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REVIEW ARTICLE

Unveiling the Potential of Nanoclays in Pharmaceuticals

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Abstract
Clay minerals or nanoclays are layered aluminosilicate nanoparticles with unique physicochemical properties, excellent biocompatibility, high surface area, lamellar structure, diverse ion-exchange capacity, tuneable surface chemistry enabling them to form different intermolecular interactions which offer supreme prospects for optimized pharmaceutical applications. This review article dives into the key characteristics of different types of nanoclays like Halloysite, Kaolinite, Sepiolite, Montmorillonite, Bentonite and Laponite, highlighting their possible exploitation within the pharmaceutical landscape. Also, the review describes the recent advances in nanoclay-based drug delivery systems and discusses the role of nanoclays as drug carriers and their use in tissue engineering and gene therapy. Nanoclays, are used to modulate drug release and stabilize the loaded active molecules as well as to enhance drug loading. Interdisciplinary research, advanced characterization techniques, and careful formulation designs are required to augment the use of nanoclays in various pharmaceutical applications. Despite the faced challenges, research on nanoclay-based drug delivery systems is growing. With targeted research and development, nanoclays are poised to redefine the biomedical domain, offering tailored and efficient therapies for a multitude of diseases.

Keywords Aluminosilicate · Controlled release · Drug delivery and therapeutics · Nanoclays · Nanolayers

Introduction

Clay, a mineral substance, has a fascinating history of human consumption dating back millions of years. Our early ancestors, Homo habilis, practiced geophagy, the intentional ingestion of earth or clay, as a nutritional strategy. The clays have been used actively for more than 25,000 years, even before Homo sapiens. The early humans also employed ocher clay diluted with water to treat various skin conditions, including wound healing. Ancient Egypt called the present-day clay minerals medicinal earth and used them for hemostasis and wound healing [1, 2]. Clay minerals are a type of hydrated aluminosilicate, rich in magnesium, potassium, calcium, sodium, and iron, that belong to the phyllosilicate family [3].

The excellent adsorption quality, high surface area, colloidal property, thixotropy, swelling capacity, and chemical inertness of clay minerals are what give them their adaptability for use in human applications. Furthermore, the Geophagy culture has also demonstrated its extremely low toxicity when taken orally [4]. Because it is non-toxic to living organisms, nanoclay can be employed as drug carrier through intermolecular interactions including hydrogen bonding, covalent bonds and van der Waals forces [5].

The pharmaceutical sector was the first to employ clay minerals as excipients for a variety of uses, including coloring, flavoring, viscosity-inducer as well as emulsifiers, lubricants, and binding agents. Clays have long been utilized in a variety of semi-solid formulations including gels, ointments,

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lotions, and pastes for cosmetic and medical applications. Apart from their application as excipients, various clays have demonstrated versatile biological effects such as the protection against pathological diseases by serving as antacid (smectite, sepiolite), antidiarrheal (smectite, sepiolite, kaolinite), dermatological protection (kaolinite, smectite), and anti-inflammatory (kaolinite). They have demonstrated their efficacy in preventing stomach ulcers [3].

Nanoclays, a diverse group of naturally occurring or synthetically derived layered aluminosilicate nanoparticles, have emerged as a captivating platform for revolutionizing pharmaceutical applications.

Their inherent nanoscale dimensions, coupled with charged and biocompatible surfaces and well-defined compositions, position nanoclays as an emerging class of biomaterials that are both abundant and cost-effective, with the added advantage of being environmentally friendly [6, 7]. As nanoparticles composed of layered mineral silicates, nanoclays encompass various classes such as montmorillonite, bentonite, kaolinite, hectorite, and halloysite, each with distinct properties that can be tailored for specific drug delivery needs. Their unique physicochemical properties, including high surface area, tailorable surface chemistry, and lamellar structure, offer unparalleled prospects for optimized drug delivery.

While nanoclays offer significant potential in pharmaceutical drug delivery, navigating the regulatory landscape and overcoming translational challenges in manufacturing and clinical evaluation are critical for their successful development and approval.

This review article dives into the key characteristics of nanoclays, highlighting their potential within the pharmaceutical landscape and possibilities to overcome challenges to realize the full therapeutic potential of nanoclay-based pharmaceuticals.

Nanoclay Configuration

Clay, a ubiquitous mineral found on Earth's surface, is primarily composed of aluminosilicates. These minerals, characterized by their layered structure and minuscule particle size, contain varying quantities of aluminum, silica, iron, and magnesium [8]. They appear in sheets, the arrangements of which impact various defining and distinguishing aspects. They may appear in two forms: first; tetrahedral sheets consisting of silicon surrounded by four oxygen atoms in a tetrahedral form; and second; octahedral sheets consisting of a metal cation surrounded by six oxygen atoms in an octahedral form [9].

Nanoclay possesses a tetrahedral structure, with silicon cations (Si^{4+}) at the core and four oxygen anions (O^{2-}) occupying the corners (Fig. 1 A-C). The three shared oxygen atoms within each tetrahedron enable interconnectivity, leading to the formation of sheets. Octahedral sheets, on the other hand, are characterized by a central metal cation (Mn^{+}/

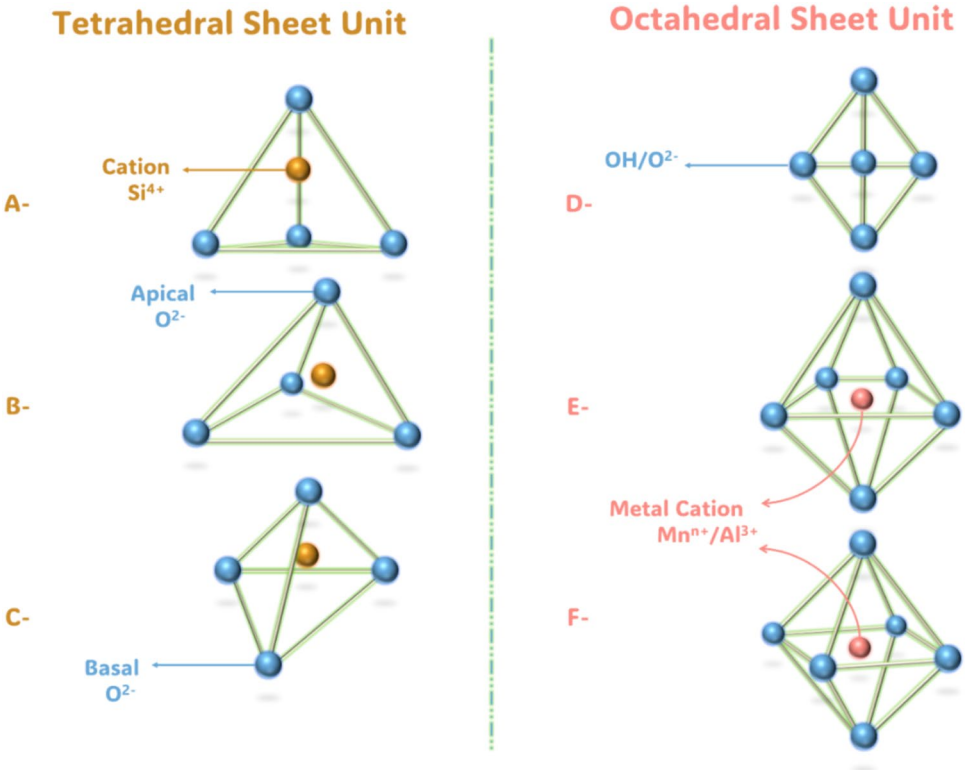

Fig. 1 Structure of nanoclays: **A**, **B** and **C** single tetrahedral sheet unit showing the central cation surrounded by four oxide anions and **D**, **E** and **F** single octahedral sheet unit showing the central metal cation surrounded by six oxide anions

Al^{3+}) surrounded by six oxygen anions at the corners (Fig. 1 D-F) [10]. It is possible to replace the central Si^{+4} and Al^{+3} ions in the tetrahedral and octahedral sheets with ions of lower valence that possess the same atomic radius under specific conditions. It has been reported that Al^{+3}, Mg^{+2}, Li^{+}, or Fe^{+2} ions can replace Si^{+4} and Al^{+3} ions [11]. The presence of pair O^{-2} or OH groups on either side supports the formation of an octahedral sheet [5].

According to their chemical composition, clay minerals can be distributed in three major groups [8], the classification is depicted in Fig. 2. In the first class, the ratio of silica: alumina is 1:1. This type of clay are identified by the arrangement of tetrahedral sheets positioned on top octahedral sheets by sharing oxygen atoms [12] (Fig. 3A). Clays like halloysite, kaolinite, and rectorite are some examples in this category [13].

In the second class, the ratio of silica: alumina is 2:1 and is called phyllosilicates/pyrophyllites. This family of clays is made up of a single octahedral sheet of alumina sandwiched between two tetrahedral sheets of silica (Fig. 3B). Furthermore, the substitution of silica and alumina cations in pyrophyllite with alumina and magnesium cations results in the formation of mica and montmorillonite, respectively [9]. Lastly, in the third category, the ratio of silica: alumina to magnesium is 2:1:1. Chlorite clay is among this category [14].

Nanoclays can be also grouped into cationic, anionic, and neutral types based on their charge characteristics (Table I), which dictate their potential applications [7]. Cationic nanoclays such as Smectite and Vermiculite exhibit a significant negative charge stemming from isomorphous substitutions in their 2:1 or 2:1:1 layer structures. The interlayer cations balancing this charge contribute to their high cation exchange capacity and swelling behavior. While, anionic nanoclays, especially layered double hydroxides (LDHs), possess a positive charge due to their octahedral sheets containing trivalent and divalent metal ions. The presence of interlayer anions (e.g., $CO3^{-2}$, $NO3^{-}$, Cl^{-}) allows them to function as effective anion exchangers, making them suitable for drug delivery. On the other side, neutral nanoclays, like those in the Kaolinite-serpentine and Pyrophyllite-Talc groups with 1:1 or 2:1 layers, have minimal charge due to their composition of $[SiO4]^{-4}$ and Mg^{+2} or Al^{+3} complexes. Their limited swelling and ion exchange capacity make them appropriate for applications where structural stability and inertness are key.

