Advanced biohybrid materials based on nanoclays for biomedical applications

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ABSTRACT

Bio-nanohybrids prepared by assembling natural polymers (polysaccharides, proteins, nucleic acids, etc) to nanosized silicates (nanoclays) and related solids (layered double hydroxides, LDHs) give rise to the so-called bionanocomposites constituting a group of biomaterials with potential applications in medicine. In this way, biopolymers, including chitosan, pectin, alginate, xanthan gum, ι-carrageenan, gelatin, zein, and DNA, as well as phospholipids such as phosphatidylcholine, have been incorporated in layered host matrices by means of ion-exchange mechanisms producing intercalation composites. Also bio-nanohybrids have been prepared by the assembly of diverse bio-polymers with sepiolite, a natural microfibrous magnesium silicate, in this case through interactions affecting the external surface of this silicate. The properties and applications of these resulting biomaterials as active phases of ion-sensors and biosensors, for potential uses as scaffolds for tissue engineering, drug delivery, and gene transfection systems, are introduced and discussed in this work. It is also considered the use of synthetic bionanocomposites as new substrates to immobilize microorganisms, as for instance to bind Influenza virus particles, allowing their application as effective low-cost vaccine adjuvants and carriers.

Keywords: biohybrids, bionanocomposites, clay minerals, sepiolite, biopolymers, medical applications, Influenza vaccines.

1. INTRODUCTION

The development of functional hybrid materials, based on the bottom-up assembly at the nanometric scale of polymeric moieties with nano- and micro-metric sized inorganic solids, is widely known especially for clay-based materials.[1] The growing relevance of the resulting nanoarchitectures is due to the possibility to obtain advanced nanostructured materials whose properties cover a wide range of applications, including electrical, electrochemical, optical, optoelectronic, magnetic, catalytic, selective molecular adsorption, ionic-molecular recognition or controlled release of bioactive species.[2-7] Not only is the hybrid materials class receiving increasing attention due to its functional properties, but also for its capacity to substantially improve the mechanical, rheological, thermal, and barrier properties as compared to conventional materials. An outstanding example of these improvements is achieved by polymer-clay nanocomposites[8-10] and more recently by the so-called bio-nanocomposites in which the polymer is of biological origin.[11-14] These materials also present an important role in the development of biomimetic and bioinspired materials.[15-18] Actually, Nature creates such fine structures based on building block combinations and self-assembly processes. Therefore, those observed structures in biological systems have been recognized as practical tools and inspiration for fabrication of advanced materials.[19] Many important advances using biomimetic approaches involve carbonates and phosphates, for instance to prepare artificial nacre, bone, and other bionanocomposites[20-27] but silica, silicates and polysiloxanes also offer a viable alternative for preparation of biohybrids since their chemistry is extremely versatile, allowing the formation of hierarchical superstructures, supramolecular materials and other multifunctional bioinspired systems.[1,11,19,27-31] The present contribution focuses on the diverse approaches to prepare biohybrid materials specifically based on clay minerals, by assembling of smectites and sepiolite to molecular and polymeric species of biological origin, with the aim of developing suitable nanostructured materials for potential biomedical applications.

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2. ASSEMBLING BIOPOLYMERS TO CLAY MINERALS: BIONANOCOMPOSITES

It is widely known that synthetic polymers derived from petroleum are commonly used for nanocomposites preparation. The use of biopolymers, instead of synthetic polymers, represents a major advantage as these materials combine diverse biopolymers (proteins, lipids, polysaccharide, etc.) with inorganic solids (silica, silicates, phosphates, carbonates, etc.) giving rise to more ecological materials. These bionanocomposites often mimic the composition, structure, and behavior of natural hybrid materials present in different living organisms.[15-18,21] Scientific research in the field of biohybrid materials, and particularly in bionanocomposites, is experiencing a remarkable growth, which is pointed out by the exponential evolution of scientific publications in the last years, as typically shown for emerging lines (Figure 1).

