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CLAY MINERALS IN SKIN DRUG DELIVERY

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Abstract—Clays have played an important role in medicine since the dawn of mankind and are still applied widely as active ingredients and/or excipients in pharmaceutical formulations. Due to their outstanding properties of large retention capacity, swelling and rheological properties, and relative low cost, they have been used widely as advanced carriers for the efficient delivery of drugs by modifying their release (rate and/or time), increasing the stability of the drug, improving the dissolution profile of a drug, or enhancing their intestinal permeability. In addition, recent studies have shed new light on the potential of clay minerals in the nanomedicine field due to their biocompatibility, beneficial effects of clay nanoparticles on cellular adhesion, proliferation, and differentiation. Use as active ingredients and excipients are exerted via the oral and topical administration pathways. Skin drug delivery represents an attractive alternative to the oral route, providing local and/or systemic drug delivery. Due to their complex structures, however, most drugs penetrate the human skin only with difficulty. Enormous efforts have been invested, therefore, in developing advanced drug delivery systems able to overcome the skin barrier. Most strategies require the use of singular materials with new properties. In particular, and on the basis of their inherent properties, clay minerals are ideal candidates for the development of intelligent skin drug delivery systems. In this article, the properties of clay materials and their use in the skin-addressed pharmaceutical field are reviewed. A brief introduction of skin physiology and biopharmaceutical features of penetration by a drug through the skin layers is also included and is designed to shed light on the optimum properties of ideal nanosystems for advanced skin drug delivery. Special attention is devoted to the pharmacological functions of clays and their biomedical applications in pelotherapy, wound healing, regenerative medicine, antimicrobial, and dermocosmetics.

Keywords—Skin · Antimicrobials · Clay Minerals · Dermocosmetics · Pelotherapy · Regenerative Medicine · Skin Engineering · Wound Healing

INTRODUCTION

The aim of this review was to provide a summary of recent research on clay mineral uses in advanced skin drug delivery; a future perspective is included to discuss challenges and prospects.

Because of their healing properties and global accessibility, use of clay minerals in the therapy of skin pathologies goes back to prehistoric times and continues to play a crucial role in the design of skin-addressed drug delivery systems. Clay minerals are used in conventional medicinal products as excipients or actives (Cornejo et al. 1990; López-Galindo & Viseras 2004; Carretero et al. 2006; López-Galindo et al. 2011; Viseras et al. 2007) as well as advanced materials developed to modify drug delivery features (Aguzzi et al. 2007, 2016; Carazo et al. 2018; Sandri et al. 2016; Viseras et al. 2008, 2010, 2015).

Clay minerals were likely used in the very first prehistoric remedies, probably including geophagy and wound treatments. Clay minerals have continued to be essential ingredients in medicinal products during human history. In Europe, ancient western medicine used "Terra sigillata" (Λημνία Γη) or Stamped earth with "trade mark" denominations (terra Armenica, Terra Florentina, terra Hierosolymitanae, terra Hispanica, terra Lemnia, terra Portugallica, terra Silesiaca, among others) (Macgregor 2013; Mantle et al. 2001). Clay minerals were mentioned in at least half of the most important historical texts constituting the European *materia medica* since the "Hippocratic Corpus" (5th–4th century BC) (De Vos 2010). During the nineteenth century, the presence of clay "simples" in western medicine continued (Medicamentarius, 1866). In the first half of the twentieth century, the major Western pharmacopoeias included clay minerals in the substances used in medicinal products. Nowadays, the terms "Bentonite," "Magnesium trisilicate," "Magnesium aluminum silicate" (or "Aluminium Magnesium silicate"), and "Attapulgite" have their own monographs in the most important worldwide pharmacopeias (USP 41, 2018; BP, 2018; EP 5.0, 2015 (Pharmacopeia 2018; British Pharmacopoeia Commission 2018; Ministerio de Sanidad y Consumo 2015)).

