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REVIEW ARTICLE

Nanoclays for wound management applications

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Abstract

Nanotechnology has been comprehensively applied as a new approach to managing wound healing. Particularly, nanoclays are being used to improve traditional wound healing approaches or new therapies. Nanoclays are nanoscale aluminosilicates with remarkable intrinsic properties, including the capacity to promote hemostatic response, anti-inflammatory effects, angiogenesis, and re-epithelization. The main purpose of the present review is focusing on skin lesions, post-surgical wounds, burn wounds, and chronic ulcer skin wounds that can be treated using nanoclays, not only as vehicles for therapeutic molecules' efficacy improvement but also alone due to their native beneficial features. A systematic search of the PubMed, ScienceDirect, Scopus, Web of Science, and Google Scholar databases revealed several studies satisfying the purpose of our study. In addition, the selected keywords were used to refine the information. Non-planar hydrous phyllosilicates have been compared with other nanoclays considering their acute specific surface area and loading capacity are strongly influenced by their structure. Nanocomposites in the powder form may be directly incorporated in polymers to form gels, biofilms, and scaffolds that may be adjustable to wound sites. Also, nanoclays can be directly incorporated into polymer mats. Regarding hydrogels/films and mats, nanoclays can improve their mechanical strength, thermal stability, viscosity, and cohesive strength. Additionally, nanoclays are able to control drug release, as well as their skin bioavailability, and seem to be promising candidates to overcome cytotoxicity problems; further in vivo toxicity studies are required.

Keywords Wound · Nanoclay · Halloysite · Laponite · Montmorillonite · Sepiolite · Palygorskite

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Abbreviations

5-FC	5-Fluorocytosine
BC	Bacterial cellulose
CMC	Carboxymethylcellulose
CLX	Chlorhexidine
CVR	Carvacrol
DA	Dopamine
EGF	Epidermal growth factor
FDA	Food and drug administration
GelMA	Gelatin methacryloyl
GG-MA	Gellan gum methacrylate
HNT	Halloysite clay nanotube
LA	Laponite
MMT	Montmorillonite
NF	Norfloxacin
PAL	Palygorskite
PEDOT:PSS	Poly (3,4-ethylenedioxythiophene):poly (styrenesulfonate)
γ-PGA	Poly (γ-glutamic acid)
PVA	Polyvinyl alcohol
QCH	Quaternized chitosan

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SEP	Sepiolite
VEGF	Vascular endothelial growth factor
SPI	Soy protein isolate

Introduction

Skin is the major organ of the human body and it is continually exposed to external insults [1]. Skin wounds are of growing importance in medical and economical fields due to an aging society and the growth of chronic diseases like diabetes and vascular diseases, beyond an increased incidence of antibiotic resistance [2]. There is still much to discover and discuss about wound treatment and care. It is a growing problem and of increasing concern, because current conventional treatments are limited in combating, especially, bacterial infections, excessive inflammation, and insufficient blood supply [3].

According to the duration and healing process, wounds can be classified as acute and chronic wounds. Acute wounds result from external factors and with proper care treatment skin heals in a short period of time. Chronic wounds are associated with chronic diseases and do not follow the normal wound healing process [4–6]. Wound healing is a dynamic and complex physiological process involving various cell types, mediators, cytokines, growth factors, components of the extracellular matrix, and proteinases. It is usually divided into concurrent but overlapping phases of hemostasis, inflammation, proliferation, and re-epithelization [3]. When skin damage occurs and causes blood loss, the hemostasis phase (vasoconstriction) starts immediately to stop bleeding and a fibrin clot is formed. The overlapping inflammatory phase is induced by the release of inflammatory mediators enhancing permeability and vasodilatation [4–6]. Neutrophils and macrophage infiltration to remove dead fibrin tissue occur while neoangiogenesis reconstructs vasculature in damaged areas. Granulation tissue yields chemotactic factors for fibroblasts and promotes mesenchymal cell proliferation [7]. The proliferative phase is defined by fibroblast migration and proliferation, where new connective tissue is built with collagen and extracellular matrix components. In the re-epithelization and remodeling phase, collagen III of the extracellular matrix is gradually replaced by collagen I with a more ordered structure and scar tissue being formed [8]. At this stage, all phases are complete. If one of the phases fails all process is compromised leading to chronic wounds [4–6].

So far, just a few treatment procedures have been approved to treat chronic wounds meaning that many patients remain untreated [4]. Wound management aims to prevent infection, enhance wound healing, and reduce scar tissue [9]. Chronic, non-healing diabetic foot ulcer management approaches include control of glycemia, topical therapeutics, and prevention of secondary infection and inflammation. Curiously topical application of insulin proved to reduce inflammation [10]. Another example regards malignant wounds arising from tumor progression on the skin where topical wound management is the main way to control signs and symptoms [11]. Topical localized therapies are preferred to manage wound healing because it avoids systemic effects and treatment is directly applied to the wound site. Moreover, with topical wound healing approaches, the wound site is covered and moisturized preventing secondary infections [10].

Conventional topical treatment strategies for wound healing have several limitations such as rapid drying of the wound area, allergic reactions, and risk of infection [5]. Therefore, nanotechnology is a promising approach to addressing the specificity and complexity of acute and chronic wounds [8]. The physicochemical properties of nanomaterials make them perfect candidates to enhance wound healing as vehicles/carriers for controlled drug delivery or even used due to intrinsic healing properties proven to be more effective than traditional approaches [6]. In comparison with non-nanomaterials, nano-size materials have a versatile use due to a higher surface/volume ratio [12]. The design of functionalized nanomaterials has aroused interest in the scientific community, regarding the development of systems with higher affinity to the main target and increased bioavailability. This may be a suitable way to overcome some drawbacks related to conventional strategies and a great advance in nanotechnology [13–15]. These materials usually need a repeated application, cannot assure the appropriate moisture and adherence to the wound, and are related to pain on application or removal [16].

Nanoclays have shown good biocompatibility and degradation properties as well as intrinsic properties that can be used to improve wound healing management. In addition, the length scale of nanoclays is relevant to wound healing processes [17]. For instance, used as topical agents, nanoclays benefit from their capacity of forming a protective film by adhering to the skin [18]. Nanoclays are nanoscale layered aluminosilicates widely distributed in nature that can be divided into planar or non-planar hydrous phyllosilicates. They can be classified by their chemical structure and composition, such as montmorillonite (MMT), bentonite, kaolinite, laponite (LA), and halloysite. These nanosystems are characterized by chemical and mechanical stability, layered structure, and environment-friendly nature [19].

Naturally, nanoscale clays can also arise in an amorphous phase, for example, Allophane [20]. The crystalline nanoclays can also be divided according to the structural organization and ratio of octahedral and tetrahedral sheets. Kaolin group has a 1:1 structure and its common nanoclays are kaolinite and halloysite. Halloysite is often presented as a nanotube — halloysite nanotube (HNT) — with an external silica surface rolled with an internal alumina surface. For the 2:1 type structure, the smectite group is the most representative. MMT,

LA, and bentonite are the most common clays of the group [20, 21]. The 1:1 type structure is characterized by having a tetrahedral and a octahedral sheet in each clay layer. On the other hand, 2:1 type structure consists of one octahedral sheet between two tetrahedral sheets of silica [21].

Bentonite is an example of clay that can promote skin adhesion of a blend active component [22]. MMT can induce a significant increase in collagen synthesis. Kaolinite proved to enhance collagen fibbers in rat skin [23]. Clay minerals, such as kaolinite and smectites, can also induce a haemostatic response [18]. They may play the main role in blood stoppage due to their ability to enhance plasma absorption and by cooperating with hemostasis pathways [24]. Nanoclays can be used as nanofillers of polymeric networks to obtain nanocomposite hydrogels, but they also work as cross-linkers during gel formation mainly because of clay-polymer interface interactions [25]. These characteristics may significantly influence wound care treatments. Despite the slow transition to clinical research, wound healing management using nanoclays is actual and novel concepts and approaches have been recently published.

Application of nanoclays in wound management

Considering the most recent publications, the nanoclays that seem to have the greatest interest and impact on the treatment and care of chronic wounds will be discussed below. We highlight in this context halloysite, laponite, and montmorrilonite as planar hydrous phyllosilicates, and sepiolite, and palygorskite as non-planar hydrous phyllosilicates, which will be presented next in detail.

Planar hydrous phyllosilicates

Halloysite

Halloysite is a common nanoclay in the kaolin group [20]. This group possesses a 1:1 structure with two aluminum octahedral sheets bound with a silicon tetrahedral sheet by hydrogen and ionic bonds. This crystalline structure is electrically neutral presenting edges that are charge pH-dependent. Halloysite can be described as a rolled hydrated kaolinite, as the most common and well-studied form of HNT is the hallow tubular morphology. HNT presents an external negatively charged and hydrophobic silicon surface and an inner positively charged hydrophilic aluminum surface. HNTs typical size includes an outer diameter of 50–70 nm inner diameter of 10–30 nm and a length of 200 nm– 1 μm [20, 26]. Moreover, in this structure, OH-groups can be between layers and in lumen surface, aluminols, or on the surface at a low density, silanol groups, configuring a hydrophobic character [27–29].

Characterized as natural and environmentally friendly, this clay material has been described with superior hemostatic properties, when compared to other clays such as montmorillonite and kaolin [26]. For better understanding, we present in Fig. 1 a schematic illustration with the detailed structure of the HNTs.

HNT has been widely investigated in life sciences applications, namely to manage wound healing [30]. They were found to have a major role in hemostasis through the following approaches. First, their hydrophilicity and tubular structure contribute to water absorption and consequently increased blood concentration. Then, HNTs trigger an intrinsic coagulation cascade by interacting with negatively

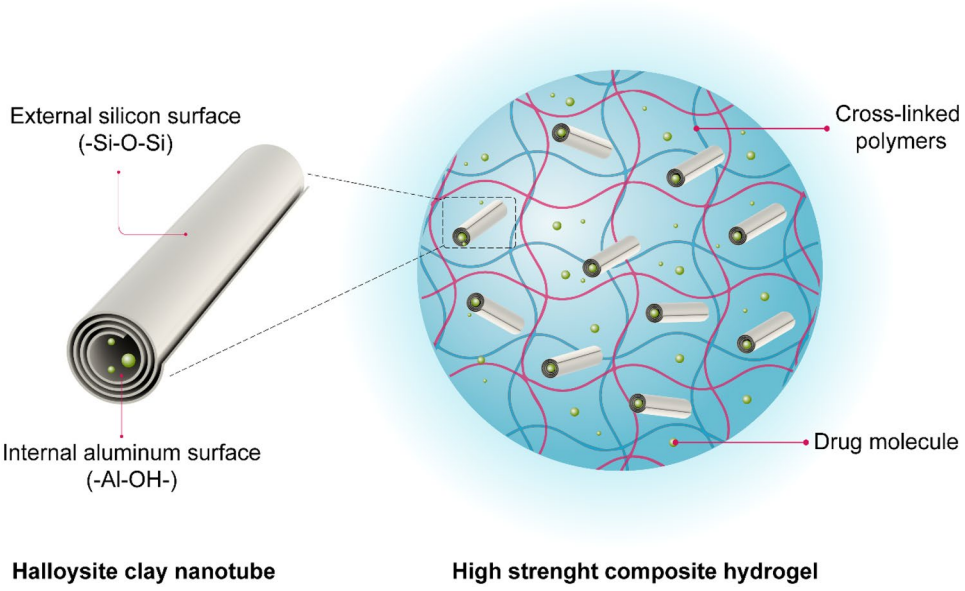

Fig. 1 Schematic illustration of a hydrogel containing halloysite clay nanotubes (HNTs), highlighting the structure of HNTs, particularly the tubular morphology, the external siloxane surface, and the internal aluminol surface

charged surfaces and, finally, contribute to accelerating clot formation by linking with platelets [31].