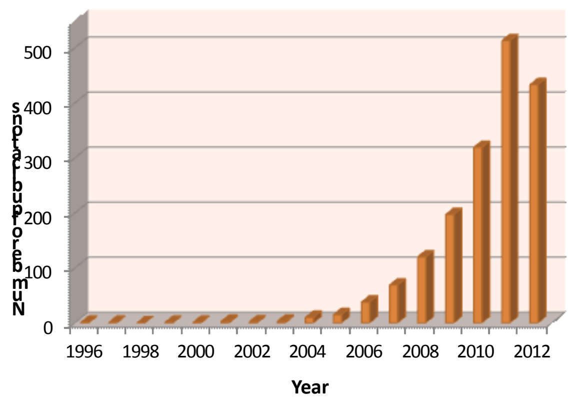

Figure 1. Evolution of the publications number of bio-hybrids/bio-nanocomposites. Data from SciVerse Scopus (September, 2012).

The bio-hybrid nanocomposites lie on the borderline between the inorganic and the living world, being of extreme importance due to their non-toxic, biocompatible, and biodegradable behavior, properties that place this class of materials as good candidates for potential biomedical applications (tissue engineering, artificial bone, genetic therapy, etc.) among other advanced applications.[5,6,11,12,32-34] Another important point refers to the benefit associated with the use of biological sources of polymers to generate the so-called *green plastics* or *green nanocomposites*, avoiding therefore the indiscriminate consumption of petroleum derivates and enhancing the biodegradability as opposed to the non-ecological conventional plastics.[35] In this context, our group has pioneered the innovative development of biohybrid materials based on layered or fibrous clay minerals combined with biopolymers such as polysaccharides (chitosan, alginate, pectin, carrageenans…), proteins (gelatin, zein), nucleic acids (DNA) and lipids (phosphatidylcholine).[11,21,36-46]

Typical clay minerals used in the preparation of these bionanocomposites are smectites (montmorillonite, hectorite, saponite, beidellite, etc.) which are 2:1 layer silicates whose structure is formed by the repetition of octahedral alumina sheets sandwiched between two tetrahedral silica sheets (Figure 2a). Isomorphic substitutions of silicon, aluminium and other metal atoms in those sheets by ions with lower charge result in a net negative charge that is compensated by cations in the interlayer region. Smectites show swelling capability and the interlayer cations can be easily exchanged with other cations, including organic cations and positively charged biomolecules. Other clay minerals that deserve more and more interest for bionanocomposite preparations show microfibrous morphologies, such as palygorskite and sepiolite (Figure 2b). Sepiolite is a hydrated magnesium silicate[47,48] whose structure is composed of ribbons of a 2:1 phyllosilicate structure with a discontinuous sheet of octahedral magnesium oxide hydroxide sandwiched by tetrahedra silica sheets. These building blocks form channels and tunnels that are accessible to water and other small molecules.[49] Sepiolite also exhibits free silanol groups (Si-OH) along the external surface of these channels, which are susceptible to interact with diverse functional groups via hydrogen bonding. This silicate shows a low cation exchange capacity related to the existence of isomorphic substitutions as occurs in smectites, being also able to interact with charged species.

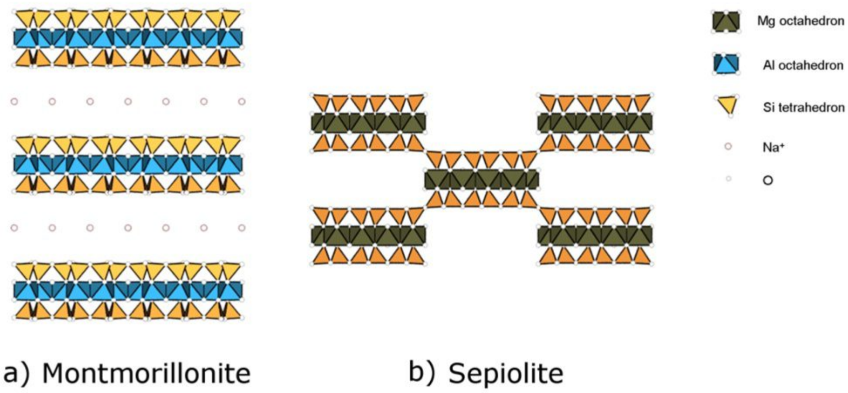

Figure 2. Structural representation of smectite layered silicate (a) and microfibrous clay sepiolite (b).