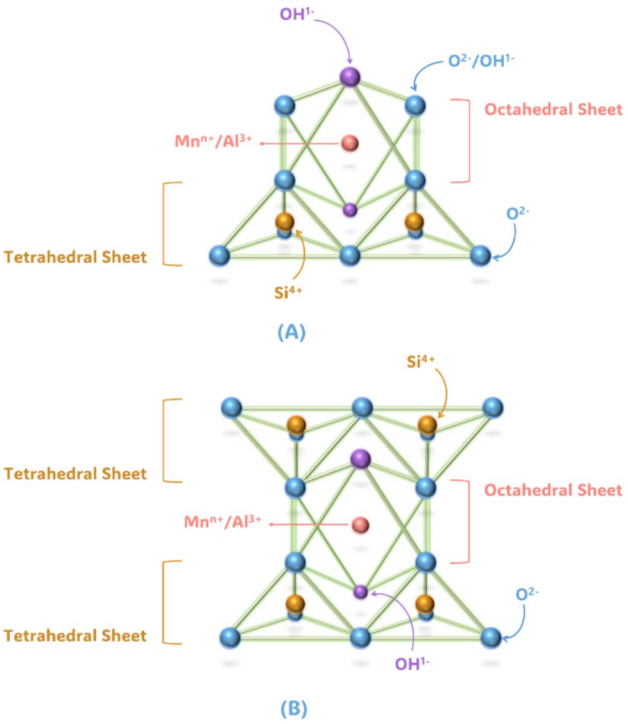

Fig. 3 Diagrammatic representation of **A** tetrahedral -octahedral sheet (1:1) and **B** tetrahedral—octahedral sheet (2:1)

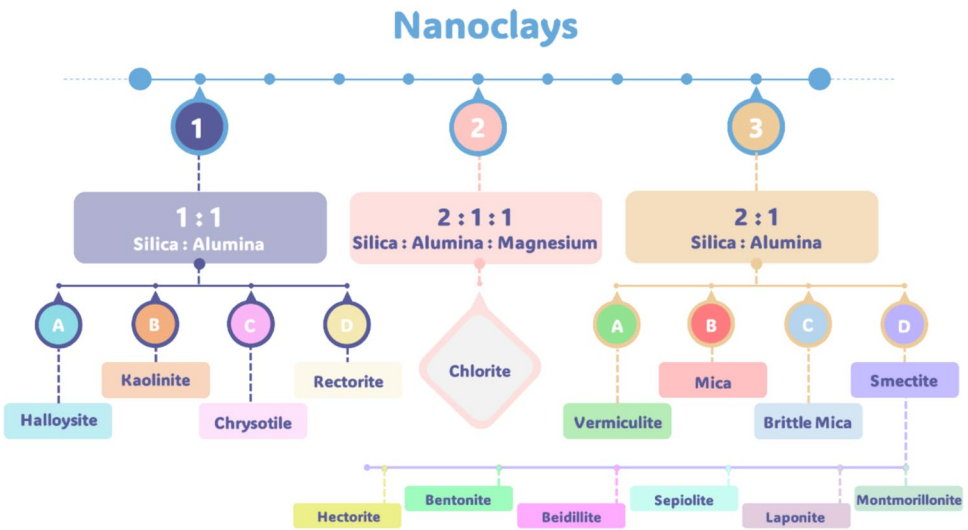

Fig. 2 Classification of nanoclays

Table I Classification of Nanoclays Based on Chemical Charge

Type	Anionic	Cationic	Neutral	Ref
Examples	Montmorillonite (MMT), Bentonite, Hectorite, Saponite	Layered Double Hydroxides (LDHs), Hydrotalcite-like compounds	Kaolinite, Halloysite Nanotubes (HNTs)	[7, 15]
Drug Loading Efficiency	Generally high for cationic drugs	Generally high for anionic drugs	Variable	[16]
Pharmaceutical Applications	Sustained/controlled release, taste masking, enhancing solubility & stability of poorly soluble drugs, preparation of semi-solid dosage forms. Complexation with cationic polymers carrying nucleic acids for gene delivery, Preparation of scaffolds for tissue engineering Adsorbents (binding toxins)	Delivery of anionic drugs, pH-dependent drug release, direct binding and protection of nucleic acids for gene delivery, Vaccine Adjuvants, bioimaging (carriers for contrast agents)	Sustained release, preparation of cosmetics, wound dressings and scaffolds	[17–19]

Types of Nanoclays Used in Pharmaceutical Application

Depending on their mineral composition, there are roughly 30 distinct types of nanoclays, each with specific applications tailored to their unique properties [20], the most important are described below.

Halloysite

Halloysite (HS) nanoclays or nanotubes are submicron aluminosilicate minerals having a double layer structure with a hollow tubular pattern [21]. Each layer of HS is composed of a combination of tetrahedral (Si–O) and octahedral (Al–OH) sheets. The outer surface of HS is characterized by Si–O-Si groups, resulting in a negative charge, while the inner layer comprises Al–OH groups, leading to a positive charge [22]. Due to its low surface energy, this structure exhibits reduced aggregation within the film matrix compared to other nanofillers. The inherent chemical properties of the elements present on the inner and outer surfaces enable HS to function as a carrier material for various active compounds [23].

Kaolinite

Kaolinite (KN), also known as kaolin clay, is a naturally abundant, non-toxic material widely employed in various industries. Chemically, it is a hydrated aluminosilicate (1:1) compound consisting of one tetrahedral (Si–O) sheet and one octahedral (Al-O) sheet. The asymmetric structure produced by this morphology allows the creation of H-bonds between succeeding layers, this greatly increases the cohesive energy amongst the layers [24]. KN can improve the mechanical, barrier, and thermal properties of the polymeric films [25].

Sepiolite

Sepiolite (SP) is a natural hydrated magnesium silicate classified as a 2:1 phyllosilicate mineral category. It is consisting of two tetrahedral sheets of silica that encircle a core composed of magnesium hydroxide-oxide sheet with an octahedral structure [26]. Sepiolite has a needle-shaped distinctive architecture with porous morphology showing alternating tunnels and block-shaped structures with a high specific surface area [27, 28]. Silanol groups (Si–OH) present in the outer surface react with organic chemicals to form organo-modified sepiolite materials [29] and can enhance interfacial interaction and formation of dispersions [26].

Montmorillonite

Montmorillonite (MMT) is formed of two tetrahedral silica sheets merged with the edge of Al $(OH)_3$ octahedral sheet [30]. This aluminosilicate smectite possesses an elevated surface to volume ratio and cation-exchange characteristics offering good water-dispersibility, active agents-carrying capacity, and miscibility with cationic polymers [31]. The layered structure and interlayer spacing of MMT contribute to its excellent adsorption properties. While both sodium MMT and calcium MMT exist, sodium MMT exhibits superior swelling ability, making it more suitable for drug delivery applications [4].

Bentonite

Bentonite (BT) is an absorbent smectite clay originating from altered volcanic ash. BT nanoclays consist of two tetrahedral silicate sheets sandwiching a central octahedral alumina sheet [32]. The surface of each sandwiched platelet exhibits a positive charge along its edges and a negative charge on its surface. To maintain overall

charge neutrality, Na^+ ions are present. These ions, known as exchangeable ions, can be readily replaced by other cations [33]. This structure facilitates the formation of H-bonds with other molecules which strengthens intermolecular interactions.

Laponite®

While all the types displayed above have a natural origin, Laponite® is a synthetic clay belonging to the smectite group, structurally similar to naturally occurring hectorite. Compared to natural clays, it offers advantages in terms of higher purity, aspect ratio, and transparency [34]. It has a two-dimensional structured nanomaterial composed of disk-shaped nanoscale crystals consisting of six octahedral Mg^{2+} ions. The Mg^{2+} ions are sandwiched between two layers of four tetrahedral Si atoms. These disks can interact with a wide range of chemical substances, including small molecules, ions, polymers, and nanoparticles, and can be easily modified to have specific properties. It is biodegradable and produces non-toxic or even beneficial breakdown products [35], and it has been classified as generally recognized as safe (GRAS) [36].

A comprehensive understanding of nanoclays' physicochemical properties is crucial for tailoring their applications in pharmaceuticals. To achieve this, some advanced analytical techniques are employed to provide insights onto their structural, morphological, thermal, and surface characteristics [37], these include:

X-ray Diffraction (XRD)

XRD is a fundamental technique for determining the crystalline structure and interlayer spacing of nanoclays. By analyzing the diffraction patterns, researchers can identify the specific clay mineral type, assess the degree of crystallinity and can be used for monitoring of surface modification and intercalation [38].

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy provides information about the functional groups present on the nanoclay surface. It helps to identify characteristic Si–O, Al-O, and OH groups, as well as intercalated or modified species [39].

Thermogravimetric Analysis (TGA)

TGA measures the weight loss of a material as a function of temperature, revealing information about its thermal stability and composition. This technique is useful for quantifying the amount of intercalated organic molecules or adsorbed water in nanoclays [40].

Dynamic Light Scattering (DLS)

DLS is employed to determine the particle size distribution and hydrodynamic diameter of nanoclays in suspension [41]. This technique is important for assessing the colloidal stability and dispersibility of nanoclays in pharmaceutical formulations.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

SEM and TEM can allow visualization of the morphology and microstructure of nanoclays.

BET Surface Area Analysis

The Brunauer–Emmett–Teller (BET) method is used to determine the specific surface area and pore volume of nanoclays and help for understanding their adsorption capacity and drug loading potential [42].

Solid-State Nuclear Magnetic Resonance (SSNMR)

SSNMR is a technique for analyzing material properties by revealing chemical structure, bonding, and molecular arrangement within different phases. It's especially valuable for studying dispersion and polymer phases in organic and inorganic systems, like clay nanocomposites, due to their impact on macroscopic properties.

Combining SSNMR with other techniques, like XRD and TEM, provides a powerful approach to characterize clay dispersion, layer structure, and interfacial properties within clay-polymer nanocomposites.

Cytocompatibility and Biocompatibility of Nanoclays

One of the first studies on cytotoxicity assessment dates back to 2012, in which the cytotoxic effects of platelet-shaped and tubular nanoclays on human lung epithelial A549 cells were evaluated for the first time using high-content automated screening combined with real-time impedance monitoring. The results showed varying cytotoxicity depending on the type of nanoclay, dose, and exposure time, with platelet-shaped nanoclays being more toxic than tubular ones. Even at low concentrations (25 µg/mL), platelet-shaped nanoclays exhibited significant cytotoxic effects, whereas tubular nanoclays inhibited cell growth only at high concentrations (250 µg/mL). Intracellular accumulation with perinuclear localization, highlighted by confocal microscopy, underscores the potential risk of nanoclays, requiring further studies to assess actual human exposure and potential health hazards. The study concluded with the need for a better evaluation of toxicity and biocompatibility, which was further investigated

in subsequent research [43]. In particular, studies on sepiolite cytotoxicity indicated that its effects vary depending on concentration, exposure time, and cell type. While sepiolite can induce intracellular ROS production and inflammatory cytokine expression in a dose-dependent manner, no significant DNA damage, cell cycle arrest, or apoptosis was observed. Moreover, at commonly used concentrations (1–10 µg/mL), sepiolite showed minimal toxicity in primary rat hepatocytes and HeLa cells, with cell survival remaining above 70% even at higher doses (450 µg/mL) [44]. On another hand, the toxicity of sepiolite appeared to be influenced by its fibrous structure, but sonication significantly reduced haemolytic activity and cytotoxic effects, restoring cell viability to levels close to untreated controls. Overall, transient exposure to sepiolite led to cellular responses but did not generate substantial genotoxic stress, suggesting limited toxicity under normal conditions [45].