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Clay minerals have been classically used in the elaboration of topical semisolid products as pastes, poultices, or liniments. Two examples, still in vogue, are "Calamine Lotion," indicated for treatment of skin irritations that include 25% w/w of "Bentonite magma" and "Titanium dioxide paste" formulated with 10% w/w of kaolin (USP 41, 2018; BP, 2018; EP 5.0, 2015 (Pharmacopeia, 2018; British Pharmacopoeia Commission 2018; Ministerio de Sanidad y Consumo 2015)).

Clay minerals are currently used mainly as excipients (any constituent of a medicinal product other than the active substance and the packaging material). Excipients represent the largest part of the medicines (up to 95%) and determine drug release and bioavailability. More than 1200 excipients from many origins (animal, vegetable, or mineral) are used in medicines. Clays account for ~5% of the global market for inorganic excipients. Most of the advances in pharmaceutical science and technology are related directly to inorganic excipients, the market for which should reach $433.7 million by 2020 (BCC Report 2016).

SKIN ANATOMY AND PHYSIOLOGY

In order to understand fully the design and development of skin pharmaceuticals, the structure, composition, and functions of human skin must be reviewed. The skin, which is considered the largest organ of the human body, is a multistratified structure (epidermis, dermis, and hypodermis) with essential functions as temperature control and barrier against physical, chemical, and thermal aggressions (Ng & Lau, 2015). The presence of appendages (hair follicles, sweat, and sebaceous glands) leads to several interesting properties. Human skin has an average surface area of 1.8 m^2 and constitutes a cellular layer, named dermis or true skin, sandwiched between the epidermis (outer layer and boundary with the exterior) and hypodermis (inner layer). The thickness of the epidermis varies between 0.05 mm on the eyelids to 1.55 mm on palms and soles. The epidermis is divided into basale, spinosum, granulosum, lucidum (only in palms and soles), and corneum strata. Continuous cell renewal in the stratum basale generates different cell types, mainly keratinocytes, melanocytes, and merkel cells (associated with terminal filaments of cutaneous nerves). Replication rates (normal full skin renewal requires ~28 days) increase during inflammation or injury. Keratinocytes move through the strata to reach the stratum corneum as corneocytes. The stratum spinosum contains a large concentration of keratin filaments appearing as a "spiny" area where Langerhans cells (antigen-presenting cells with an immunologic role) are also frequent. Langerhans cells and melanocytes are connected to adjacent cells by desmosomes in the same way keratinocytes are connected to one another. In the stratum granulosum the keratinocytes become flattened, lose their nuclei, and secrete their contents to form a lipid barrier. One of the most determining layers of the skin in terms of permeation by control drugs is the stratum corneum (SC). The SC is the hardest barrier of the skin, comprising rows of corneocytes (matured keratinocytes lacking nuclei and having elongated and flattened shapes) organized on a "brick and mortar" structure: corneocytes ("bricks") immersed in a lipid matrix ("mortar") (Prow et al. 2011). The space between adjacent corneocytes is occupied by a mesophase (lyotropic liquid crystal) formed by phospholipid bilayers and with the presence of proteins. The dermis layer is much thicker than the epidermis. Blood vessels, nerves, and various appendages (sweat glands, hair follicles, and sebaceous glands) are also found, providing nutritional and structural support to the epidermis. The hypodermis obeys the main functions of energy supply and thermal insulation.

ROUTES AND TARGETS ON, INTO, AND THROUGH SKIN DRUG DELIVERY

Delivery of drugs on/into/through the skin enables either local or systemic actions and improvement of poor biopharmaceutics profiles of drugs administered via other administration paths, and becomes a useful strategy in situations in which other administration routes are not possible or inadvisable (Aulton & Taylor 2017). With these backgrounds, the main goals of advanced skin drug delivery systems are improving drug biopharmaceutics and pharmacokinetics and obtaining targeted drug delivery based on interaction with skin appendages and skin lipids leading to a facilitated, sustained, and/or stimuli-induced release.