In 2017, Sandri et al. studied the application of a pour powder with HNT and chitosan oligosaccharide with the purpose to treat cutaneous non-healing lesions. The pour powder was prepared based on the spontaneous electrostatic interaction of HNT negatively charged outer surface and chitosan oligosaccharide cation amine groups. In addition, HNTs Si–O groups allow hydrogen bindings to chitosan oligosaccharide amine and hydroxyl groups [7, 32]. To confirm the structure of hybrid nanocomposites, advanced electron microscopy techniques were considered. Furthermore, the internal diameter of HNT/chitosan nanocomposite decreased, and the external diameter increased compared to pristine HNTs due to chitosan coating. The self-assembled nanocomposite proved to be biocompatible in vitro. After 24 h, the percentage of cell viability was greater than the one of the control group, HNT, and chitosan. Of those viable cells, the nanocomposite presented the highest percentage of cells in the proliferative phase. Chitosan presented better results when compared to HNT, and thereby, it is assumed chitosan contribution enhances the biocompatibility of the nanocomposite. Animal experiments in male rats were done to understand the application of this nanocomposite in wound management. Although in vivo HNT profile of lesion reduction vs time is the most promising when compared with chitosan and to the nanocomposite where there was not a significant reduction in the wounded area after 7 days of treatment, the re-epithelialization process started earlier but was limited to lesion borders. Hemostasis and cell migration had occurred. Granulation tissue was also detected in the lesion that had been in contact with the nanocomposite. The pour powder proved to promote advanced angiogenesis and after 18 days, the skin even showed hair follicles. This study showed that HNTs/chitosan nanocomposite can be considered a therapeutic option to enhance skin re-epithelization and reorganization in skin lesions and burns [7].

Halloysite nanotubes loaded with polymyxin B sulfate were combined with ciprofloxacin into a gelatin elastomer to develop an ideal biomaterial with antibacterial activity [33]. The main results of this study are shown in Fig. 2. Both ciprofloxacin and polymyxin B sulfate-loaded HNT were uniformly distributed into the gelatin matrix as represented in Fig. 2B. While several techniques were used to study HNTs,

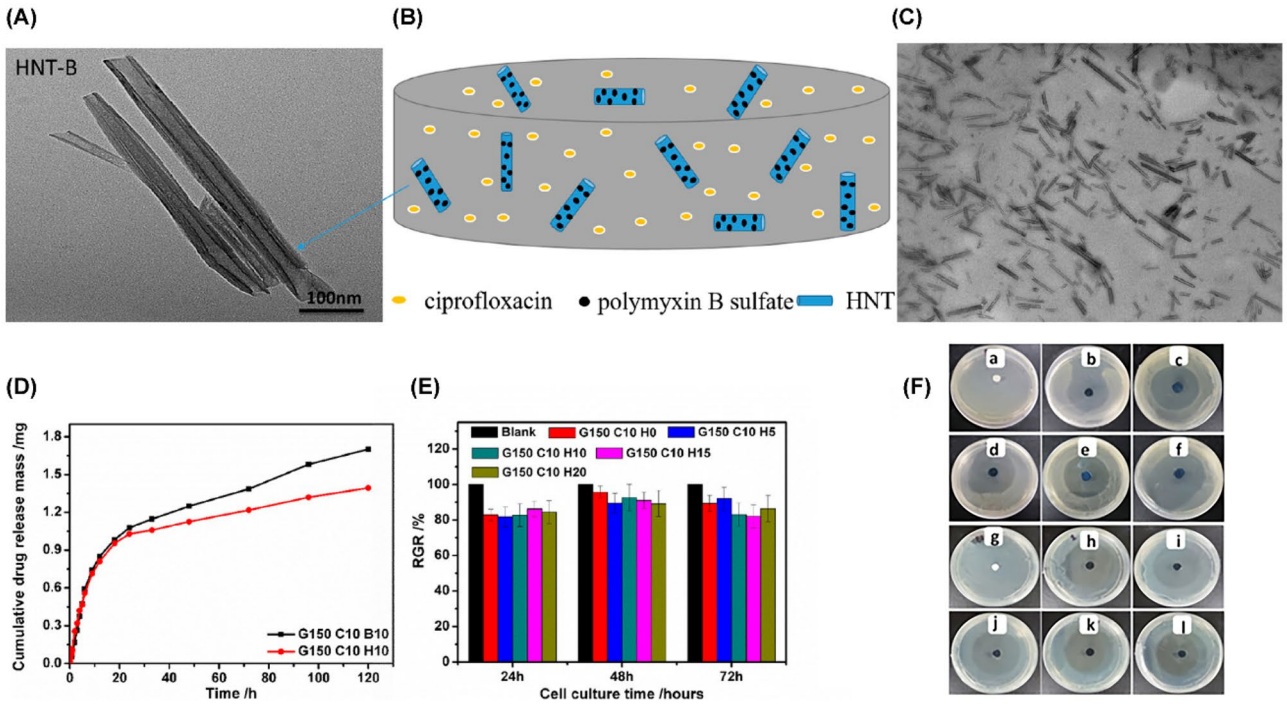

Fig. 2 **A** TEM image of the halloysite clay nanotubes (HNTs) loaded with polymyxin B sulfate. **B** Structural representation of the double drug co-delivery elastic antibacterial nanocomposite. **C** TEM micrographs of gelatin-based bio-elastomer nanocomposites plasticized by polymyxin B sulfate-loaded halloysite clay nanotubes (HNTs-B). **D** Cumulative drug release profiles of the polymyxin B sulfate released from the nanocomposite with G150 C10 B10 (nanocomposite composed of 150 g of glycerin, 10 g of ciprofloxacin, and 10 g of polymyxin B sulfate — parts per hundreds of gelatin) and G150 C10 H10 (nanocomposite composed of 150 g of glycerin, 10 g of ciprofloxacin, and 10 g of HNTs-B — parts per hundreds of gelatin) nanocomposites. **E** Results of the in vitro cytotoxicity: relative growth rate (RGR) of L929 fibroblast cells cultured in extract substrates of nanocomposite membranes. **F** Inhibition of bacterial (**a**–**f**: *Staphylococcus aureus* and **g**–**l**: *P. aeruginosa*) growth on agar plates of the drug-loaded gelatin nanocomposites (adapted from [33])

the structure of these nanocomposites was confirmed through transmission electron microscopy (TEM) images, as observed in Fig. 2A, C. The matrix tensile strength and the thermal stability were boosted by increasing the amount of HNT, which was also useful to slow the release rate of polymyxin B sulfate, a high dissoluble drug. This double-drug co-delivery gelatin-based nanocomposite holds ideal properties for wound management as water absorbency, adaptable biodegradability, low cytotoxicity, and elasticity. Also, double drug release allows antimicrobial activity against both gram-positive and gram-negative bacteria. The antimicrobial effect was tested with and without HNT incorporating polymyxin B directly into the gelatin. In this regard, results of in vitro cytotoxicity were obtained through the evaluation of the relative growth rate (RGR) of L929 fibroblast cells cultured in extract substrates of nanocomposite membranes. In Fig. 2E, it is observed that, although the blank control has shown high biocompatibility and nontoxicity, all nanocomposites exhibited a lower RGR.

The nanoclay can maintain the antimicrobial effect for 7 days due to a slower drug release. The evaluation of the cumulative drug release profile suggests that HNTs play a major role in promoting a sustained release. Although results were similar in the first 10 h, it was observed a slower degradation in the following time in the group using the nanocomposite (Fig. 2D). Even though in the first 4 days the inhibition halo was greater with polymyxin not loaded in HTN, it decreased gradually while with HNT, the inhibition halo increased (Fig. 2F). This novel nanoclay-based biopolymer can be used in chronic wounds, post-surgical wounds (adhesion), and tissue engineering [33].

An elastomeric nanocomposite was used to create a wound dressing to deliver topical antibiotic treatment, in this case, minocycline [34]. This broad-spectrum antibiotic was loaded in HNT modified with a silicon agent. Then, HNT was added to polyvinyl alcohol (PVA) creating an HNT/minocycline/PVA nanocomposite film intended for wound healing management. The inhibition of *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* (gram-negative) growth on agar plates was evaluated. HNT could retain the antibacterial effect for up to 7 days. The antibacterial inhibitory effect was greater in gram-positive bacteria. During the days of treatment, the biofilm showed absorption of scarring/secretions by its ability to absorb water and the nanocomposite had an acceptable degradability. The film presented a slow and controlled drug release in vitro compared to films without HNT and thereby antibacterial effects over time. Another important point taken care of in this study was the photostability of the antibiotic, as minocycline is not stable by light. Trapping minocycline molecules in the nanotube was enough to increase photostability allowing this drug to be used more securely. This nanocomposite may be a candidate for dressing to treat more infection-prone wounds such as burns [34].

Other studies regarding the use of HNTs and antibiotics loaded into biopolymer matrices support the benefits of using nanoclays to enhance wound healing. Furthermore, the long-term efficacy of HNT-based topical treatments was tested in humans [30].

The studies presented show that HNTs enhance lesion reduction over time. The nanoclay can enhance tensile strength and thermal and photostability in the polymer matrix. HNT can also be used for sustained drug release. As an example, HNT's slow release of antibiotics can enhance their antimicrobial effect over time. HNT-based nanocomposites proved to enhance angiogenesis and skin re-epithelization and reorganization processes [7, 33].

Laponite

Trioctahedral LA occurs in thin lath-like shape strips with 25 nm diameter and 1 nm thickness [20, 35–38]. LA presents a unique anisotropic structure [39] that forms a clear dispersion in the presence of water [40]. LA shows a high specific surface area [20] and absorptive and cation exchange capacity [25]. Although LA has a highly sorptive nature, it also has limited porosity which can limit LA biomaterials [41]. This mineral nanoclay can support cell proliferation, differentiation, and attachment and promotes type I collagen formation in vivo and it also has haemostatic properties [39, 40]. The long history of the use of LA suggests that it is well-tolerated and non-toxic even at high doses [41].

Figure 3A represents a schematic illustration of laponite nanodisks, showing how drugs can be incorporated there. Figure 3B shows how laponite can be integrated into hydrogels for wound-healing applications.

Due to the stated characteristics, Kim et al. elected LA for their study to increase the mechanical strength of catechol-modified hydrogels and thus improve the adhesion strength of hydrogel-based adhesives [40]. The aim was to control adhesive properties through variation of LA concentrations. The natural anionic poly (γ-glutamic acid) (γ-PGA) was first modified with dopamine (DA). After, PGADA was added to a LA dispersion. Then horseradish peroxidase and H_2O_2 solutions were added and mixed forming a PGADA-LA hydrogel. Tissue adhesive properties of the nanocomposite hydrogel were studied in vivo and in vitro. In vitro, with porcine skin tissue, a higher LA concentration improves cohesive strength and the hardness of hydrogels. In vivo results presented a better regeneration capacity for LA 2% rather than LA 4%. The haemostatic ability was also measured by the amount of blood loss in a rat liver tissue indicating an excellent haemostatic capacity and a good gelation time with a 2% concentration of LA even though the adhesive strength in the bovine liver model was lower with this concentration. In vivo, LA has proven to speed the closure of an open wound in rat skin with minimal inflammation. The actual

