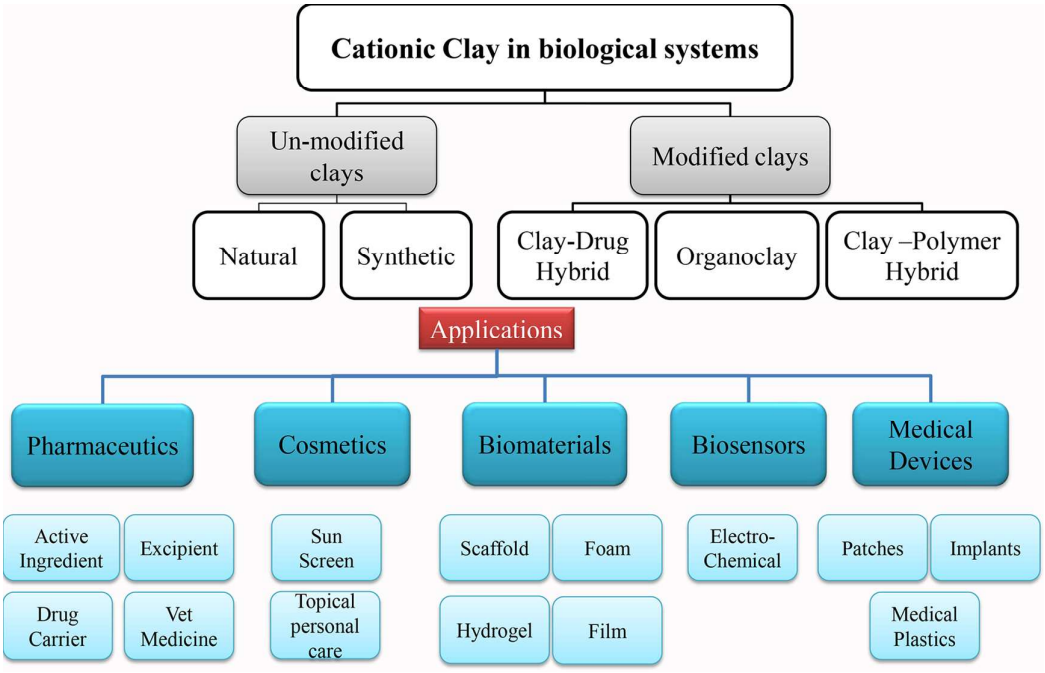
RSC Advances

Biomedical Applications of Cationic Clay Minerals

Journal:	*RSC Advances*
Manuscript ID:	RA-REV-12-2014-016945.R1
Article Type:	Review Article
Date Submitted by the Author:	09-Mar-2015
Complete List of Authors:	Ghadiri, Maliheh; University of Sydney, Faculty of Pharmacy Chrzanowski, Wojciech; University of Sydney, Rohanizadeh, Ramin; University of Sydney, Faculty of Pharmacy

SCHOLARONE™
Manuscripts



Different types of cationic clay minerals and their applications in various biological systems

Biomedical Applications of Cationic Clay Minerals

M. Ghadiri,[a] W. Chrzanowski[a, b] and R. Rohanizadeh[a]*

Clay minerals have been a subject of interest owing to their easy availability in nature, a wide range of applications in various industries, and particularly their current and potential biomedical applications. They have been widely used for curative and protective purposes by humans since ancient times. Cationic clay minerals possess specific physicochemical characteristics such as high surface reactivity (high adsorption, cation exchange, colloidal or swelling capacity), good rheological behavior, high acid-absorbing capacity, and high dispersibility in water, which renders them suitable for various biomedical applications. Only a few reviews have exclusively discussed the biomedical applications of clay minerals, and to our knowledge, there is no updated review focusing on cationic clay minerals and their applications in pharmaceuticals, cosmetics, and regenerative medicine. In this review, we provide a brief introduction on natural, synthetic, and hybrid cationic clay minerals followed by a detailed discussion about their applications in biological systems.

This journal is © The Royal Society of Chemistry 2013

I. Introduction

Clay minerals are one of the oldest earth materials used for healing purposes in traditional medicine.[1] Indigenous people around the world have been using clay minerals for curative and protective purposes. In traditional medicine, clay minerals have been primarily used in external applications such as clay baths to cure skin diseases and in simple gastrointestinal ailments like diarrhea.[2] Additionally, clay minerals have also been employed in animal dietary and medicine.[3-5] The healing properties of clay minerals, passed on since ancient cultures, continue to be applied in modern life for the treatment of various topical and internal ailments. With emerging modern technologies, the advantages of using clay minerals in several industries have also been explored. Clay minerals possess specific physicochemical characteristics such as high surface reactivity (adsorption and cation exchange capacity), colloidal and swelling capacity, optimal rheological behavior, and high water dispersibility, which render them suitable for different biological applications including pharmaceutics, cosmetics, veterinary medicine, biomaterials, and biosensors.

This review aims at discussing the recent advances in biomedical applications of cationic clay minerals (Cationic-CM) based on their specific physicochemical and biological properties. The use of either natural or synthetic cationic clay minerals, or their composites in pharmaceuticals, cosmetics, biomaterials, biosensors, and other medical devices is discussed in the review (Figure 1).

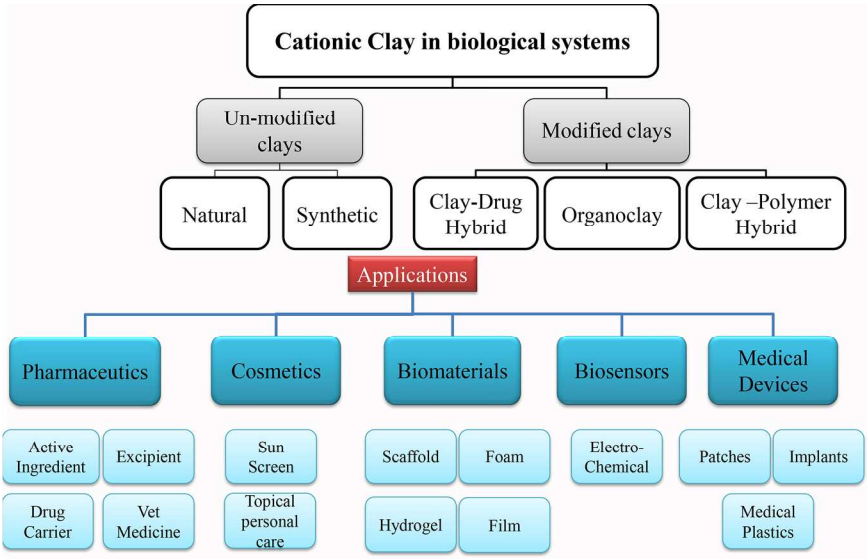


Figure 1. Applications of modified and unmodified cationic clay minerals in various biological systems.

Clay minerals: natural origin and basic properties

Clay minerals are members of the phyllosilicate or sheet silicates family (which form parallel sheets of silicates) consisting of hydrated alumina-silicates. Phyllosilicates are an important group of minerals that include micas, chlorite, serpentine, talc, and the clay minerals. The basic building blocks of clay minerals are tetrahedral silicates and octahedral hydroxide sheets (Figure 2). The arrangement of tetrahedral and octahedral sheets gives rise to various classes of clay minerals such as 1:1 and 2:1. The 1:1 clay consists of one tetrahedral and one octahedral sheet (e.g., kaolinite and serpentine) whereas the 2:1 clay consists of an octahedral sheet sandwiched between two tetrahedral sheets (e.g., smectite, chlorite and vermiculite) (Figure 3). Further, the octahedral sheets contain metal ions and based on these ions, clay minerals are divided into two groups: dioctahedral and trioctahedral. Divalent metal ions such as Fe^{2+} and Mg^{2+} lead to formation of a trioctahedral clay and trivalent metal ions such as Al^{3+} form a dioctahedral clay.

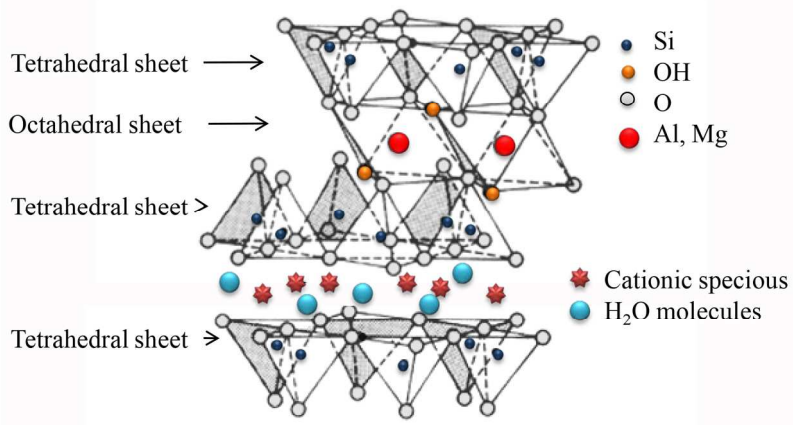


Figure 2. Smectite structure of a 2:1 clay mineral showing two tetrahedral sheets sandwiched between one octahedral sheet within the clay-stacking pattern.

Clay minerals are usually either positively charged or negatively charged, which is the main reason for their ion exchange capacity. Further, there are two types of charges. The first is a permanent or structural charge arising due to the substitution of Al^{3+}, Si^{4+}, Mg^{2+}, and Fe^{3+} ions, whereas the second charge is caused by broken edges of clay, and is pH-dependent and originates from the surface reactivity of clay.[6] This charge accounts for less than 1% of the total charge in 2:1 clay minerals. However, in the 1:1 class such as kaolinite, where no charge due to ion substitution is present, the pH dependent charge provides the major proportion of total net charge. Based on their charge, clay minerals can generally be divided into two groups. The cationic clay minerals (cationic-CM) possess a negative charge and are widespread in nature (e.g., smectite). The anionic clay minerals or layered double hydroxides (LDH) possess a positive charge and are relatively uncommonly found but rather simple and economical to synthesize.

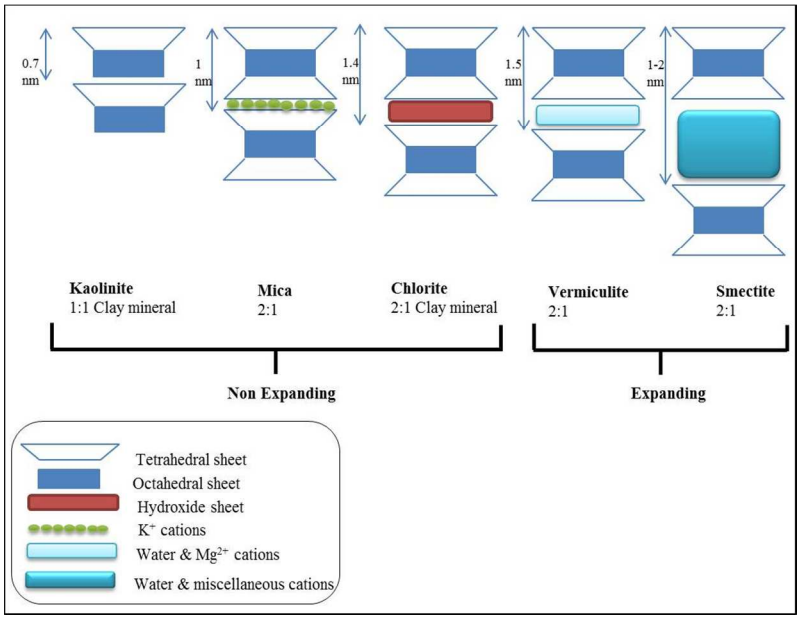


Figure 3. Expanding and non-expanding cationic-CMs: the arrangement of tetrahedral sheets with octahedral sheets gives rise to various classes of clay minerals.

