacterial cellulose, impregnated by phenol resin and compressed at 100 MPa. Amiloid fibers are one of the widespread important self-assembled molecular nanobiomaterials (nanowires, layered materials, gels, etc.) obtained by the bottom-up strategy [304].

Many companies produce and use biocomposites beginning from wood powder to vegetable fibers (such as *Ananase rectifolius*, *Cocos nucifera* L., *Agave* sp. and *Corchorus* sp.), for example, as components of seats, doors, panels, or in interior parts or in car boots: in Mercedes-BenzA-Class car there are 27 components consisting of vegetable fibers [305]. Information about synthesis, structure and properties of these modified natural materials is very expansive (see, for example, [306–308]). We shall just note that nanocellulose, nanostructural materials (such as halloysite clay nanotubes, modified betonites and montmorillonites) are examples of commercialized organic-inorganic hybrid bionanomaterials [309, 310]. Nanocomposites based on cellulose acetate, commercial organic clay (Cloisite30B), triethyl-acetate with different contents of antimicrobial agents (thymol and cinnamaldehyde) are cast from solution. Antimicrobial activity depends on content of ether oils in a nanocomposite, significant impact on antimicrobial activity also has organic clay [311]. Significant plasticizing effect was observed on thymol and cinnamaldehyde in cellulose nanocomposite. At last, active nanocomposites have shown a significant antimicrobial activity with respect to L. innocua, which was higher for the nanocomposite containing cinnamaldehyde, than in that containing thymol. Besides, the nanocomposite containing thymol showed higher antimicrobial activity than acetate cellulose films without nanofiller. Presence of a surfactant, organic clay, can contribute to increase in antimicrobial activity of cellulose acetate, propionate and butyrate. Cellulose acetate is most interesting for its biodegradation in combination with high optical transparency and high hardness, and with ability to obtain cast films [312].

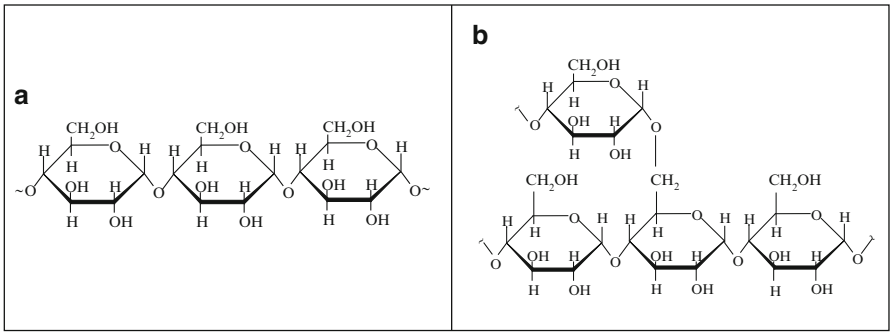


Fig. 7.21 Chemical structure of (**a**) amylose, (**b**) amylopectin

Nanofibers of bacterial cellulose (BC) are used as strong biotemplates for simple one-staged production of new nanocomposites: Au—bacterial cellulose fiber. The BC nanofibers are uniformly coated with Au nanoparticles from water suspension with usage of poly(ethylenimine) as reducing and binding by hydrogen bonds agent [313]. The possible mechanism of formation of Au-BC nanocomposites with different thickness of Au shell is proposed. The Au-BC nanocomposites are promising carriers for immobilizing ferments and for production of biosensors.

Starch grains are partially crystalline and consist mainly of two polysaccharides, glucopyranose homopolymers: (a) amylose and (b) amylopectin (Fig. 7.21). Processes of starch conversions are: acid hydrolysis, oxidization, dextrinization or pyro-conversion, and fermentative hydrolysis.

Into interplanar distance of MMT only linear polymer, amilose, can penetrate, oppositely to huge globules of branched amylopectin. At that increment of tearing load and edge wetting angle is higher for exfoliated than for intercalated nanocomposite, which is caused with higher degree of interaction of biomacromolecules with MMT particles in it (Fig. 7.22) [314–317].

