Binding of DNA to Natural Sepiolite: Applications in Biotechnology and Perspectives

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Clays and Clay Minerals

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Clays & Clay Minerals

Binding of DNA to Natural Sepiolite: Applications in Biotechnology and Perspectives

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ABSTRACT

DNA manipulation is crucial for many biotechnological prospects and for medical applications such as gene therapy. This requires the amplification and extraction of DNA from bacteria and the transfer of these DNA molecules into cells, including bacterial and mammalian cells. The capacity of the natural magnesium silicate clay mineral sepiolite to bind to DNA makes it a potentially useful tool for biotechnological/medical strategies. In addition, sepiolite is inexpensive and classified as non-toxic and non-carcinogenic. This review will first describe the physico-chemical interactions between sepiolite and DNA. Then, the leverage of sepiolite/DNA interactions for DNA extraction from bacteria, to optimize DNA transfer into bacteria and DNA transfection into mammalian cells, are presented. Finally, the putative toxicity of sepiolite and its advantages and perspectives for future prospects, such as the improvement of immunotherapy, are also discussed.

Keywords: Sepiolite; dna interaction; bacterial transformation ; DNA plasmid extraction; DNA transfection in mammalian cells

INTRODUCTION

Genome engineering is a major strategy for the development of new biological models of interest in academia and the applied sciences, such as biotechnological, biomedical, and agronomic research. Remarkably, this constitutes the basis of gene therapy aiming to correct an endogenous, mutated, defective gene and to restore normal physiological functions. DNA is the central biomolecule bearing the genetic information that is transmitted from one generation to another. Hence, methods aimed at extracting and purifying DNA and/or transferring DNA into living cells represent central issues in biotechnology and biomedical applications.

Notably, the development of vectors based on biohybrid materials for DNA transfer, which allows the avoidance of virus-based vectors, represents an appealing approach for the treatment of different genetic disorders (Choy et al., 2000; Lin et al., 2006; Shi et al., 2011; Wu et al., 2014; Choi et al., 2014). Among the different micro/nanoparticles, sepiolite represents an alluring solution.

Sepiolite is a natural magnesium silicate clay mineral with a micro-fibrous morphology. The size of sepiolite fibres varies according to geographical origin. For example, the length of sepiolite fibres from Taxus Basin deposits in Spain ranges from 0.2 to 0.8 μm (Castro-Smirnov et al., 2016; Piétrement et al., 2018). Interestingly, sepiolite is able to bind different kinds of biological molecules, including polysaccharides (Alcântara et al., 2014), lipids (Wicklein et al., 2010), proteins (Alcântara et al., 2012), and virus particles (Ruiz-Hitzky et al., 2012). These capacities make sepiolite a promising micro/nanovector for the non-viral transfer of biomolecules (Piétrement et al., 2018). Particularly, combining analyses from physics, chemistry, and biology, sepiolite has been shown to bind to DNA (Castro-Smirnov et

al., 2016). These capabilities can be leveraged for biotechnology applications for future promising biomedical strategies.

This aim of this review was to discuss the potential biotechnological and biomedical uses of sepiolite, based on the physico-chemical characterisation of sepiolite/DNA interactions. Such interactions allow consideration of sepiolite as a promising substrate for DNA extraction from bacteria, for improving sepiolite-mediated bacterial transformation, and for mammalian cell DNA transfection. A further objective was to envision the putative toxicity of sepiolite *versus* its advantages and future perspectives.

SEPIOLITE AND ITS INTERACTION WITH DNA

Sepiolite Structure.

DNA/sepiolite interactions depend mainly on the sepiolite nanostructure and surface properties. Sepiolite belongs to a family of clays called pseudo-layered hydrated fibrous magnesium phyllosilicates. Its chemical formula is $Si_{12}Mg_8O_{30}(OH)_4(H_2O)_4(H_2O)_8$ and it has a trioctahedral structure: two layers of tetrahedral silica sandwiching a central octahedral magnesium oxide-hydroxide layer (TOT family). Unlike other phyllosilicates, sepiolite, like palygorskite, exhibits a three-dimensional crystalline organization with surface channels and internal tunnels. The crystal structure of sepiolite was studied by Brauner and Preisinger (Brauner & Preisinger, 1956) and is shown in Fig. 1. The internal channels have dimensions of close to 0.37 × 1.06-nm^2.