Studies on the cytotoxicity of halloysite nanotubes (HNTs) indicated that their effects depend on cell type, dose, and exposure time. Generally, at low doses and short exposure times, HNTs exhibit low cytotoxicity [46]. However, prolonged exposure (72 h) and higher concentrations (e.g., 600 µg/mL) can reduce cell viability, particularly in HeLa, HepG2, and A549 cells [47]. The IC50 values for HNTs decreased over time, indicating increased toxicity with prolonged exposure. Nevertheless, some cell lines, such as HUVECs, MCF-7, and Raw 264.7, maintained high viability even at elevated concentrations [48]. At extreme doses (1000 µg/mL), HNTs inhibited lymphocyte proliferation, but in most cases, they demonstrated high biocompatibility. Even modified HNTs showed good tolerance, making them promising candidates for drug delivery applications [49]. To further reduce potential side effects, factors such as surface functionalization, size, and specific surface area must be carefully considered.

Numerous studies have reported positive results regarding the *in-vivo* biocompatibility of nanoclays. For example, Thorpe and colleagues used a subcutaneous rat model to evaluate the safety of a Laponite® crosslinked pNIPAM-co-DMA implant, finding no inflammatory responses or systemic toxicity. Similarly, Naumenko *et al*. demonstrated the biosafety of HNT-based systems in a subcutaneous rat study, observing no abnormalities or toxic effects in major organs after 3 and 6 weeks post-implantation [50]. Furthermore, Zhai and collaborators found no inflammatory reactions or adverse effects around nanocomposites implanted in a tibia defect model, concluding that the produced hydrogels were biocompatible [51]. Finally, Wang *et al*. evaluated the *in vivo* biosafety of a Laponite® RD-based bioceramic through acute systemic toxicity tests and intramuscular stimulation tests, obtaining positive results [52].

However, some studies have highlighted a certain degree of toxicity. For instance, Baker *et al*. excluded scaffolds containing Cloisite 30B (modified montmorillonite) due to their inflammatory response [53], while Boyer and collaborators observed that Laponite® RD, when prepared as a suspension, can exhibit toxicity at concentrations around or above 10 µg/L [54].

Although nanoclays offer promising properties for pharmaceutical applications, however, like many nanomaterials, there are some concerns about their potential cytotoxicity. Some strategies can mitigate these concerns, such as surface passivation and alteration (by silanization and PEGylation), because unmodified clay surfaces can have sharp edges, reactive sites (like silanol groups), and surface charges that can interact with cell membranes, proteins, or DNA [55–57], surface modification can create a"stealth"effect, reducing interactions that trigger inflammation, oxidative stress, or direct membrane damage. Biopolymeric coating, using chitosan, alginate or cellulose, is also another strategy [58]. These biopolymers have been widely used in the modification of pharmaceutical formulations [59–61]. The polymer layer acts as a physical barrier between the nanoclay surface and the biological environment. It can mask reactive sites and modify the overall surface properties of the nanocomposite [17, 62].

Also, designing nanoclay-based systems to degrade into non-toxic components within the body after fulfilling its therapeutic function can limit possible cytotoxic effects by reducing the risk of long-term bioaccumulation, which is a major concern for persistent nanomaterials. Some specific nanoclay types or modified versions might be engineered for enhanced degradation under specific physiological conditions [8, 15, 63].

In conclusion, there is still no clear determination regarding the *in-vivo* toxicity of nanoclays. A more in-depth evaluation of the dose-dependent toxicity and inflammatory response across different animal models and over prolonged exposure periods is necessary to confidently assert that nanoclays are safe for the human body at clinically relevant concentrations and during extended exposure times.

Factors Affecting Drug Delivery from Nanoclay-Based Formulations

The main factors affecting drug delivery from nanoclay-based formulations can be summarized as follows:

Size and Morphology

Clay nanoparticles come in a variety of shapes, including tubes, plates, and films. They can be as thin as 10 A° or as thick as 2 mm. Their average size can be measured using a number of techniques, such as dynamic light scattering,

light microscopy, and scanning and transmission electron microscopy [64]. It has been shown that clay nanoparticles up to 200 nm in size are particularly effective for drug delivery. The shape of clay nanoparticles is also important; for example, tubular clay nanoparticles can increase tensile and bending strength [17].

Pore Size and Volume

The entrapment or loading of drug primarily depends at the pore/cavity size of nanoclay. For example, in halloysite nanotubes, the loading capacity is influenced by the lumen diameter. As the lumen increases in diamter from 10–15 nm to 30–40 nm, the loading capacity then increases, reaching up to 30–40% [65].

Zeta Potential and Charge Density

The charge on clay nanoparticles can be determined by measuring their zeta potential using differential light scattering. Different types of nanoclay exhibit varying charge densities. For example, montmorillonite and halloysite nanotubes typically possess negative surface charges. This negative charge, which predominates at physiological pH levels (> 2), suggests significant potential for binding cationic drugs [66]. While, halloysite nanotube has a positively charge lumen allowing it to bind anionic molecules [67].

Thermal and Mechanical Properties

Nanoclay-based drug formulations exhibit Nanoclays enhance mechanical, thermal, and other critical properties, crucial for the stability of drug formulations. Tabak *et al*. demonstrated that the thermal stability of organic molecules encapsulated within nanoclay increases to 750°C, likely due to interactions between the aromatic rings of the molecules, the oxygen planes of the clay sheets, and the protective aluminosilicate layers [68].

Entrapment Efficiency

Entrapment efficiency is a critical factor for the therapeutic use of nanoclay in pharmaceutical applications. The low entrapment of water-soluble drugs in polymers presents a challenge for developing drug delivery systems. Water-insoluble polymers like nanoclay have demonstrated superior entrapment efficiency and controlled drug release, overcoming these limitations [69].

The drug release mechanisms from nanoclay-based drug delivery systems are complex, including: intercalation, diffusion, swelling-controlled, and stimuli-responsive drug release.

The intercalation-based drug release mechanism involves incorporating the drug between the layers of nanoclays through interactions such as ion exchange or hydrogen bonding. The release occurs when the drug is desorbed from the nanoclay interface into the biological medium. Factors that influence this process include the pH of the environment, the concentration of competitive ions, and the properties of the drug (such as hydrophobicity and charge). For example, Dexamethasone, when intercalated in nanoclays, has shown prolonged release, making it useful for bone regeneration [70].

The diffusion-based drug release mechanism involves the drug being adsorbed onto the surface of the nanoclays or within their pores, from where it gradually diffuses into the surrounding medium based on the concentration gradient. For example, Mafenide, an antibiotic used for burns, incorporated into a nanoclay-alginate composite, demonstrated sustained release over time [19].

The swelling-controlled drug release mechanism involves nanoclays combined with hydrophilic polymers that swell in the presence of water or biological fluids, creating channels that facilitate the drug's release; for example, nanoclay-based hydrogels are used for controlled drug delivery of antibiotics, taking advantage of polymer swelling to modulate drug diffusion [71].

The stimuli-responsive drug release mechanism involves drug release being triggered by external stimuli such as pH changes, temperature, enzymes, or magnetic fields; for example, pH-sensitive systems can be engineered with nanoclays to release the drug in acidic environments, such as those found in tumors, enhancing treatment specificity [70].

Nanoclays Applications in the Pharmaceutical Field

Nanoclays are widely investigated for their pharmaceutical applications, these spin around the following uses.

Applications as Drug Carriers

The applications of nanoclays as drug delivery systems stem from their biocompatibility as well as their capacity to bind and release diverse compounds (based on the surrounding environment), thereby enhancing dissolution characteristics and, consequently, the bioavailability of poorly water-soluble drugs [72].

Based on the capabilities of nanoclays outlined above, numerous studies have been conducted regarding their applications in this field. This review highlights publications concerning the utilization of nanoclays as delivery systems, both alone and in combination with other ingredients.

In the study conducted by Maestrelli *et al*., various combinations of carriers composed of cyclodextrins and nanoclays were tested to enhance the bioavailability of hydrochlorothiazide, a poorly water-soluble drug [72]. The cyclodextrins analyzed were β-cyclodextrin, hydroxypropyl-β-cyclodextrin, amorphous randomly methylated-β-cyclodextrin, hydroxyethyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin. While, the used nanoclays were Sepiolite, Attapulgite and Bentonite. In the first part of the study, binary systems were prepared between the individual carriers (cyclodextrins or nanoclays) and drug. The best results were obtained with randomly methylated-β-cyclodextrin and hydrochlorothiazide, as this cyclodextrin enhanced the dissolution properties of hydrochlorothiazide due to its superior complexation and/or solubilization capacity, as well as the complete amorphization of the drug achieved through co-evaporation. The best binary system composed of nanoclays and the drug was found to be between Sepiolite and hydrochlorothiazide in a 1:4 w/w ratio; this system exhibited the most effective interactions with the drug among the three types of nanoclays tested.

Ternary systems prepared by co-evaporation with both selected carriers (randomly methylated-β-cyclodextrin and Sepiolite) demonstrated a synergistic effect in enhancing drug dissolution, resulting in significant increases in solubility compared to binary formulations. Tablets formulated with these ternary systems showed a markedly improved dissolution profile compared to a commercial formulation, with approximately 60% more drug dissolved at 60 min, indicating their potential to enhance the dissolution and oral absorption of hydrochlorothiazide.

Another study highlighted the capability of nanoclays to enhance the efficacy of poorly soluble drugs. The study demonstrated that the increased solubility of the poorly water-soluble drug carbamazepine (CBZ) significantly improves its anticancer efficacy when administered *in-vitro* to tumor cells, either encapsulated within layered double hydroxide (LDH) nanoclays or incorporated into magnetic nanoparticles embedded within LDH nanoclays. LDHs are negatively charged nanoclays composed of layers containing positively charged metal ions (in this study, a mixture of Mg^{2+} and Al^{3+} ions were used), separated by an aqueous interlayer containing negatively charged molecules. Upon heating to 450°C, LDHs transform into mixed oxides, and when exposed to an aqueous environment, they can revert to their original LDH structure due to the"memory effect."During this process, the layers of positively charged ions can trap water molecules and anions from the surrounding medium [73]. Additionally, as reported in a previous study, neutral molecules like CBZ can be incorporated into the interlayer during reconstruction [74]. LDHs are appealing for drug delivery applications due to their biocompatibility, ability to penetrate cell membranes, and capacity to protect attached drugs during circulation in the bloodstream (at pH 7.4) while releasing them within cancer cell lysosomes (at pH 4.8) through disintegration [75].