Nanocomposites based on starch plasticized with glycerin are obtained during intense stirring using MMT natural smectite clay, kaolinite, hectorite or modified (by quaternary amines of fat acids of hydrated tall oil) hectorite. In all cases clay additions increase Young modulus and shear modulus. MMT and non-modified hectorite provide far higher increase in these parameters, than do kaolinite and treated hectorite, whose particles have lower aspect ratio and higher specific surface than MMT. Impact of a type of mineral on mechanical properties of a composite shows that highly hydrophylic starch molecules cannot interact with clay, and its particles cannot disperse properly [318–321].

Young modulus increases linearly with increase in intergallery space (Fig. 7.23).

Great gallery space allows large starch molecules to diffuse between layers and thus promotes more interphase interactions, which causes more intense strengthening effect [318].

Dynamic rheological properties of starch mixtures depend on their types (wheat, potato, and wax starch grains), and on origin of clays modified by different Cloisite -C: CNa^+, C30B, C10A and C15A used during gelatining of starch

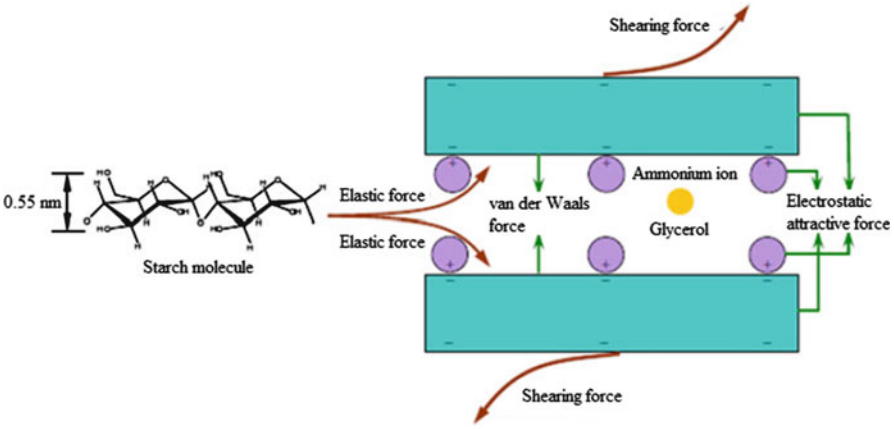


Fig. 7.22 Scheme of intercalation in the starch/clay nanocomposite [319]

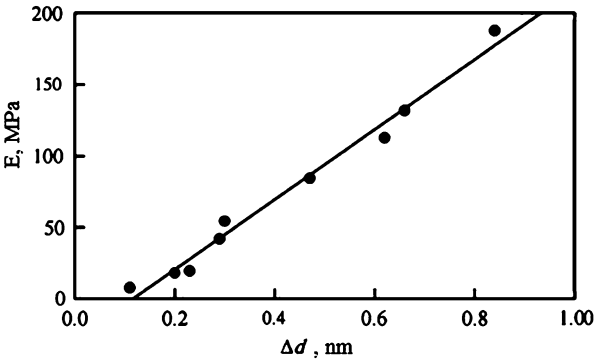


Fig. 7.23 Correlation between Young's modulus and gallery spacing (Δd) of nanocomposites with 5 wt% of various clays [318]

Table 7.6 Mechanical properties of different starches and clays [323]

Sample	Young modulus, MPa	Stress at peak, MPa	Strain at break, %
Wheat starch	28.3 ± 1.8	2.24 ± 0.04	31.7 ± 1.5
Wheat starch + 3 wt% MMT-Na	35.6 ± 0.6	2.32 ± 0.08	27.3 ± 0.6
Wheat starch + 6 wt% MMT-Na	39.2 ± 1.4	1.90 ± 0.06	21.0 ± 0.8
Wheat starch + 3 wt% SEP-Na-sepiolite	45.3 ± 1.8	2.91 ± 0.06	36.5 ± 2.1
Wheat starch + 6 wt% Na-sepiolite	67.3 ± 2.3	2.99 ± 0.04	31.0 ± 1.0