One of the focus of this review is to discuss current novel investigations on cationic clay mineral applications in biological systems. Cationic clay minerals contain negatively charged aluminosilicate sheets, which further contain cations in their interlayer space to balance the charge and also contain interstitially located water molecules.[7] Cationic clay minerals are generally found in crude forms in nature and are then purified, whereas anionic clay minerals are mostly synthetic.[8] A wide variety of 2:1 class clay minerals such as hectorite, bentonite, and smectite, and 1:1 class minerals such as kaolinite have been exploited for their potential biomedical applications. Among the different cationic-CM subgroups, smectites have been the subject of several studies and have

been applied in different biological systems. The vast range of interest in the smectite stems from their physicochemical properties such as swellabilty, cation exchange capacity, gel formation ability, and adsorptive capacity. These unique properties are the result of (1) extremely small particle size, (2) chemical composition, (3) large cation exchange capacity, (4) large surface area (layered structure), (5) different types of exchangeable ions and surface charge, and (6) interaction and intercalation with inorganic and organic substances. It is due to these properties that smectites have been the subject of more studies than any other subgroups and have been widely used in various applications.

Cationic-CMs have been combined with different organic substances in order to optimize their characteristics for specific applications.[9] These additives can be adsorbed on the surface, edges, or interlayer spaces of clay particles (Figure 4). Since cationic-CMs have high ion exchange capacity and large total surface areas, they can be easily hybridized with drugs, polymers, and organic substances. Based on the nature of these substances, modified clay minerals can be further categorized into different sub-groups such as clay-drug hybrids, clay-polymer hybrids, and organoclays.[10]

Synthetic cationic-CM

Despite the abundance of clay minerals in nature, various synthetic clays, which provide homogenous structural and compositional properties as well as lower rates of chemical and microbial contaminations, have been synthesized to meet specific industrial requirements.[11] Pure clays are not found in nature and often contain chemical contaminants such as heavy metals (e.g., arsenic, lead, chromium, and titanium)[11] or they are blend of different clays and sediments. Clay minerals used in the formulation of pharmaceutical and cosmetic products should be highly consistent in terms of particle size, chemistry, water content, and free from microbial contaminations.[12] Based on the application, synthetic cationic-CMs have been produced to obtain specific chemical compositions by enriching them with mineral elements. For example, enriched cationic-CM were synthesized by adding elements such as Zn, Co, and Ni.[13] Cobalt-substituted smectite synthesized for clay-based electrodes in new generations of biosensors,[14] or Laponite enriched with magnetic iron oxide was produced to enhance clay's super-paramagnetic behavior, which showed the potential to adsorb and separate a wide range of pollutants from liquids.[15] Synthetic cationic-CM have also been used as a catalyst because of their high reactivity.[16] Apart from producing purer clay minerals and enriching them with different elements, synthetic clays can provide higher consistency in terms of clay particle size. Although natural clay minerals have been used for decades as micro-fillers in composite materials, reducing their size to nanometer scales substantially improved their performance in some applications and led to new applications for clay minerals.[17]

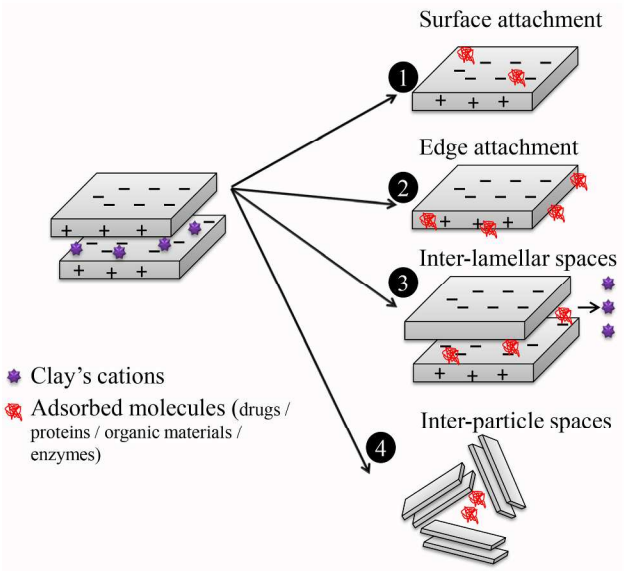


Figure 4. Different biomolecule and drug adsorption sites on a cationic clay mineral: (1) surface site, (2) edge site, (3) inter-lamellar site, and (4) inter-particle site

Modified cationic-CM

Several physical and chemical methods have been employed to modify the characteristics of clay minerals in order to ameliorate their performance and widen the range of their applications.[18] Modified cationic-CM containing organic materials such as surfactants and polymers have been widely studied. Cationic-CM can be modified in various ways: (1) chemical modification in

 This journal is © The Royal Society of Chemistry 2012

order to promote surface reactivity (homoionic clays), (2) interaction with organic substances in order to increase hydrophobicity (organoclays),[19] and (3) incorporating clay nanoparticles into polymers to create new composite materials (clay-polymer composites).[20]

a) Organoclays: Clay minerals are naturally hydrophilic and most polymers are hydrophobic. The hydrophilic structure of clay minerals makes them poorly miscible with most polymer matrices. Therefore, clay must be modified and treated with certain organic substances to render it miscible with a hydrophobic polymer. Given their high ion exchange capacity and large surface areas, cationic-CMs readily interact with many substances, particularly with organic materials. Prepared organoclay has the ability to mix homogenously with hydrophobic polymers. The most common organic cations used for organoclay preparation are cationic surfactants, which are adsorbed on the surface of clay minerals and their alkyl chains render the interlayer space organophilic. As a result, the organoclay acquires excellent adsorption properties towards poorly water-soluble organic species. Hybridizing organoclays with polymers can create novel materials suitable for different applications.[9, 21,22]
Clay-substance interaction is mainly driven by physical interactions such as hydrogen bonds, ion-dipole interaction, co-ordination bonds, acid base reactions, charge-transfer, and van der Waals forces.[22, 23] Apart from these physical forces of interaction, complexation and covalent bonds also exist between organic substances and clay minerals.[24] Further, for ion exchange in cationic clay minerals, the nature of substances should be cationic to be exchanged with the inter-layer cations of the clay minerals.[25]

Although organoclays have mainly been used in the oil industry as adsorbents or in other industries as viscosity modifiers, recently they have been employed in biological applications, either individually or in combination with polymers, in advanced formulations for drug delivery or tissue engineering.[26, 27] Many organoclays have been commercially synthesized for use in combination with hydrophobic polymers. Some of the commercially available organoclays are Closite 30A®, Closite 30B®, Tixogel®, and Garamite®.[28] Commercial organic modified montmorillonite (OMt), available under the trade name of Cloisite 30B, was blended with polymer poly (dimethyl siloxane) and used for metronidazole delivery.[29] Researchers have also used organically modified montmorillonite with methyl, tallow, and bis-2-hydroxyethyl quaternary ammonium salt and mixed it with Poly(N-isopropylacrylamide) for drug delivery applications.[26] In another study, the ammonium salt of the amino acid L-methionine (Met) was used as a novel chiral bio-surfactant for organo-modification of natural cloisite by ion exchange method to enhance the biodegradability of PVA film.[30] Shaikh et al. also synthesized a pressure-sensitive adhesive organoclay (montmorillonite/octadecylamine) and polydimethyl siloxane for transdermal delivery of drugs.[31]

The use of organoclays in biomedical applications should be accompanied with great consideration for the cytotoxicity of released organic compounds. Styne et al. reported that quaternary ammonium compounds, common organic compounds used in organoclay preparation, exerted cytotoxicity and inhibited the growth of fibroblasts.[32] Incorporation of aminoundecanoic acid did not hinder cellular growth on the polyurethane-montmorillonite surface in the same study.[32]

b) Clay-polymer hybrid: Clay-polymer hybrids are used in many industries and a few examples with specific applications in pharmaceutics are mentioned in Table 1. Extensive studies have focused on clay-polymer hybrids in different forms such as hydrogels, scaffolds, and films in regenerative medicines (Table 2). The four main methods for the preparation of clay-polymer hybrids include *in-situ* synthesis, solution intercalation, *in-situ* intercalative polymerization, and melt intercalation.[32] Due to the hydrophilic nature of clay and the hydrophobic nature of most polymers, the preparation of clay-polymer nanocomposites with good dispersion of clay nanoparticles within the polymer matrix is not possible by physical mixing of polymer and clay.[18] However, a few hydrophilic polymers such as poly(ethylene oxide)[33] and poly(vinyl alcohol)[34] can be mixed with cationic-CMs. Since most clays are hydrophilic, clay must be treated with organic substances to make it miscible with a hydrophobic polymer. Montmorillonite (Mt) and Laponite (Lap) have been mainly used in clay-polymer hybrids for biomedical applications. Among the vast number of polymers, the commonly used varieties for biomedical applications are poly(ethylene glycol) (PEG)[33], poly(ethylene oxide) (PEO)[34], poly(lactic-co-glycolic acid) (PLGA)[35], and poly(lactic acid) (PLA)[36] (Table 1 and 2). PLGA/Mt nanoparticle was specifically formulated for the drug delivery of paclitaxel (an anticancer drug). This mixture improved the bioavailability and reduced the side effects of the encapsulated drug. [35, 37]

Table 1. Cationic-CMs hybridized with polymers for drug delivery applications

Clay	Polymer	Application	Ref.
Mt	polyurethane	Ocular drug delivery	38
Mt	PLGA	Drug carrier for Paclitaxel	39
Hallyo site	Chitosan /poloxamer	Drug carrier for Tetracycline	40
Lap	Pluronic based multi-block copolymer	Delivery of low-molecular-weight proteins	41
Mt	PLA	Oral Drug delivery of Docetaxel	36
Mt	Polyurethane	Topical Triamcinolone delivery	42

Abbreviations: poly(lactic-co-glycolic acid) (PLGA); poly(lactic acid) (PLA); montmorillonite (Mt); Laponite (Lap)

II. Biological applications of cationic-CM

Biological applications, namely, pharmaceutical, cosmetic, animal health, biomaterials, and biosensors applications of cationic-CM are discussed in details in this section.