The capability of nanoclays to sustain the drug release was highlighted in a previous study. In this study, gentamycin was incorporated into Montmorillonite (Cloisite®-Na^+) through an ion-exchange process, resulting in a stable hybrid material where gentamycin^{5+} cations form a monolayer that compensates for the negative charge of the silicate layers. The Gibbs energy for this interaction, determined from the adsorption data, was –18.6 kJ mol^{-1}, which is comparable to values observed for the adsorption of other antibiotics onto clay minerals. This strong interaction between gentamycin and the silicate ensures a controlled and prolonged release of the drug in aqueous and buffer solutions, which is beneficial for developing controlled-release systems. Moreover, the nanoclay hybrid material was then combined with HPMC (hydroxypropyl methylcellulose) at various concentrations to create bio-nanocomposite films. These films showed improved mechanical strength compared to pure HPMC films, although higher amounts of the added nanoclay hybrid material decreased their elasticity. Despite this, the addition of the nanoclay hybrid material did not affect the hydrophilic properties of HPMC, as the water absorption characteristics remained consistent. Furthermore, the presence of the nanoclay reduced the films'tendency to swell, thus preventing disintegration. Antimicrobial tests conducted against Staphylococcus aureus (S. aureus) demonstrated that the films exhibited significant antibacterial activity, even with low concentrations of gentamycin. This effectiveness suggested that these films were well-suited for wound-healing applications. The low gentamycin content allowed for the maintenance of film elasticity, while still providing strong antibacterial properties. Additional testing on agar plates with various microorganisms, including Escherichia coli, Pseudomonas aeruginosa, S. aureus, methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococcus faecium, Acinetobacter baumannii, Klebsiella pneumoniae, confirmed that the films had inhibition zones in all cases except for MRSA, where the effect was only partial [76].

Nanoclays can address some of the challenges faced in hydrogel-based drug delivery systems. As is well-known, hydrogels often struggle to effectively modulate drug release. A recent study by Khachani *et al*. [77] demonstrated that incorporating nanoclays into a polyethylene glycol (PEG) hydrogel can slow down the release of active ingredients. Specifically, the authors investigated the release of three small molecules with different charges: Acridine orange (positively-charged), Doxorubicin (neutral), and Alexa (negatively-charged) from three different nanoclays: Laponite, Montmorillonite, and Halloysite. The results showed that the three molecules were released rapidly from PEG-only hydrogels, with over 90% of the encapsulated

molecules released within 2 h regardless of their net charge, however due to PEG's inert nature, where release is driven solely by diffusion. Overall, PEG – Laponite and PEG – Montmorillonite hydrogels exhibited a much slower release compared to both the PEG-only control and PEG – Halloysite hydrogels. In case of Acridine orange, it was manifested that after one day, 16% of the encapsulated molecules were released from PEG – Laponite hydrogels, while only 7.5% were released from PEG – Montmorillonite. PEG – Halloysite hydrogels showed minimal improvement over PEG-only hydrogels, with a release of 77% after one day. The authors thus demonstrated that the type of nanoclay in the hydrogel significantly influenced Acridine orange release. This prolonged release was due to electrostatic adsorption on the silanol groups of the clay nanoparticles. In Laponite and Montmorillonite samples, Acridine orange was exchanged with interlayer sodium ions, resulting in a tightly bound compound that was released slowly. Conversely, in PEG – Halloysite hydrogels, Acridine orange was primarily adsorbed on the outer surface, allowing for faster release through cationic exchange. Although the study monitored release for up to 15 days, not all the active molecules were released in this period. Specifically, 24% and 12% of Acridine Orange were released after 15 days from PEG – Laponite and PEG – Montmorillonite, respectively, suggesting that significantly longer release times are achievable compared to PEG-only systems. To further illustrate the role of electrostatic interactions between nanoclays and small molecules in determining release rates, the authors also studied the effect of the charges of the small molecules on the release rate. Positively charged Acridine orange was released more slowly from PEG – Laponite, PEG – Montmorillonite, and PEG – Halloysite hydrogels. In comparison, negatively charged Alexa was released much more quickly, with 63%, 49%, and 26% more Alexa released after one day from PEG – Laponite, PEG – Montmorillonite, and PEG – Halloysite hydrogels, respectively, compared to Acridine orange from the same hydrogels. This faster release of Alexa is attributed to its interaction with nanoclays, which have a lower positively charged surface area for binding. Doxorubicin, with a neutral net charge, showed an intermediate release rate, with 31%, 29%, and 20% more release compared to Acridine orange from PEG – Laponite, PEG – Montmorillonite, and PEG – Halloysite hydrogels, respectively. This intermediate release rate is due to doxorubicin's neutral net charge, allowing binding to various surfaces of the nanoclays, although steric hindrance may limit efficient binding, resulting in a quicker release compared to positively charged molecules like Acridine orange. Obviously, both the incubation time of molecules with nanoclays prior to hydrogel encapsulation and buffer salt concentration and pH influenced release rates by modulating nanoclay-small molecule interactions. These findings suggest the potential for highly tunable nanocomposite hydrogel delivery systems to achieve prolonged small molecule release compared to traditional hydrogels.

Despite the widespread utilization of nanoclays in drug delivery systems, certain challenges persist. For instance, Montmorillonite has often been employed as a carrier for drugs. However, the significant tendency of Montmorillonite to aggregate under physiological conditions has limited its effectiveness in drug delivery applications. In response to the challenge of Montmorillonite aggregation, one study introduced PEGylated chitosan (PEG-CS) adducts to stabilize Montmorillonite dispersion [78]. These adducts, synthesized to exploit electrostatic interactions, were incorporated within Montmorillonite layers. The resulting nanosheets, rich in PEG on their surfaces, exhibited remarkable dispersion, even in serum-containing environments. Increasing the mass ratio of PEG-CS to Montmorillonite transitioned nanosheets from multilayered to exfoliated configurations. Notably, nanosheets with a mass ratio of 8.0 in freeze-dried state displayed a hierarchical lamellar structure, with enhanced loading capacity for doxorubicin. Importantly, internalized doxorubicin-loaded nanosheets in TRAMP-C1 cells released the drug gradually within acidic organelles, inducing cellular apoptosis. This study introduces a novel method for controlling Montmorillonite dispersion stability, expanding its potential in drug delivery applications [78].

Particular insights are needed to understand the interactions between nanoclays and drugs. Here, the mechanisms of doxorubicin delivery by unmodified nanoclays (Halloysite, Kaolinite, Montmorillonite) were thoroughly investigated. Both experimental and theoretical studies have shown that these nanoclays, characterized by high loading capacity (> 50%) and encapsulation efficiency (> 90%) of doxorubicin, owe their performance to the exposed hydroxyl groups and Lewis base sites on their surfaces. Density functional theory calculations confirmed that doxorubicin preferentially adsorbs on Al–OH surfaces, while adsorption on Si–O surfaces is less favored. Additionally, the pH-responsive release profiles of doxorubicin from nanoclays are associated with the protonation of negatively charged nanoclays in weakly acidic solutions, facilitating dissociation from positively charged doxorubicin. This comprehensive mechanistic approach underscores the potential of nanoclays as efficient nanocarriers for various biomedical applications [79].

It is evident that in recent years there has been significant interest in synergistic formulations that combine nanoclays with polymers, liposomes, or nanoparticles to enhance drug delivery efficiency. This aspect is particularly highlighted in the work of Han (2024), which presents an innovative nanobactericidal platform based on lithium magnesium silicate (LMS) nanoclay, modified with copper ions through a dopamine-mediated metallophenolic network [80]. This sandwich-like structure, encapsulated in GelMA hydrogel

microspheres, demonstrated potent bactericidal activity against *Staphylococcus aureus* (S. aureus), excellent biocompatibility with bone marrow-derived mesenchymal stem cells (BMSCs) and human umbilical vein endothelial cells (HUVECs), and the ability to induce osteogenic differentiation and angiogenesis. *In-vivo* tests confirmed the system's ability to effectively eliminate bacteria and promote vascular regeneration and tissue repair, showing great potential in treating complex infections. More recently, a novel study highlighted the development of KaoliniteMeOH-Dox (KMD), a novel formulation for doxorubicin (Dox) delivery, which combines nanoclays with other materials to enhance drug delivery. KMD demonstrated superior characteristics such as optimal size, charge, shape, high loading efficiency (90.16%), and pH-dependent release. Compared to free Dox, KMD showed enhanced anti-cancer effects, with an 86.075% tumor suppression rate *versus* 60.379% for Dox monotherapy. Additionally, KMD minimized toxicity to normal tissues and reduced tumor size, metastasis, and oxidative stress. These advantages make KMD a promising candidate for breast cancer treatment, demonstrating improved selectivity and efficacy in both *in- vitro* and *in- vivo* studies [81].

Applications in Gene Therapy

Gene therapy aims to treat diseases caused by human genetic disorders. Approaches such as CRISPR, siRNA-delivery, and the delivery of nucleic acids into cells facilitate genetic modification and targeted modulation of gene expression. However, DNA and RNA are vulnerable to degradation and struggle to penetrate cell membranes, necessitating protective carriers [82–85]. Viral and non-viral vectors are used for delivery, emphasizing the crucial need for improved vector systems to protect nucleic acids during delivery without causing adverse effects [86]. The potential applications of nanoclays in gene therapy are of particular interest due to their well-known biocompatibility and their ability to interact with charged molecules such as nucleic acids [87]. They, indeed, thanks to the presence of cations in their interlayer spaces [86], can interact with nucleic acids. These cations can electrostatically stabilize the negatively charged groups on the nucleotides of nucleic acids. This charge-charge interaction promotes the adhesion and absorption of nucleic acids onto the surface of nanoclays, allowing them to be transported and protected during delivery. Additionally, the nanostructured nature of nanoclays enables them to easily traverse biological barriers, such as cellular membranes, further facilitating the delivery of nucleic acids into target cells [86]. This capability, in gene therapies, is crucial for successful treatment. Nanoclays have been shown to increase the uptake of nucleic acids by cells, thereby improving the transfection efficiency. Their ability to form complexes with nucleic acids and protect them from degradation contributes to this enhanced efficiency. This aspect was demonstrated in a study conducted by Liu *et al*. (2019), which showed that the natural Halloysite nanotube (HNT)-assisted delivery of an active small interfering RNA (siRNA) targeting receptor-interacting protein kinase 4 (RIPK4) effectively silenced its expression to treat bladder cancer. The HNTs/siRNA complex increased the serum stability of the siRNA, prolonged its circulation time in the blood, and promoted cellular uptake and tumor accumulation of the siRNA [88]. The incorporation of nanoclays increased the efficiency of gene transfection and the expression of the desired therapeutic genes. Nanoclays not only enhance transfection efficiency but can also deliver nucleic acids to selected targets, such as specific tissues or cells. They can be functionalized with targeting ligands, such as peptides or antibodies, to improve specificity and reduce the systemic side effects of gene therapy.