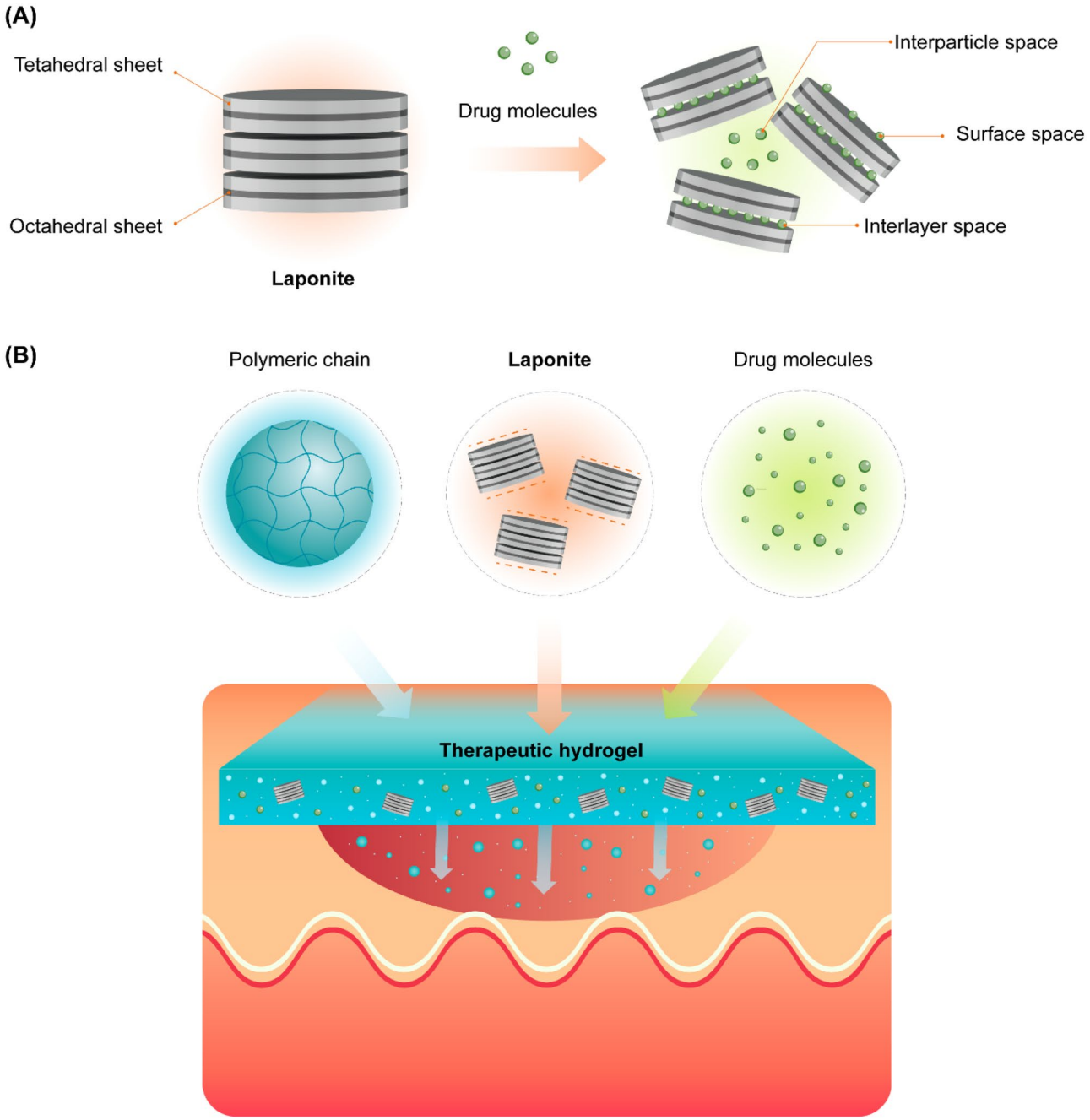

Fig. 3 Laponite for wound healing applications. **A** Schematic illustration of laponite nanodisks for drug molecules delivery, highlighting various possible interactions between drug molecules and laponite surfaces. **B** Representation of the development of hydrogels containing Laponite for wound healing applications

effect on skin and wound regeneration properties was tested against Tisseel® and Dermabond® presenting rapid wound closure, effective re-epithelization, minimal scarring, and hair regrew. A controlled concentration of LA can enhance the cohesive force of the hydrogel and thereby its haemostatic, regeneration, and adhesive abilities. The PGADA-LA hydrogel has proven to be biocompatible and so it could be applied as a biomaterial, especially for tissue bioadhesive applications, tissue engineering, injectable hydrogels, and haemostatic materials [40].

A gellan gum methacrylate (GG-MA) was used to create an injectable photocrosslinkable hydrogel combined with LA to design a novel wound dressing material [25]. The integration of this clay in GG-MA network increases

its viscosity forming a stronger hydrogel that can handle sterilization. After thermal treatment, and depending on the concentration of LA, hydrogels were able to maintain their mechanical characteristics. This point simplifies the use of sterile systems when LA is applied to design nanoclay-based wound dressings. The swelling properties are also altered in the presence of LA and are strongly influenced by the pH and ionic strength of the medium. The influence of LA on drug diffusion was observed through the characterization of diffusion profiles. As represented in Fig. 4A, LA can slow down the diffusion of loaded drugs due to its interaction with the therapeutic molecule. According to the measurement of the diffusion coefficient (D_{app}) of ofloxacin, the highest value was obtained in the sample with 0% of laponite (Fig. 4B), thus supporting the previous statement. In addition, this LA-based hydrogel was considered biocompatible [25].

LA was also studied to be incorporated in injectable nanoclay gels to stabilize vascular endothelial growth factor (VEGF) and retain its active form at the delivery site [41]. Although VEGF has a promising potential in the growth and regeneration of blood vessels, it is intrinsically labile which compromises its action in an inflammatory microenvironment. The in vitro cell culture tubulogenesis assays showed that VEGF/LA gels can enhance tubulogenesis in a dose-dependent manner although the morphology of the vessels was not addressed. For in vivo angiogenesis, assays gels were subcutaneously injected in male rats. LA was retained for 3 weeks increasing the blood vessel formation and cell invasion without VEGF release. LA enhances VEGF efficacy due to a strong interaction that proved to retain the growth factor at the implementation site preserving its activity for longer periods of time in vivo [41].

A polymer prepared with gelatin methacryloyl (GelMA) and LA was used to create a scaffold intended for wound healing management [39]. First, LA was exfoliated in deionized water and mixed with epidermal growth factors (EGF)

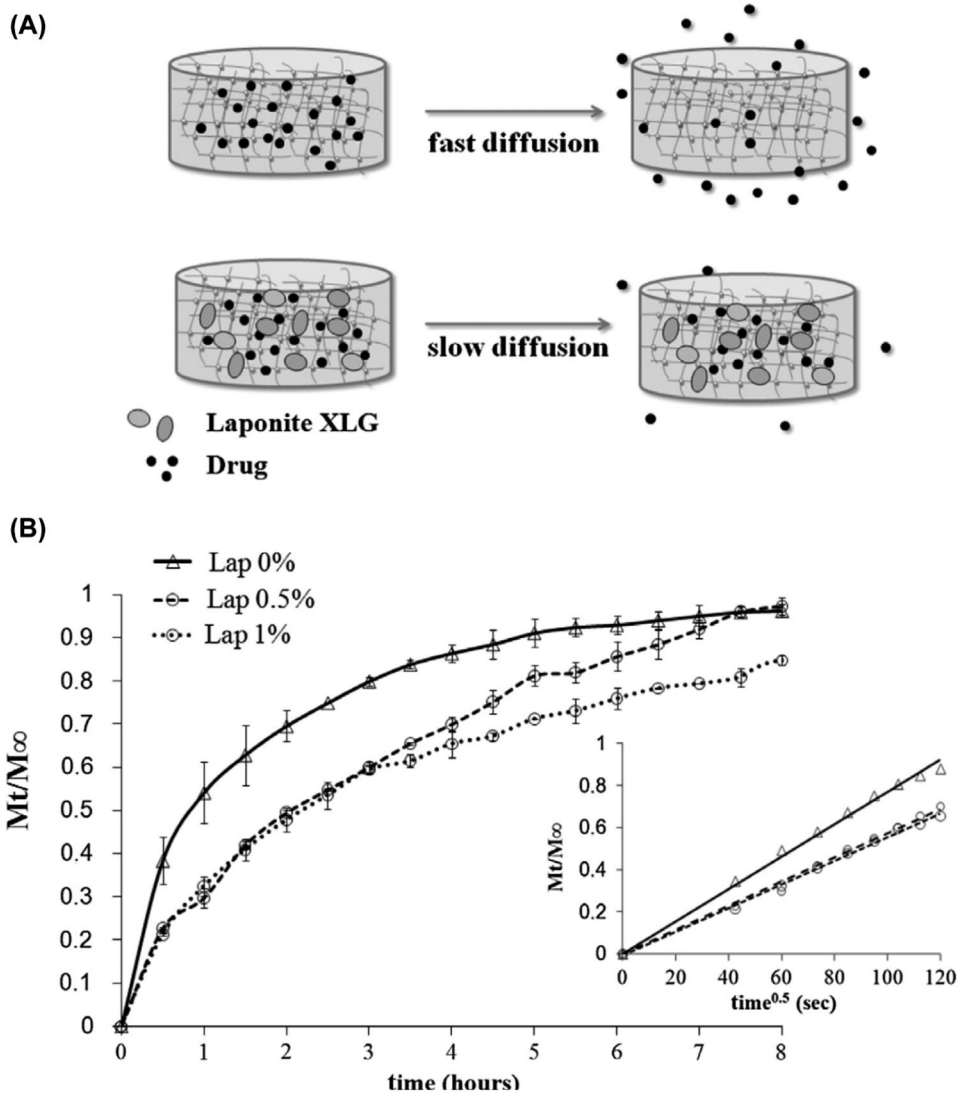

Fig. 4 Diffusion profiles of a model drug from the nanocomposite photochemical hydrogels. **A** Representation describing the influence of laponite on the diffusion of ofloxacin loaded in the polymeric networks. **B** Diffusion profiles of ofloxacin in PBS pH 7.4 for 8 h for the different systems in the presence of different concentrations of laponite. Measurements were carried out in triplicates at 37.0 ± 0.1 °C. The same release values were plotted in function of the square root of time to calculate the diffusion coefficient of the drug (adapted from [25])

to load LA with the growth factor. Secondly, GelMA was added, and an EGF-loaded GelMA/LA hydrogel precursor was created. Then, a fibrous gelatin and glucose mat previously prepared by electrospinning is placed above this hydrogel precursor, and the UV crosslinked is used to create a photocrosslinking hydrogel bilayer scaffold [39, 42]. Electrospinning is one of the most common methods involved in nanomaterial development, due to its simplicity and easy application on industrial scale production [43]. The fibrous sheet performs as the dermis layer and EGF-loaded GelMA/LA hydrogel functions as the epidermis matrix. The gelatin electrospun film functionalized with GelMA/LA hydrogel loaded with EGF has proven potential to be used in wound management as a mat for sustained release of EGF, helping to enhance full-thickness cutaneous wound healing, as it mimics skin structure and facilitates oxygen and fluids transport for wound dressing applications [39, 42]. LA was used to improve the mechanical strength and extensibility of the hydrogels. The layered silicate nanostructure of LA, its high specific surface area, and its positive and negative surface charges allow LA to crosslink within GelMA matrix and form networks that present a shear-thinning behavior [39].

Moreover, LA was considered a good therapeutic carrier to enhance the stability of EGF and its controlled delivery. The biomimetic nanoengineered bioactive bilayer scaffolds were evaluated in vivo. Due to their flexibility, these mats can be cut to fit the wound area [44]. The wound was evaluated for granulation tissue, skin appendage, wound closure, and collagen metabolism. While the nanofibrous layer provides a platform to remove wound exudates, the GelMA/LA hydrogel layer provides a moist environment for the wound. The moist environment, the fibrous matrix, and the sustained release of EGF continuously stimulate the proliferation and differentiation of keratinocytes and fibroblasts improving the rate of wound closure [39]. Moreover, due to this warm and moist wound environment, partial re-epithelization was noticed on day 7 in treated groups. After 21 days, the bilayer adhesive scaffold achieves more percentage of wound closure than the control group (untreated), the group treated with nanofibrous mat, and the one treated with GelMA/LA hydrogel. LA enhanced tissue adhesive and haemostatic properties of the scaffolds containing EGF for full-thickness skin repair. As silicate nanoplatelets are negatively charged scaffolds that show a haemostatic activity, and the sustained release of EGF has also been demonstrated showing a dose-dependent reduction of blood clotting time. The bilayer scaffold allows a sustained drug release, and by mimicking the skin's natural microenvironment, full-thickness wound healing is accomplished in vivo [39].