Cationic-CM in pharmaceutical products

Clay therapy, in form of clay baths for external use or as clay-eating habit (geophagy), has been practiced among indigenous people all around the world since ancient times to treat certain ailments.[1] Clay minerals are also used for similar treatments in current times and have been employed in modern pharmaceutical systems as active ingredients (having therapeutic properties) or excipients. As an active ingredient, cationic-CMs have been used externally for skin therapy or are ingested for gastrointestinal diseases. Oral consumption of cationic-CMs has been used to cure infection, balance the body pH, regulate gastrointestinal problems, counter poisoning, and augment nutrition. As an excipient, cationic-CMs have been applied in the formulation of many conventional and novel drug delivery systems. Although natural forms of purified cationic-CMs are generally used in the pharmaceutical industry, the production of newly synthesized clay minerals with controlled and better properties are more promising. In addition to the benefits of unmodified clays in pharmaceutics, new applications have emerged from the combination of organic materials (surfactants or polymers) with clays (organoclays and clay-polymer composites). The pharmaceutical applications of cationic-CMs as active ingredients or excipients will be discussed in the following sections.

a) As active ingredient: Cationic-CMs have been utilized as active ingredients in pharmaceutical products in oral or topical forms owing to their extraordinary adsorptive properties. Their oral administration originated from the habit of clay eating (geophagia), which has been in practice among humans and animals since ancient times to solve medical problems with natural materials available around them. In Mediterranean areas, holy clay tablets were widely consumed as cures for poison and diarrhea.[1, 43, 44] For example, Kaopectate was formulated using kaolinite as the main ingredient to treat diarrhea and to reduce toxic effects of bacteria in the digestive system.[45] Several mechanisms such as (1) antacid property, (2) adsorption of toxic chemicals, (3) adhering to mucus and improving the protective barrier, and (4) release of ions, have been suggested for gastrointestinal protection upon oral ingestion of cationic-CMs.[46] Their antacid property is attributed to the interaction of cationic-CMs with gastric acid and surface adsorption of protons, H^+.[46, 47] Reduction in gastric acidity protects the gastrointestinal system from excess acids. The high adsorption capacity and large surface area of cationic-CMs enable them to adsorb and remove toxins, excess water, gases and bacterial toxins.[44, 48] Particularly, smectite has been widely used as an anti-diarrheic since it undergoes degradation upon contact with gastric acid and produces silica gel with a high adsorption capacity, which synergistically contributes to anti-diarrheic

 This journal is © The Royal Society of Chemistry 2012

properties.[49] Furthermore, smectite adheres to the gastric and intestinal mucus membrane and diminishes irritation and gastric secretion. In addition, smectite is capable of removing gases, toxins, bacteria, and also viruses due to its high adsorptive capacity. Furthermore, smectite can increase the viscosity of mucus, the stability of glycoprotein in the mucus, and reduce the effect of pepsin on gastrointestinal membranes.[50, 51] Dioctahedral smectite has been shown to be an efficient drug in alleviating the severity of acute diarrhea in children.[52]

Topical administration of clay for curative purposes has also been practiced since ancient times and the use of cationic-CMs for dermal applications have been extensively studied by Viseras, who demonstrated the involvement of clay in absorption of soluble or exchangeable cationic species through or from skin.[12]

Cationic-CMs have been also investigated as active ingredients in antibacterial compounds. It is claimed that some clays display specific effects on bacteria due to their chemical or physical properties.[43] These effects can range from potential inhibition of microbial growth to complete sterilization. Physical properties of clay mineral that render them as antibacterial compounds are: (1) high sorptive properties, (2) large surface area, (3) mineral content, (4) pH and redox state, and (5) crystalline structure that facilitates the adsorption of essential nutrients, and/or disrupts bacteria cell envelopes, and/or impair efflux of metabolites to bacteria.[53] Clay minerals can kill bacteria by leaching out mineral compounds, toxic to bacteria, in solution.[13] The pH and oxidation state buffered by the surface of cationic-CMs are the key to control solution chemistry and redox related reactions occurring at the bacterial cell walls. In general, the antibacterial effects of cationic-CMs occur by exchange of soluble clay constituents, which are toxic to bacteria.[43, 53, 54] For example, French green clay (rich in Fe-smectite) has been used for healing Buruli ulcer, a necrotizing fasciitis. Free Fe^{2+} ions led to redox related oxidation and killed the bacteria.[43] Additionally, Fe^{2+} and P^{3+} ions leaching out from clay minerals have been shown to destroy *E. coli* in aqueous solutions. For instance, Williams and Haydel[54] found that a specific type of iron-rich French green clay (smectite) could completely destroy pathogens such as *Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*. This clay was also able to kill an antibiotic-resistant *E. coli* strain and also inhibited methicillin-resistant *Staphylococcus aureus*, which is a potential threat for nosocomial infections. Other clays such as allophane and imogolite have demonstrated antibacterial effects through chemical sorption of known bactericidal elements such as Ag^{+}, Cu^{2+}, Co^{2+}, and Zn^{2+} onto certain crystallographic sites of the mineral surface and are known to release soluble antibacterial metal oxides.[55] Furthermore, organoclays possessing antibacterial activity have also been synthesized.[56] Cationic-CMs have also attracted great interest as antibacterial and antifungal delivery devices.[57] For example, the intercalation of tetracycline, mafenide, and itraconazole into clays is shown to hold potential to treat bacterial and fungal infections.[58, 59]

In the context of modern medicine, the application of clay minerals has expanded to the treatment of several ailments such as hemorrhoids,[60] viral infections,[61] mucus colitis,[62] open wounds,[63] and acne.[64] Limited studies have focused on the use of cationic-CMs in the treatment of contemporary gastrointestinal disorders such as IBS (irritable bowel syndrome). IBS is a highly prevalent gastrointestinal motility disorder, characterized by the symptoms such as abdominal pain and discomfort.[65] Chang et al. reported abdominal pain and discomfort relief upon use of di-octahedral smectite on IBS patients.[52]

b) As an excipient: Excipients are substances that are incorporated into pharmaceutical formulations in order to improve physicochemical characteristics of active ingredients or to enhance the formulation process. Apart from their direct consumption, clay minerals have also been used as excipients in many pharmaceutical products. Cationic-CMs have been utilized as excipients via. oral administration particularly as lubricants, diluents, binders, and isotonic agents to provide better formulations of drug.[66] They have also been used as excipients to slow down or for controlled release of drug.[67] Other applications of cationic-CMs are drug taste masking, increasing drug solubility, and protecting drugs from harsh digestive system conditions.[2] Clay minerals such as palygorskite, smectite, and kaolinite are commonly used as excipients in pharmaceutical and cosmetic formulations for various purposes, among which smectite is the most commonly used cationic-CM excipient.[2] Cationic-CMs have been used as flavor correctors because drugs such as metronidazole and sildenafil can be intercalated between inter-layers of clay, which masks the unpalatable taste of these drugs.[68, 69] Cationic-CMs have also been used as disintegrates because of their high swellability and dispersibility, as thickeners or emulsifiers, as anti-caking agents because of their thixotropic and rheological properties, or as diluents and binders because of their alkaline reaction and plasticity. As isotonic agents, cationic-CMs are naturally hydrophilic and can thereby freely disperse in water, resulting in solutions with an osmotic pressure similar to that of body fluid.[70-72]

Table 2. Cationic- CMs hybridized with polymers in regenerative medicine

Polymer/s	Clay	Application	Form	Ref.
Chitosan/PGA	Mt	Bone tissue engineering	Scaffold	73
PLGA	Mt	Improved toughness and elongation of the nano-composites	Film	74
PLA	Mt	Increased tensile modulus compressive strength, hydrophilicity and degradation rate.	Scaffold	75-77
PNIPA	Lap	Enhanced elongation with almost complete recovery, increased swell-ability	Hydrogel	78-80
Chitosan/Gelatine	Mt	Reduced degradation rate and enhanced cell adhesion		81
PNIPA	Lap	Cell sheet easily detached by changing temperature		82
Gelatine/cellulose	Mt	Enhanced biodegradation and improved cell proliferation	Scaffold /film	83
PEO	Lap	Tunning cell adhesion	Film/scaffold/ hydrogel	84, 85
Gelatine	Sepiolite	Improved mechanical property	Foam	86
PEG	Lap	Improved mechanical property	Hydrogel	33, 87
Pluronic	Lap	Tough nano-composite hydrogel	Hydrogel	88
PVA	Lap	Multilayered composite films were found to be hydrated, flexible and transparent with high tensile strength, modulus and toughness.	Film/hydrogel/scaffold	89
Chitosan-g-Lactic acid	Mt	Improved Young's modulus and strength with expense of elongation by Mt reinforcement	Film /scaffold	90
Gelatine	Sepiolite	Improved mechanical properties	Foam	86
DMMA/ MEA	Ht/Lap	Soft stimuli-responsive nano-composite gel	Hydrogel	91
PNIPA	Hectorite	Improved mechanical, optical, and swelling-de-swelling properties	Gel	80

Abbreviations: polygalacturonic acid (PGA); montmorillonite (Mt); poly(lactic-co-glycolic acid) (PLGA); poly(lactic acid) (PLA); poly(N-isopropylacrylamide) (PNIPA); Poly(ethylene oxide) (PEO); N,N-dimethylacrylamide (DMMA); methoxyethyl acrylate (MEA); poly(vinyl alcohol) (PVA); poly(ethylene glycol) (PEG); poly(methyl methacrylates) (PMMA); Laponite (Lap); hectorite (Ht)

c) Enhanced pharmaceutical formulations: Studies concerning the direct hybridization of clay minerals and drugs are rapidly increasing due to the superior characteristics of clay minerals necessary for improving the formulation of pharmaceutical products.[92] Pharmaceutical product improvement includes controlled release of drugs, enhanced drug water solubility, improved drug dispersibility, and better skin absorption of the drugs.[26, 93, 94] Two methods of clay-drug hybridization are known, namely, (1) in solution intercalation, where the clay is dispersed in solubilized drug solutions followed by equilibration and finally subjected to recovery by drying in the solid phase and (2) *in-situ* melting intercalation, where clay and drug are both maintained at the melting point temperature of the drug.[21] Ito et al.[93] studied the direct intercalation of indometacin, an anti-inflammatory and analgesic drug, with montmorillonite, which was found to increase the penetration of indometacin into the skin. Enhanced drug permeability was attributed to the increase in both the stability of amorphous indometacin and also its water solubility.[93] In another study a Laponite-tetracycline hybrid was generated for the *in-situ* delivery of tetracycline for treatment of periodontal diseases.[59] The colon cancer drug, 5-fluorouracil, was intercalated in montmorillonite to decrease the side effects of this drug.[95] Park et al. studied the controlled release of donepezil, an anti-Alzheimer drug, by the hybridization of the drug into three different types of smectites: montmorillonite, synthetic Laponite, and saponite.[96] A similar study reported that the release of ranitidine was slowed down in a drug-clay hybrid compared to the formulation without a clay carrier.[67] Intercalation of a poorly water soluble drug, itraconazole, in smectite also remarkably improved its bioavailability.[97] Studies reporting the direct intercalation of clay-drug hybridization are listed in Table 3. Direct clay-drug hybrids have been used in both conventional drug delivery systems such as tablets, suspensions, topical creams and gels, as well as in novel drug delivery systems such as gene therapy,[98] wound dressing and transdermal delivery patches.[31] The effects of clay minerals on drug delivery characteristics from different formulations are discussed in the following sections,