[322]. The composite obtained by combination of wheat starch and CNa + (heated to 95 °C) has shown the highest modulus [323] (Table 7.6, Fig. 7.24). This behavior is caused by two factors: interaction between clay and amilose destroys during gelatining, and gel-like material forms. However, position of diffraction maxima does not change, which points to the fact that the clay plates remains in a pack

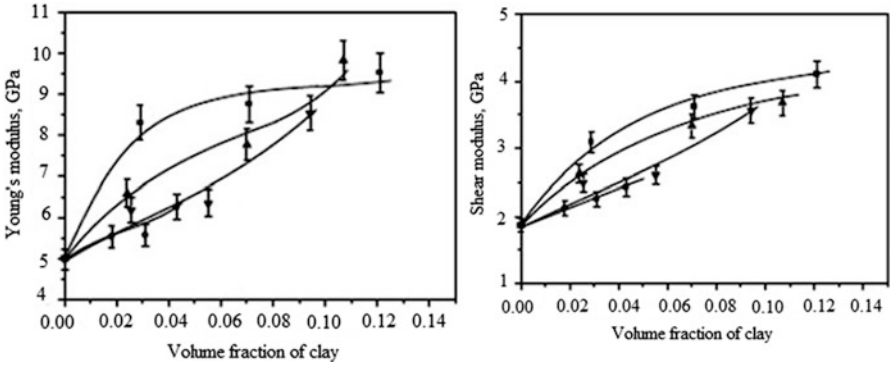


Fig. 7.24 Dependence of physical-mechanical properties of starch/clay composite on the fraction of clay (■ – starch/MMT; ▲ – starch/nonmodified hectorite; • – starch/modified hectorite; ▼ – starch/caolinite) [324]

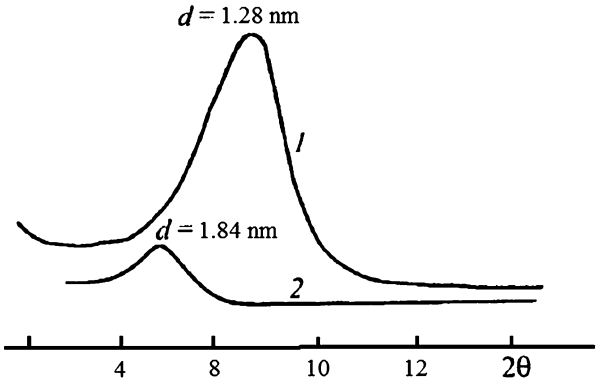


Fig. 7.25 Diffractograms of: (*1*) MMT, (*2*) starch/MMT nanocomposites [326]

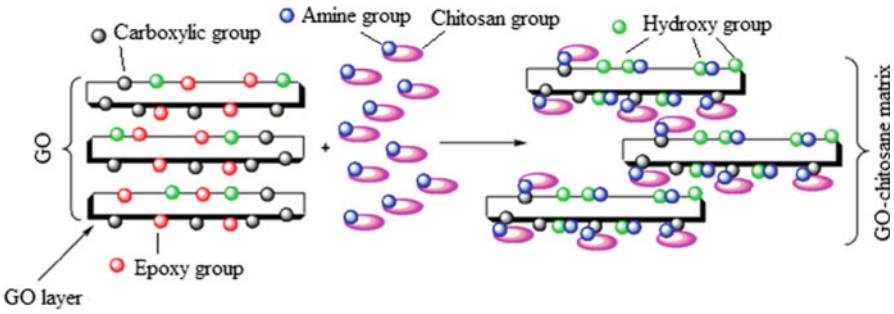
configuration, and interaction with starch molecules is only at the surface level [324].