While all topical and transdermal compounds are applied to the skin, it is necessary to accentuate the fact that skin drug delivery can provide local (topical) or systemic (transdermal) therapeutic effects. The two principal routes of penetration are transappendageal (via the pores and shafts embracing sweat glands and hair follicles with their associated sebaceous glands) and transepidermal (diffusion through the stratum corneum). The transappendageal pathway is minor but preferred by ions and large polar molecules because the stratum corneum is not involved, whereas the transepidermal route is the dominant one and comprises two routes: transcellular, also known as intracellular, and intercellular (Fig. 1). Via the intracellular route, drug molecules repeatedly diffuse through corneocytes (keratin-filled; of an aqueous environment) and then partition into the intercellular lipid domains. This pathway is preferred by hydrophilic molecules. In contrast, the intercellular route implies that drug molecules diffuse via a tortuous route within the continuous lipid domain. Lipophilic molecules opt for this route. All drug molecules might use the three available routes; their physicochemical properties, however, determine the preferred pathway for finally reaching the capillaries at the epidermal–dermal junction.

QUALITY AND PERFORMANCE OF TOPICAL DRUG PRODUCTS

Topically administered drug products include those applied for local action (exert their actions on the stratum corneum and/or modulate the function of the epidermis

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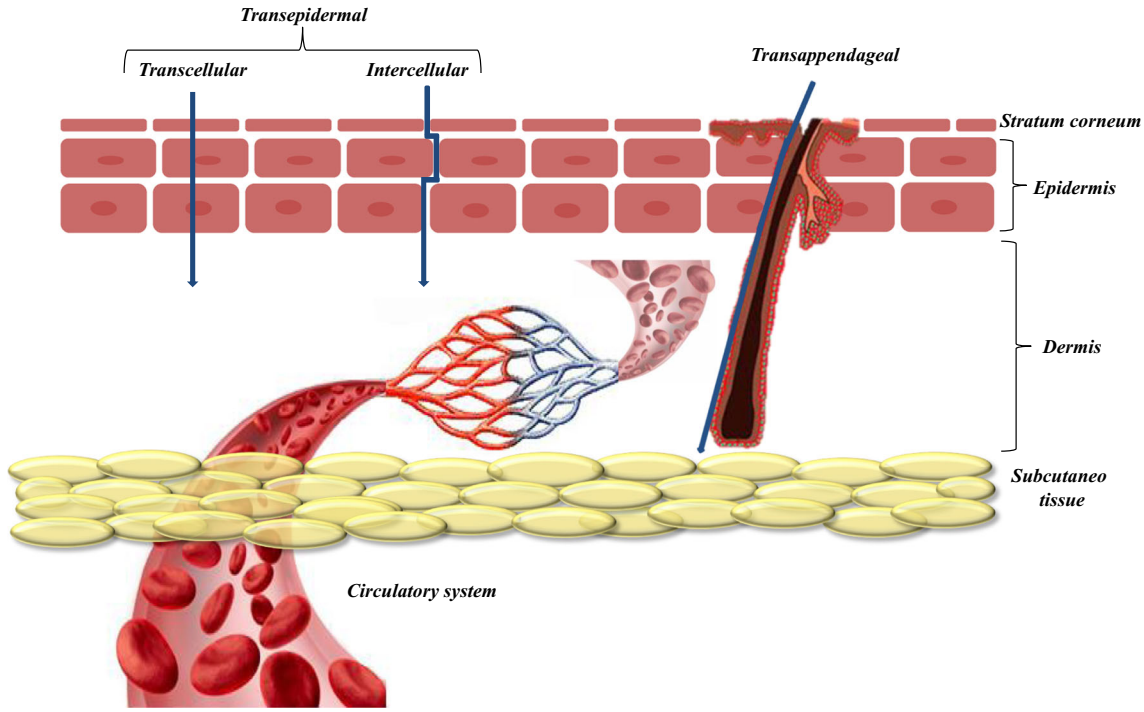

Fig. 1 Skin layers and diverse routes of penetration

and/or the dermis) and those applied for systemic effects (transdermal drug delivery systems). Forms of topical dosage include solutions (for which release testing is not indicated), suspensions, emulsions (e.g. lotions), semisolids (e.g. foams, ointments, pastes, creams, and gels), solids (e.g. powders), and sprays (e.g. aerosols).