Table 3. Cationic clays used in clay-drug hybrids

Clay	Drug	Purpose	Origin	Ref.
Mt	Buspiron HCl	Controlled release	N	99
Mt	Glutathione	Enhanced bioavailability	N	100
Lap	Tetracycline	In-situ delivery	S	59
Mt	Nifedipine	Increased solubility and biocompatibility	N	101
Mt	Metronidazole	Colon drug delivery	N	102
Mt	Sildenafile	Taste masking	N	103
Lap	Mafenide	Topical antibiotic delivery	S	104
Mt	5-Flouracil	Decrease side effects	N	95
Lap	Itraconazole	Increased solubility	S	58, 97
Mt, Lap	Donepezile	Controlled release	N, S	96
Mt	Vitamin B6	Controlled release	N	105
Mt	Docetaxel	Oral delivery	N	36
Rectorite	Ibuprofen	Decreased side effects	N	106
Mt	Timolol	Controlled release	N	107
Mt	Tramadol hydrochloride	Controlled release	N	108
Mt	Chlorhexidine	Controlled release	N	109
Mt	Vitamin B1	Controlled release (pH sensitive)	N	110

Montmorillonite (Mt); Laponite (Lap); natural (N); synthetic (S)

Slow release drug delivery: The utilization of clay minerals in modulating the bioavailability of drugs began with studies which showed a decrease in the oral absorption of drugs upon co-administration with clays.[111] Since this discovery, the intercalation of cationic drugs in cationic-CMs has been successfully employed to prolong the therapeutic action of such drugs.[96] MacGinity reported that the concentration of amphetamine sulfate in urine was much lower upon oral administration in combination with montmorillonite compared to its administration individually.[112] The stimulating effect of amphetamine reached a maxima several hours after administration of the drug, indicating a prolonged release of amphetamine from the formulation.[112] Another study on clindamycin showed that the formulations containing montmorillonite demonstrated prolonged therapeutic drug action.[113]

Hemostatic dressing: Some cationic-CMs act as anti-hemorrhagic agents due to their high swellability and capacity for water absorption.[114, 115] These lead to the formation of a paste-like substance when clay comes in contact with blood or body fluids, thereby acting as a bleeding barrier with strong adhesiveness to the surrounding tissue.[116] Commercial anti-hemorrhagic products formulated using cationic-CMs are currently available. For example, WoundStat®[115] made from granular smectite was approved by FDA for pre-hospital topical hemorrhages. Clay granules absorb water and swell upon exposure to blood and thereby increase the local concentration of clotting factors, platelets, and red blood cells necessary for stimulating clot formation. However, few reports suggested that WoundStat® may cause injury to the surrounding blood vessels.[115] Because of this potentially serious side effect,

new products such as QuikClot® have been introduced in the market. QuikClot® gauze is a wound dressing gauze impregnated with synthetic zeolite.[117, 118] Zeolite was reported to achieve hemostasis within 3 minutes during severe bleeding. Zeolite I is a micro-porous alumina-silicate with high adsorptive capacity. It possesses excellent hemostatic properties because of its micro-porous structure, which acts as a molecular sieve and adsorbs water. The adsorption of water along with trapping of platelets within the micro-pores leads to concentration of clotting factors and platelets, and promotes clot formation. Recently Combat Gauze™,[115, 118] a non-woven gauze impregnated with kaolin, has replaced other clay-based hemostatic dressing products and is the only product endorsed for use in the first line treatment of life threatening injuries. In a recently published study, Laponite and gelatin nanocomposites were formulated as an injectable hydrogel for hemorrhage treatment.[119] The hydrogel displayed enhanced *in vitro* and *in vivo* coagulation properties.

Ocular delivery systems: The treatment of posterior segment ocular diseases such as uveitis is challenging due to the natural barrier provided by the eye for drug penetration. The use of eye drops or oral medications are usually not effective and therefore placement of ocular implants has been investigated as an alternative treatment for uveitis.[120] Da silva et al. prepared polyurethane/closite implants and incorporated dexamethasone acetate, a corticosteroid used in the treatment of uveitis, into this composite.[38] Closite, (trade name for natural Mt) mixed with polyurethane resulted in significant increase in release of dexamethasone. This can be attributed to the increase in swellability and water absorption as well as polymer hydrolysis due to the presence of closite.

Gene delivery: Gene therapy is the delivery of oligonucleotides, especially DNA (deoxyribonucleic acid), used as drugs to target cells in order to treat genetic deficiencies and life-threatening diseases such as cancer.[121] Gene therapy is a novel therapeutic approach and recently has gained significant attention in bio-pharmaceutics. A suitable carrier system is required for the effective delivery of DNA into cells. Cationic-CM nanoparticles have been recently investigated as DNA delivery systems. It has been shown that montmorillonite, a cationic clay mineral, protects DNA and prevents its degradation by the action of the nuclease enzyme.[122, 123] Protective effects of montmorillonite on plasmid DNA via oral gene delivery method has also been studied.[98] The penetration of cell membranes by DNA carrying clay nanoparticles, presumably through phagocytosis or endocytosis, and delivery to cell nucleus has been successfully demonstrated.[124, 125]

A major challenge in using cationic-CM as DNA carriers is the negative charge on both clay and DNA, which results in a repulsive electrostatic force that makes the intercalation of DNA into inter-layer spaces of cationic-CM difficult to achieve. However, DNA is capable of adsorbing to some extent, to the positive charges around the edges of clay nanoparticles without undergoing any modification.[123] Organoclays have been formulated to make clay more hydrophobic and therefore more suitable to carry organic macromolecules in order to overcome repulsion between clay nanoparticles and DNA. Lin et al.[124] developed an organoclay by combining montmorillonite with hexadecyltrimethylammonium (HDTMA) and illustrated successful intercalation of DNA into the inter-layer spaces of modified montmorillonite.

Cationic-CM in cosmetics

A cosmetic product is any substance or formulation intended for external application on human body (skin, teeth, hair or lips) for the purpose of cleaning, beautification, or alteration of appearance without affecting the basic body structure or function.[64] A broad range of topical personal care, cosmetic, and personal hygiene products are made from cationic-CMs for cleansing and moisturization of the skin and to combat compact lipodystrophies, acne, and cellulite.[126] Cationic-CMs are inert and biocompatible, and therefore do not affect the skin structure or function. Moreover, physical properties of cationic-CMs make them suitable for use in cosmetic products. Physical properties such as particle size, shape, high specific surface area, texture, color, and brightness as well as chemical properties like surface chemistry, charge, and chemical composition of clays are the main reasons for their use in cosmetic products. As an active ingredient, cationic-CM adsorbs unwanted substances, like sebum oils and residues generated by the sebaceous glands, toxins, greases and odors, generated by the skin or by the natural skin microbiota. Because of its high sorptive capacity, optimum rheological characteristics, and large specific surface area,[127] cationic-CMs are employed in myriad products such as antiperspirants, skin cleansers, odor absorbers, sports and athletic sprays and powders, foot and body powders, body sprays, and deodorants. Unlike heavily perfumed products that mask odors and can cause skin irritation, cationic-CMs are generally non-irritable to the skin and in fact soothe the skin. Some of the cationic-CMs commonly used in cosmetics are kaolinite, montmorillonite, saponite, hectorite, palygorskite, and sepiolite. These have also been used as excipients in cosmetics due to their high cation exchange capacity, excellent swelling properties, hydration ability, and suitable rheological behavior. The fine texture of clay and its thixotropic behavior (plasticity) allows easy application of makeup and increases its durability after application on skin. Due to their excellent coverage, absorption, and adhesion properties, cationic-CMs have been used for creating a flawless finishing effect to hide imperfections and fine lines, even at medium coverage, in facial treatments such as black-spots, acne, and seborrhea. Cationic-CMs possess an oil control property that allows the makeup to withstand

 This journal is © The Royal Society of Chemistry 2012

perspiration and wet or humid conditions. However, they do not allow the skin to dry by adsorbing oil because clay particles adhere to and form a protective film over the skin surface and keep it moist. This protective film also provides a mechanical layer of protection against external physical or chemical agents.[127]

The clays that are currently used in cosmetic products are often unmodified natural products. However, the use of modified clays in cosmetic formulations are also increasing in order to impart new functionalities and improved properties. New composite materials that modify the initial hydrophilic character of clay have been developed. This has widened their applications and further increased their already wide utilization in cosmetics. Synthetic clays are also being used in cosmetic products for different purposes including topical administration of active molecules and heat-therapy or protection against environmental agents. For example, the intercalation of vitamin C, antioxidants, and therapeutic elements such as vitamin B1 and vitamin B6 in cationic-CMs has been investigated in order to deliver these elements to the skin for cosmetic purposes.[105, 128, 129]

Clay minerals are widely used in sunscreen formulations due to their protective effect against UV rays. Sunscreens are cosmetics which have health related applications and clay minerals possessing a high refractive index and good light scattering properties can block UV radiation and be used as solar protectors. The sunscreen properties of clay minerals are specifically attributed to their ability to scatter and absorb radiant energy and thus protect DNA in skin cells from UV damage. The effectiveness of clay minerals as sunscreen agents also stems from their stability against UV-degradation as well as non-allergic and non-toxic degradation products.[130] Bentonite and hectorite organoclays are increasingly recognized as effective sunscreen agents.[131,12]

Cationic-CM in regenerative medicine

Regenerative medicine encompasses a broad category of approaches involving replacement or regeneration of cells, tissues, and organs to restore their natural functions. Clay particles were initially utilized as fillers for enhancing the mechanical strength of biopolymers and have recently also been employed as biomaterials in regenerative medicine and tissue engineering as (1) mechanical property enhancers, (2) biomolecule carriers, (3) for improvement of cell adhesion, and (4) proliferator and differentiator substances for progenitor cells. [132, 133] All four applications of clay minerals in regenerative medicine are illustrated in figure 5. For all these applications, hybrids made from clay minerals and a variety of biodegradable and biocompatible polymers have been fabricated as 3D scaffolds, hydrogels and thin films. Naturally-derived polymers and polysaccharides such as collagen, gelatin, alginate, and chitosan[134-136] or synthetic polymers such as polyethylene glycol (PEG), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) have been combined with many nanoclays, mostly montmorillonite (Mt), and synthetic nanoclays such as Laponite. The resulting nanocomposites are tougher, are improved in bio-responsiveness, display improved viscoelastic behavior, and enhanced electroconductivity.[137]