GelMA was again mixed with LA and a poly (3,4-ethyl enedioxythiophene):poly (styrenesulfonate) (PEDOT:PSS) solution was then added to create an aqueous mixture of GelMA/LA/PEDOT:PSS (GLP) [45]. The GLP was then vigorously sheared to yield an air-in-water emulsion. Polymerization of GPL resulted in a biocompatible macroporous nano-enabled hydrogel sealant (Fig. 5A). The sealant dressing demonstrates adjustable pore size, good electrical conductivity attributed to PEDOT:PSS, robust mechanical properties, and wet-adhesiveness as well as cell adhesion and proliferation. The sealant adapts to wound sites, presents a haemostatic effect in vivo, and firmly adheres to biological tissue. The sealant with 1% LA was chosen to evaluate wound healing efficiency in rat skin. SD rats were the animal model used for in vivo wound healing evaluation of the GPL sealants in full-thickness cutaneous wounds. LA was used to enhance prepolymer viscosity, accelerate blood clotting, and facilitate platelet aggregation, by activating coagulation factors due to the negative surface charge of LA (Fig. 5B) [45]. According to photographs of wounds on days 3, 7, and 14, by day 3, the GPL sealant-treated animals had the highest wound closure. In contrast to other groups, after 14 days, these wounds were almost completely closed (Fig. 5C). The highest percentage of wound contraction was detected for GPL sealant being almost complete after 14 days (Fig. 5D) revealing signs of collagen regular deposition and a new thick epidermis (Fig. 5E) connected to the dermis. The inflammatory reaction was not as noticeable in the GPL-treated group. This novel dressing proved to have haemostatic, adhesive, and mechanical properties to realize a proper wound-healing process [45].

LA is mainly used due to its haemostatic inherent properties. Blood clotting, platelet aggregation, and activation of coagulant factors were also described [46–48]. LA also enhances tissue adhesiveness, speed of wound closure, regeneration, and re-epithelization of biological tissue [46, 49, 50]. LA nanocomposites were able to provide a moist environment essential for proper wound healing. When applied to form gels, LA alters their swelling properties and enhances cohesive strength, viscosity, and shear thinning behavior with good gelation time [45]. LA is also used to stabilize growth factors by providing their controlled release [51].

Montmorrilonite

MMT is a natural nanoclay with a 2:1 hydrous aluminasilicate structure that belongs to the smectite group. This planar-layered structure defines a high specific surface area, and therefore, excellent absorption capability is associated with MMT [20]. MMT has attracted great interest for several therapeutic applications in wound management due to bacterial adsorption, cleansing and skin protection, and haemostatic properties [52]. This nanoclay can be modified due to its high cation exchange capability and the ability to form thick layers by exfoliation to enhance its distinctive wound healing capabilities [17].

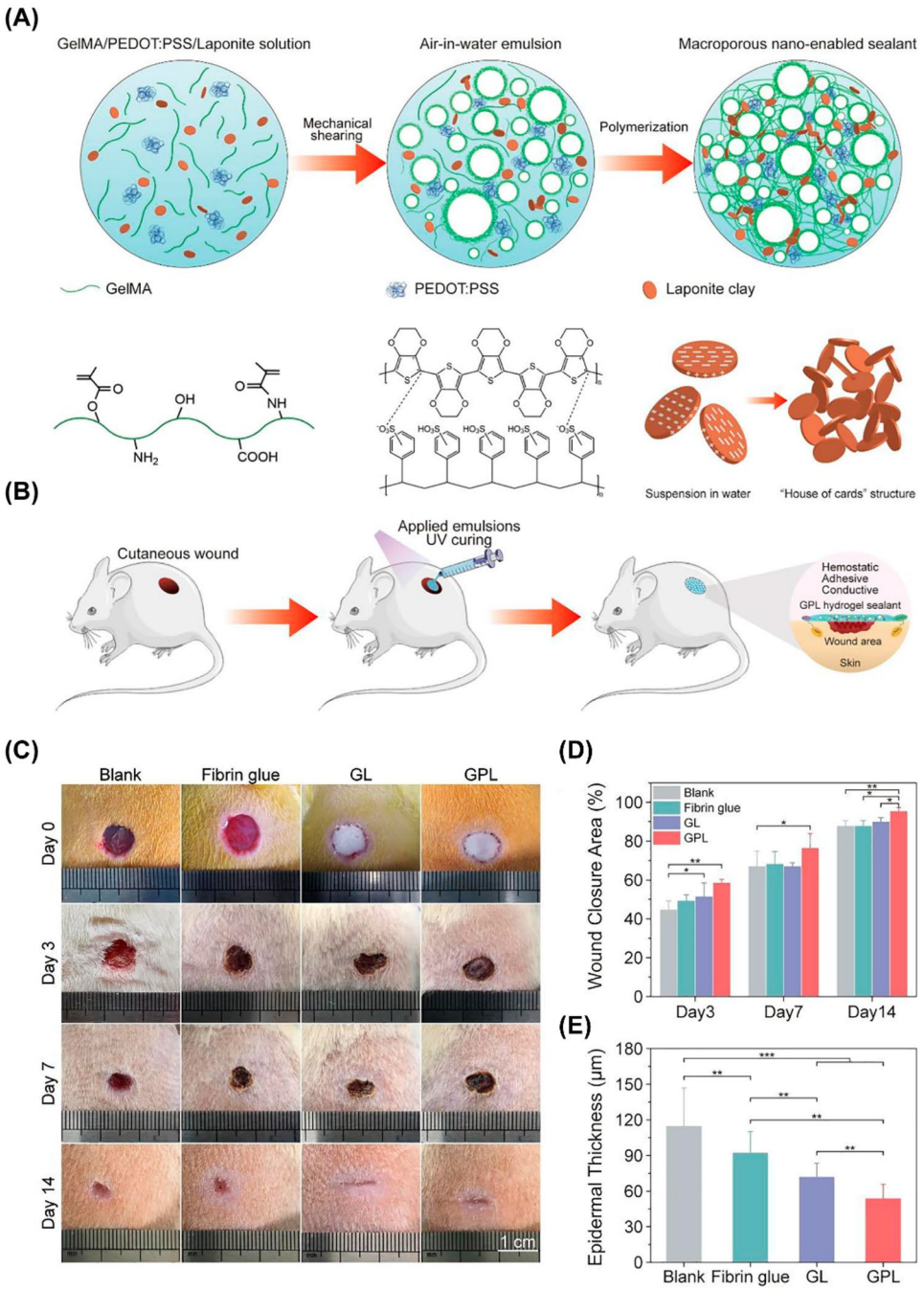

Fig. 5 The preparation of macroporous nano-enabled sealants based on air-in-water emulsions. **A** Schematic illustration of the sealant fabrication process. **B** Applying the sealants for wound healing application in an animal model. In vivo wound healing evaluation of the GPL sealants applied in SD rats with full-thickness cutaneous wounds. **C** Photographs of wounds at the 3, 7, and 14 days treated with fibrin glue, gelatin methacryloyl/aponite (GL), and aqueous gelatin methacryloyl/poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate)-laponite (GPL) emulsion sealants, with an untreated wound serving as the control group. Quantitative data of the **D** wound closure area and **E** epidermal thickness in each group (adapted from [45]). Abbreviations: GL, gel/laponite; GPL, gel/laponite/poly(3,4-ethylenedioxythiophene): poly (styrenesulfonate)

Figure 6A represents a schematic illustration of drug-loaded montmorrilonite and Fig. 6B shows drug-loaded montmorillonite (MMT) nanohybrid hydrogel for promoting wound healing.

MMT was used to lower chlorhexidine (CLX) cytotoxicity which impairs its use in wound management despite its good antimicrobial activity [53]. The characterization of the CLX intercalation suggests that CLX forms a monolayer in the interlayer space of MMT and that can also be adsorbed to MMT negatively charged surface.

Two procedures were carried out to prepare chitosan/MMT/CLX films. The first procedure was carried out to evaluate if CLX is maintained in the interlayer space of MMT, and therefore, chitosan was later added to the intercalation product MMT-CLX. In this film, the expansion of the interlayer distance of MMT is assigned to the occupation of the interlayer space for both chitosan and CLX. In a different procedure, CLX, MMT-Na and chitosan were dispersed at the same time. For this film, although intercalation of both chitosan and CLX in the interlayer space

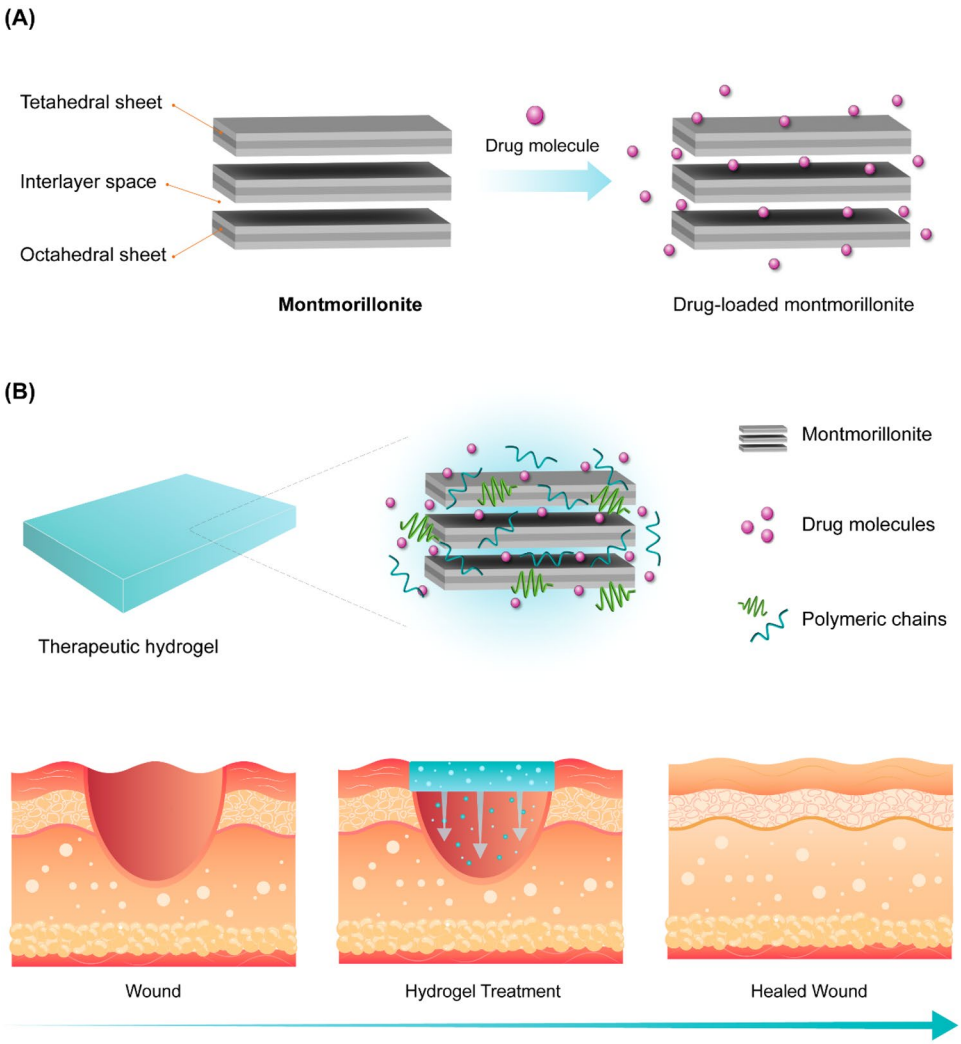

Fig. 6 Montmorillonite for wound healing applications. **A** Schematic representation of the preparation of drug-loaded montmorillonite (MMT) via electrostatic interactions. **B** Possible components of drug-loaded montmorillonite (MMT) nanohybrid hydrogel for promoting wound healing

cannot be excluded, the X-Ray diffractogram shows a pattern like the one of film containing only MMT-Na and chitosan where the polymer chains are intercalated between MMT layers. CLX was released faster from the film where CLX was loaded free reaching 40% of drug release after an initial 30-min burst effect. From chitosan/MMT-Na/CLX film, CLX was released more slowly but in the film in which CLX was first loaded in MMT, the release was even slower, and it only began after a lag time of 30 min. MMT does slow CLX release and it can retard its diffusion through chitosan. All films showed antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermis* but were less effective against *Pseudomonas aeruginosa* and *Candida Albicans*. When CLX is first loaded into MMT, the resulting films present a non-statistically significant decrease in antimicrobial activity. Films with only chitosan showed low antimicrobial activity but films with chitosan and MMT-Na show no antimicrobial activity suggesting that intercalation of chitosan into MMT reduces chitosan availability. Films with MMT and CLX previously intercalated or not showed good antibiofilm activity. Only the MMT/CLX/chitosan film (1%CLX) showed no significant antibiofilm activity against *S. aureus*.