Two categories of tests, product quality tests and product performance tests, are performed with topical drug products. Product quality tests are performed to assess attributes such as assay, identification, content uniformity, pH, and microbial limits.

Product performance tests are conducted to assess drug release from the finished dosage form.

Quality tests ensure safety and efficacy (ICH guidelines Q6A, www.ich.org) and include general tests such as identification, assay, content uniformity, impurities, pH, water content, microbial limits, antimicrobial preservative content, antioxidant preservative content, and sterility (in some cases), and specific tests such as viscosity and particle-size determinations (USP 41, 2018; BP, 2018; EP 5.0, 2015).

Performance tests are particularly interesting and are designed to measure drug release from the finished dosage form and detect changes in drug release related to formulation and manufacturing variables as well as storage and aging effects.

The intercellular route represents the principal mode of entry for permeation of both hydrophilic and lipophilic drugs. A drug that penetrates the SC can reach the dermis and enter the bloodstream by passive diffusion which is considered to be the rate-limiting step for the transdermal transport of drug molecules and depends on the physicochemical properties of the substance (Couto et al., 2014). This transport can be described by Fick's First Law of Diffusion (Eq. 1).

$$J = -D\delta C/\delta x \quad (1)$$

where J is the flux, C is the concentration of diffusing drug, x is the space coordinate, and D is the diffusion coefficient of the drug. Fick's Law assumes that diffusion occurs through an isotropic material, with the same structural and diffusional properties in all directions. Skin, however, is a heterogeneous structure so Fickian diffusion laws lead to approximations from transdermal drug delivery data.

In vitro protocols aim to mimic the in vivo situation. Several diffusion-type cell devices have been proposed as potential apparatus for drug release testing from topical drug products. However, only vertical diffusion cell systems (VDC, also named Franz Cells) have been normalized to measure drug release from semisolid dosage forms (<1724 > Semisolid Drug Products – Performance Tests. In the United States Pharmacopoeia and National Formulary USP 37–NF 32; the United States Pharmacopoeial Convention, Inc.: Rockville, Maryland, 2014, pp. 1273–84).

VDCs are made of a membrane (synthetic, animal, or human epidermis) separating two compartments. The drug in a vehicle is then applied to the uppermost membrane surface ('donor' solution). The other compartment contains a 'receptor' solution that provides sink conditions (near zero concentration) allowing a concentration gradient to exist between the donor and receptor phase, which is required for diffusion across the membrane.

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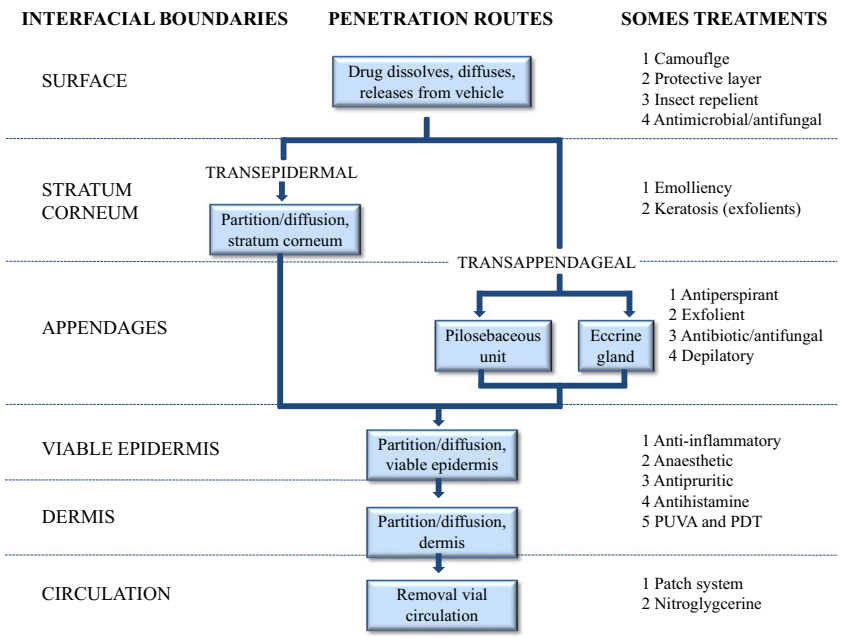