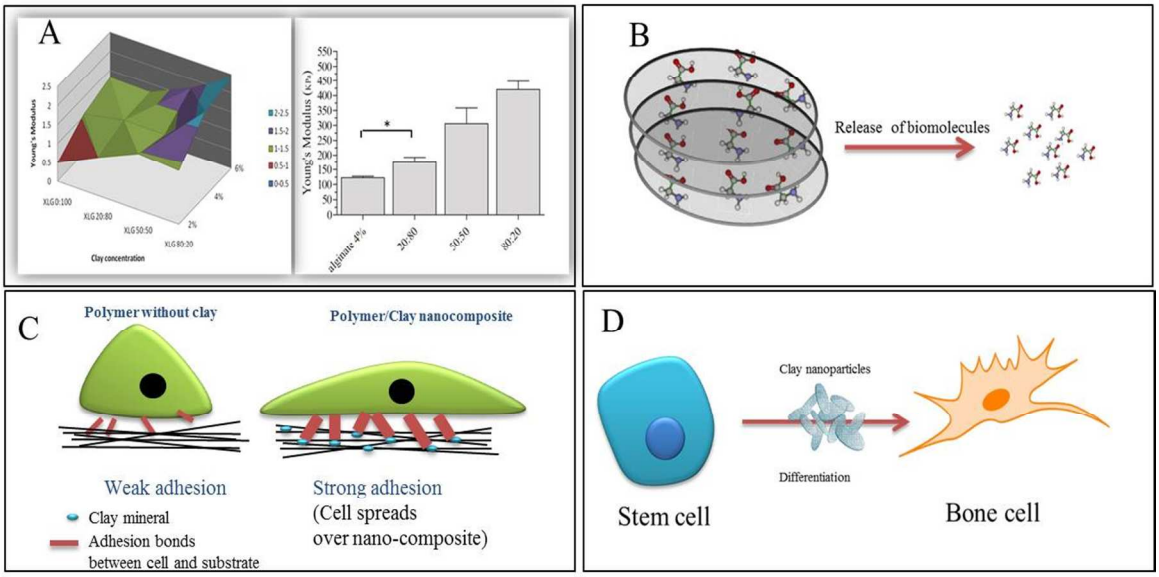


Figure 5. Application of cationic clay minerals in regenerative medicine: enhancing the mechanical property of polymers (A), delivering biomolecules (B), improving cell adhesion (C), and facilitating the proliferation and differentiation of stem cells to bone cells (D).

In tissue engineering a three-dimensional support (scaffold) is required for initiating cell attachment and for subsequent tissue growth.[138] Tissue engineering scaffolds must possess adequate mechanical strength, suitable porosity, permeability as well as provide an appropriate extracellular matrix (ECM) for cells to allow biological and molecular signaling for regulating the tissue reparative capacity and vascularization.[138, 139] Cationic-CMs have been studied in combination with different polymers to achieve these two characteristics. The structural aspect of scaffolds was addressed by using cationic-CM as fillers to improve the mechanical properties of synthetic and natural polymer scaffolds.[140] For instance, a PLGA/Mt nanocomposite showed improved toughness and PLGA elongation when clay minerals were added.[141] Combining Mt with PLA enhanced the rate of nanocomposite biodegradation compared to that of PLA alone.[142] Further, PLGA mixed with Laponite resulted in nanocomposites in the form of films, which were highly extensible at low silicate concentrations and mechanically strong at high silicate concentrations.[143] A similar study focusing on the viscoelastic behavior of PEG/Laponite hydrogel demonstrated an improved hydrogel mechanical strength along with an increase in tensile modulus and fracture strength after addition of Laponite. The hydrogel preserved the ability to recover from deformation once the compressive load was removed. Photo cross-linking of poly(ethylene oxide) - poly(propylene oxide) - poly(ethylene oxide); PEO-PPO-PEO; tri-block copolymer diacrylates (Pluronic F127 diacrylate) in the presence of Laponite improved the toughness of the tri-block compared to their hydrogel counterparts without Laponite.[88] Another study demonstrated a reduction in permeability while the biocompatibility and mechanical properties of polyurethane were maintained after organically modified Laponite nanoparticles were added to this polymer. The resulting nanocomposite showed a five-fold lower permeability towards water vapor when compared to polyurethane alone.[144] Zheng et al. demonstrated that a gelatin/montmorillonite/chitosan nanocomposite scaffold possessed a suitable pore structure, where montmorillonite led to improvement in the mechanical property as well as in the adhesion and proliferation of stem cells onto the scaffold.[81] A similar study on gelatin-montmorillonite-cellulose scaffold created especially for tissue engineering showed that the amount of montmorillonite incorporated in the scaffold controlled the rate of degradation and improved the osteoblast cytocompatibility of the composite.[83] It has also been shown that the cellular responses of biomaterials are enhanced upon incorporation of silicate-based particles.[145] The presence of silica in Laponite was reported to provide sites for cell adhesion and allowed cell spreading and proliferation.[143] For example, the addition of synthetic cationic clay (Laponite) to alginate not only enhanced its mechanical toughness but also enhanced cell attachment on this nanocomposite.[136] Addition of Laponite to cross-linked PEO was shown to modulate cell adhesion and mineralization for bone tissue engineering application.[146] This study suggested that cellular adhesion and proliferation could be modulated by altering the concentration of clay in nanocomposites. Similarly, Gaharwar et al. demonstrated that the incorporation of Laponite into PEG promoted cell adhesion.[147] Poly(N-isopropylacrylamide), PNIPA, hydrogel generally does not support cell adhesion and spreading. However, the nanocomposite of PNIPA hydrogel and cationic-CM showed improved cell adhesion and proliferation depending on clay concentration in the hydrogel.[82] Enhanced cell adhesion and proliferation in clay/PNIPA nanocomposite was attributed to (1) higher protein absorption on the surface of clay nanoparticles, (2) surface flatness, and (3) hydrophilization of PNIPA chains, favorable for cell attachment and growth, due to negatively charged clay particles. Similar studies on clay/PMEA (Poly(2-Methoxyethyl Acrylate)) nanocomposites also showed that clay nanoparticles played a significant role in increasing cell attachment and cell proliferation.[148, 149]

Cationic-CM has also been intercalated with proteins, micronutrients, and other bioactive macromolecules, which can promote cell signaling and growth in the scaffold. Dawson et. al[132] demonstrated the *in-situ* self-assembling ability of cationic-CM in response to physiological saline when materials were incorporated with regenerative micronutrients such as vascular endothelial growth factor (VEGF) to stimulate angiogenesis. The hydrogel clay (Laponite) scaffold has the capability to transport proteins and nutrients necessary for the modulation and differentiation of progenitor cells.[132, 140] In a similar study, Gaharwar et al. demonstrated that synthetic cationic-CM (Laponite) induced osteogenic differentiation of stem cells without the presence of any additional osteoinductive agents.[133]

Although three-dimensional scaffolds and hydrogels are commonly utilized in tissue engineering, two-dimensional films are also being employed as coatings in regenerative medicine. Polymer-clay films have been developed for regenerative applications such as wound dressing or medical device coatings. For example, a chitosan-montmorillonite hybrid was grafted onto polydimethylsiloxane (PDMS) chains and the resulting film showed increased flexibility, water retention, and swelling behavior, which are beneficial for improved cell growth. Similar results were obtained using a lactic acid grafted chitosan/montmorillonite hybrid.[90] Preparation of polyurethane foam mixed with alginate/bentonite hydrogel created a pH sensitive wound dressing that provided a pH-dependent bovine serum albumin (BSA) release profile.[150]

Cationic-CM in biosensors

A sensor is defined as a device that can transfer chemical or biochemical changes arising due to chemical interactions between the medium around the analyte and the sensor device, into a measurable and analytically informative signal. In the context of biosensors, a receptor is any biological module such as an enzyme or an antibody within the cell and where a biological

 This journal is © The Royal Society of Chemistry 2012

mechanism substitutes a chemical process (Figure 6).[151] Electrochemical sensors are most desirable among all sensors due to their high sensitivity, experimental simplicity, and low cost production. In such sensors, the measured signal can either be current-based (amperometry), voltage-based (potentiometry), or impedance-based change (conductometry).[152] Electrochemical sensors are generally composed of an electro-active polymer and an inorganic two-dimensional solid such as layered silicate substances (e.g., clays, sol-gel silicates, layered double hydroxides). Cationic-CMs have attracted considerable attention among all layered inorganic substances owing to their unique properties such as high cation-exchange capacity, large surface area, and chemical inertness. Although, cationic-CMs are naturally non-conductive,[153] they can serve as matrices for electro-active ion transfer due to their cation exchange ability. Clay mineral-based biosensors are generally called clay-modified electrodes and are prepared by depositing thin films of clay onto conductive substances (Figure 6).[154] Most clay minerals used in clay-based biosensors are smectites, for example Laponite, montmorillonite, nontronite[155], and have been applied in analytical applications.

One of the key steps during biosensor designing is the effective immobilization of bio-molecules (e.g., enzymes) on a suitable electrode. The process of immobilization should preserve the biological activity of bio-molecules, increase their stability, and provide accessibility to the sample analyte.[156] Cationic-CMs are inert substances and hence do not deactivate bio-molecules. Further, owing to their cation exchange capacity, cationic-CMs can efficiently immobilize several cationic bio-molecules in their structure.[157] Immobilization of biological components on cationic-CMs has been performed using various methods such as covalent bonding, chemisorption, intercalation, or physical entrapment.[158] Proteins and enzymes can be immobilized both within the interlayer space of the clay and also on the surface of clay particles.[159] In this regard, smectites are suitable cationic-CMs for the immobilization of cations (cation exchange process) and also anions (attached to the edges of clay particles).[155]

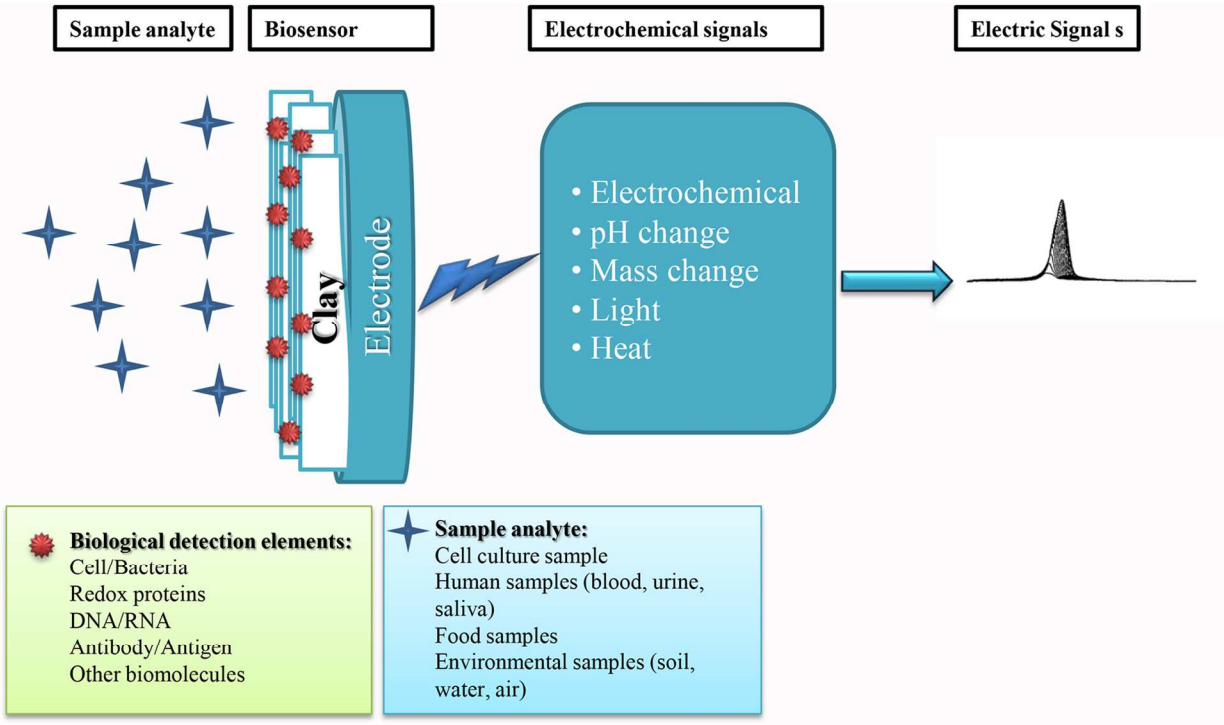


Figure 6. Representation of a clay-based material in biosensors.