Cytotoxicity was evaluated in human keratinocyte (NCTC2544) cell lines and human dermal fibroblast cells (HuDe). All films with 5% CLX were cytotoxic. Regarding fibroblasts, the film containing CLX interlayered in MMT resulted in less toxicity than films containing free CLX or MMT-Na and CLX. Films containing 1% CLX and MMT (CLX-MMT or MMT-Na and CLX) did not show toxicity possibly due to the slow and modified release of CLX. This proved that films based on MMT and chitosan loaded with CLX can be potentially used as a wound dressing as CLX release can be localized and prolonged; concentrations can be reduced maintaining antibiofilm and antimicrobial activities with improved cytotoxicity [53].

Two other studies were conducted with norfloxacin (NF) and MMT. This fluoroquinolone has antimicrobial activity against a broad spectrum of aerobic bacteria [54, 55]. In the first study, the application of MMT and norfloxacin to form

a powder was accomplished. The intercalation of NF into MMT occurs by isotherm adsorption in one single process as protonated NF interacts with negatively charged active sites of MMT [54]. In the second study, the MMT-NF nanocomposite was encapsulated in three different types of scaffolds that are based on polysaccharides [55]. One type with only chitosan, another with chitosan and chondroitin sodium sulfate and the third one with chitosan and hyaluronic acid. In addition, all polymeric blends contain pullulan and citric acid as a crosslinking agent and were made by electrospinning in a one-step process. In contrast, the three types of scaffolds were also prepared with NF-loaded free [55]. The presence of NF in MMT was confirmed and disclosed great stability. The adsorption method caused an expansion of MMT by forming a homogeneous monolayer of NF in an amorphous state between MMT interlayer spaces [54].

Physical and chemical characterization of scaffolds evidenced that scaffolds loaded with MMT-NF nanocomposite present an irregular surface with ribbons more evident as more MMT-NF is loaded. In fact, the lamellar structure of MMT was identified in the broadened parts of all three types of scaffolds. Silicon content of MMT-NF scaffolds was also reported and was coherent with MMT-NF concentration. Without MMT, fibers are homogeneous structures with 500 nm diameter but when added, MMT gives surface roughness and doubles fibers' diameter probably due to the interaction between MMT and the biopolymer matrix [55]. Drug release of MMT-NF nanocomposite was tested with liquid chromatography coupled to diode-array ultraviolet detector method showing advantages to controlling the release profile of NF highlighting that without MMT, 100% of the drug is released in 2 h but when added, MMT can release NF for 48 h [54]. When NF release properties were studied in saline solution, independent of biopolymer composition, scaffolds loaded with the nanocomposite reached plateau values at a percentage of drug release lower than the one presented by scaffolds loaded with the free drug. The release profiles of NF (loaded in MMT) were higher when scaffolds were exposed to lysozyme degradation but show no relevant differences when the composition of scaffolds and drug loading are taken into concern. Without glycosaminoglycans, chitosan scaffolds loaded with NF as free drug showed a high degree of degradation presenting no visible nanofibrous structure (Fig. 7A). This suggests that the presence of MMT allowed the slowing loss of nanofibrous structure which is confirmed by scanning electron microphotographs, where MMT appears covered in part by lysozyme particles tied to the polymer matrix (Fig. 7C) [55]. Probably, MMT interacts with chitosan limiting lysozyme degradation activity and conferring a higher enzymatic resistance to scaffolds enhanced when chondroitin and hyaluronic acid are added. Mechanical properties of scaffolds were tested in

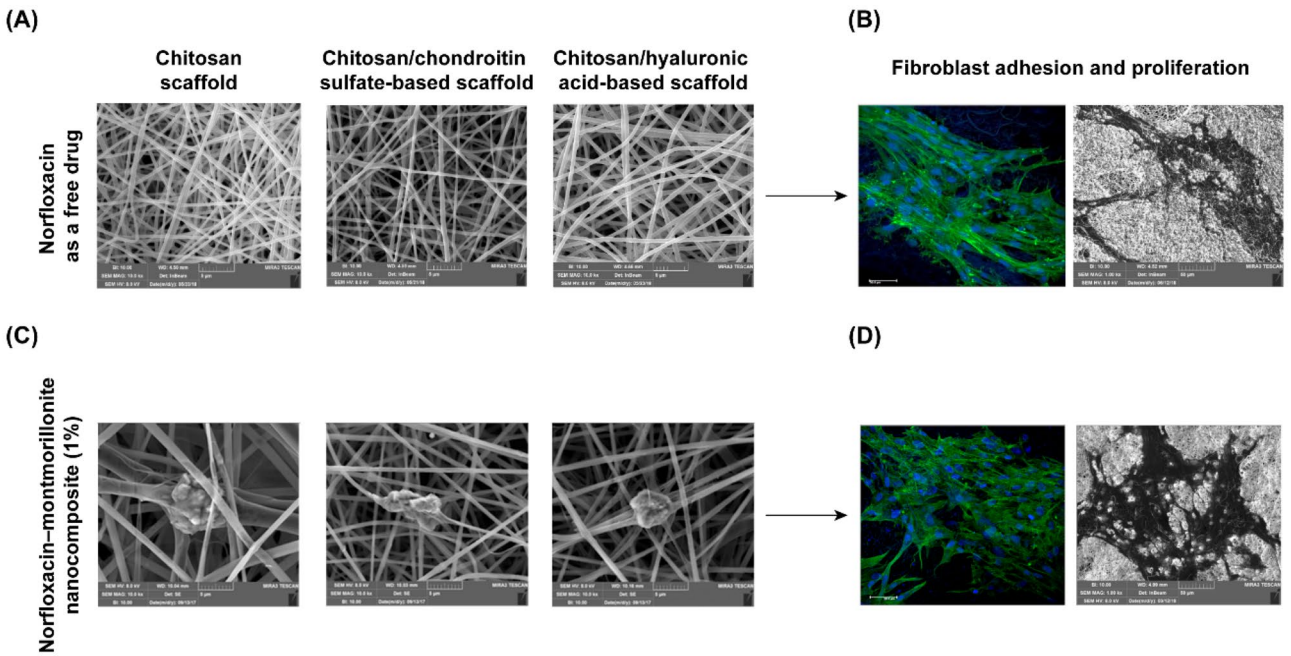

Fig. 7 Scanning electron microscopy (SEM) image of chitosan-based, chitosan/chondroitin sulfate-based, and chitosan/hyaluronic acid-based scaffolds loaded with norfloxacin as a free drug (1% norfloxacin) (**A**), or in norfloxacin-montmorillonite nanocomposite (**C**). Confocal laser scanning microscopy (CLSM) and SEM microphotographs of fibroblasts grown for 6 days chitosan/chondroitin sulfate–based scaffolds loaded with norfloxacin as a free drug (1% norfloxacin) (**B**) and or in norfloxacin-montmorillonite nanocomposite (**D**) (adapted from [55]). Abbreviations: MMT, montmorrilonite; NF, norfloxacin

dry and wet conditions. Regarding fibroblast adhesion and proliferation, confocal laser scanning microscopy (CLSM) and SEM microphotographs were used to study their grown for 6 days in chitosan/chondroitin sulfate-based scaffolds loaded with norfloxacin as a free drug (1% norfloxacin) (Fig. 7B) or in norfloxacin-montmorillonite nanocomposite (Fig. 7D). Results are in agreement with the literature, considering the highest fibroblast proliferation and biocompatibility observed in the norfloxacin-montmorillonite nanocomposite [55]. The dry state is necessary for the maintenance of integrity through application. Hydration works as if scaffolds were applied on the lesion site. In both states, the introduction of MMT caused more deformability and less elasticity making the scaffold less resistant.

In vitro cytotoxicity was tested using fibroblasts and although the nanocomposite does lower the optical density, the difference is not significant because tests were assessed with concentrations higher than the concentration needed for antimicrobial activity against *P. aeruginosa* and *S. aureus*, and thereby, MMT/NF nanocomposite was characterized by acceptable biocompatibility in vitro [54].

NF/MMT scaffolds also increase cytocompatibility of norfloxacin-loaded free. By measuring the microbiocidal effect, it was possible to conclude that MMT/NF nanocomposite substantially increases antimicrobial action of NF probably by increasing the contact area of NF with bacteria. Using confocal laser scanning microscopy and scanning electron microscopy, fibroblasts grow for 6 days onto scaffolds containing glycosaminoglycans and when loaded with MMT-NF nanocomposite showing the preservation of its filaments and its fusiform structure. On the contrary, when norfloxacin is loaded as a free drug, fibroblasts form aggregates and do not appear homogeneous [55].

While many studies rely only on single-target bacteria, the development of a double-treatment nanomaterial can be considered an option to manage a mixed-infected wound [56]. A powder with the antibiotic 5-fluorocytosine (5-FC) and copper ions in the interlayer space of MMT nanosheets involved by quaternized chitosan (QCH) was conceived [56]. The nanocomposite indicates a significant activity against *Candida albicans* that improves as the concentration of 5-FCCu increases. Antimicrobial properties were studied in vitro against *Escherichia coli* and *S. aureus* showing great improvement when QCH is added even at low concentrations of MMT/5-FCCu. The coordinated action of 5-FC with antibacterial metal copper ions gives not only an initial high concentration of 5-FCCu but also a sustained release and a microbial inhibition long-acting. Moreover, the deposition of QCH on MMT surface leads to an acceleration of wound healing and promising biocompatibility. The surface of the nanocomposite is not homogeneous as a QCH layer is deposited on the surface of MMT. 5-FC appears to be intercalated between the interlayer spaces of MMT and coordinated with Cu. The resulting nanocomposite QCH/MMT/5-FCCu proved to have good thermal stability and a high loading capacity. In the presence of QCH, drug release from MMT is slower, maintaining an initial burst release to establish a therapeutic dose and an afterward continuous sustained release.

A mice wound model was used to evaluate the inhibition of inflammation and cytotoxicity in vivo. Wounds were infected with *S. aureus*. The control group presented severe infection and inflammation after 3 and 5 days. Inflammation decreased even in wounds treated with only MMT/5-FCCu. The wounds subjected to QCS/MMT/5-FCCu, for the same number of days, were treated with no signs of inflammation cells (neutrophil granulocyte) (Fig. 8A). Wound recovery is illustrated in Fig. 8B. It was observed that the QCS/MMT/5-FCCu group line was higher than the others over time. The novel nanocomposite exhibits antimicrobial activity in vitro and in vivo and epidermal regeneration so it can be indicated as a dressing for infected wound management. This group has shown the lowest percentage of bacteria growth (Fig. 8C). Cell toxicity of QCS/MMT/5-FCCu was also studied in L929 fibroblasts. MTT assay showed a high decrease in cell viability for 5-FC and 5-FCCu groups. As for the groups with MMT, with or without QCH, cell viability was maintained above 90% regardless of concentration which means MMT is responsible for lower cytotoxicity. Regarding histological studies of skin tissues, it was observed that QCS/MMT/5-FCCu-treated group had almost no inflammatory reaction (Fig. 8D). Other studies were carried out and demonstrated that the nanomaterial would not cause damage to living organisms [56].

A silver/MMT/bacterial cellulose (BC) film was prepared intended to prevent microbial infections and help with tissue regeneration using silver and MMT [57]. By ion change, it is possible to incorporate silver in MMT using a solution of silver nitrate. The resulting suspension was dried to create a potential scaffold with membranes of BC. Water uptake (%), expressed in terms of relative mass increase after rehydration, is higher for BC-MMT, followed by BC-MMT-Ag and then native BC. The antimicrobial activity of the modified membrane was tested in *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* (gram-negative) cultures based on a live-dead assay. The biocidal activity is attributed to silver look upon membranes with BC and BC-MMT that did not impair cell growth. BC-Ag membranes showed a bigger inhibition halo as its release is faster without MMT reflecting the importance of the nanoclay to lessen the side effects of silver. BC-MMT-Ag membrane showed cytotoxicity depending on silver concentrations. To determine the cytotoxicity of the nanocomposites, biocompatibility in vitro was demonstrated using L929 fibroblast cells. In total, 1–25% BC-MMT-Ag showed to be nontoxic for 24 h, and no morphological cell changes were observed for 12 h. Cytotoxicity studies in vivo are desirable [57].