Many active sites on the sepiolite surface consist of both silanol groups (SiOH) located at the periphery of the fibers and magnesium hydroxyl type (Mg-$(H_2O)_2$) groups located on either side of the channels (see Fig. 1). These sites play an essential role in DNA adsorption (Castro-Smirnov et al., 2016) and allow the grafting of numerous chemical groups (García et al., 2011; Moreira et al., 2017; Undabeytia et al., 2019) and are responsible for the remarkable

74 physico-chemical properties of sepiolite. Sepiolite also has a sorption capacity mainly due to
75 the edge surfaces: the tunnels can accept molecules and retain them in a three-dimensional
76 space, whereas the surface channels interact with the outside. Both participate in very
77 different sorption mechanisms.
78 Like all clays, sepiolite has the property of retaining and exchanging cations with its
79 environment, which is called the cation exchange capacity (CEC). However, the structure of
80 sepiolite makes the internal faces of the channels difficult to access, and its three-
81 dimensional structure prevents swelling, unlike many of the two-dimensional phyllosilicate
82 clays. Sorption is, therefore, limited to the capacity of binding to the faces of the edges of
83 the sheets that emerge at the periphery of the fibres. For sepiolite, the CEC is quite low (20
84 to 30 meq/100 g of mineral) compared to other clays, but nevertheless plays an essential
85 role in the ability of sepiolite to adsorb various biomolecules, such as polysaccharides
86 (Darder et al., 2006), lipids (Wicklein et al., 2012), proteins (Alcântara et al., 2012), and
87 viruses (Ruiz-Hitzky et al., 2009), giving rise to a great diversity of bio-nanocomposites.
88 Finally, as a consequence of its structure, sepiolite has a large interaction surface that can be
89 split into two contributions; the internal surface is close to 300 m^2/g, and the external
90 surface ranges from 200 to 300 m^2/g (mainly depending on the fineness of the fibres). This
91 large surface of interaction (both internal and external) partly gives an understanding of how
92 sepiolite can interact strongly with biomolecules.
93 This ability to adsorb cations has helped to develop the theory that clays could be the cradle
94 of life on Earth, or, more specifically, of complex biochemical compounds that made life
95 possible (Yang et al., 2013). The hypothesis is that the clay hydrogel, thanks in part to its
96 structure organized in microcavities, plays a containment role for basic chemical elements
97 that could have carried out the complex reactions at the origin of the formation of proteins,

98 DNA, and ultimately all of the machinery involved in the functions of a living cell. The clay
99 hydrogels could also then have confined and protected these chemical processes until the
100 formation of the membrane surrounding the living cells.
101 In addition, the adsorption of DNA on mineral surfaces might favor horizontal gene transfer
102 into living organisms, which may play a significant role in the evolution of living species. All
103 these studies suggest a specific interaction between clays and nucleic acids.
104 *Sepiolite and DNA*
105 With the aim of using sepiolite as a new DNA vector, a study have thus shown that DNA
106 could efficiently bind to sepiolite, notably highlighting the role of multivalent cations as
107 enhancers of the DNA binding process (Castro-Smirnov et al., 2016). However, while
108 monovalent cations reduce the adsorption of DNA molecules to mica (Pastre et al., 2003),
109 surprisingly, these cations promote the adsorption to sepiolite fibres. While monovalent
110 cations are less effective than multivalent cations, they unequivocally contribute to the
111 adsorption process (see Fig. 2). Interestingly, the presence of polyvalent cations (Mg^{2+}, Ca^{2+},
112 spermidine, or spermine) strongly stimulates the adsorption of DNA depending on the
113 valence of the cation (see Fig. 2). These results suggest an adsorption mechanism mediated
114 by an electrostatic bridge through hydrogen bonds between the DNA phosphate groups and
115 the silanol groups located on the outer surface of the sepiolite, which is unlike the mica
116 surface, where counterion correlations occur (Pastre et al., 2003; Pastré et al., 2006).
117 Fourier-transform infrared (FTIR) spectroscopy analysis thus showed that, indeed, the
118 interaction of DNA with sepiolite was mediated by only the external silanol groups, whereas
119 the hydroxyl groups of the octahedral sheets of sepiolite play no role (Castro-Smirnov et al.,
120 2016).