In turn, the starch/clay mixtures are prepared in different ways. However, the most important procedures (casting, intense stirring, extrusion) relate to two different mechanisms of clay and starch mixing [317], as in synthetic polymers. Most often for this is used an extruder, since it provides a combination of shear and tensile flows. However, this instrumentation in combination with calendaring or blow molding can bring additional tension during extrusion. By this way clay nanoparticles can also be oriented in a certain direction [325].

Under conditions of intense mechanical impact in rotor-pulsation device nanocomposites are obtained based on maize starch and Na-montmorillonite (biopolymer:glycerine:MMT = 69:27.5:3.5) [326]. Interlayer distance is expanded from 1.28 to 1.84 nm (Fig. 7.25). This provides conditions for decomposition of the crystal structure of the clay mineral and formation of single plates (paste dispersion) and its distribution in biopolymer.

Aspect ratio of clay is also important factor for formation of mechanical properties. In order to reach percolation level, higher aspect ratio is needed, lower critical lengths and lower concentration of nanoparticles. Moreover, the higher degree of interlamellar exfoliation (d), the higher is elasticity modulus. A degree of intercalation/exfoliation depends on chemical modification of clay and its compatibility with starch molecules.

Chitosan (Ch) is multifunctional polymer based on polysaccharides including active hydroxyl and highly reactive amino groups. It is considered as optimal starting material for absorption [327–329]. Scheme of chitosan integration between layers of oxidized graphite can be presented as (Scheme 7.3):



Scheme 7.3 Intercalation of chitosan into interlayer space of graphite oxide [329]

We do not consider here a range of nanocomposites based on other polysaccharides, including those of dextran range (reserve polysaccharides of yeast and bacteria formed by remains of glucose are used as substitutes for blood plasma), etc.

7.3.3 *Biomedical Application of Hybrid Nanocomposites*

The considered nanocomposites are important in medicine (drugs), genetic engineering (DNA, RNA), biotechnologies, (proteins, individual cells), and in food industry [330–333].

Lipidic bi-layer vesicles are well known materials, which are intensely used as supramolecular ensembles for structuring molecular devices, widely known are liposomes coated with ceramics, so called "cerasomes" [334]. Cerasomes are novel organic-inorganic hybrids consisting of liposomal membrane with ceramic surface. Cerasomes were obtained from organoalkoxysilane pro-amphiphiles (**1** and **2** in Fig. 7.26) under conditions of sol-gel reaction [335]. Diameter of nanoparticles is 70—300 nm and 20—100 nm for **1** and **2**, respectively (see above Fig. 7.26). The results show that neither cerasome-plasmid complex responsible for transfection (~70 nm), nor cerasomes without DNA (60–70 nm) are toxic. Different additional functionalities (magnetic, luminescent, sensor) can be assigned to a capsule shell by integration of nano-Fe_3O_4, q-CdS, etc. [336, 337].

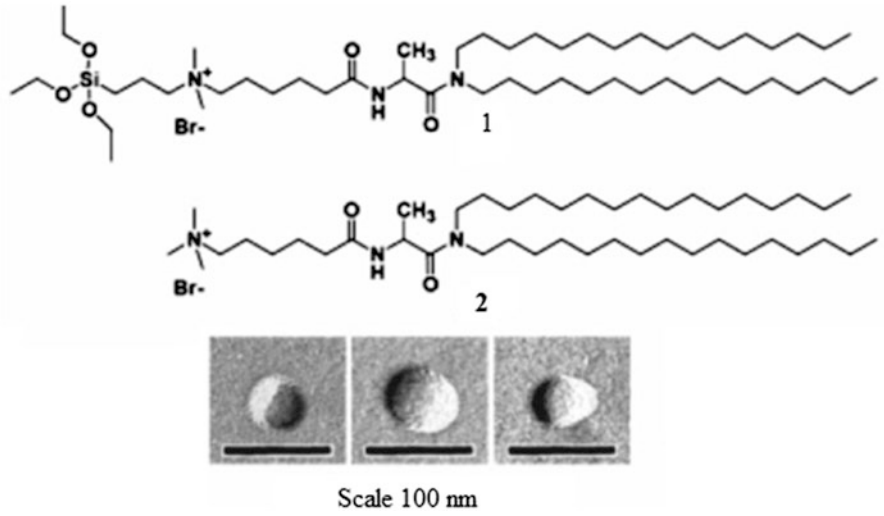


Fig. 7.26 Structure of lipids **1** and **2** and TEM microphotographs of liposomes formed with lipid **1** in water at 12.5 μM