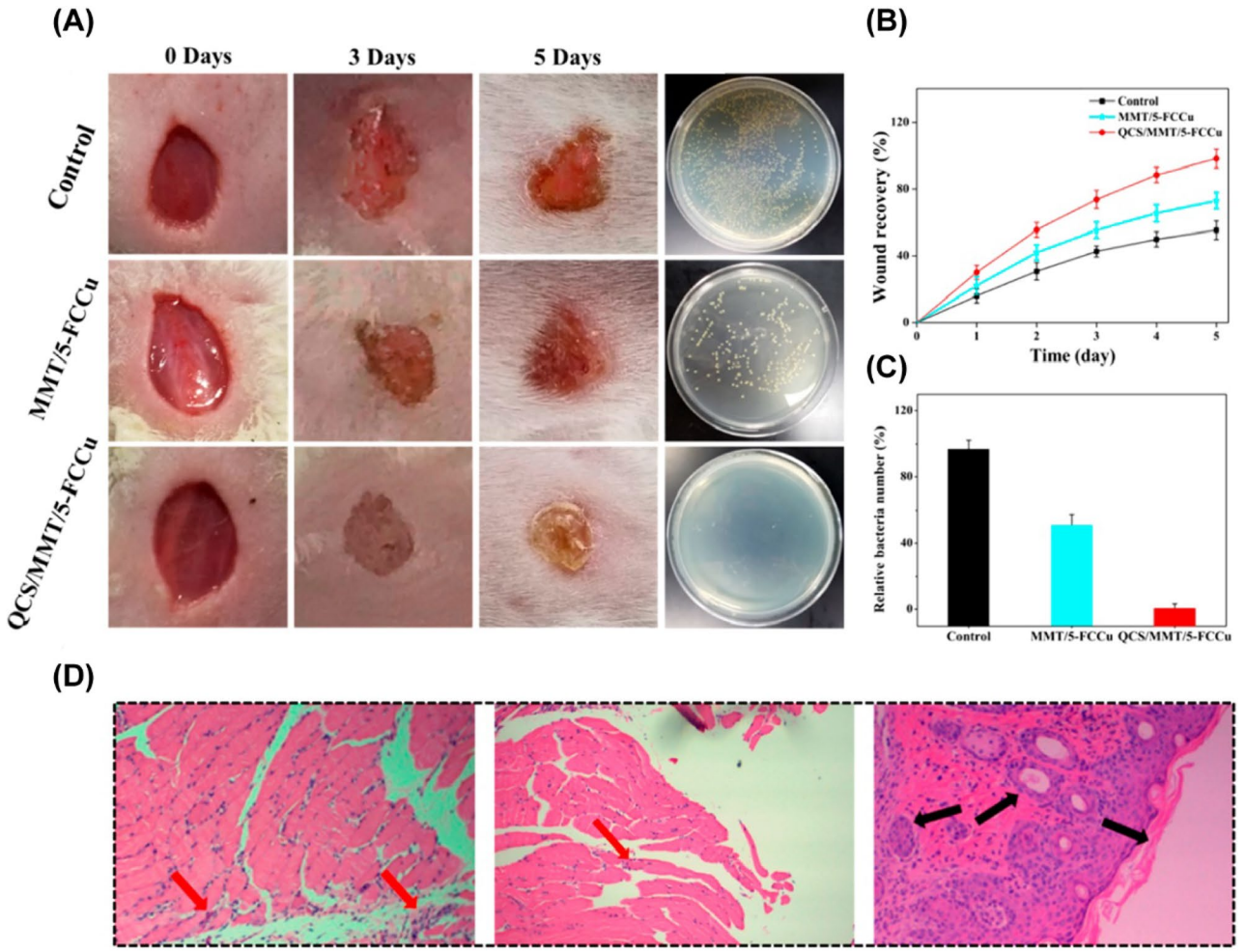

Fig. 8 **A** In vivo assessment of materials with antibacterial effects in mice wound model. All of the wounds were injected with 20 μL of 1×10^6 colony-forming units $mL.^{-1}$ *Staphylococcus aureus* to build the infection model. **B** Wound recovery of the control, montmorillonite/antibiotic 5-fluorocytosine (MMT/5-FCCu), and quaternized chitosan (QCS)/MMT/5-FCCu groups. **C** Bacteria separated from wound tissue after treatment were cultured on agar plates and statistical numbers of the surviving bacteria in the wound tissue were measured. **D** Histological studies of skin tissue slices by hematoxylin and eosin (H&E) staining with different groups (control, MMT/5-FCCu, and QCS/MMT/5-FCCu from left to right, respectively) (adapted from [56])

MMT is mainly used due to its excellent absorption capacity and high cation exchange capability. MMT proved to slow drug release and drug diffusion throw polymer matrix. MMT can also increase the water uptake of biopolymers. The nanoclay proved to down cytotoxicity and preserve fibroblast form. MMT seems to be responsible for the loss of homogeneity and nanofibrous structure of scaffolds resulting in deformability, less resistance, and elasticity.

Non-planar hydrous phyllosilicates

PAL and SEP are both non-planar hydrous phyllosilicates with a unique shape of flexible elongated needles. SEP and PAL are clay minerals of a 2:1 structure with an octahedral sheet layer into two tetrahedral sheets forming blocks and tunnels alternated. Due to the parallel channels, these nanoclays have a high specific surface area, a high viscosity, and a high absorption capacity [23]. Also, this group of clays proved to be stable at different pH conditions. For SEP, the external surface channels and silanol groups enhance its adsorption properties that can also be loaded into tunnels coordinated with Mg cation. PAL has a more rigid crystal structure than SEP which impairs its rheological behavior [23]. Although, when compared to MMT or LAP (smectite group), PAL and SEP present a lower cation exchange capacity and do not show properties of lamellar expansion.

SEP and PAL showed edema inhibition and migration of neutrophils. SEP was also shown to have higher anti-inflammatory activity compared to HNT, as a higher edema inhibition has been demonstrated [58].

In comparison to the previously described nanoclays, the latest publications with SEP and PAL will be highlighted next, realizing the possible contribution of non-planar hydrous phyllosilicates to improve wound healing management.

Sepiolite

Sepiolite (SEP) is a natural silicate characterized by a microfibrous morphology with a structure of two tetrahedral sheets (continuous) and one octahedral sheet (discontinuous). This structure and the interruptions formed in the octahedral sheet constitute a promising way for molecule encapsulation. In addition, their absorption abilities and stability under different pH conditions constitute a great advantage to a possible topical application [20, 59].

Another bionanocomposite based on carboxymethylcellulose (CMC) polysaccharide and zein protein was designed with MMT and SEP, both clays as support for topical drug delivery. Neomycin was used in the following study. To create SEP-neomycin hybrids, different concentrations of neomycin were added to SEP suspension to measure the adsorption capacity of SEP. To prepare the MMT-neomycin hybrid, an MMT/neomycin suspension was maintained under magnetic stirring for 1, 2, and 3 days to assess the influence of ionic exchange reaction time. Then the required amount of hybrid was added to a CMC solution, and finally, zein protein was added to create a CMC-zein bionanocomposite film with MMT or Sep nanoclay. For MMT, it is expected to have neomycin into the layers, and for SEP, the positively charged neomycin tends to penetrate in the clay tunnels neutralizing the negative charges of SEP and thereby creating a stronger interaction that makes drug release difficult. For MMT studies of neomycin, adsorption indicates that the adsorption of neomycin increases the interlayer distance of MMT although its content does not change in time suggesting a reorganization of neomycin within the interlayer space of MMT forming a more stable conformation. The study of the isotherm adsorption curve at 25 °C shows that neomycin molecules' adsorption on SEP occurs progressively increasing the zeta potential of SEP-neomycin material when compared to neat SEP. The difference between the internalization of the drug is pointed out as the major aspect of the marked difference between the inhibitory activity for hybrid SEP-neomycin and for MMT-neomycin, which is more effective with MMT — neomycin. Although both nanocomposite biofilms showed a delayed drug release when compared with a biopolymer matrix where the drug was incorporated directly. As more zein protein is incorporated in the biofilm and depending on the type of hybrid, more antimicrobial activity is present. The total zein protein present in the bionanocomposite imparts the capacity to resist humidity environments and consequently delays drug release. Both systems are shown to have the ability for sustained long-time drug release downing side effects and making the topical administration more comfortable [60].

PVA, soy protein isolate (SPI), and SEP were used to produce nanofiber mats where ketoprofen was loaded [61]. Three types of mats were prepared. The first one was without SEP (PVA/SPI/ketoprofen), the second one with SEP (PVA/SPI/ketoprofen/SEP), and a third one where ketoprofen was preloaded in SEP (PVA/SPI/Keto/Sep) (Fig. 9A). For the third mat, it is expected for the ketoprofen to be distributed inside SEP channels, on the SEP surface and free in the polymer matrix as the mixing process can remove some drugs from the clay. SEP increased the mechanical strength of the mats when SPI could no longer do it. A fiber diameter analysis was developed through scanning electron microscopy (SEM) images of the three nanofiber mats were compared in Fig. 9B. Mat viscosity and conductivity increased and shear thinning behavior was enhanced by adding SEP and making them possibly longer-lasting and easier to handle. The PVA/SPI/SEP-ketoprofen mat presented the lowest pore size and the highest surface area. The release profile of the three mats in the first 16 h is illustrated in Fig. 9C. All three mats showed an initial burst release. Depending on whether the drug was preloaded into sepiolite or not, PVA/SPI/Keto/SEP showed the highest release rate and the greatest percentage of cumulative release. On the contrary, PVA/SPI/SEP-ketoprofen showed the lowest release rate and the smallest percentage of cumulative release demonstrating that the incorporation of SEP clay could either accelerate or delay the drug release. This was attributed to the disposition of the drug in the polymer matrix and due to its interaction with SEP. When directly added, SEP accelerates drug release by its volume exclusion effect [61].

In PVA/SPI/Keto/Sep, the drug is in greater concentration in the polymer matrix and mainly distributed closer to the surface due to the presence of SEP. When preloaded, ketoprofen is encapsulated on SEP channels and coated on the outer surface. Preloaded ketoprofen resulted in the slowest drug release and improved thermal stability of ketoprofen. The long-term release profiles for the three mats are illustrated in Fig. 9D. Those electrospun nanofiber mats demonstrated the potential to be used as controlled-release drug delivery vehicles [61]. Other studies where SEP and drug molecules are incorporated separately but also preloaded would help to trace an interesting release profile curve.