Fig. 2 Places and routes of skin treatments and penetration with examples of clay mineral functions (modified from Barry 1983)

CLAY MINERAL FUNCTIONS IN TOPICAL PRODUCTS

Inorganic excipients and in particular clay minerals may be used to overcome the traditional difficulties derived from topical drug administration and provide advanced functionalities (Carazo et al., 2018). Clay minerals have traditionally been included in topical products to improve technical properties and to increase the stability of emulsions and the viscosities of suspensions (Viseras et al. 2007). In addition, clay minerals show advanced functionalities that made them essential ingredients in anti-inflammatory, antibacterial, and wound-healing products. Clay minerals also provide specific functions in some dermocosmetics. Figure 2 attempts to clarify the different locations, pathways, and advanced functions of clay minerals in topical products. The scope and uses of clay minerals administered on/into/through the skin, are listed in Table 1 and explained further in the text below.

Anti-inflammatory

Pelotherapy is the topical administration of hot-muds known as peloids. Peloids are inorganic gels with optimal rheological and thermal properties composed of clay minerals and mineral-medicinal water aimed at treating arthro-rheumatic issues, bone-muscle traumatic damage, and dermatological pathologies. The optimum characteristics of a peloid depend on the required treatment and are related not only to the components of the peloid (mineromedicinal water and clay minerals) but also to the process of maturation (contact between the solid and water medium over a prolonged time period) (Veniale et al., 2007). Baschini and coworkers (2010) used natural peloids from Copahue: "clayey–sulphurous mud," a special type of therapeutic mud, the thermal properties of which are similar to those of other peloids but, due to the presence of sulfur, having special possibilities for the treatment of various pathologies. Portuguese clayey materials for medical hydrology applications were selected as candidates to be used in the preparation of tailored peloids (Rebelo et al., 2011). Regulations and quality criteria for suitable therapeutic applications of peloids were reviewed (Quintela et al., 2012). The influence of "maturation" conditions (time and agitation) on aggregation states, gel structure, and rheological behavior of peloids made with a pharmaceutical-grade smectite, a sepiolite, and a medicinal mineral water from a Spanish thermal spring (Graena, Granada, Spain) were investigated (Aguzzi et al., 2013). A concise definition and a classification of peloids as well as a complete glossary of all the mud-therapy terms were proposed in order to compile the different terminology used in the course of time (Gomes et al., 2013). Five clay samples used in various spa centers of the southern European/Mediterranean area were subjected to ethnopharmaceutic research aimed at ascertaining the compositional characteristics that enable the establishment of quality attributes and corresponding requirements for peloids, including identity, purity, richness, and safety (Sánchez-Espejo et al., 2014). The suitability of eleven clay samples (green and brown) from five Tunisian medina markets, traditionally used in home-made mud-packs, was fully investigated (Khiari et al., 2014). Maturation increased the release of cations from therapeutic muds but did not improve their thermal properties, indicating that maturation could explain the differential chemical effects associated with the use of therapeutic muds compared to other thermotherapeutic agents (Sánchez-Espejo et al., 2015). Therefore, the bacterial community in peloids changed mostly during the early stages of maturation and