Magnetic resonance tomography, hyperthermia, controlled drug delivery [129, 338], regeneration of bone tissue, prosthodontic treatment are accessed with usage of additional specific chemical agents: Fe_2O_3 magnetite for tomography and hyperthermia, functioning of organic silicates (binding agents) for drug delivery, for biomodifiers. One of promising applications of bionanocomposites is clinical Nuclear Magnetic Resonance Imaging (MRI). Antineoplastic chemotherapeutical drugs (chemical, biological, genetic, radiological) cause a metabolic imbalance of nucleic acids, impede biosynthesis processes and DNA functions, suppress haemopoiesis, digestion, are cardio- neuro- and nephro-toxic. The problem of development of the target drug delivery systems to a tumor cells (see Sect. 7.2.2), including magnetic carriers, is very urgent. Fixation and deposition of drugs in a tumor zone allows a significant decrease in therapeutic dose of a medicine and reduction to minimum of toxic-allergic reactions of an organism [339, 340] as compared to non-target delivery. To the methods of passive immune therapy in oncology belongs also usage of monoclonal antibodies, whose efficiency is comparable with chemotherapy at lower level of toxicity. A special place has development of magnetically controlled drugs of chemotherapeutical, diagnostic and hyperthermal activity. Two mechanisms are known by which a cell becomes multidrugresistant: increase in drugeffluxpumps through a cell membrane and increase in anti-apoptosis ways. Usage of nanotechnologies for TDDS development allowed researchers to overcome limitations of antineoplastic drugs due to increase in solubility of drugs and decrease in toxicity for healthy cells. Encapsulation of drugs in nanoparticles makes it possible to avoid drugefflux thus increasing intercellular concentration of a drug. SiRNA (small interfering RNA participating in suppression of genetic expression at the stage of transcription

and translation) can disturb work of signal cellular paths blocking genetic expression and inhibiting anti-apoptosis ways with respect to tumor therapy. Usage of nano-carriers for delivery of siRNA simplifies both kidney purification and degradation of protected siRNA chains, increasing their half-lifetime in blood. Co-delivery of drugs and siRNA together in one system can be more efficient in overcoming of cancer cell resistance, than is treatment of cancer cells by separate systems carrying either siRNA or drugs. Progress in nanometer systems of co-delivery in overcoming of cellular resistance to many drugs is analyzed in a latest review [341].