Biopolymer-based nanocomposite hydrogel was prepared with CMC, polyvinylpyrrolidone, agar, 5-fluorouracil, and SEP to study the FU release and thermal properties [62]. Polymer–clay nanocomposite hydrogel films were prepared with different nanoclay concentrations. Increasing SEP concentration increases the amorphous nature and decreases the semi-crystalline properties of the systems probably due to

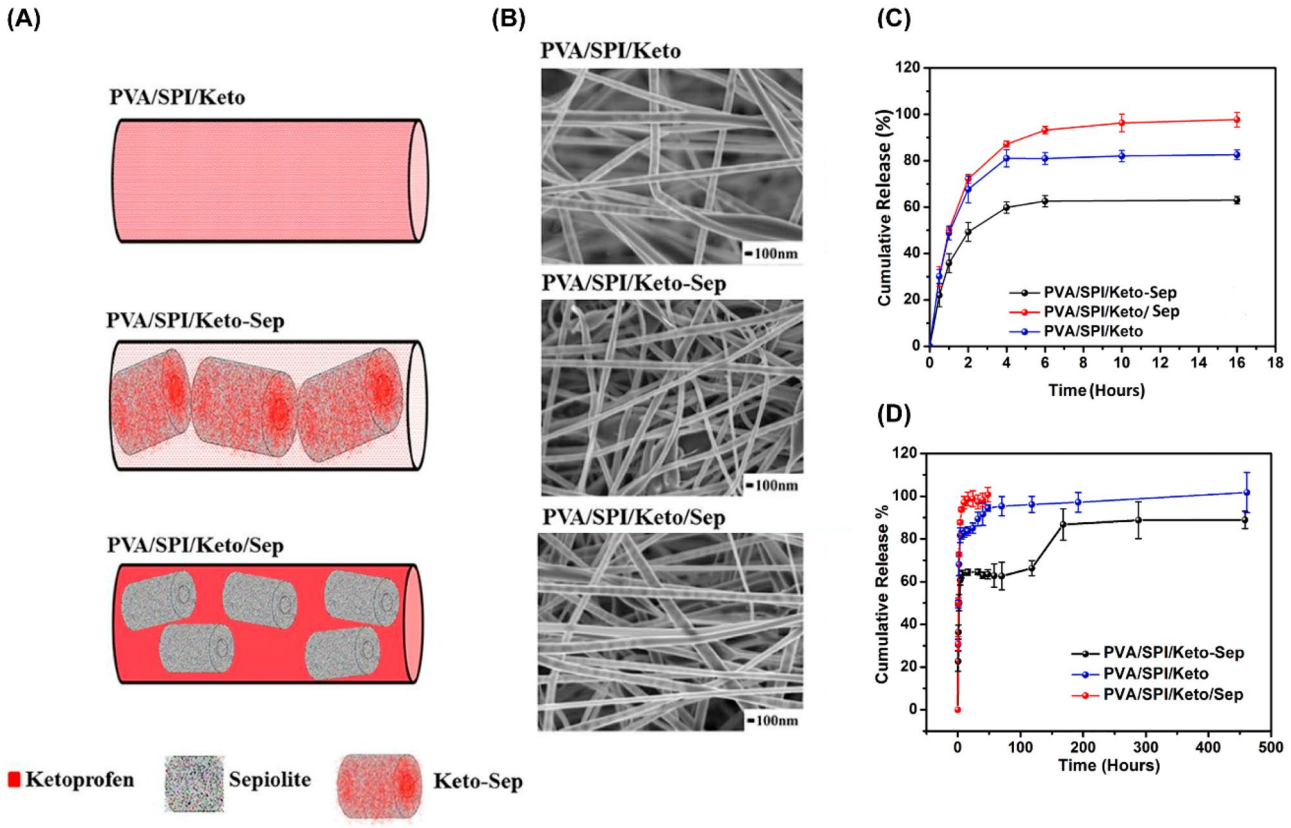

Fig. 9 **A** Schematic diagrams of electrospun nanofibers showing the distribution of the sepiolite and ketoprofen in poly (vinyl alcohol)/soy protein isolate/ketoprofen (PVA/SPI/Keto), PVA/SPI/Keto-sepiolite (Sep), and PVA/SPI/Keto/Sep nanofibers. **B** Scanning electron microscopy (SEM) images of electrospun nanofiber mats of PVA/SPI/Keto, PVA/SPI/Keto- Sep, and PVA/SPI/Keto/Sep nanofibers. **C** Release profile of the three mats in the first 16 h. **D** Release profile of the three mats up to 460 h (adapted from [61]). Abbreviations: Keto, ketoprofen; PVA, polyvinyl alcohol; SPI, soy protei isolate; SEP, sepiolite

the interaction of CMC and polyvinylpyrrolidone with SEP layers. FU is also reported to be intercalated in SEP layers influencing SEP reinforcement of the crystal structure of the hydrogel. The physical crosslinking network between SEP and polymers is properly connected proving SEP reinforcement of the nanocomposite [62].

SEP reinforcement of the physical hydrogel network depends on its concentrations affecting surface morphology. SEP incorporations increase the melting temperature of the polymer and reduce crystallinity. The immersion time and SEP increase display an important role in swelling properties, increasing as both increase. Hydrophilicity is pointed out as the major aspect influencing the percentage of swelling rate. SEP highest concentration incorporated revealed high water adsorption capacity, significant improvement in mechanical strength, reasonable flexibility, and fracture resistance properties. The interaction between SEP and the functional polymer enhances porosity increasing penetration of the drug and giving an advantage during hydrogel formation. But this point also enhances controlled drug release as pH is increased. SEP increases mechanical properties in the studied concentrations although further reinforcement beyond that can have the opposite effect probably due to the uneven distribution of the clay in high concentrations. This pH-sensitive nanocomposite hydrogel proved to be suitable for prolonged and sustained drug release in tissue engineering and other biological applications regarding its good chemical stability, mechanical properties, ease of the process and non-toxic nature. Cytotoxicity was tested in fibroblasts presenting an excellent percentage of cell viability after 72 h. Moreover, cell morphology in the live dead assay also indicates good cytocompatibility for hydrogel films. These films can be used to provide physical support, control drug release, and improve the survivability of normal cells [62].

Palygorskite

Tenci et al. compared the ability of three different clays to load carvacrol (CVR), a phenol with antioxidant, antifungal, and antimicrobial properties. PAL presented the highest loading capacity when compared to MMT and HNT. Films with the three different nanoclays were prepared

by shear mixing technique and by adsorption in the saturated atmosphere for 48 h at different temperatures. In both techniques, PAL presented the highest loading capacity, which was related to the structure of nanoclays, as PAL is a fibrous clay, HNT is a tubular clay and MMT is a lamellar clay as illustrated in Fig. 10 [63].

Furthermore, all clays increased the percentage of loading capacity by increasing temperature, but HNT was able to adsorb CVR only at temperatures above 40 °C. The percentage of loading capacity for the three clays prepared by shear mixing was greater than that determined for the second technique. PAL as a thermostable clay can reduce CVR volatility by forming a stable film when temperatures are lower than 80 °C. The film also showed to be stable for 1 month at 20 °C in a desiccator without CVR evaporation. When loaded in the nanoclays, CVR did not show cytotoxicity and even maintained its antioxidant activity. In addition, the nanocomposite was able to decrease minimum inhibitory concentration and minimum bactericidal concentration when compared to pure carvacrol. The higher antimicrobial activity was attributed to the reduced volatility and the prolonged release of CVR when loaded in PAL. Films prepared with PVA, vinylpyrrolidone, chitosan glutamate, sericin, PAL, and glycerol were optimized to present optimal properties. Upon hydration, a viscoelastic gel able to slow CVR release is formed suggesting these films are promising candidates for the treatment of chronic skin ulcers [63].

Chitosan, glycyrrhizic acid, ZnO, and PAL were used to create a wound dressing film able to inhibit wound infections during tissue damage [64]. First, a ZnO was easily loaded on PAL blocks. The nanocomposite revealed excellent antibacterial activity, and thereby, it was used to reinforce chitosan-based films. The antimicrobial activity of the nanocomposite is mainly attributed to ZnO although PAL structure may also cause mechanical damage to the bacterial cell membrane. The nanocomposite was found not to be uniformly distributed in the polymer matrix which can negatively influence its mechanical properties although it enhances the thermal stability of the films. The presence of PAL in the wound dressing improved its mechanical properties by increasing tensile strength and decreasing elongation at break to a favorable value, optimizing ZnO/PAL. The presence of PAL increases the percentage of water content and swelling degree and decreases water solubility. Water content relates to empty spaces of the nanocomposite. The higher the swelling degree, the better films adsorb wound exudates, and the lower the water solubility, the better for biodegradable film applications. The synergistic effect of chitosan, glycyrrhizic acid, and ZnO/PAL results in remarkable antimicrobial activity worth for wound healing applications although ZnO/PAL did not meaningfully affect the antimicrobial activity of the film. Films proved to be nonhemolytic and therefore hemocompatible. More in vivo studies are mandatory to observe wound area changes, antibacterial performance, and biocompatibility [64].

Figure 11 shows the applicability of palygorskite in wound healing applications, evidencing its structure (Fig. 11A), the development of a hydrogel consisting of palygorskite loaded with bioactive molecules (Fig. 11B) and the synergistic effects on wound healing resulting from the administration of a hydrogel consisting of fibrous palygorskite and bioactive molecules (Fig. 11C).

On the hole, the structure of non-planar hydrous phyllosilicates impairs its loading capacity creating stronger drug interactions when compared with the previously described nanoclays. In consequence, drug release is also strongly influenced by PAL and SEP morphology. When the drug is first loaded in PAL or SEP drug release is slow and it can be too slow but, when directly incorporated together with

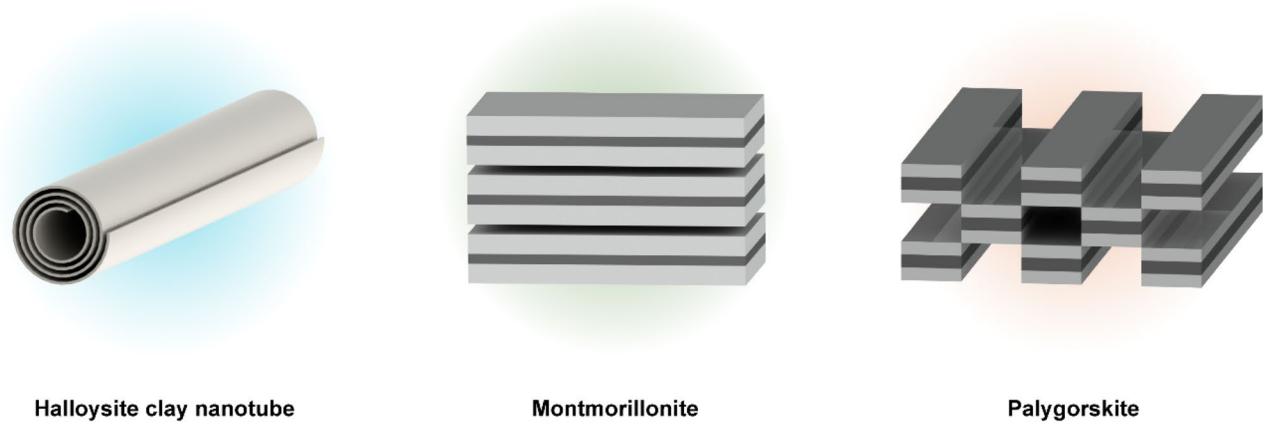

Fig. 10 Schematic illustration of the different morphologies of planar hydrous phyllosilicates (i.e., halloysite clay nanotubes and montmorillonite) and non-planar hydrous phyllosilicates (i.e. palygorskite)