If the DNA is adsorbed onto the sepiolite fibres, the question of its desorption also arises in the context of a project to create a nano-cargo for cell transfection. In cellulo, the mechanisms that could induce this desorption remain unknown, but the possibility of DNA desorption *in vitro* has been tested through the desorption of DNA from the bionanocomposite by incubating it with EDTA, a well-known metal ion chelating agent. By using different DNA plasmids, the quality of the DNA recovered after its attachment to sepiolite was examined and the different DNA isoforms (super-coiled, open circle, or linear) were unmodified after their binding to sepiolite. This shows that sepiolite does not alter the quality of DNA and that this method can be considered a new method of DNA purification (Castro-Smirnov et al., 2016).

All these results confirmed the potential of sepiolite as an efficient cargo for DNA transfer into cells. Moreover, the capacity of desorption suggests the possibility of using sepiolite for DNA extraction.

EXTRACTION OF PLASMID DNA FROM BACTERIA

DNA extraction and purification, especially of plasmids, is the basis of molecular biology. Plasmids are circular DNA molecules that autonomously replicate in bacteria and are essential tools in molecular biology and biotechnology, from bacteria to mammalian cells. Indeed, they are used as DNA vectors/backbones in gene transfer technology. Hence, fusion of an exogenous DNA (for example, a piece of a human DNA) allows replication and amplification into bacteria. After this replication and amplification process, their extraction is necessary for subsequent experiments.

Several methods of plasmid extraction are available commercially. These methods are more or less expensive and time-consuming. The DNA binding capability of sepiolite, associated with the possibility of DNA release, makes sepiolite a simple and inexpensive alternative

method (Castro-Smirnov et al., 2020). Indeed, the binding of DNA onto sepiolite requires cations, notably divalent cations such as Mg^{2+} or Ca^{2+} (Castro-Smirnov et al., 2016). The chelation of cations with EDTA, therefore, enables the release of DNA from sepiolite, which is not covalently bound (Castro-Smirnov et al., 2020). The general protocol (Castro-Smirnov et al., 2020) can be summarized as follows:

a- Bacteria bearing plasmids are grown in the appropriate medium with the suitable selection of antibiotics.

b- At the mid-saturation state (as monitored by spectrophotometry), bacteria are pelleted by centrifugation and then re-suspended in specific buffers. Bacteria are lysed, and the DNA is denatured (the two complementary strands are separated) in alkaline buffers.

c- The addition of neutralizing buffer allows plasmid DNA (which are small DNA molecules) to renature (return to the bicatenary DNA structure), remaining soluble. In contrast, bacterial genomic DNA (which are long DNA molecules) cannot properly renature, and so they precipitate.

d- Centrifugation precipitates genomic DNA pellets and bacterial debris.

e- The supernatant (containing the plasmid DNA) is recovered. Sepiolite plus Mg^{2+} or Ca^{2+} is added, allowing plasmid DNA to bind to sepiolite, allowing the subsequent washing steps to eliminate contaminants.

f- Sepiolite-bearing DNA is centrifuged and washed with Tris buffer.

g- Then, EDTA is added to the sepiolite/DNA pellets, releasing the plasmid DNA from the sepiolite.

h- Plasmid DNA and sepiolite are then separated by centrifugation (sepiolite in the pellet and plasmid DNA in the supernatant).

Steps (a) to (d) are common to almost all methods of plasmid extraction. Importantly, the structures of plasmid DNA molecules are not altered by this procedure, as shown by electrophoresis and transmission electron microscopy analyses. Moreover, plasmid DNA can be processed by enzymes such as restriction enzymes (Castro-Smirnov et al., 2020). These facts attest to the good quality of the plasmid DNA extracted by the sepiolite-based method.

IMPROVEMENT OF THE YOSHIDA EFFECT ON BACTERIAL TRANSFORMATION

In bacteria and non-animal eukaryotic cells, the non-viral transfer of DNA is called transformation. In animal cells, it is called transfection.

Efficient transformation requires first rendering the bacteria to be competent for DNA transfer. However, the protocols to prepare competent bacteria are generally laborious and time consuming. One alternative is to purchase commercially competent bacteria, but this method is expensive. One advantage of the Yoshida effect protocol for bacterial transformation is that it does not require the use of competent bacteria.