Clinical implementation of tomography in the early 1980s revolutionized diagnostics. Development of MRI accelerated designing of contrast agents. Characteristics of contrast agents and methods of their detection widely vary [342]. Thus, dendrimers are involved in biomedical studies as diverse platform for production of nanomaterials with required properties [343]. Hybrid superparamagnetic nanocomposites are used in tomography for localization and diagnostics of brain and cardiac infarction, liver damages or tumors, where nanoparticles have a tendency to accumulation in high concentrations due to a difference in tissue compositions and/or endocytosis and channel processes [344]. For MRI usually superparamagnetic contrast agents are used, which consist of nanoparticles having a maghemite/magnetite core, encapsulated into polysaccharides of dextran family with trademark *superparamagnetic iron oxides* (SPIOs), available at market. Colloid nanocomposites are also known as ultrafine superparamagnetic iron oxides (USPIO) due to extremely small hydrodynamic sizes (20–30 nm) coated with dextran, trademark Sinerem®. Significant efforts are made for development of new synthetic ways of development of contrasting agents with improved properties. For example, biocompatible hybrid magnetic dispersions are obtained from Fe nanoparticles (12 nm) by continuous laser pyrolysis of $Fe(CO)_5$ vapor [345]. A new one-pot way for production of USPIO contrasting agents by thermal decomposition of iron (III) precursors has been developed [346, 347] involving magnetic nanoparticles (10 nm) with covalent coating of polyethylene glycol modified by terminal monocarboxylic groups. Hydrodynamic size of this particle is 20 nm. MRI experiments performed on rats have shown that these particles have good biocompatibility and can potentially be used as contrasting agents. Also hybrid magnetic nanoparticles were tested for MRI [348], which were obtained by thermal decomposition of iron (III) acetylacetonate in hot organic solution and then modified by 2,3-dimercaptosuccinic acid (particle sizes vary from 4 to 12 nm). Moreover, this material with conjugated ligand of a shell has shown a perfect selectivity in cancer diagnostics with tumor selective antibodies Herceptin. Magnetic particles encapsulated in liposomes (magnetoliposomes[6]) [349] are used as MRI contrasting agents ([350] and references therein). We shall consider

[6] Advantage of magnetic liposomes as compared to USPIOs is in that various biomedical functions can be provided by conjugation with biological ligands [349].

some reviews on composites based on magnetic nanoparticles,[7] [351–358] concerning their synthesis, properties, functioning and applications, including biology [359–362]. As an example we shall describe synthesis of magnetic chitosan (Chm). Pure chitosan, 2 g, was dissolved in 400 ml of acetic acid solution. Than 0.75 g of magnetic particles were added of the chitosan solution and the mixture for subjected to ultrasonic treatment for 30 min. Then glutaraldehyde was added (as linking agent) to the mixture solution for chitosan linking, in order to prevent high degree of chitosan swelling in water solution. Adsorption of synthetic color by adsorbents is considered as a simple and economic method of its elimination from water and water deposits.

Magnetic Hyperthermia is a form of local hyperthermia with a purpose to heat very local part of a body. More than 50 years ago local magnetic hyperthermia with usage of fine magnetic particles was described [363]. Being exposed to alternate magnetic field these particles can act as a local source of heat in some place of a human body, a target.

A response of magnetite nanoparticles dispersed is compared in two different media: water solutions and 2 % agarose gel modeling bone-like thermal properties [364, 365]. A significant decrease in thermal effect was observed in the case of agarose gel, because Brownian motion in it is impeded. Thermal effect of Fe_2O_3 -SiO_2 and non-capsulated Fe_2O_3, immersed in agarose is similar for both cases, which points to preserving of magnetic properties of magnemite nanoparticles at encapsulation into mesoporous microspheres.

In the recent years a hope appears to realizing of efficient method of medical treatment of cancer. Heating of magnetic oxide particles with low electric conductivity in external alternate magnetic field proceeds due to loss of paired spins in a particle during re-magnetization, or due to friction losses if particles rotate in a medium with a certain viscosity. Induction heating of magnetic oxide particles (by eddy current) is insignificant due to low electric conductivity [366]. Acceptability of magnetic nanoparticles coated with dextran for hyperthermia of mouth cavity [367] is tested in combination with genetic therapy and hyperthermia with usage of cation liposomes, i.e. liposomes filled with magnetic nanoparticles [368]. Hybrid nanocomposites with bimodal antineoplastic functionality are prepared [369] on the basis of iron and porphyrin oxides nanoparticles and are active in combined medical treatment by photodynamic therapy and hyperthermia.