In another study, a potential method for utilizing Halloysite nanotubes to deliver DNA was developed. This study addressed the main issue encountered in using this material which is the combination of DNA and Halloysite through a high-speed milling process, which proved to be too destructive. Instead, the study proposed an interaction between DNA and Halloysite in solution in the presence of $MgCl_2$ to obtain DNA-modified Halloysite nanotubes. Furthermore, the bound DNA could potentially be partially released from the nanotubes under appropriate conditions, with the percentage of released DNA depending on the amount of $MgCl_2$ present during the binding process (the optimal $MgCl_2$ concentrations are between 40 and 60 mM and the DNA release is about 90%). Therefore, the modification of Halloysite nanotubes with DNA could potentially be used to transfer information encoded in nucleic acids for gene therapy purposes [87].

The first study, focusing on the utilization of a nanocarrier constructed from Halloysite nanotubes labeled with fluorescent switchable halochromic oxazine molecules, for transporting single-stranded peptide nucleic acids tetramer (PNA_{ts}) into living cells, was published in 2022 [89]. The PNA_{ts} were covalently bonded to Halloysite nanotubes (HNTs-PNA). Cellular uptake was evaluated using confocal laser scanning microscopy on both normal and tumoral cell lines. This research underscores the efficacy of the covalent strategy in fabricating HNTs-PNA nanomaterials, suggesting their potential utility for targeting specific nucleic acids in living cells. Such advancements may pave the way for innovative applications in theragnostic and gene therapy. This same application was subsequently investigated in which the possibility of combining PNA with Halloysite using the supramolecular approach was analyzed to scale up the synthesis of these nanomaterials for potential future clinical applications of these systems and to design diverse PNA molecules with specific sequences capable of interacting with Halloysite surfaces for the treatment of various pathologies [90].

The study by Falanga *et al*. [91] proposed the potential use of Halloysite nanotubes (HNTs) as delivery systems for gene therapy, with potential future applications in the treatment of solid tumors or other pathologies. In this research, two 12-mer PNA molecules with distinct charges, designed to specifically recognize a 12-mer DNA sequence mimicking a purine-rich region of the neuroglobin gene, were synthesized and loaded onto HNTs through electrostatic interactions. Following characterization, their release behavior was analyzed under conditions simulating a physiological environment. Resonance light scattering experiments confirmed their capacity to bind complementary single-stranded DNA. Additionally, their cellular uptake was investigated using confocal laser scanning microscopy in live MCF-7 cells incubated with fluorescein isothiocyanate (FITC)-labeled PNA and HNTs tagged with a molecular probe. The results indicated that these nanomaterials efficiently crossed both the cell membrane and the nuclear envelope. Lastly, it is essential to emphasize that the HNTs/PNA complex effectively downregulated neuroglobin gene expression, as verified through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis.

A recent study assessed the ability of HNTs and Kaolinite particles to deliver both small and large nucleic acids. Vacuum treatment enhanced the binding of small nucleic acids to HNTs, a process further facilitated by the addition of divalent metal ions (Mg^{2+}). In contrast, Kaolinite efficiently bound only small RNAs in the presence of Mg^{2+} ions. Large linear double-stranded DNAs and circular plasmid DNAs exhibited weak binding to Kaolinite under all conditions but formed strong associations with HNTs. These findings indicate that the interactions between nucleic acids and clays are significantly influenced by both the type and conformation of the nucleic acid, as well as the specific properties of the clay [86].

A very recent study was focused on the development of a novel nanomedicine strategy for targeted drug delivery in Alzheimer's disease (AD) treatment. The researchers designed a nanocarrier system using halloysite nanotubes (HNTs) functionalized with RVG29, a brain-penetrating peptide, and loaded with Ripk1 siRNA (siRipk1) to target amyloid-beta (Aβ) plaques in the brain. The halloysite nanotubes (SH) were engineered to absorb and inhibit the formation of toxic Aβ aggregates while promoting their clearance. The RVG29 peptide facilitated the delivery of the siRNA to the brain by targeting Aβ plaques specifically. The system successfully reduced Aβ plaque accumulation, alleviated memory impairment, and protected neurons from necrosis by decreasing Ripk1 expression around the plaques in AD mouse models [92]. This approach demonstrates a promising targeted drug delivery strategy for treating AD through nanomedicine.

It is important here to mention the work of Niu *et al*. (2020), who demonstrated the use of montmorillonite nanoclays to deliver CRISPR/Cas9 components into mammalian cells. The study investigated gene delivery for local therapy in the vaginal tract of pigs using two formulations: PBAE-plasmid polyplex nanoparticles (NPs) combined with montmorillonite (mMMT) or hectorite (HTT) gels. The PBAE (poly (β-amino ester) -GFP-NPs-mMMT gel was more efficient than the PBAE-GFP-NPs-HTT gel for intravaginal DNA delivery, as demonstrated through flow cytometry of cervical cells. Specific CRISPR/SpCas9 sgRNAs targeting porcine endogenous retroviruses (PERVs) were used to assess genome editing *in vivo*. The PBAE-SpCas9/sgRNA NPs-mMMT gel significantly reduced the porcine endogenous retroviruses (PERV copy number in vaginal epithelium. High-fidelity versions of SpCas9, such as SpCas9-HF1 and eSpCas9, also yielded comparable results when used in the mMMT gel. The expression of SpCas9 was localized to the vagina/cervix and did not spread to nearby organs, ensuring low toxicity. The PBAE-NPs mMMT vaginal gel proved to be an effective formulation for local gene therapy, with potential applications in treating sexually transmitted diseases in the genital tract. The presence of mMMT played a critical role in enhancing the delivery efficiency and genome editing outcomes [93].

Applications in Tissue Engineering

Nanoclay biomaterials are emerging as an innovative approach in regenerative medicine, leveraging the inherent benefits of their composite structure to repair various musculoskeletal disorders [94]. With a distinct combination of chemical, mechanical, biological, and physical properties, as these materials closely mimic the complexity of natural tissues. Providing substantial mechanical reinforcement and easy chemical modification, nanoclays show great potential as a versatile element in creating orthopedic devices, as well as scaffolds, tailored for effective tissue integration and regeneration. Indeed, they are able to improve cellular adhesion, proliferation, and differentiation, particularly in osteogenesis (bone tissue formation), acting as bioactive additives. Recent studies have highlighted the potential of 2D nanoclay particles for applications in regenerative medicine. These particles possess unique properties such as exceptional surface area to volume ratios and high modulus values, which make them valuable for enhancing the mechanical properties of biopolymers and hydrogels in biomedical nanocomposite formulations [95]. Furthermore, 2D nanoclay particles have shown biocompatibility at higher doses compared to other nanomaterials and their degradation products are non-toxic and absorbable [96].

Bone Reconstruction

Nanoclays are important in bone reconstruction as their unique characteristics, such as high surface area and ability to mimic the natural structure of bone on a nanoscale,

allow them to improve bone cell attachment, proliferation, and differentiation. In particular, nanoclays can be regarded as optimal reinforcing biomaterials, due to their high specific surface area, dual charge distribution, and excellent biocompatibility. These features make them promising for bone tissue engineering application, being able to promote bone regeneration due to their ability to simultaneously provide mechanical reinforcement, bioactivity increment, controlled delivery of bioactive molecules, such as drugs and growth factors. Indeed, these three elements are considered pivotal for promoting bone regeneration [97]. In particular, the osteogenic differentiation and bone tissue growth are promoted by the release of ions such as lithium, magnesium, sodium, and silicon from nanoclays. Moreover, the nanoclays structure allows them to encapsulate and gradually release therapeutic molecules essential for effective bone healing in a controlled manner [98].

Different types of nanoclays, including Montmorillonite (MMT), Halloysite nanotubes (HNTs), Laponite RD, Kaolinite, Talc, Pyrophyllite, Illite, Vermiculite, Smectites and Sepiolite, are promising for bone engineering applications. Among them, MMT, HNTs, and Laponite RD are mainly used in bone regeneration. MMT demonstrated to be able to increase cell proliferation and differentiation while suppressing osteoclast formation [99], while HNTs are appropriate for bone tissue engineering due to their ability to provide high mechanical strength and biocompatibility [100]. Also, Laponite RD has been commonly used as fillers within bone tissue engineering scaffolds due to its exfoliation capacity and ability to improve mechanical properties and bioactivity requested for tissue regeneration [98]. Several studies have investigated scaffolds-loaded with nanoclays, evidencing that they can enhance mechanical strength, support controlled degradation, and exhibit bioactive properties beneficial for bone tissue formation. These features promote better integration with the biological environment and help in healing and regenerating bone tissue. In the work performed by Hu *et al*., the role of nanoclays (nanosilicates) on the osteoblastic differentiation and bone formation was studied using mouse calvarial pre-osteoblasts (MC3 T3-E1), by comparing the performance of gelatin nanofibrous scaffold and a scaffold modified with nanosilicates added in low-dose (0.5 µg per scaffold) besides bone morphogenetic protein (BMP2) [101]. To examine the effects of nanosilicates and BMP2 on the osteogenic differentiation of MC3 T3-E1 cells, the alkaline phosphatase (ALP) activity and degree of mineralization were assessed *in-vitro*. After 7 days of culture, the groups that received a single dose of BMP2, multiple doses of BMP2, and a combined BMP2/NS treatment demonstrated significantly higher ALP activity compared to the scaffold lacking their addition. However, the group treated with a single dose of nanosilicates did not show a significant increase in ALP activity. To further investigate the effects of nanosilicates on the osteogenic differentiation of MC3 T3-E1 cells, the mineralization of the cultured cells was measured using Alizarin Red S staining and CPC quantification after 3 weeks. Consistent with the ALP activity results, a much higher level of mineralization was observed in the BMP2 multiple-treatment group and BMP2/nanosilicates combined samples if compared to the control group lacking the addition of nanosilicates. There was no significant difference between the BMP2 multiple-treatment group and BMP2/nanosilicates single-treatment group. These findings suggest that the interaction between nanosilicates and BMP2 is crucial in BMP2-induced osteogenic differentiation. Moreover, a comparison between scaffolds based on neat gelatin nanofibers and those based on nanosilicates-loaded gelatin nanofibers was performed, demonstrating the ability of nanosilicates to enhance and promote the osteoblastic differentiation. This experimental evidence was mainly ascribed to the nanosilicates capability to improve the gelatin nanofibrous scaffold mechanical stiffness, considering the pivotal role of the biomaterials mechanical properties played in stem cell osteogenic differentiation *in vitro* and bone formation *in vivo* [102, 103]. In details, Yao *et al*. reported that the gelatin nanofibrous/nanosilicates scaffolds presented a compressive modulus significantly higher than that of the neat gelatin nanofibrous scaffolds (around 2.75 MPa *versus* around 1.5 MPa), even if characterised by comparable macroporous and nanofibrous structures [104]. This interesting result was justified with the tight interactions between the gelatin chains and nanosilicates, due to their anisotropic nature and plate-like shape with high specific surface area [105, 106].