In the Yoshida effect protocol, the solution containing the bacteria and the acicular material forms a colloidal solution. Upon spreading on solid agar medium in Petri dishes using a solid spreader, the sliding friction stimulates bacterial transformation (Yoshida et al., 2001; Yoshida, 2007; Yoshida & Sato, 2009). However, the Yoshida effect was first described with asbestos, thus raising health and toxicity concerns for its use. Sepiolite, which is considered non-toxic and non-carcinogenic (see below), can circumvent this health worry. Indeed, the fibrous nature of sepiolite favors bacterial transformation through a Yoshida-like effect (Yoshida, 2007; Yoshida & Sato, 2009; Tan et al., 2010; Wilharm et al., 2010; Rodríguez-Beltrán et al., 2012; Rodriguez-Beltran et al., 2013).

The Yoshida effect does not absolutely require the pre-assembly of DNA with the fibers. However, combining different capacities of sepiolite (i.e. the use of sonicated sepiolite, the capacity to bind DNA and bacteria), a 100-fold improvement in transformation efficiency has been observed (Castro-Smirnov et al., 2020). The general protocol can be summarized as follows.

a- The use of sonicated sepiolite (sSep). Indeed, sepiolite spontaneously aggregates and can be dissociated by sonication, increasing its spreading efficiency.

b- Pre-assembly of DNA and sSep, using the characteristics (incubation in the presence of divalent cations) then centrifugation) described above, before adding the bacteria.

c- Pre-incubation of the sSep/DNA biohybrid material with bacteria (at the mid-phase of growth) before spreading. Indeed, sepiolite can spontaneously bind to bacteria (Castro-Smirnov et al., 2016, 2020) and it can thus be used for liquid decontamination. In addition, this procedure favors bacterial transformation (Castro-Smirnov et al., 2020). However, the Yoshida effect is still necessary because sepiolite-bacteria assembly alone is not sufficient for efficient internalization.

Adapting these 3 conditions to the Yoshida effect, transformation efficiencies reached close to 10^6 transformants per microgram of DNA (Castro-Smirnov et al., 2020). Such transformation efficiency is lower than that of the commercially available competent bacteria but is largely sufficient for most molecular biology applications at a lower cost. This method is also much less time-consuming than the classical protocols for preparing competent bacteria.

TRANSFECTION OF MAMMALIAN CELLS

Transfer of DNA into mammalian cells is an essential issue in biotechnology and biomedical applications. Indeed, this constitutes the basis of strategies aimed at designing new model

organisms for academic, biomedical, or agronomical research and gene therapy. However, mammalian cells do not efficiently incorporate exogenous DNA.

The Yoshida effect cannot work with mammalian cells because frictional forces kill them. However, sepiolite can be spontaneously internalized into mammalian cells without requiring friction, mainly through endocytosis and macropinocytosis pathways that involve the invagination of the cell membrane around the sepiolite fibers, resulting in its incorporation in the endosomes (intracellular organelles embedded into membranes) of the cells (Castro-Smirnov et al., 2017). Interestingly, this process has a low effect on viability (Castro-Smirnov et al., 2017; Ragu, Dardillac, et al., 2020). Given that sepiolite can bind DNA, it can serve as a vector for stable DNA transfection into mammalian cells (Castro-Smirnov et al., 2016). This application relies on the binding of DNA to sSep prior to the addition of the sSep/DNA bio-hybrid material to the cell media. The uptake of the sSep/DNA bio-hybrid also acts through the endocytosis pathway, and the intracellular sSep/DNA bio-hybrid is then embedded in endosomes. The addition of drugs that destabilize endosome membranes, such as chloroquine, results in a 2-fold increase in the transfection efficiency (Castro-Smirnov et al., 2016).

These methods of cell transfection are somewhat less efficient than the use of commercial reagents but are much less expensive. Different strategies can be envisioned to increase the efficiency of intake of the nanoparticles into the cell. These strategies might be based on the structure of the cell membrane, which is constituted of a bi-layer of lipids. Moreover, techniques that could favor the delivery from the endosomes, such as favoring endosome collapse into the cells, are now address; this might favor DNA delivery into cells. Moreover, the pH of the endosome is very acid, and thus might lead to hydrolysis of the DNA. Methods controlling this issue are, therefore, under investigation. The injection of sepiolite in vivo

should be precisely analyzed. Indeed, it could be injected into the blood micro-vessel and/or accumulated in the liver. Moreover, the production of ROS (reactive oxygen species) and of inflammatory cytokines might generate unwanted inflammation and/or premature ageing. Therefore, the behavior and the fate of sepiolite, as well as the consequences of sepiolite injection (through different procedures such as in blood versus intra-peritoneal), should be addressed and analyzed in depth and in vivo in a mouse model before attempting this transfer in humans.