A significant role in structuring of ensembles based on biomodified nanoparticles play specific bimolecular interactions [370–373]. Thus, DNA can be used for assembling of gold nanoparticles in dimers, trimers or higher

[7] In the last decade technique of preparation of magnetic microspheres has been developed and optimized, including *in situ* formation of core-shell structures, different types of emulsion polymerization, linking, etc. Most often low-dimensional magnetic particles are coated during suspension polymerization. However, these particles have a wide distribution by shape and sizes of magnetic fractions. Commercial magnetic microspheres are obtained by deposition of magnetic nanoparticles into porous polymeric latex and insulating them by a polymer layer. Though this method is laborious and time consuming, the obtained nanocomposites have high homogeneity and high saturation magnetization, and they meet biotechnological demands [351–358].

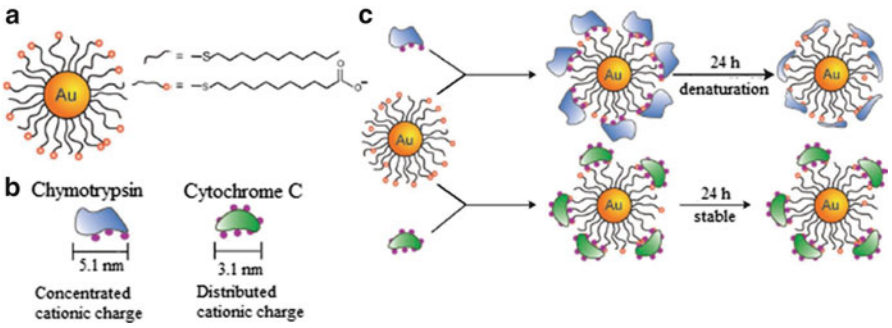


Fig. 7.27 (**a**) Mixed monolayer gold nanoparticles featuring a hydrophobic interior with carboxylate end groups. (**b**) Schematic depiction of protein electrostatic surfaces (**c**) Protein–particle assembly of gold nanoparticles with chymotrypsin and cytochrome C [374]

aggregates. Similarly, gold nanoparticles with chemosorbed antibodies [372, 373] or disulfide biotin analogues [389] can be linked by introduction of bivalent antigens and streptavidin, respectively, for formation of aggregated structures of nanoparticles.

Ensemble of nanoparticles provides access to spatial-dependent modulation of optical, electronic and magnetic properties of the ensembles for biological applications. For example, nanoparticle-protein ensemble can be used for control of interparticle space [374] and morphology by choice of respective protein size, its shape and charge. Thus, two types of proteins, cytochrome C and chymotrypsin (ChT) used for structuring composites (Fig. 7.27) show different behaviors on the surface of carboxylate functioned Au nanoparticles. ChT is developed on surface of a nanoparticle and works as linear polymer, while CC preserves its native conformation on the surface of the nanoparticle, this behavior remains in a solid state (Fig. 7.27c).

Brief analysis of the problem explains interest to action of molecular mechanisms in bionanocomposites expressed not only by biologists, but also chemists, specialists in synthesis of novel materials. A possibility of using organic-inorganic nanoparticles for synthesis of new composite biomaterials of different morphologies has been studied in detail. Stabilization of the obtained permolecular structures is realized due to multiple hydrogen, and often donor-acceptor bonds with participation of surface groups and donor atoms of organic polymers. Encapsulated ceramic nanoparticles can be used as biosensor devices and for development of vaccines, etc. The most important sides of this problem concern biomineralization of mixed-valent poly-cored structures and clusters in biology (especially iron-oxo, molybdenum-oxo, and manganese-oxo structures), the way of small molecules activation with their participation, biosorption, environment control, biomedicine [375]. Special attention of researchers is focused on biosensors with optical, electrochemical and magnetic detection systems. Magnetic properties are used in nuclear magnetic resonance and hyperthermia. Different kinds of nanocapsules are applied for drug delivery. In our opinion, usage of hybrid nanoparticles and