The firstly reported clay-based bionanocomposites refer to the intercalation of chitosan in layered silicates.[38] At low concentrations of chitosan in slightly acid aqueous solutions the intercalation of the polymer progresses by a cation-exchange reaction until incorporation of a monolayer of the biopolymer in the interlayer region of montmorillonite. The use of high amounts of polysaccharide exceeding the cation-exchange capacity (CEC) may lead to the uptake of chitosan in a bilayer configuration, being the excess of intercalated biopolymer accompanied of counter-ions in order to compensate the excess of positively charged groups of chitosan chains. In this situation the initial CEC of the clay is reversed to an anion-exchange capacity (AEC) in the resulting bio-nanocomposite. These AEC properties are of special interest for the development of active phases in electrochemical sensors for anions detection.[50] In the same way, it is possible to prepare bionanocomposites based on the assembly of chitosan to sepiolite.[39] The resulting materials show improved mechanical properties and also functional properties related to their anionic exchange capacity, which could be of interest for electroanalytical applications.

Another group of bionanocomposites refers to the assembling of collagen and gelatin to different types of inorganic substrates.[40] In the case of clay-based bionanocomposites it has been observed that sepiolite can control the cristallinity of the gelatin matrix, having an important impact on the mechanical properties of the resulting bionanocomposites. At 20% mass fraction of sepiolite the elastic modulus of the bionanocomposite increased by a factor of 2.2 as a result of both the mechanical reinforcement of the silicate inside the polymer matrix, but also as a result of the crystallinity variation induced by the clay particles.[51,52] This last group of bionanocomposites can be of interest for biomedical applications especially in tissue engineering related to the development of scaffolds for bone regeneration.[40] For these types of applications it is required that the bionanocomposites are provided of adequate macroporosity and good mechanical properties. The development of such types of bionanocomposite materials exhibiting a hierarchical porosity, with micro and mesoporosity, together with macropores of tens and hundreds of micrometers requires their conformation as foams. Amongst the diverse foaming techniques the most common procedure in the case of bionanocomposites consists in the application of freeze-drying or supercritical drying processes to biopolymer-clay hydrogels.[46] In this context, we have applied this methodology to the development of diverse ultra-light foams based on bionanocomposites that combine polysaccharides and proteins with clay minerals, mainly sepiolite. Several patents[53-55] based on these results indicate the different fields of application of this class of materials, including acoustic and thermal insulation, and packaging materials. In biomedical applications these biohybrids can be of interest as carrier of pharmaceutical and biological species. In this way, biohybrid foams can serve as support of biological entities or even living organisms to cellular solids as for instance microalgal cells.[23,46] Functional proteins and enzymes may also be introduced in the bionanocomposite foam by mixing them with the starting components prior to freeze-drying. In this last case, the biocompatibility of the foams helps to preserve the catalytic activity of entrapped enzymes allowing the development of bioreactors. A recent example of this application is a macroporous PVA foam in which the reinforcing agent is a phospholipid-modified sepiolite used as support of urease enzyme, which introduces the catalytic functionality in the system.[56]