Another study highlighted the significance of nanosilicates in the field of bone regeneration [107], examining the influence of different concentrations (0, 1, 2.5, 5, and 10%) of nanosilicate on the mechanical properties of poly (glycerol sebacate) (PGS) scaffolds. The authors concluded that nanosilicate incorporation significantly enhanced the compressive modulus of the scaffolds in a concentration-dependent manner. Compressive modulus increased from 27.9 ± 5.1 kPa with plain PGS to 45.9 ± 4.8, 67.2 ± 16.1, 88.6 ± 9.0, and reached 130.8 ± 15.3 kPa with 1, 2.5, 5 and 10% nanosilicate, respectively. Figure 4 illustrates the stress–strain curves for uniaxial cyclic compression (8 cycles) obtained for different concentrations of nanosilicates (0, 2.5 and 10% of concentration), where it is possible to evidence that the addiction of 10% of nanosilicate improved the compression properties compared with pure PGS scaffolds 4 to 5 times. These results indicate that the mechanical properties of PGS/nanosilicate scaffolds could be tailored by modulating the nanosilicate concentrations, whose increase allowed to raise the strength while preserving elasticity. Energy dissipation during mechanical deformation also was improved by increasing nanosilicate concentration. During the first

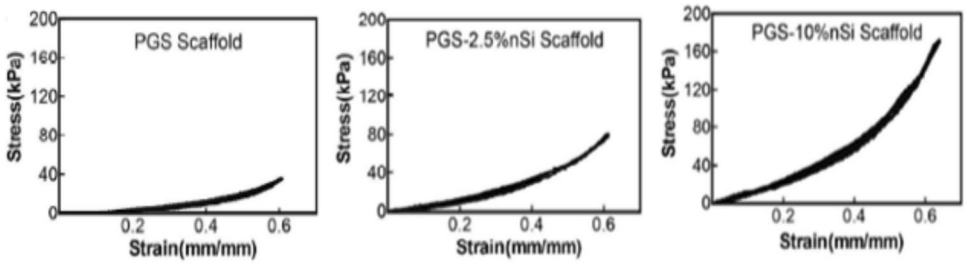

Fig. 4 Stress–strain curves of pure poly (glycerol sebacate) scaffolds (PGS) and PGS loaded with different concentrations of nanosilicate (nSi; 2.5 and 10%) scaffolds. Reprinted with permission from "Kerativitayanan, P., *et al*., Nanoengineered osteoinductive and elastomeric scaffolds for bone tissue engineering. ACS Biomaterials Science & Engineering, 2017. 3(4): p. 590–600 [107]. Copyright 2024 American Chemical Society

compression cycle, the energy absorbed by the scaffolds was 1.2 ± 0.4 kJ/m^3 for pure PGS, 1.8 ± 0.3, 2.6 ± 0.8, 2.1 ± 0.4 for 1, 2.5 and 5% nanosilicate, and 3.1 ± 1.6 kJ/m^3 for 10% nanosilicate where a more than 2.5-fold increase compared to plain PGS. Despite these enhancements in strength and energy dissipation, the scaffolds maintained their elasticity, with no significant impact on network recovery, which remained high at 98.4 ± 1.4% for pure PGS and at 97.6 ± 3.4% for PGS loaded with 10% nanosilicate after 8 compression cycles. Moreover, the nanoclays addition allowed to regulate the scaffolds degradation rate, considering that both durability and flexibility are important for proper tissue integration and regeneration. Finally, adding nanosilicates to the scaffold improved the ALP activity and promoted the matrix mineralization, without any signs of cytotoxicity [107]. Such scaffolds show promise for applications like craniofacial bone repair, where both strength and flexibility are crucial. Future efforts may focus on further increasing nanosilicate concentration or modifying the PGS network to achieve mechanical properties closer to those of cancellous bone.

A new biomaterial composed of polylactic acid (PLA) and Halloysite nanotubes loaded with zinc nanoparticles (Zn NPs) was developed in one study by fused deposition modelling (FDM) [108]. The Zn NPs -loaded Halloysite nanotubes were mixed with PLA in order to create a thermoplastic filament, which was subsequently moulded to make the scaffold, with enhanced mechanical properties. In this work, the scaffold was successively coated with three layers starting with fetal bovine serum (FBS), sodium hydroxide and another time FBS, resulting in an increase in the hydrophilicity of the material (contact angle from $66.4° \pm 9.8°$ for uncoated PLA to less than 5° (thus, not recordable) for coated PLA) with a consequent increase in cell adhesion. Moreover, the study evaluated the impact of Halloysite nanotubes on the mechanical properties of 3D-printed PLA scaffolds by analyzing their compressive strength. Scaffolds containing Halloysite nanotubes, both without and with Zn NPs, exhibited a higher strain percentage (23%) and an increased average compressive modulus (around 0.3 MPa) with respect to PLA scaffolds without Halloysite nanotubes (20% and 0.24 MPa for the strain percentage and the compressive modulus, respectively) (Fig. 5). On the other hand, the produced scaffolds presented osteoinductive capability where the pre-osteoblasts (MC3 T3-E1) seeded on these scaffolds differentiated into osteoblasts, without the addition of exogenous osteogenesis agents, and produced a type I collagen matrix (one of the markers of osteogenic differentiation), and subsequent calcium deposition, It was demonstrated by mineralization-Alizarin red staining and Picrosirius red staining, to detect the calcium deposition, a sign of bone matrix mineralization, and the production of collagen fibers, respectively. From these results, it was concluded that the implementation of Halloysite nanotubes for the production of scaffolds via 3D printing is possible [108].

Mechanical reinforcing was also found in a study published by Guo *et al*. [109], where silica (SiO_2) nano-structures were spontaneously formed on Halloysite nanotubes (HNT) through the hydrolysis of tetraethoxysilane (TEOS) and subsequent condensation with the surface O–H groups of HNT. Subsequently, these modified HNTs (HNT@SiO_2 composite) were incorporated into poly (L-lactide) (PLLA) scaffolds, which were fabricated using laser-based additive manufacturing techniques. When HNT@SiO_2 composite was incorporated into PLLA scaffold, the SiO_2 nano-structures protrusions enhanced the interaction with the polymer matrix, leading to stronger interfacial adhesion and better dispersion of HNT@SiO_2 within the polymer. This resulted in PLLA composite scaffolds with higher tensile strength (13.81 and 18.12 MPa for PLLA/HNT and PLLA/HNT@SiO_2, respectively) and higher Young modulus (548.25 and 637.46 MPa for PLLA/HNT and PLLA/HNT@SiO_2, respectively) compared to neat scaffolds (10.53 MPa for tensile strength and 420.65 MPa for Young modulus), thus improving the overall mechanical strength of the material. Besides, the produced

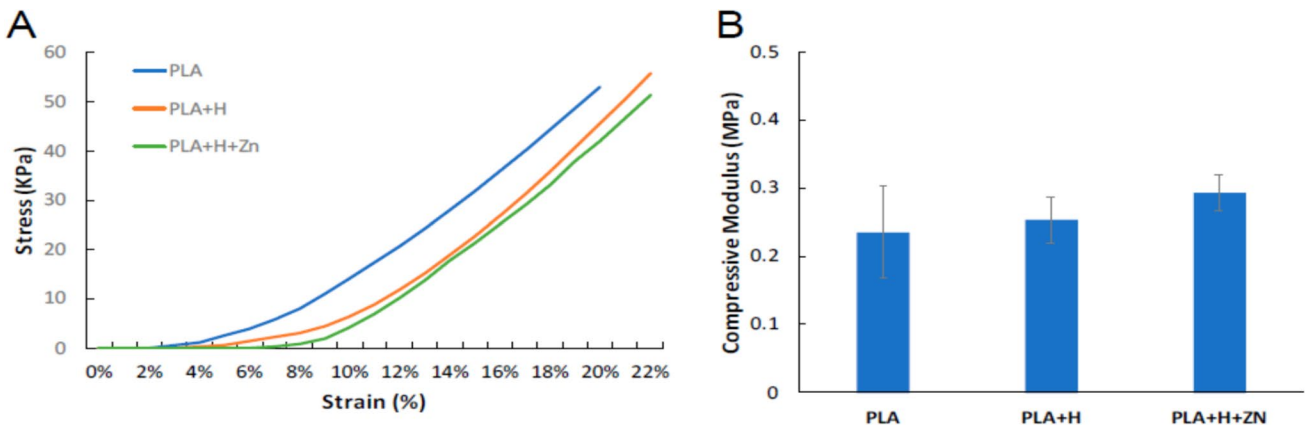

Fig. 5 Stress–strain profiles (**A**) and average compressive modulus values (**B**) of neat PLA and PLA loaded with Halloysite nanotubes (H) without (PLA + H) and with zinc nanoparticles (PLA + H + Zn) based scaffolds [108]

composite scaffold (PLLA/HNT@SiO_2) showed improved response of stem cells and capability of apatite formation (*in vitro* bioactivity). Similarly, another study demonstrated the fabrication of a composite scaffold was made of alginate and Halloysite nanotubes in different alginate: HNTs weight ratios (2:1, 1:1, 1:2 and 1:4 w/w), in order to improve both mechanical and cell-attachment properties. Scaffold preparation involved dissolving alginate in pure water then mixing it with a suspension of HNTs to form a homogeneous mixture, and then freeze-drying the mixture to remove the solvent and form a well interconnected porous structure with a uniform dispersion and partial orientation of HNTs within the matrix. The results of the study indicated that the HNTs incorporation into alginate matrix significantly enhanced the compressive strength from 10 to 47 kPa and modulus from 8.4 to 43.7 kPa for neat alginate scaffolds and composite scaffolds prepared using alginate: HNTs weight ratio of 1:4, respectively. Additionally, the presence of HNTs led to an increase in the scaffold density and a decrease in water swelling ratio, implying that the HNTs improved the alginate scaffolds stability against enzymatic degradation. HNTs also enhanced the alginate thermal stability, acting as thermal barrier. From a biological perspective, mouse fibroblast cells showed better attachment to the alginate/HNTs composites than to pure alginate scaffolds, suggesting good cytocompatibility, which is crucial for tissue engineering applications [110].