TOXICITY OF SEPIOLITE IN HUMAN CELLS

Because of the multiple potential uses of sepiolite, including its biomedical applications, the question of its toxicity has become an important issue. Its fibrous nature has led to concerns of asbestos-like effects. This can be supported by the fact that, like asbestos, sepiolite can generate a Yoshida effect and, in addition, generate DNA damage in bacteria (González-Tortuero et al., 2018). However, translating these conclusions to mammalian cells would represent an over-interpretation. Indeed, bacteria are much smaller than mammalian cells (approximately 1000-fold). Therefore, while the length of a sepiolite fibre is similar to that of a bacterial cell, the sepiolite fiber is much smaller than that of a human cell (Castro-Smirnov et al., 2017; Ragu, Dardillac, et al., 2020). In addition, the genome of eukaryotes (including human cells) is embedded into a nuclear compartment, while in prokaryotes (bacteria) no nucleus exists, and DNA is contained directly in the cytoplasm. Therefore, a sepiolite fiber that penetrates bacteria can directly interact with its genomic DNA to alter it. In contrast, in mammalian cells, the sepiolite fibre that enters the cell will be in the cytoplasm and not in the nucleus. Hence, the sepiolite is not in contact with the genomic DNA (Castro-Smirnov et al., 2017; Ragu, Dardillac, et al., 2020). For DNA interaction, the sepiolite fiber should be transported into the nucleus, which appears to be an infrequent process (Castro-Smirnov et

al., 2017; Ragu, Dardillac, et al., 2020). In agreement with this observation, the interaction of human cells with sepiolite does not trigger the DNA damage response (Ragu, Dardillac, et al., 2020), supporting the fact that sepiolite does not attack genomic DNA in mammalian cells. Coherently, sepiolite appears to be weakly toxic in cultured mammalian cells (Castro-Smirnov et al., 2017; Ragu, Dardillac, et al., 2020), especially at the doses used for DNA transfection, which are much lower than other classical transfection methods.

Sepiolite also induces the production of reactive oxygen species (ROS) in cells (Ragu, Dardillac, et al., 2020). Because ROS can potentially alter any biological component, including DNA, and lead to mutagenesis, this has raised some concerns about the potential toxicity of sepiolite. The respiratory chains that synthetize ATP and provide the energy required for various metabolic pathways generate ROS as by-products. However, cells can also control the production of ROS that act as secondary messengers in physiological processes (Ameziane-El-Hassani et al., 2016). In this situation, ROS are beneficial to the organism. Notably, while antioxidants have been proposed to protect against tumorigenesis, in contrast, treatment with antioxidants favors the development of lung carcinomas and metastasis (Breau et al., 2019; Le Gal et al., 2015; Wiel et al., 2019), thus underlying the potential benefit of ROS production. In addition, ROS can also trigger the induction of apoptosis, eliminating stressed cells, to the benefit of the whole organism. Hence, an intracellular increase in ROS can result from the detection of sepiolite by cells and their response but does not automatically imply that the fate of the cell or organism will be jeopardized.

Finally, ROS can oxidize lipid cell membranes, leading to lipid peroxidation (LP). However, sepiolite represses LP from the supernatants of rat brain homogenates, suggesting a putative antioxidant role for sepiolite (Cervini-Silva et al., 2015). One hypothesis is that cells respond

to sepiolite interaction through the production of controlled ROS but, in parallel, sepiolite could also scavenge ROS, thus protecting against potential noxiousness.