3. BIOMIMETIC & SUPPORTED MEMBRANES

Inspiration from naturally occurring structures is an interesting source of active and multifunctional smart materials systems. In this context, the biological cell membrane is of special interest because it hosts a great variety of molecules acting as receptors in the interaction with the external environment and therefore, can inspire diverse functional architectures for the development of novel biotechnological tools. Thus, bioinspired materials based on solid supported bilayer lipid membranes are applied in technological fields such as biosensors, artificial photosynthesis, or drug delivery. The key to their success lies in the close resemblance to cell membranes and the accompanied capacity to accommodate biological entities with diminished loss of biological activity. Our group has developed a new class of biohybrid materials based on the interaction of phospholipids with clay minerals that results in biomimetic interfaces which allow for the association of different types of biological species such as enzymes, proteins, virus particles, or toxins and their exploitation in technological relevant areas such as biosensing, influenza vaccination, or as toxin sequestrants. Lipid adsorption on montmorillonite can be performed either from liposome suspensions or from organic solutions (Figure 3). The acidic character of interlayer water bonded to exchangeable cations may interact with the phosphate group of the PC molecule and turn the entire lipid molecule cationic which would enable the cation exchange mechanism.[44] In the case of sepiolite the assembling with the PC molecules occurs via hydrogen bonding with the silanol groups on the external surface of this fibrous clay, making possible the incorporation of PC as mono- or bilayer that cover the external silicate surface.[44] The resulting materials can be used as biomimetic interfaces (biointerfaces) for adsorption of enzymes, such as the cytoplasmic enzyme urease and the membrane-associated enzyme cholesterol oxidase. The excellent enzyme stabilization properties of the sepiolite supported lipid matrices were explored for the construction of urea sensors and cholesterol bioreactors.[45] In the case of the cholesterol bioreactor, the presence of superparamagnetic magnetite nanoparticles previously assembled to sepiolite,[57] allows the easier recovery of the bioreactor from the reaction media with the help of a magnet.[58]

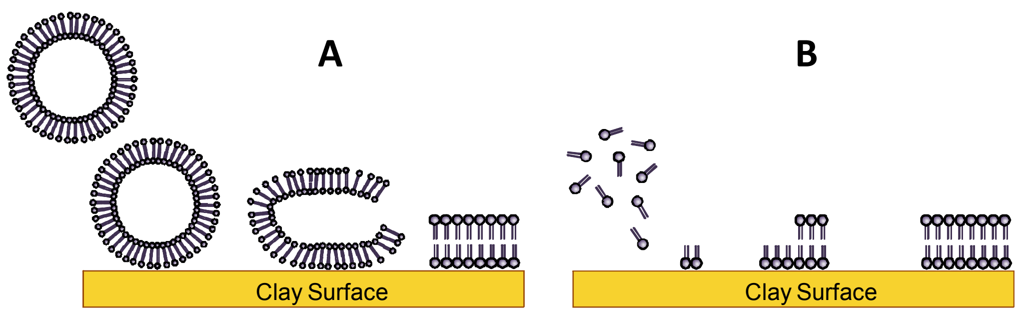

Figure 3. Scheme of PC adsorption on a clay surface from aqueous solution by liposome deposition (A) and from ethanol phase by molecular self-assembly (B). Adapted from Ref. 59.

4. DNA AND VIRUSES SUPPORTED ON CLAYS

A further step in the development of clay-based materials at the frontier of biological and mineral worlds refers to the interaction of DNA and virus particles with clay minerals. Thus, nucleic acids (low molecular weight DNA from salmon sperm) can be spontaneously adsorbed on the external surface of microfibrous sepiolite through hydrogen-bonding interactions with external silanol groups present on this silicate following a L-type adsorption isotherm (Figure 4A).[60] These interactions provoke the agglomeration of the fibrous clays and the DNA biopolymer as shown in figure 4B. We have observed a protection of the adsorbed DNA towards the degradation by the action of DNase enzyme. This type of supported DNA-clay systems is potentially of interest in view to carry out non-viral gene transfection.