The effect of adding bioactive calcium silicate (BG) and Halloysite nanotubes (HNTs) on a nanocomposite hydrogel composed of polyacrylamide (PAM) and polyvinyl alcohol (PVA) was investigated in one study. Morphological analysis confirmed the presence of a highly porous and interconnected structure of the obtained nanocomposite hydrogel. The increase in the biomineralisation capacity of the nanocomposite hydrogel loaded with BG and HNT was demonstrated through the *in vitro* bioactivity test, observing the formation of a calcium phosphate layer (Ca/P: 1.21 ± 0.14) on the material surface after the immersion in the simulated body fluid. The study also examined the dynamic mechanical properties of the obtained nanocomposite hydrogels, focusing on their compressive strength and stiffness, particularly under different levels of strain. The nanocomposite hydrogel exhibited a slightly lower maximum compressive strength of 87.14 kPa but at a lower deformation of 57.5% strain with respect to PAM hydrogel (maximum compressive strength of 89.88 kPa at a deformation of 72.5% strain). This improvement in strength at lower deformation is attributed to enhanced physical interactions and chemical crosslinking within the hydrogel network. Moreover, the nanocomposite hydrogels loaded with 40% of BG and different percentages of HNTs from 2, 5 and 10 wt % were characterised by improvements in compressive strength. Where the recorded compressive strength values when using 2, 5 and 10% wt of HNTs were 90.83 kPa at 50% strain, 94.24 kPa at 45% strain and 102.1 kPa at 45% strain, respectively. In conclusion, the incorporation of BG and HNT in the nanocomposite hydrogel allowed to increase compressive strength and stiffness. The results of the biological tests showed a substantial increase in the growth of hFOB1.19 osteoblastic cells on nanocomposite hydrogels compared to those lacking the addition of HNTs after 14 days. These results lead to the assumption that the nanocomposite hydrogel could have a promising application in the bone tissue regeneration [111].

Another study highlighted the beneficial combination of PLLA with nanoclays by combining a nanocomposite technique with the electrospinning process [112]. The study reported the incorporation of Montmorillonite (MMT) nanosized platelets into a PLLA solution, which was then electrospun and mechanically entangled by cold compression molding process in order to provide a dual porosity to the formed fibers and create a 3D scaffold structure. A comparison of

scaffold morphology after 6 weeks of hydrolysis reaction was made on neat PLLA scaffold and PLLA/MMT composite. As evident from the acquired SEM micrographs (Fig. 6), neat PLLA showed larger holes and advanced degradation, resulting in a structural integrity loss, whereas PLLA/MMT samples maintained an integrated structure with micro and nanometrics pinholes, facilitating the transport of metabolic nutrients and wastes through its nano-sized pores and supporting the cell implantation and blood vessel invasion through its micro-sized pores. Moreover, the presence of tiny holes led to a better structural integrity during the biodegradation process, ascribed to the incorporation of MMT nanosized platelets into PLLA nanofibers, providing a better framework and a great potential in terms of mechanical and biological characteristics for tissue engineering applications [112].

A shear thinning nanocomposite hydrogel was successfully produced by the electrostatic reaction of the silicate nanodiscs (0.25, 0.5 and 1%) with the phosphate ions backbone of DNA. The formulated nanocomposites were biocompatible at all silicate nanodiscs concentrations, as demonstrated by MTS assay performed at 24 and 72 h on human adipose-derived stem cells (hASCs) in contact with the nanocomposite hydrogels (Fig. 7a), with no-significant differences in terms of cell viability by varying the concentration of silicate nanodiscs, and by the investigation at fluorescence microscopy of hASCs after 72 h of contact with the hydrogels (Fig. 7b). Also, it was found that higher concentrations of the added silicate nanodiscs led to smaller pore diameter followed by a slower release rate of dexamethasone (Dex) from DNA hydrogel scaffold (Fig. 7c & d). The sustained release behavior of the nanocomposite hydrogels was confirmed by the half-release time ($t_{1/2}$) of Dex, which varied with nanosilicate concentration (Fig. 7d). The retention of shear-thinning behavior following Dex incorporation was confirmed by the plots of viscosity *versus* shear rate for drug-loaded hydrogels, as shown in Fig. 7e [113].

Cartilage Regeneration

Articular cartilage purposes are to cover bones joint, protecting them from damage and allowing a smooth movement between them. However, cartilage has limited self-repair capacity due to low presence of blood vessels [114]. Articular cartilage regeneration continues to be a medical challenge, increasing interest for innovative strategies on tissue engineering focusing on creating scaffold that mimic native cartilage. In this framework, nanoclays are also regarded as ideal candidates for cartilage regrowth applications, particularly due to their ability to provide bioactive properties through ionic dissolution products, promoting cartilage regeneration. In the work conducted by Eslahi *et al*. [115], different amounts (0, 3, 6% wt) of Laponite (LP) were incorporated in a hydrogel of Pluronic® F127 copolymer conjugated with chitosan and crosslinked with keratin using Genipin as a reinforcing agent in order to create a novel injectable nanocomposite hydrogel for cartilage tissue engineering application. The LP addition led to a reduction in the scaffold pore size from 100 to 5 µm, and enhanced up the elastic modulus from 2.5 to 11.6 kPa as well as biostability to as high as 6 times without inhibiting cell regrowth, as demonstrated by the investigation of the viscoelastic properties in simulated physiological conditions. Indeed, cytotoxic tests, conducted with articular chondrocytes for 14 days, demonstrated the non-toxicity of the produced scaffolds, as well as an increased cell culture proliferation, comparing tested material with pure culture medium (RPMI and FBS) used as control element. Furthermore, incorporating silicate nanoparticles into the hydrogels improved the adhesion of articular chondrocytes.

In the same context, another study demonstrated the preparation of injectable poly-ethylene glycol (PEG)-based hydrogels loaded with LP particles as scaffolds that closely replicate the mechanical properties of natural articular cartilage (Fig. 5). The nanocomposites were fabricated through imide ring-opening reactions utilizing synthesized copolymers containing PEG

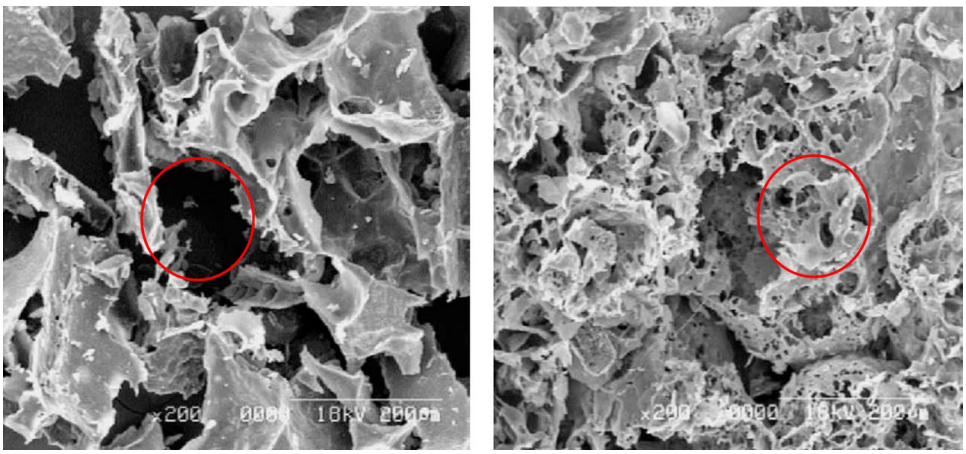

Fig. 6 SEM micrographs of neat PLLA (left) and MMT/PLLA (right) structures after 6 weeks of hydrolysis treatment. Reprinted with permission from "Lee, Y.H., *et al*., Electrospun dual-porosity structure and biodegradation morphology of Montmorillonite reinforced PLLA nanocomposite scaffolds. Biomaterials, 2005. 26(16): p. 3165–3172. [72]. Copyright 2024 Elsevier

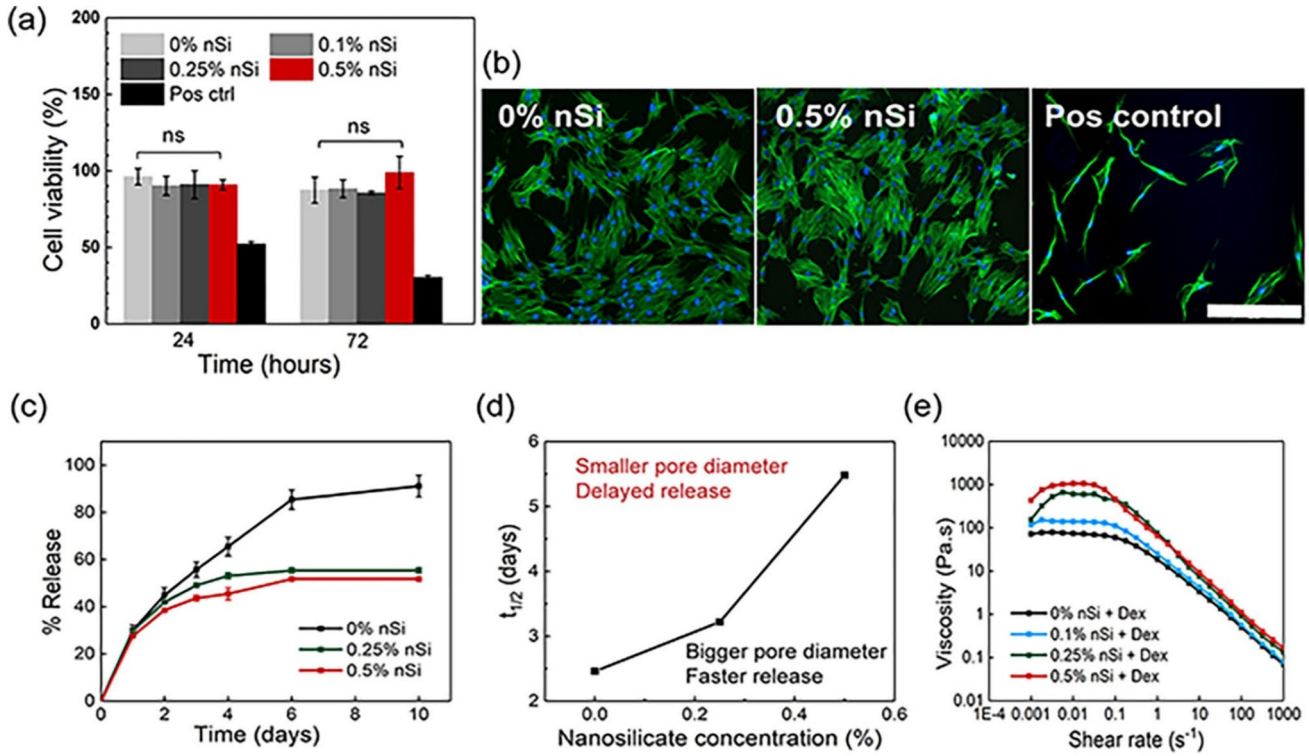

Fig. 7 **a** MTS assay on human adipose-derived stem cells (hASCs) treated with the nanocomposite hydrogels. **b** Fluorescence images of hASCs after 72 h of contact with the hydrogels (actin stained with Alexa Fluor 488 Phalloidin (green) and nuclei stained with DAPI (blue). **c** Dexamethasone (Dex) release profiles from DNA hydrogels without nSi and from nanocomposite systems over a 10-day period. Data are presented as the mean ± standard deviation (n = 3). **d** Half-release time (t1/2) of Dex *vs* nSi concentration. **e** Plots of viscosity *versus* shear rate for Dex-loaded hydrogels. Adapted with permission from "Basu, S., *et al*., Harnessing the noncovalent interactions of DNA backbone with 2D silicate nanodisks to fabricate injectable therapeutic hydrogels. ACS nano, 2018. 12(10): p. 9866–9880 [113]. Copyright 2018 American Chemical Society

blocks and nanoclays (LP), via a two-step thermal poly-(amic acid) process. Butanediamine was selected as the nucleophilic reagent. This process resulted in the creation of a scaffold with interconnected porosity, with pore sizes ranging from 100 to 250 nm. The addition of LP significantly enhanced the viscoelastic properties by 50%, thereby approaching the reference values of the original cartilage tissue (380–650 kPa). The physical crosslinking, caused by the adsorption of NH_3^+ ions of amino-terminated poly-(ethylene glycol) during the *in-situ* synthesis, resulted in the creation of crosslinking sites on the LP surface. This process enhanced the yield stress by making the chemical linking sites more resistant to detachment. From the MTT assay conducted cultivating human mesenchymal stem cells (hMSCs) for 7 days, it was revealed that the nanoclays particles addition decreased the cell viability, due to the entrapment of the butanediamine molecules used during crosslinking. However, an *in vivo* investigation should be required to better explore the potential of the hydrogel loaded with nanoclays. The main issue identified in the case of the cartilage is its lack of spontaneous regeneration [116].