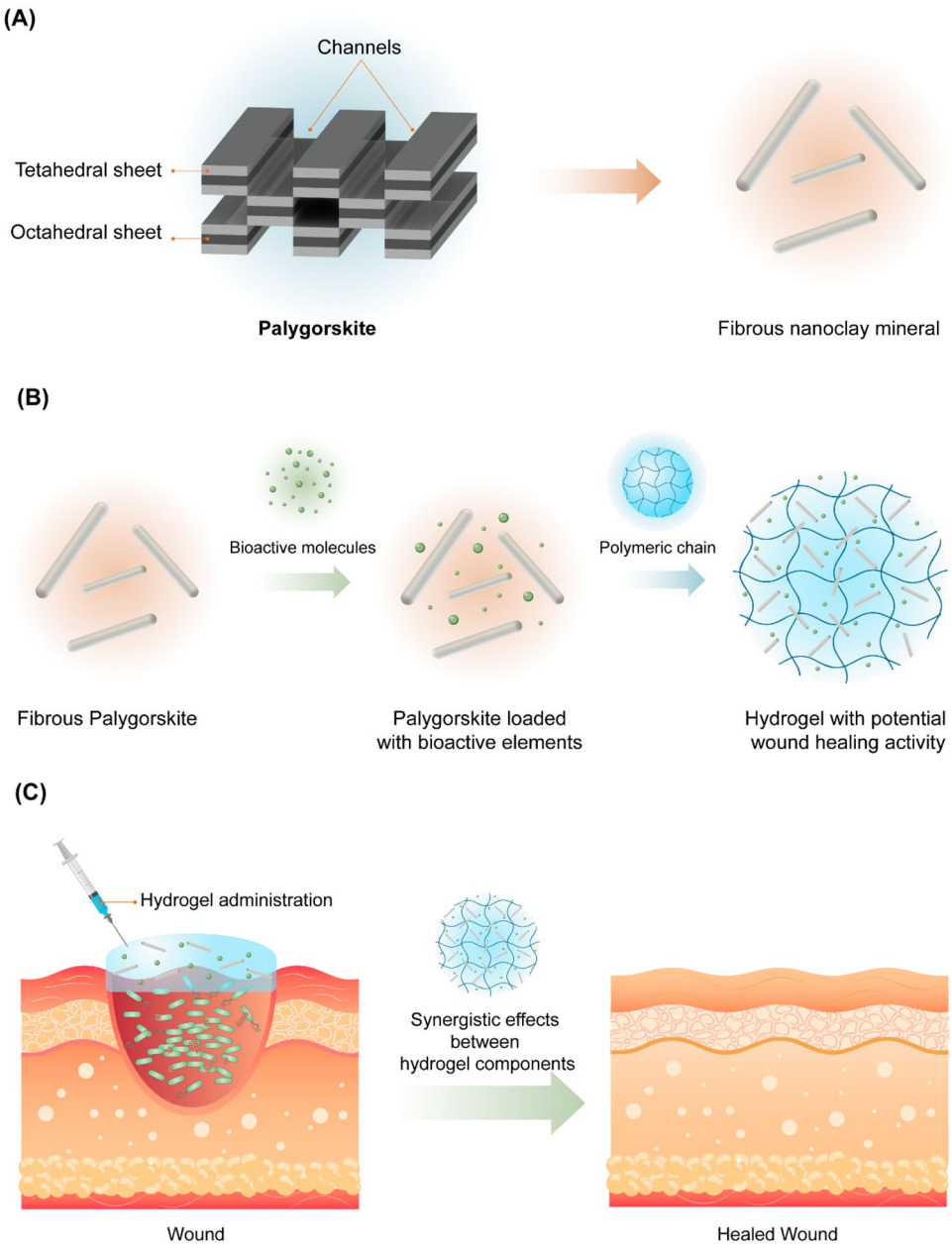

Fig. 11 Palygorskite for wound healing applications. **A** Schematic illustration of palygorskite structure. **B** Representation of the fabrication process of a hydrogel containing palygorskite loaded with bioactive molecules. **C** Administration of an inorganic hydrogel formulated with bioactive molecules and fibrous palygorskite used to treat skin affections

drugs into a polymer matrix, non-planar clays can cause a volume expansion effect making drug release faster. PAL as a thermostable clay can reduce the volatility of drugs but more studies on its antimicrobial mechanism are needed.

Regarding its technological properties, SEP and PAL enhance the mechanical strength, viscosity, conductivity, and shear-thinning behavior of films/mats. They can also increase water content and swelling degree and reduce water solubility of scaffolds although with PAL, a decrease in elongation at break was observed. SEP can enhance the water-resistance of biopolymer films by reinforcing the polymer matrix of various composites like CMC, chitosan, and agar nanocomposite films. The high surface area of non-planar clays improves the compact packing of the biopolymer matrix with the nanoclays, decreasing its sensitivity to water.

Relevant features regarding the application of nanoclays for wound healing management are summarized in Table 1.

Toxicity

The toxicity of nanomaterials is one of the major concerns for human and environmental health as they are enforced daily [65]. Toxicity of nanomaterials includes, but is not

Table 1 Main features of nanoclays as delivery systems for wound healing management with an emphasis on their properties, polymer matrix interaction, drug interaction and physicochemical characteristics

Nanoclays	Wound healing properties	Polymer matrix interaction	Drug interaction	Physicochemical characteristics	References
Halloysite	• Control of inflammation phase • ↑ Cell proliferation • ↑ Angiogenesis • ↑ Skin re-epithelization • Lesion size reduction over time	• ↑ Tensile strength • ↑ Thermal stability • ↑ Photostability	• ↓ Drug release	• Electrically neutral rolled structure • pH-dependent charged edges • Hydrophobic character	[7, 20, 33, 34]
Laponite	• Adhesiveness • Better regeneration • Haemostatic capacity • Speed wound closure • Re-epithelization • Minimal scarring • Hair growed • Blood clotting • ↑ Moist environment	• ↑ Cohesive strength • ↑ Hardness • Good gelation time • ↑ Viscosity • Allows sterilization • Alters swelling properties • ↑ Shear thinning behavior	• Retains growth factors • ↑ Stability of growth factors and its controlled release	• ↑ Specific area • Positively or negatively charged surfaces	[20, 25, 39–41, 45]
Montmorrilonite	• Preservation of fibroblast forms	• ↑ Water uptake • ↑ Deformability • ↓ Elasticity • ↓ Resistance • ↓ Homogeneously • Loss of nanofibrous structures of nanocomposite	• ↓ Cytotoxicity • Double drug loading • ↓ Drug release • ↓ Diffusion through the polymer matrix • ↑ Drug controlled release	• High contact area • Absorption capacity • Cation exchange capability	[17, 20, 53–57]
Sepiolite + Palygorskite	• Edema inhibition • Migration of neutrophils	• ↑ Mechanical strength • ↑ Viscosity • ↑ Conductivity • ↑ Shear thinning behavior • ↑ Swelling degree • ↓ Water solubility • ↓ Elongation at break	• Structure strongly influences loading capacity • Strong drug interaction	• ↑ Specific surface area • Absorption capacity • ↓ Cation exchange capability** • No lamellar expansion capacity	[20, 23, 60–64]

*Some are only possible regarding nanoclays functionalization

**Compared to semectite group

only limited to, dermal toxicity, pulmonary toxicity, liver toxicity, carcinogenic toxicity, and cytotoxicity [66], despite skin cell lines are not as used as liver and intestine human cell lines to study nanoclays toxicity [67].

Regarding in vitro and in vivo toxicity studies of nanoclays, the results can seem different from the expected. Many times, in vitro results, are not promising but for animal and human data, toxicity showed to be lower [67]. Regarding cosmetic application, Food and Drug Administration (FDA) recommends subchronic toxicity and repeated dose evaluations of the nanomaterial, as well as skin sensitization, photoirradiation, mutagenicity, and genotoxicity testing [68]. However, most reported studies based on nanomaterials intended for wound healing applications are based on in vitro studies, and more knowledge is needed for in vivo models that report variable results. Different nanoclay structures, concentrations and the type of modifier used can influence the toxicity of nanoclays [20]. To the best of our knowledge, in the last years, the toxicity mechanisms of nanoclays have been barely investigated. Controlling drug release and drug diffusion nanoclays can impair the cytotoxicity of some drugs allowing their therapeutic use. Also, nanoclays do not impair cell viability, and cell morphology and possibly enhance cell migration and proliferation. For example, in the previously described QCH/MMT/5-FCCu nanocomposite, an in vivo toxicity evaluation was performed in addition to cell toxicity tests. For the routine blood tests, the results are in line with normal indicators. Vital organs were treated with hematoxylin and eosin, demonstrating that the nanomaterial would not cause damage to living organisms [56].

Table 2 Examples of patented systems with nanoclays for application in wound management

Patented systems	Composition	Main functions	References
Essential oil loaded mucoadhesive nanocomposite delivery system for gastrointestinal system	Chitosan and nanoclay spheres	• Avoid the toxic effects of synthetic drugs • Provide a controlled release of the essential oil, with antibacterial activity • Regenerate damaged gastric tissue • Prevent the bacteria adhesion to gastric mucosa	[72]
Hydrogel nanocomposite wound dressing and a method of synthesizing the same	MMT, peptide chain, reinforcing agent and gentamicine	• Release of gentamicine during the microbial infection • Prevent of long-term effects of antibiotics • A replacement or new application is not necessary	[73]
Nanocomposite hyaluronic acid-clay based hydrogels	Hyaluronic acid and laponite	• Accelerated healing of wounds and burns by promoting tissue formation • High porosity and suitable pore size of the nanocomposite facilitate cell seeding and diffusion	[74]

HNT have been used for centuries, and thereby, it is considered safe and have proved high biocompatibility in vivo. Although SEP and PAL morphology can be an advantage, it also raises concerns about toxicity [20]. HNT toxicological response seems to strongly depend on clay concentration [30]. The research for biological and chemical interactions of nanoparticles and their kinetics is crucial to comprehend nanoparticle accumulation and its possible harmful effects on the environment and water [66].

Regarding their ecotoxicity, chemical modification of clays, use of surfactants, and non-toxic reagents have been described as a way to overcome the environmental contaminants produced during their preparation [69]. Moreover, nanotherapies can only evolve with the establishment of international criteria on toxicology and biocompatibility [70]. The development of novel nanoclay-based approaches for wound healing management demands physical–chemical stability analyses, particularly in vivo and in vitro cytotoxicity assays, permeability tests, and analyses of the interaction with solar radiation, in order to clarify its efficiency and therapeutic safety [23].

Patented systems

Patented systems of nanoclays with wound management applications have been reported in the literature. In this regard, medicinals and medical devices have been used and developed to overcome the drawbacks of conventional treatments. Compounds with medicinal properties can also be included in medical devices, designed and produced for healthcare use and not only for nutritional or medicinal purposes [71].

It is well known that infection of wound sites is many times the cause of impairing a normal wound healing process. Therefore, antibiotics are as well many times used to design novel approaches for wound management. While many studies rely only on single-target bacteria, the development of a mixed treatment nanomaterial that includes, for example, gram-positive and gram-negative antibacterial properties and or antifungal properties, has been a concern for those who intend new wound healing applications using nanomaterials. In this regard, due to the lack of results of antibiotic in H. pylori infection, an essential oil was loaded into gastroretentive chitosan/nanoclay spheres. The main purpose of this local treatment was to avoid the toxic effects of synthetic drugs, provide a controlled release of the essential oil, with antibacterial activity, regenerate damaged gastric tissue, and prevent the bacteria adhesion to gastric mucosa [72]. Hydrogels have been also reported as a suitable choice for wound management applications. A hydrogel nanocomposite was developed to improve wound dressing materials by promoting a slow release of a drug and avoiding possible bacterial infection. The composition included MMT, a peptide chain, a reinforcing agent to provide a slow drug release and gentamicine. The main advantages of this nanocomposite are the ability to release gentamicine during microbial infection, avoiding the long-term effects of antibiotics, and the fact that it does not replacement or new application [73]. In addition, other patented system consisted of the crosslinking of hyaluronic acid and a nanoclay, which resulted in stable homogeneous rigid hydrogels. Hyaluronic acid-clay-based hydrogels were described as biodegradable and viscoelastic, and the preferred clay was laponite. The main objective of this system was to promote accelerated healing of wounds and burns, by enhancing tissue engineering. While hyaluronic acid has a major role in granulation tissue formation, nanoclays provide the necessary high porosity and suitable pore size to facilitate cell seeding and diffusion [74].

Table 2 shows some examples of patented systems with nanoclays for wound management.

Conclusions and future perspectives

The unique characteristics of nanoclays make them perfect candidates to enhance current wound healing approaches or to be considered for novel nano-based materials intended for wound healing management. The ability to create a nanomaterial with therapeutic properties and supply all healing phases is an increasingly important, at the same time, challenge. The previously described investigations are examples of how different types of nanoclays can be used to create powders, films, scaffolds, and gels intended to enhance wound healing management. Nanoclays are described as modifiers of the rheological behavior of biopolymers, and as modifiers of drug release but they are also used due to their intrinsic possible therapeutic properties. Oher terapeutic molecules should be considered and swelling tests are required, for example, to facilitate granulation tissue removal or to enhance the formation of connective tissue and contraction of the wound area. Despite the great interest of the pharmaceutical and cosmetic industries in nanoclays in various applications, little information is out on the effects of this type of material on the human body, especially when they are applied to the skin. In vivo studies, cell culture, neuroprotective, and anti-inflammatory effects may be investigated to develop novel wound healing approaches. Although there are exciting and enormous potential and feasible applications for nanoclays, more than a few challenges stay unsolved. An intensive study on nanomaterials toxicity for humans and the environment is necessary, as well as nanomaterial toxicity after the end-of-life, namely for nanoclays. Active laws, regulation, and harmonization are also mandatory. An increase in industrial capacity and economic evaluation may also be necessary in order to understand the cost–benefit of using these new therapeutic strategies.

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Declarations

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