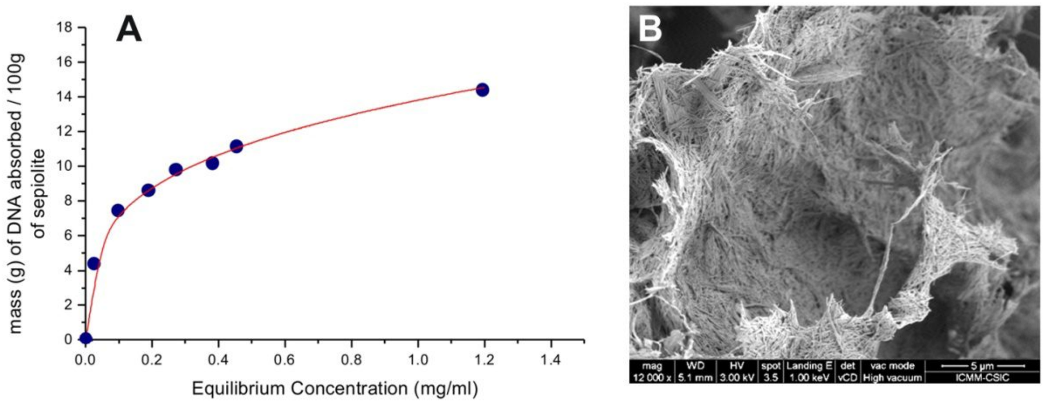

Figure 4. Adsorption isotherm (298 K) of DNA on sepiolite from aqueous solution (A) and FE-SEM image of the DNA-sepiolite nanocomposite (B).

Unmodified sepiolite is not able to retain viral particles, as the virus agglomerated in large clusters on the mineral surface. However, bionanocomposite consisting in the assembling of negatively charged natural polysaccharides (e.g., xanthan) or phospholipids (e.g., PC) with sepiolite allows for the incorporation of Influenza virus particles resulting in novel bio-hybrid systems. For instance, xanthan-sepiolite bionanocomposites are able to assemble Influenza viral particles with a homogeneous distribution on the modified fibres.[43] The presence of negatively charged sites from the xanthan biopolymer assembled to the silicate sepiolite facilitates its binding to virus particles. The integrity and bioactivity of these supported viral particles are preserved and water dispersions can be directly used as intranasal or intramuscular flu vaccines and experiments carried out in mice demonstrate that the virus/bio-nanocomposites induce the formation of specific antibodies and protect against Influenza virus infection acting as a new and efficient co-adjuvant of vaccines.[43] In an alternative approach, sepiolite-PC biohybrids can be also used as biomimetic interfaces for the immobilization not only of the entire viral particle but also of specific surface antigens as the haemagglutinin (HA) or neuraminidase (NA) from influenza virus.[61] It could be shown that the sepiolite supported lipid membrane afforded an environment where those surface proteins showed comparable activity to its native environment and therewith, one can speak about biomimetic association of HA and NA on the sepiolite-lipid hybrid.[59] A more practical issue in vaccine manufacturing is the thermal stability of the associated antigenic compound, which may become especially critical in countries of the Third World, where the cold-chain for vaccine preservation is not always assured. It could be shown, that influenza virus adsorbed on the sepiolite biohybrid preserved its neuraminidase activity as well as its immunogenic potential until 50 ºC in a higher extent than the free virus and the viruses stabilized on the conventional carriers based on aluminum hydroxide gels.[61] Moreover, these results may be of interest for other novel biomedical applications of these biohybrids such as uses in the development of biosensors, bioreactors, or drug-delivery systems.

5. CONCLUDING REMARKS

Biohybrids resulting from the assembly of biopolymers and particulate inorganic solids such as clay minerals, are versatile materials that show biocompatibility, which is of great interest in view of diverse potential biomedical applications. For instance, bionanocomposites can be conformed as highly porous and bio-compatible hybrid materials showing good mechanical properties, which may be of potential interest as scaffolds in tissue engineering for bone repair. In other cases, they can be used as support of different biological entities including cells and viruses. Clay-lipid biohybrids are versatile, safe, non-toxic, and biocompatible materials that behave as biomimetic lipid interfaces resembling cellular membranes, having a possible contribution in the development of biosensors, enzymatic bioreactors, or as adjuvant of vaccines. Sepiolite spontaneously adsorbs DNA protecting it against degradation by the action of DNase enzyme, which can be of potential interest in applications as non-viral agents for gene-therapy. However, this type of biohybrid materials is still far from actual uses in biomedicine. The interaction mechanisms between the biological entities and the substrates are still largely unknown and both fundamental and applied (eg. *in vivo* assays) research are required to prove their activity, effect and efficiency.

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