Besombes et al. [155, 160, 161] reported that the presence of Laponite within a poly (amphiphilic pyrrole derivative) matrix enhanced the sensitivity and stability of a cholesterol biosensor. This study also demonstrated the possibility of cholesterol quantification using the given matrix, which was not feasible with a poly (amphiphilic pyrrole derivative) biosensor without Laponite. Urea,[162] phenol,[163] hydrogen peroxide,[164] and lactic acid are among the several biological factors that can be detected by these biosensors. Laponite also provides an excellent biocompatible microenvironment for the immobilization of oxidases. For example, an amperometric galactose biosensor was developed by immobilizing galactose oxidase (GAOx) within a Laponite film coated on a platinum electrode surface.[165] Apart from the conventional use of clays in biosensors, customized synthetic cationic-CM with conductive ions for electrochemical biosensors are being researched recently. For example, two types of smectite containing cobalt[166] or copper[167] in their structures were synthesized and tested for electro-conductivity for use as electrochemical sensors.

Cationic-CM in medical devices

A current major trend in the development of medical devices is the use of nanocomposites to create drug delivery patches, implantable or insertable medical devices, and laboratory plastics for cell culture. Biodegradable polymers hybridized with inorganic nanoparticles (nanocomposites) have improved the mechanical and thermal properties of polymers. Nanocomposites may also degrade at a faster rate due to the lower degree of crystallinity of the polymer in nanocomposites and the intercalation of polymer degradation initiators into inter-layer spaces of the clay.[168]

Clay nanoparticles used as fillers in polymers improve polymer characteristics, enabling fabrication of advanced plastic medical devices. The improved characteristics include (1) enhanced mechanical properties and reduced shrinkage, (2) decreased gas permeability, (3) advanced thermal retardancy, (4) improved chemical resistance, (5) modified surface characteristics, and (6) improved electrical conductivity.[168] These materials can be shaped into various forms such as thin films, rods, spheres, and hollow tubes. One of the currently explored biomedical applications is the use of thermo-responsive polymers for the production of enzyme-free cell-harvesting systems.[82] A non-allergic composite material fabricated from nanocomposites with block copolymers, such as polystyrene-b-poly(ethylene-co-butylene)-b-polystyrene mixed with polyolefins, and clays has been suggested for the replacement of latex rubber and poly(vinyl chloride), PVC, in medical tubings such as catheters.[169] Another example of medical grade nanocomposites is flexible PVC-Mt, which has been utilized in a wide range of medical devices ranging from examination gloves and tubings to blood bags and dialysis equipments.[170] Enhanced barrier properties would be of potential benefit, both in terms of keeping air and moisture out and keeping plasticizers in, in many of these applications.

Cationic-CM in animal health

There is an intimate relationship between the environment, specifically between geological materials and animal health.[3] Clay consumption (geophagy) is commonly observed in reptiles, birds and mammals.[171,146] Several theories such as removal of toxins present in the diet, treatment of gastrointestinal ailments such as diarrhea via the antimicrobial components of clay minerals, mineral supplementation, and alleviating stomach hyperacidity have been suggested for the geophagic behavior of animals.[47] In many cases of geophagy, minerals from the kaolin group were identified as major components consumed among the clay minerals. Cationic-CMs, particularly smectites, with a 2:1 structure and high adsorptive capacity are consumed by animals as an antitoxin agent, while 1:1 clay minerals such as kaolinite tend to be more effective against gastric disorders [44, 172] and have been used as veterinary medicine to prevent diarrheal infections. Clay minerals have been used in farm animal feeds as formulation excipients (binder to pellet the animal food) and also as adsorbents for mycotoxins and heavy metals.

III. Conclusion

The benefits of cationic clay minerals for human and animal health are well known and have been used in traditional medicine since ancient times. Cationic clay minerals have recently found way in a diverse range of applications in various biomedical fields such as pharmaceutics, cosmetics, biosensors, load bearing implantable devices, drug delivery systems, tissue engineering and several more. In particular, the combination of cationic clay minerals with polymers and organic compounds has resulted in the development of novel hybrid materials tailored for specific biological applications. Cationic clay minerals offer novel perspectives in biomedical applications of inorganic-based materials.

References

1. P. W. Abrahams, *Applied Geochemistry*, 2012, **27**, 954-968.
2. M. I. Carretero, C. S. F. Gomes and F. Tateo, in *Developments in Clay Science*, eds. B. Faïza and L. Gerhard, Elsevier, 2013, vol. Volume 5, pp. 711-764.
3. R. Slamova, M. Trckova, H. Vondruskova, Z. Zraly and I. Pavlik, *Applied Clay Science*, 2011, **51**, 395-398.
4. E. Rivagli, A. Pastorello, M. Sturini, F. Maraschi, A. Speltini, L. Zampori, M. Setti, L. Malavasi and A. Profumo, *Journal of Environmental Chemical Engineering*, 2014, **2**, 738-744.
5. N. Klein, F. Frohlich and S. Krief, *Naturwissenschaften*, 2008, **95**, 325-331.
6. E. P. Eslinger, D, in *Clay Minerals for Petroleum Geologists and Engineers.*

, Society of Economic Paleontologists and Mineralogists, 1988.
7. W. T. Reichle, *Solid State Ionics*, 1986, **22**, 135-141.
8. J. J. Bravo-Suárez, E. A. Páez-Mozo and S. T. Oyama, *Química Nova*, 2004, **27**, 601-614.
9. L. B. de Paiva, A. R. Morales and F. R. Valenzuela Díaz, *Applied Clay Science*, 2008, **42**, 8-24.

 This journal is © The Royal Society of Chemistry 2012

10. Seema and M. Datta, *Applied Clay Science*, 2013, **80–81**, 85-92.
11. Y. N. Vodyanitskii, *Eurasian Soil Science*, 2014, **47**, 255-265.
12. C. Viseras, C. Aguzzi, P. Cerezo and A. Lopez-Galindo, *Applied Clay Science*, 2007, **36**, 37-50.
13. E. Pazdziora, K. Matejova, M. Valaskova, S. Holesova and M. Hundakova, *Nanocon 2010, 2nd International Conference*, 2010, 460-464.
14. Y. Xiang and G. Villemure, *Clays and Clay Minerals*, 1996, **44**, 515-521.
15. S. Tamás, B. Aristides, T. Vassilios, P. Szilvia, K. László, G. Gábor, M. Kuanyshbek, B. Didara, P. Dimitris and D. Imre, *Nanotechnology*, 2007, **18**, 285602.
16. J. Herney-Ramirez, M. A. Vicente and L. M. Madeira, *Applied Catalysis B: Environmental*, 2010, **98**, 10-26.
17. A. A. Yehia, A. M. Akelah, A. Rehab, S. H. El-Sabbagh, D. E. El Nashar and A. A. Koriem, *Kgk-Kautschuk Gummi Kunststoffe*, 2009, **62**, 580-588.
18. S. Ganguly, K. Dana, T. K. Mukhopadhyay, T. K. Parya and S. Ghatak, *Transactions of the Indian Ceramic Society*, 2011, **70**, 189-206.
19. D. Zhang, C.-H. Zhou, C.-X. Lin, D.-S. Tong and W.-H. Yu, *Applied Clay Science*, 2010, **50**, 1-11.
20. S. Sinha Ray and M. Okamoto, *Progress in Polymer Science*, 2003, **28**, 1539-1641.
21. H. A. Patel, R. S. Somani, H. C. Bajaj and R. V. Jasra, *Bulletin of Materials Science*, 2006, **29**, 133-145.
22. W. H. Yu, N. Li, D. S. Tong, C. H. Zhou, C. X. Lin and C. Y. Xu, *Applied Clay Science*, 2013, **80–81**, 443-452.
23. M. Olteanu, D. Achimescu, Z. Vuluga and V. Trandafir, *Revue Roumaine De Chimie*, 2008, **53**, 157-163.
24. J. A. S. Rausell-Colom, J. M., in *Monograph, Mineralogical Society* 1987, pp. 371-422.
25. F. Bergaya and G. Lagaly, *Applied Clay Science*, 2001, **19**, 1-3.
26. W. F. Lee and Y. T. Fu, *Journal of Applied Polymer Science*, 2003, **89**, 3652-3660.
27. S. P. Vasilakos and P. A. Tarantili, *J Biomed Mater Res B Appl Biomater*, 2012, **100B**, 1899-1910.
28. G. Rytwo, H. Varman, N. Bluvshtein, T. N. Konig, A. Mendelovits and A. Sandler, *Applied Clay Science*, 2011, **51**, 43-50.
29. S. P. Vasilakos and P. A. Tarantili, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2012, **100B**, 1899-1910.
30. S. Mallakpour and M. Dinari, *Journal of Applied Polymer Science*, 2012, **124**, 4322-4330.
31. S. Shaikh, A. Birdi, S. Qutubuddin, E. Lakatosh and H. Baskaran, *Annals of Biomedical Engineering*, 2007, **35**, 2130-2137.
32. K. E. Styan, D. J. Martin and L. A. Poole-Warren, *Journal of Biomedical Materials Research Part A*, 2008, **86A**, 571-582.
33. C.-W. Chang, A. van Spreeuwel, C. Zhang and S. Varghese, *Soft Matter*, 2010, **6**, 5157-5164.
34. A. K. Gaharwar, V. Kishore, C. Rivera, W. Bullock, C. J. Wu, O. Akkus and G. Schmidt, *Macromolecular Bioscience*, 2012, **12**, 779-793.
35. Y. Dong and S.-S. Feng, *Biomaterials*, 2005, **26**, 6068-6076.
36. S. S. Feng, L. Mei, P. Anitha, C. W. Gan and W. Zhou, *Biomaterials*, 2009, **30**, 3297-3306.
37. B. Sun, B. Ranganathan and S. S. Feng, *Biomaterials*, 2008, **29**, 475-486.
38. G. R. da Silva, A. da Silva-Cunha Jr, F. Behar-Cohen, E. Ayres and R. L. Oréfice, *Materials Science and Engineering: C*, 2011, **31**, 414-422.
39. Y. Dong and S. S. Feng, *Biomaterials*, 2005, **26**, 6068-6076.
40. H. M. Kelly, P. B. Deasy, E. Ziaka and N. Claffey, *International Journal of Pharmaceutics*, 2004, **274**, 167-183.
41. V. K. Garripelli and S. Jo, *Journal of Bioactive and Compatible Polymers*, 2012, **27**, 198-209.
42. F. C. H. Pinto, A. Silva-Cunha, G. A. Pianetti, E. Ayres, R. L. Orefice and G. R. Da Silva, *Journal of Nanomaterials*, 2011.
43. L. B. Williams and S. E. Haydel, *International Geology Review*, 2010, **52**, 745-770.
44. N. J. Dominy, E. Davoust and M. Minekus, *Journal of Experimental Biology*, 2004, **207**, 319-324.
45. Z. H. McMahan and H. L. Dupont, *Alimentary Pharmacology & Therapeutics*, 2007, **25**, 759-769.
46. P. S. Charles Banenzoue, Jean-Aimé Mbey* and Daniel Njopwouo and *Journal of Chemical and Pharmaceutical Research*, 2014, **6**, 668-676.
47. M. J. Wilson, *Journal of Chemical Ecology*, 2003, **29**, 1525-1547.
48. A. A. Madkour, E. M. H. Madina, O. E. Z. El-Azzouni, M. A. Amer, T. M. K. El-Walili and T. Abbass, *Journal of Pediatric Gastroenterology & Nutrition*, 1993, **17**, 176-181.
49. I. Novak and B. Cicel, *Clays and Clay Minerals*, 1978, **26**, 341-344.
50. A. López-Galindo and C. Viseras, in *Interface Science and Technology*, eds. W. Fernando and S. Kestur Gundappa, Elsevier, 2004, vol. Volume 1, pp. 267-289.
51. A. Leonard, M. T. Droy-Lefaix and A. Allen, *Gastroenterologie clinique et biologique*, 1994, **18**, 609-616.
52. F.-Y. Chang, C.-L. Lu, C.-Y. Chen and J.-C. Luo, *Journal of Gastroenterology and Hepatology*, 2007, **22**, 2266-2272.
53. L. B. Williams, D. W. Metge, D. D. Eberl, R. W. Harvey, A. G. Turner, P. Prapaipong and A. T. Poret-Peterson, *Environmental Science & Technology*, 2011, **45**, 3768-3773.
54. S. E. Haydel, C. M. Remenih and L. B. Williams, *Journal of Antimicrobial Chemotherapy*, 2008, **61**, 353-361.
55. Y. Onodera, T. Iwasaki, A. Chatterjee, T. Ebina, T. Satoh, T. Suzuki and H. Mimura, *Applied Clay Science*, 2001, **18**, 123-134.
56. H. He, D. Yang, P. Yuan, W. Shen and R. L. Frost, *Journal of Colloid and Interface Science*, 2006, **297**, 235-243.
57. K. Malachová, P. Praus, Z. Pavlíčková and M. Turicová, *Applied Clay Science*, 2009, **43**, 364-368.