One important component of the potential toxicity of fibres (such as asbestos) relies on the fact that they remain stuck in tissues, generating chronic inflammation, which can become pathogenic with time. These concerns connect two issues: the size of the fibres and the exclusion capacity of the cells to the fibres. Indeed, small fibres should more easily be excluded, thereby avoiding a potential asbestos-like effect. The large majority of the sepiolite fibres from Vallecas-Vicalvaro deposits in Spain range in length from 200 to 800 nm (Castro-Smirnov et al., 2016), values that are lower than those reported in the literature (Bellmann et al., 1997). Such sizes are much smaller than those of mammalian cells; therefore, the capacity of the cells themselves to exclude such sepiolite fibres thus becomes prominent for its harmlessness. Interestingly, the stable natural intrinsic fluorescence of the sepiolite used in the present study(from Vallecas-Vicalvaro deposit) enabled examination of its fate in cells (Castro-Smirnov et al., 2017; Ragu, Dardillac, et al., 2020). In particular, time-lapse fluorescent video microscopy analysis revealed the spontaneous exclusion of these sepiolite fibers from mammalian cells, likely through an exocytosis process that involved the membranes of endosomes (Castro-Smirnov et al., 2017). These data can account for the low cellular toxicity of sepiolite (Denizeau et al., 1985; Castro-Smirnov et al., 2016; Ragu, Dardillac, et al., 2020). More generally, and in agreement with the size effect hypothesis, both epidemiological studies and in vitro and in vivo analyses have led to the conclusion that sepiolite, especially with fiber lengths less than 5 µm, does not present a health risk (Maisanaba et al., 2015). Moreover, previous biological and epidemiological analyses have led the International Agency for Research on Cancer (IARC) to classify sepiolite as non-

311 hazardous and non-carcinogenic (McConnochie et al., 1993; Santarén & Alvarez, 1994;
312 Wagner et al., 1987).

313 ## PERSPECTIVES FOR IMPROVEMENT OF IMMUNOTHERAPY

314 Additional bio-medical applications can be derived from the interaction of sepiolite with
315 cells, such as immunotherapy. This strategy takes advantage of the capacity of the immune
316 system to eliminate stressed cells. It has been applied to cancer therapy (Esfahani et al.,
317 2020) and was recognized by the Nobel Academy (Nobel prize of Physiology or Medicine to
318 James Allison and Tasuku Honjo, in 2018). This process is triggered by the production and
319 excretion of inflammatory cytokines that modify the microenvironment of the cell. This leads
320 to activation of the innate immune response, which recruits immune system cells (for
321 instance, natural killer cells, macrophages, and lymphocytes). Tumors are classified as hot or
322 cold, depending on whether they respond to immunotherapy. Unfortunately, the majority of
323 tumors are cold. Therefore, strategies that are able to change the status of cold tumors to
324 hot tumors should represent important progress in immunotherapy (Duan et al., 2020).
325 Sepiolite is internalized into cells and recognized, leading to the production of inflammatory
326 cytokines (Ragu, Dardillac, et al., 2020). Hence, this particularity opens a hopeful perspective
327 to turn cold tumors into hot tumors for immunotherapy.
328 Additionally, grafting DNA onto sepiolite can be expected to optimize the inflammatory
329 response. Indeed, cytoplasmic DNA is recognized by cells that induce inflammatory cytokines
330 (Ragu, Matos-Rodrigues, et al., 2020). Thus, one hope would be that the bio-hybrid
331 sepiolite/DNA material that delivers DNA into the cytoplasm of the cell might synergize the
332 efficiency of cytokine production and thus the immune response.

333 ## CONCLUSIONS

DNA is negatively charged, and the first obstacle is the cell membrane, which is made of lipid bilayers that have charges incompatible with the spontaneous DNA uptake by cells. Non-viral methods use physical and chemical strategies to bypass the membrane barrier but are generally toxic to the cell. One limitation of both viral and physicochemical methods is the fact that they can transfer DNA only. One ideal strategy would be based on transport systems that can co-convey different kinds of molecules. The development of novel nanocarriers using biohybrid materials for non-viral gene transfer constitutes thus a promising approach. In this context, sepiolite is an enticing candidate as a nanocarrier for the non-viral vectorization of DNA, because it is biocompatible and is spontaneously internalized and excreted by mammalian cells. Moreover, thanks to its spontaneous fluorescence, sepiolite can be detected in cells (and maybe hopefully in animals) without requirement of fluorescent chemical grafting and can be followed in cells. Importantly, sepiolite is weakly toxic to mammalian cells and is classified as non-hazardous and non-carcinogenic by the IARC (WHO). Strategies based on natural internalization/externalization capacities of the mammalian cells are preferable to limit cell toxicity. These strategies implied that DNA should first bind to sepiolite to form a sepiolite/DNA (Sep/DNA) biohybrid. Indeed, sepiolite can adsorb different molecules very efficiently, thus allowing the development of many elaborate strategies, and, as a natural clay, sepiolite represents an answer to societal concerns regarding challenging nanotechnologies.