On the other hand, in the work done by Boyer *et al*. [54], a hydrogel composed of silated hydroxylpropylmethyl cellulose (Si-HPMC) reinforced with a small amount of nanoclay (LP XLG) was developed. The nanoclays incorporation into the hydrogel resulted in the formation of a hybrid interconnected network with significantly enhanced mechanical properties. Biological tests indicated no contraindications or cytocompatibility issues with XLG. Additionally, there was a notable self-organization of chondrogenic cells into clusters, promoting the regeneration of the extracellular matrix.

Similarly, one study investigated the structures and mechanical properties of physically and chemically cross-linked nanocomposite hydrogels composed of Pluronic diacrylate and different concentration of LP nanoparticles (2, 4, 8% w/v). The resulting thermo-responsive hydrogels exhibited greater elongation and toughness with a maximum stress of 80 kPa and an elongation break more than 1250% for samples prepared with higher concentration of LP compared to their unreinforced polymer hydrogel counterparts. Also, the thermo-responsive hydrogels with higher concentration of LP possessed maximum tensile stress of 20 kPa and maximum elongation of 250%. Shear and creep tests demonstrated that LP nanoparticles interact with the covalent bonds of the cross-linked polymer network, imparting viscoelastic properties to the hydrogel [117].

Generally, chemical and structural modifications can be performed to nanoclays to improve their biocompatibility and targeting [118]. Chemical modification involves altering the inherent chemical structure or surface properties of nanoclays aiming to improve drug loading efficiency, control drug release, enhance stability, and reduce potential cytotoxicity. Successful strategies for enhancing the inherent properties of nanoclays involve functionalization, notably the intercalation of ions into their interlayer spaces and surface modification [119]. For example, to achieve effective drug release and better binding with pharmaceuticals, naturally occurring halloysite nanotubes were modified with various chemical agents. A common strategy is surface modification, with (3-aminopropyl) triethoxysilane (APTES) due to its low toxicity. APTES acts as a linker, facilitating the attachment of desired drugs by introducing silanol groups that form hydrogen bonds with the hydroxyl groups on the halloysite nanotube surfaces. For instance, APTES modification increased aspirin loading efficiency to 11.8 wt% compared to 3.84 wt% for unmodified nanotubes. The confined space within the halloysite nanotubes also induced a change in aspirin from an amorphous to a nanocrystalline state, leading to faster disintegration and burst release within the first hour [17, 120]. Also, Kaolinite was used as a host material for the loading of the anticancer agent 5-fluorouracil. Methoxy modification of the kaolinite structure substantially augmented drug loading efficiency, achieving 55.4 wt% (147.3% improvement over the unmodified material). This enhanced performance is likely due to the increased interlayer distance resulting from the modification, which provides more accessible sites for 5-fluorouracil intercalation, coupled with the creation of high-affinity external surfaces and improved thermal robustness [121].

Patents

The use of nanoclays spans a wide range of industries and applications resulting in the existence of thousands of patents worldwide. However, the number of patents specifically addressing the use of nanoclays in the biomedical field is significantly lower, reflecting a more specialized and targeted area of research and innovation. Recognizing the importance of understanding the latest advancements in this domain, a comprehensive search was conducted to address this point. Some of the biomedical applications of nanoclays across various sectors are presented below.

Skin Cancer

This patent focuses on the use of specific nanoclays, such as Palygorskite and Montmorillonite, to reduce the viability and proliferation of melanoma cells. The nanoclays are intended for use in therapeutic and prophylactic preparations aimed at treating skin cancer, particularly melanoma. The treatment involves mixing melanoma cells with a nanoclay-containing formulation, this significantly reduced cell viability. Various application methods are proposed, including topical, oral, or injection. Additionally, the invention suggests the use of these nanoclays in combination with other anti-cancer [122].

Dialysis

This patent proposes an innovation in dialysis treatment by employing nanoclay sorbents, including Montmorillonite and Bentonite, to enhance the absorption of bodily wastes like ammonium, phosphate, and creatinine. Nanoclays are shown to absorb more waste than conventional sorbents, making them suitable for use in wearable dialysis systems where the dialysis fluid is filtered through the nanoclays and then recycled back into the patient's peritoneum. The nanoclays facilitate ion exchange, replacing waste ions in the fluid with beneficial ions such as calcium, magnesium, and bicarbonate, thereby reducing the amount of dialysis fluid required for hemodialysis [123].

Another patent describes a device designed to remove toxic substances from dialysate fluid, blood, or blood plasma. The device incorporates a sorption filter made of nanostructured materials, a porous polymer matrix, or both, to eliminate toxins including small and middle-sized molecules and protein-bound toxins. The design features an inlet for fluid entry, an outlet for purified fluid removal, and a conduit that connects the inlet and outlet, forcing the fluid through the sorption filter for effective purification. The composition of the device is based on the combination of various materials, such as nanoparticles or nanocrystalline materials, such as exfoliated nanoclay, double hydroxides like nano-hydrotalcite, or pure metal oxide nanoparticles; nanoporous materials (zeolites); nanocomposites and nanofibers [124].

Antimicrobial

This patent involves the development of antimicrobial nanoclays to address the growing problem of antimicrobial resistance and healthcare-associated infections. Existing clay-based antimicrobial coatings are limited due to their reliance on a single antimicrobial agent. This invention seeks to prepare nanoclays that combine multiple antimicrobial agents from different classes, thereby enhancing their effectiveness against a wide range of microbes, including antibiotic-resistant bacteria. Additionally, it aims to improve synthesis methods for the production of novel nanoclay-based materials with enhanced properties [125].

These results highlight the diverse potential uses of nanoclays in biomedical applications, ranging from cancer treatment to medical device innovation and beyond.

Regulatory and Translational Aspects

Nanoclays, with their unique properties, hold promise for advanced drug delivery systems due to their chemical inertness, large surface area, and biocompatibility. However, similar to other nanomedicines their translation into pharmaceutical applications faces several regulatory and translational challenges [18, 126, 127].

From a regulatory standpoint, the FDA has not established a formal definition for nanomaterials but considers engineered materials with dimensions up to 1000 nm that exhibit size-dependent properties. Their guidance emphasizes risk identification and management, requiring thorough characterization of the nanoclay material, understanding its intended use, and demonstrating how its attributes relate to the drug product's safety and efficacy. Critical quality attributes (CQAs) such as chemical composition, particle size distribution, morphology, and stability are crucial for FDA approval [128, 129]. The EMA defines a pharmaceutical nanoparticle as a manufactured system with at least one component in the nanoscale, exhibiting specific properties that provide a clinical advantage. Similar to the FDA, the EMA focuses on pharmaceutical quality, requiring standardized characterization methods and the definition of CQAs. Non-clinical studies generally follow conventional toxicological approaches, but specific methods may be needed for certain nanomaterials [128] [130].

Translational challenges in scale-up manufacturing include maintaining consistent quality and physicochemical properties at industrial scales. The manufacturing process significantly impacts nanoclay characteristics, necessitating tight control over physicochemical properties like particle size and morphology. Strategies to maintain stability and prevent agglomeration, such as using stabilizing agents and optimizing storage conditions, are critical. The scale-up of nanoclay formulations for pharmaceutical applications has seen less development compared to polymer-based systems [127, 131].

Clinical trial considerations for nanoclay-based pharmaceuticals involve thorough biocompatibility and toxicity evaluations. While some studies suggest low toxicity, others indicate potential toxic effects depending on the type and modification of the nanoclay. The high aspect ratio of some nanoclays can lead to increased cellular uptake and interactions, potentially contributing to toxicity. Understanding the pharmacokinetic (PK) and pharmacodynamic (PD) properties of nanoclay drug delivery systems is crucial for optimizing drug delivery and therapeutic outcomes. Nanoclays can improve drug bioavailability, overcome biological barriers, and provide controlled drug release. Clinical trial designs must account for these unique PK/PD properties.

Nanotherapeutics are generally facing multiple challenges at the time of their commercialization [132]. Viewing their novelty, currently there are limited available nanoclay-based pharmaceuticals that have received full regulatory approval from regulatory agencies. However, general challenges for nanomaterial-containing products include demonstrating bioequivalence for generic versions and addressing regulatory uncertainties for complex nanomedicines. Differences in regulatory requirements between the FDA and EMA can also complicate global drug development programs.

Challenges Facing the Use of Nanoclays in Pharmaceutical Applications

Nanoclays, with their unique properties, have gained significant attention in pharmaceutical formulations. However, their application faces several challenges related to manufacturing and scale-up to assure consistency, reproducibility and cost effectiveness. Also, obtaining regulatory approval for nanoclay-based pharmaceuticals requires extensive testing, analysis and documentation to demonstrate their safety and efficacy. Besides that, the novel nature of nanoclays may necessitate additional regulatory hurdles and considerations.

Overcoming these challenges requires interdisciplinary research, advanced characterization techniques, and careful formulation design. By addressing these issues, nanoclays can offer significant advantages in various pharmaceutical applications, including drug delivery, controlled release, and improved bioavailability.

In conclusion, nanoclays represent a versatile and promising resource driving advancements in biomedicine, offering innovative solutions across critical domains including pharmacotherapy, tissue engineering, medical diagnostics, gene therapy, and biomedical sensing. Their burgeoning potential continues to stimulate scientific inquiry, heralding a new era of transformative healthcare solutions aimed at ameliorating human health and well-being.

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Declarations

Conflicts of interest The authors declare none.

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