58. H. Jung, H. M. Kim, Y. Bin Choy, S. J. Hwang and J. H. Choy, *International Journal of Pharmaceutics*, 2008, **349**, 283-290.
59. M. Ghadiri, H. Hau, W. Chrzanowski, H. Agus and R. Rohanizadeh, *Rsc Advances*, 2013, **3**, 20193-20201.
60. *USA Pat.*, 2009.
61. G. Rytwo, A. Mendelovits, D. Eliyahu, J. Pitcovski and E. Aizenshtein, *Applied Clay Science*, 2010, **50**, 569-575.
62. M. T. Droy-Lefaix and F. Tateo, in *Developments in Clay Science*, eds. B. K. G. T. Faïza Bergaya and L. Gerhard, Elsevier, 2006, vol. Volume 1, pp. 743-752.
63. M. Sirousazar, M. Kokabi and Z. M. Hassan, *Journal of Biomaterials Science-Polymer Edition*, 2011, **22**, 1023-1033.
64. E. L. Toombs, *Dermatologic Clinics*, 2005, **23**, 575-581.
65. I. Nastaskin, E. Mehdikhani, J. Conklin, S. Park and M. Pimentel, *Digestive Diseases and Sciences*, 2006, **51**, 2113-2120.
66. M. I. Carretero and M. Pozo, *Applied Clay Science*, 2009, **46**, 73-80.
67. G. V. Joshi, B. D. Kevadiya and H. C. Bajaj, *Drug Development and Industrial Pharmacy*, 2010, **36**, 1046-1053.
68. J. H. Lee, G. Choi, Y. J. Oh, J. W. Park, Y. Bin Choy, M. C. Park, Y. J. Yoon, H. J. Lee, H. C. Chang and J. H. Choy, *International Journal of Nanomedicine*, 2012, **7**, 1635-1649.
69. R. Shrivastava, S. R. Jain and S. G. Frank, *Journal of Pharmaceutical Sciences*, 1985, **74**, 214-216.
70. H. Patel, A. Stalcup, R. Dansereau and A. Sakr, *International Journal of Pharmaceutics*, 2003, **264**, 35-43.
71. C. Viseras, G. H. Meeten and A. Lopez-Galindo, *International Journal of Pharmaceutics*, 1999, **182**, 7-20.
72. G. Lagaly, *Applied Clay Science*, 1989, **4**, 105-123.
73. A. H. Ambre, K. S. Katti and D. R. Katti, *Journal of Nanotechnology in Engineering and Medicine*, 2010, **1**, 031013-031013.
74. W. Xu, S. Raychowdhury, D. D. Jiang, H. Retsos and E. P. Giannelis, *Small*, 2008, **4**, 662-669.
75. J. H. Lee, T. G. Park, H. S. Park, D. S. Lee, Y. K. Lee, S. C. Yoon and J. D. Nam, *Biomaterials*, 2003, **24**, 2773-2778.
76. Y. H. Lee, J. H. Lee, I. G. An, C. Kim, D. S. Lee, Y. K. Lee and J. D. Nam, *Biomaterials*, 2005, **26**, 3165-3172.
77. I. Tsivintzelis, E. Pavlidou and C. Panayiotou, *Journal of Supercritical Fluids*, 2007, **40**, 317-322.
78. K. Haraguchi and T. Takehisa, *Advanced Materials*, 2002, **14**, 1120-1124.
79. K. Haraguchi, H. J. Li, K. Matsuda, T. Takehisa and E. Elliott, *Macromolecules*, 2005, **38**, 3482-3490.
80. K. Haraguchi and L. Y. Song, *Macromolecules*, 2007, **40**, 5526-5536.
81. J. P. Zheng, C. Z. Wang, X. X. Wang, H. Y. Wang, H. Zhuang and K. D. Yao, *Reactive and Functional Polymers*, 2007, **67**, 780-788.
82. K. Haraguchi, T. Takehisa and M. Ebato, *Biomacromolecules*, 2006, **7**, 3267-3275.
83. A. Haroun, A. Gamal-Eldeen and D. K. Harding, *Journal of Materials Science: Materials in Medicine*, 2009, **20**, 2527-2540.
84. A. K. Gaharwar, P. J. Schexnailder, B. P. Kline and G. Schmidt, *Acta Biomaterialia*, 2011, **7**, 568-577.
85. A. K. Gaharwar, V. Kishore, C. Rivera, W. Bullock, C.-J. Wu, O. Akkus and G. Schmidt, *Macromolecular Bioscience*, 2012, **12**, 779-793.
86. M. Frydrych, C. Wan, R. Stengler, K. U. O'Kelly and B. Chen, *Journal of Materials Chemistry*, 2011, **21**, 9103-9111.
87. A. K. Gaharwar, C. P. Rivera, C. J. Wu and G. Schmidt, *Acta Biomaterialia*, 2011, **7**, 4139-4148.
88. C. J. Wu, A. K. Gaharwar, B. K. Chan and G. Schmidt, *Macromolecules*, 2011, **44**, 8215-8224.
89. T. U. Patro and H. D. Wagner, *Nanotechnology*, 2011, **22**.
90. D. Depan, A. P. Kumar and R. P. Singh, *Acta Biomaterialia*, 2009, **5**, 93-100.
91. K. Haraguchi, K. Murata and T. Takehisa, *Macromolecules*, 2011, **45**, 385-391.
92. L. A. d. S. Rodrigues, A. Figueiras, F. Veiga, R. M. de Freitas, L. C. C. Nunes, E. C. da Silva Filho and C. M. da Silva Leite, *Colloids and Surfaces B: Biointerfaces*, 2013, **103**, 642-651.
93. T. Ito, T. Sugafuji, M. Maruyama, Y. Ohwa and T. Takahashi, *Journal of Supramolecular Chemistry*, 2001, **1**, 217-219.
94. J. L. White and S. L. Hem, *Industrial & Engineering Chemistry Product Research and Development*, 1983, **22**, 665-671.
95. F. H. Lin, Y. H. Lee, C. H. Jian, J. M. Wong, M. J. Shieh and C. Y. Wang, *Biomaterials*, 2002, **23**, 1981-1987.
96. J. K. Park, Y. Bin Choy, J. M. Oh, J. Y. Kim, S. J. Hwang and J. H. Choy, *International Journal of Pharmaceutics*, 2008, **359**, 198-204.
97. H. Jung, H. M. Kim, Y. B. Choy, S. J. Hwang and J. H. Choy, *Applied Clay Science*, 2008, **40**, 99-107.
98. M. Kawase, Y. Hayashi, F. Kinoshita, E. Yamato, J. Miyazaki, J. Yamakawa, T. Ishida, M. Tamura and K. Yagi, *Biol Pharm Bull*, 2004, **27**, 2049-2051.
99. G. V. Joshi, B. D. Kevadiya and H. C. Bajaj, *Microporous and Mesoporous Materials*, 2010, **132**, 526-530.
100. M. Baek, J. H. Choy and S. J. Choi, *Int J Pharm*, 2012, **425**, 29-34.
101. I. J. Marques, P. D. Vaz, A. C. Fernandes and C. D. Nunes, *Microporous and Mesoporous Materials*, 2014, **183**, 192-200.
102. I. Calabrese, G. Cavallaro, C. Scialabba, M. Licciardi, M. Merli, L. Sciascia and M. L. Turco Liveri, *International Journal of Pharmaceutics*, 2013, **457**, 224-236.
103. J. H. Lee, G. Choi, Y. J. Oh, J. W. Park, Y. B. Choy, M. C. Park, Y. J. Yoon, H. J. Lee, H. C. Chang and J. H. Choy, *Int J Nanomedicine*, 2012, **7**, 1635-1649.
104. M. Ghadiri, W. Chrzanowski and R. Rohanizadeh, *J Mater Sci Mater Med*, 2014, **25**, 2513-2526.
105. G. Joshi, H. Patel, H. Bajaj and R. Jasra, *Colloid and Polymer Science*, 2009, **287**, 1071-1076.
106. H. Y. Wang, G. K. Zhang, H. H. Gan and Y. Y. Gao, *Journal of Wuhan University of Technology-Materials Science Edition*, 2009, **24**, 654-657.
107. G. V. Joshi, B. D. Kevadiya, H. A. Patel, H. C. Bajaj and R. V. Jasra, *Int J Pharm*, 2009, **374**, 53-57.