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545

546 Fig. 1. Sepiolite structure (adapted from (Brauner & Preisinger, 1956)).

547

548 Fig. 2. (A) The effects of the presence of cations with various valences on DNA adsorption.
549 Reaction conditions: 10 mM Tris-HCl pH 7.5, salmon sperm DNA and sepiolite concentrations
550 of 615 ng/µl and 1 mg/mL, respectively (from (Castro-Smirnov et al., 2016)). TEM images of
551 DNA condensed with (B) spermidine and (C) the interaction with sepiolite fibers.
552 Transmission electron microscopy (TEM) imaging: DNA molecules (5 nM) were condensed in
553 10 mM Tris-HCl pH 8, 50 mM NaCl, and 1 mM spermidine. 5 µL of reaction mixture was
554 deposited onto a 600 mesh copper grid coated with a thin carbon film previously activated
555 by glow discharge in the presence of 1-aminopentane (Merck, Saint Quentin Fallavier,
556 France) (Dupaigne et al., 2018). After 1 min, the grids were washed with aqueous 2 wt.%
557 uranyl acetate (Merck, Saint Quentin Fallavier, France) and then dried with ashless filter
558 paper (VWR, Rosny sous bois, France). TEM observations were carried out on a Zeiss 912AB
559 transmission electron microscope in bright field mode. Electron micrographs were obtained
560 using a ProScan 1024 HSC digital camera and iTEM software (Olympus, Soft Imaging
561 Solutions, Munster, Germany).

Figure 1

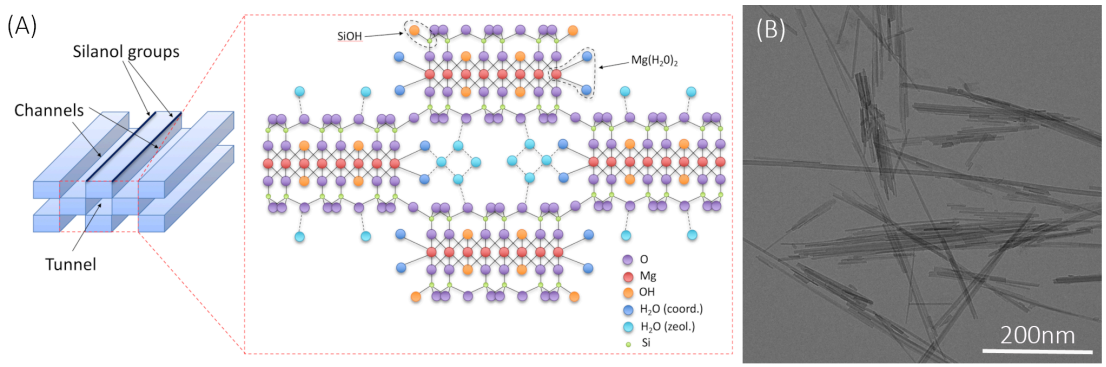

Figure 2

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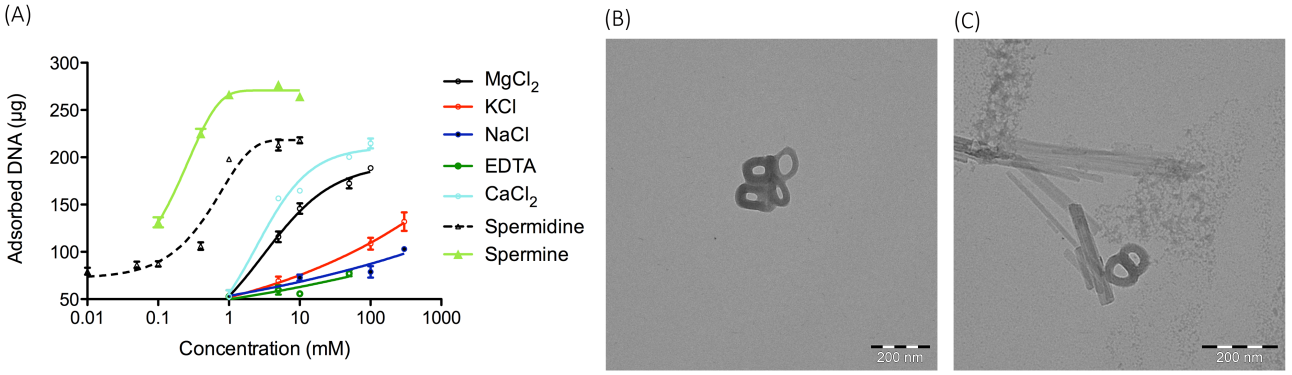

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