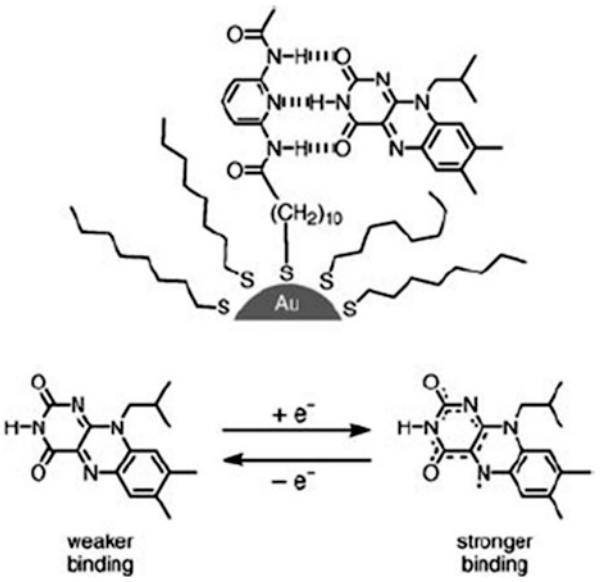


Fig. 7.28 The electrochemically controlled recognition of flavin by a pyridinediamide-functionalized nanoparticle [378]

nanocomposites in biocatalytic processes, one of mainstreams of development of bionanocomposites.

Graphene is the newest uni-atomic 2D graphite carbon system, ascending star in materials science and physics of condensed matters [376]. It was noted on the example of chitosan immobilizing in interlayer distance of low-layered graphene oxide, that this material is promising also for biological application.

Combination of metal nanoparticles with biomolecules can provide electronic or optical transformation of a biological phenomenon for development of new biosensors [377].

Due to ability of biomaterials to complementary recognition, functioning of nanoparticles with biomolecules can bring to specific interaction nanoparticle—biomolecule, i.e. to self-assembling and complex architecture. Variation of chemical properties of a biomolecule can be used for control over interaction of a modified nanoparticle with environment. Thus, a break of a bind between a bioorganic molecule and functioned surface of a gold nanoparticle can be electrochemically controlled, because during electric reduction of a derivative flavine more stable hydrogen binds are created (Fig. 7.28) [378].

Development of integrated materials based on biomolecules and inorganic nanoobjects and incorporation of these systems in functional devices is a basis of nanobiotechnology (Fig. 7.29) [22].

Thus, substantial progress has been made in this direction, however much remains to be learned. There is no general methodology for construction of bionanocomposites hierarchically organized in terms of structure and functions and true understanding of mechanism of their operating, etc.

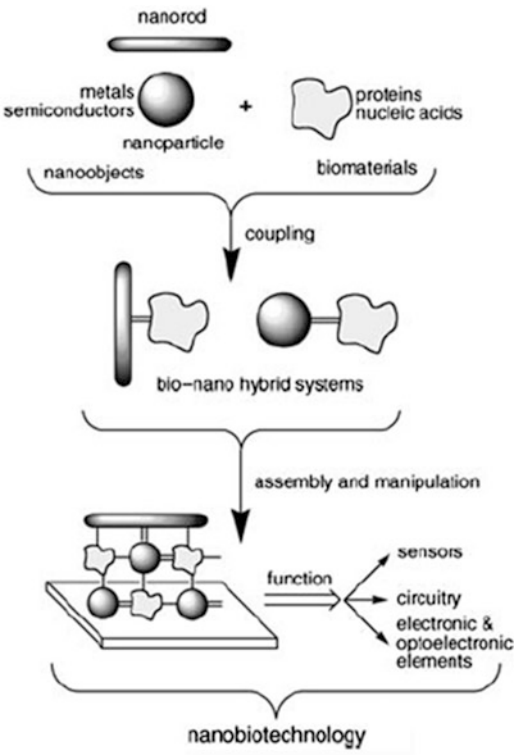


Fig. 7.29 Generation of biomolecule–nanoparticle conjugates to yield functional devices [22]

Future research will be devoted to the development of novel nano-structured, bio-inspired hybrid materials with predetermined physical and bio-mimetic properties, including the use of novel methods of synthesis, the improvement of mechanical behavior, accompanied by the application of simulation and other theoretical studies.

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