 This journal is © The Royal Society of Chemistry 2012

108. Y. M. Chen, A. N. Zhou, B. Liu and J. Liang, *Applied Clay Science*, 2010, **49**, 108-112.
109. N. Meng, N. L. Zhou, S. Q. Zhang and J. Shen, *International Journal of Pharmaceutics*, 2009, **382**, 45-49.
110. G. V. Joshi, H. A. Patel, B. D. Kevadiya and H. C. Bajaj, *Applied Clay Science*, 2009, **45**, 248-253.
111. C. Aguzzi, P. Cerezo, C. Viseras and C. Caramella, *Applied Clay Science*, 2007, **36**, 22-36.
112. J. W. McGinity and J. L. Lack, *Journal of Pharmaceutical Sciences*, 1977, **66**, 63-66.
113. L. S. Porubcan, C. J. Serna, J. L. White and S. L. Hem, *Journal of Pharmaceutical Sciences*, 1978, **67**, 1081-1087.
114. T. Peng, *Trends Biomater. Artif. Organs,* , 2010, **24**, 27-68.
115. B. S. P. Kheirabadi, J. E. M. D. Mace, I. B. M. S. Terrazas, C. G. M. S. Fedyk, J. S. D. V. M. Estep, M. A. P. Dubick and L. H. M. D. Blackbourne, *Journal of Trauma-Injury Infection & Critical Care*, 2010, **68**, 269-278.
116. H. T. Peng and P. N. Shek, *Expert Review of Medical Devices*, 2010, **7**, 639-659.
117. D. Johnson, B. Gegel, J. Burgert, J. Gasko, C. Cromwell, M. Jaskowska, R. Steward and A. Taylor, *ISRN Emergency Medicine*, 2012, **2012**, 6.
118. S. Gebauer, D. Hoopes and E. Finlay, *Journal of Pain and Symptom Management*, 2013, **46**, E6-E8.
119. A. K. Gaharwar, R. K. Avery, A. Assmann, A. Paul, G. H. McKinley, A. Khademhosseini and B. D. Olsen, *Acs Nano*, 2014, **8**, 9833-9842.
120. G. J. Jaffe, R. M. McCallum, B. Branchaud, C. Skalak, Z. Butuner and P. Ashton, *Ophthalmology*, 2005, **112**, 1192-1198.e1191.
121. Anonymous, in *Science*, 2014, vol. 344, pp. 950-950.
122. S. Demanèche, L. Jocteur-Monrozier, H. Quiquampoix and P. Simonet, *Applied and Environmental Microbiology*, 2001, **67**, 293-299.
123. M. G. Lorenz and W. Wackernagel, *Applied and Environmental Microbiology*, 1987, **53**, 2948-2952.
124. F. H. Lin, C. H. Chen, W. T. K. Cheng and T. F. Kuo, *Biomaterials*, 2006, **27**, 3333-3338.
125. J.-M. Oh, S.-J. Choi, S.-T. Kim and J.-H. Choy, *Bioconjugate Chemistry*, 2006, **17**, 1411-1417.
126. F. Veniale, A. Bettero, P. G. Jobstraibizer and M. Setti, *Applied Clay Science*, 2007, **36**, 141-147.
127. Amy R. Elmore, *International Journal of Toxicology*, 2003, **22**, 37-102.
128. G. V. Joshi, H. A. Patel, B. D. Kevadiya and H. C. Bajaj, *Applied Clay Science*, 2009, **45**, 248-253.
129. J. H. Choy, S. J. Choi, J. M. Oh and T. Park, *Applied Clay Science*, 2007, **36**, 122-132.
130. E. Biondi, S. Branciamore, M.-C. Maurel and E. Gallori, *BMC Evolutionary Biology*, 2007, **7**, S2.
131. K. B. Hongjie Cao in *Cosmetic Science Technology* Elementis Specialties Inc, Hightstown, New Jersey, USA, 2006.
132. J. I. Dawson, J. M. Kanczler, X. B. B. Yang, G. S. Attard and R. O. C. Oreffo, *Advanced Materials*, 2011, **23**, 3304-+.
133. A. K. Gaharwar, S. M. Mihaila, A. Swami, A. Patel, S. Sant, R. L. Reis, A. P. Marques, M. E. Gomes and A. Khademhosseini, *Advanced Materials*, 2013, **25**, 3329-3336.
134. A. Cojocariu, L. Porfire, C. Cheaburu and C. Vasile, *Cellulose Chemistry and Technology*, 2012, **46**, 35-43.
135. E. Ruiz-Hitzky, P. Aranda, M. Darder and G. Rytwo, *Journal of Materials Chemistry*, 2010, **20**, 9306-9321.
136. M. Ghadiri, W. Chrzanowski, W. H. Lee, A. Fathi, F. Dehghani and R. Rohanizadeh, *Applied Clay Science*, 2013, **85**, 64-73.
137. C. J. Wu, A. K. Gaharwar, P. J. Schexnailder and G. Schmidt, *Materials*, 2010, **3**, 2986-3005.
138. A. J. Salgado, J. M. Oliveira, A. Martins, F. G. Teixeira, N. A. Silva, N. M. Neves, N. Sousa and R. L. Reis, in *International Review of Neurobiology*, eds. I. P. P. T. Stefano Geuna and B. Bruno, Academic Press, 2013, vol. Volume 108, pp. 1-33.
139. Y. C. Liu, J. Lim and S. H. Teoh, *Biotechnology Advances*, 2013, **31**, 688-705.
140. J. I. Dawson and R. O. C. Oreffo, *Advanced Materials*, 2013, **25**, 4069-4086.
141. W. Xu, S. Raychowdhury, D. D. Jiang, H. Retsos and E. P. Giannelis, *Small*, 2008, **4**, 662-669.
142. S. S. Ray, K. Yamada, M. Okamoto and K. Ueda, *Nano Letters*, 2002, **2**, 1093-1096.
143. S. G. Wang, H. Tomas and X. Y. Shi, *Journal of Controlled Release*, 2013, **172**, E139-E139.
144. A. K. Mishra, P. R. Rajamohanan, G. B. Nando and S. Chattopadhyay, *Advanced Science Letters*, 2011, **4**, 65-73.
145. P. J. Schexnailder, A. K. Gaharwar, R. L. Bartlett, B. L. Seal and G. Schmidt, *Macromolecular Bioscience*, 2010, **10**, 1416-1423.
146. A. K. Gaharwar, P. J. Schexnailder, Q. Jin, C. J. Wu and G. Schmidt, *Acs Applied Materials & Interfaces*, 2010, **2**, 3119-3127.
147. A. K. Gaharwar, C. Rivera, C. J. Wu, B. K. Chan and G. Schmidt, *Materials Science & Engineering C-Materials for Biological Applications*, 2013, **33**, 1800-1807.
148. k. Haraguchi, *Journal of stem cells and regenerative medicine*, 2012, **8**, 12-20.
149. K. Haraguchi, S. Masatoshi, N. Kotobuki and K. Murata, *Journal of Biomaterials Science-Polymer Edition*, 2011, **22**, 2389-2406.
150. S. T. Oh, W. R. Kim, S. H. Kim, Y. C. Chung and J. S. Park, *Fibers and Polymers*, 2011, **12**, 159-165.
151. A. P. F. Turner, *Chemical Society Reviews*, 2013, **42**, 3184-3196.
152. C. Forano, S. Vial and C. Mousty, *Current Nanoscience*, 2006, **2**, 283-294.
153. D. Wang, W. Yu and B. Zhu, *Solid State Ionics*, 1989, **34**, 219-223.
154. T. E. Mallouk and J. A. Gavin, *Accounts of Chemical Research*, 1998, **31**, 209-217.
155. C. Mousty, *Applied Clay Science*, 2004, **27**, 159-177.
156. L. Gianfreda, M. A. Rao, F. Sannino, F. Saccomandi and A. Violante, *Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health, Volume 28b*, 2002, **28B**, 301-327.
157. S. Gopinath and S. Sugunan, *Applied Clay Science*, 2007, **35**, 67-75.

158. M. A. Rao, L. Gianfreda, F. Palmiero and A. Violante, *Soil Science*, 1996, **161**, 751-760.
159. C. Mousty, *Analytical and Bioanalytical Chemistry*, 2010, **396**, 315-325.
160. J. L. Besombes, S. Cosnier, P. Labbe and G. Reverdy, *Analytica Chimica Acta*, 1995, **317**, 275-280.
161. A. Sassolas, L. J. Blum and B. D. Leca-Bouvier, *Biotechnology Advances*, 2012, **30**, 489-511.
162. J. V. de Melo, S. Cosnier, C. Mousty, C. Martelet and N. Jaffrezic-Renault, *Analytical Chemistry*, 2002, **74**, 4037-4043.
163. Q. Fan, D. Shan, H. Xue, Y. He and S. Cosnier, *Biosensors and Bioelectronics*, 2007, **22**, 816-821.
164. D. Shan, Q.-B. Li, S.-N. Ding, J.-Q. Xu, S. Cosnier and H.-G. Xue, *Biosensors and Bioelectronics*, 2010, **26**, 536-541.
165. F. Charmantray, N. Touisni, L. Hecquet and C. Mousty, *Electroanalysis*, 2013, **25**, 630-635.
166. Y. Xiang and G. Villemure, *Clays and Clay Minerals*, 1996, **44**, 515-521.
167. J. Xiao and G. Villemure, *Clays and Clay Minerals*, 1998, **46**, 195-203.
168. Q. H. Zeng, A. B. Yu, G. Q. Lu and D. R. Paul, *Journal of Nanoscience and Nanotechnology*, 2005, **5**, 1574-1592.
169. H. M. Tiggemann, D. Tomacheski, F. Celso, V. F. Ribeiro and S. M. B. Nachtigall, *Polymer Testing*, 2013, **32**, 1373-1378.
170. A. Sarfraz, M. F. Warsi, M. I. Sarwar and M. Ishaq, *Bulletin of Materials Science*, 2012, **35**, 539-544.
171. A. M. Panichev, K. S. Golokhvast, A. N. Gulkov and I. Y. Chekryzhov, *Environmental Geochemistry and Health*, 2013, **35**, 133-152.
172. A. G. Davies and I. C. Baillie, *Biotropica*, 1988, **20**, 252-258.

 This journal is © The Royal Society of Chemistry 2012