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Table 1 Applications of clay minerals in skin drug delivery

Actions	Outcomes	References
Anti-inflammatory	Optimum characteristics of peloids	(Veniale et al., 2007)
	Advanced uses of "clayey–sulfurous muds"	(Baschini et al., 2010)
	Portuguese tailored peloids	(Rebelo et al., 2011)
	Regulations and quality criteria for suitable therapeutic applications of peloids	(Katti et al., 2008; Popryadukhin et al., 2012)
	The influence of 'maturation' conditions on peloid behavior	(Aguzzi et al., 2013)
	Definition, classification and glossary of 'mud-therapy' terms	(Gomes et al., 2013)
	Establishment of quality attributes and requirements for peloids	(Sánchez-Espejo et al., 2014)
	Suitability of Tunisian clay samples for pelotherapy	(Khiari et al., 2014)
	Maturation time increased the release of cations	Sánchez-Espejo et al., 2015)
	Bacterial community in peloids changed mostly during the early stages of maturation	(Otto & Haydel, 2013b; Otto et al., 2014, et al., 2016)
	Suitability of kaolinite-rich samples from Egypt for pelotherapy	(Awad et al., 2017)
	Optimum maturation time of peloids from Lanjaron spa (Granada, Spain)	(Fernández-González et al., 2017)
Wound healing and treatment of skin lesions	Revision of the role of clay minerals in the design of advanced wound dressings	(Sandri et al., 2016)
	Ability of clays to physically adsorb and remove bacterial cells, toxins, and debris from the wound	(Otto & Haydel, 2013a, 2013b)
	Epidermal growth factor immobilized on montmorillonite stimulate cell growth and migration	(Vaiana et al., 2011)
	Montmorillonite-chitosan nanocomposite with silver sulfadiazine: bacteriostatic and bactericidal properties	(Sandri et al., 2014)
	Solid state characterization of montmorilllonite-chitosan-silver sulfadiazine nanocomposite	(Aguzzi et al., 2014)
	Brazilian clay allowed greater formation of collagen fibers when tested in rat animal models	(Dário et al., 2014)
	Functionalized layered clays with aminoacids promoted fibroblast proliferation	(Ghadiri et al., 2014)
	Antibacterial activity of clay–ciprofloxacin composites	(Hamilton et al., 2014)
	Methyl cellulose–sodium alginate–montmorillonite bionanocomposite films	(Mishra et al., 2014)
	Polymers/clay minerals composite scaffolds used for skin tissue engineering	(Ninan et al., 2015)
	Montmorillonite-chitosan films loaded with clorhexidine for wound dressing	(Ambrogi et al., 2017)
	Silicate (tourmaline)/chitosan composite films for wound healing applications	(Zou et al., 2017)
	Chitosan oligosaccharide/halloysite nanocomposite with re-epithelialization activity	(Sandri et al., 2017)
	Carvacrol/clay hybrid for skin ulcer treatment	(Rangappa et al., 2017)
	Montmorillonite-betaine hydrochloride silver nitrate for burn wounds	(Rangappa et al., 2017)
	Poly(vinyl alcohol)/chitosan/honey/clay nanocomposite hydrogel as novel wound dressing	(Noori et al., 2018)
	Burn ointment including montmorillonite for tissue regeneration and skin growth	(Zhang et al., 2018)
Cell adhesion, proliferation and differentiation: skin engineering and regenerative medicine	Cell proliferation and adhesion properties of clay minerals	(Sandri et al., 2016)
	Clay nanoparticles on cellular adhesion, proliferation and differentiation	(Mousa et al., 2018)

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Table 1 (continued)

Actions	Outcomes	References
	Sepiolite-collagen complexes and fibroblast proliferation from explants	(Lizarbe et al., 1987; Olmo et al., 1987)
	Fibroblast attachment and spreading improved by montmorillonite and halloysite	(Kommireddy et al., 2005)
	The addition of montmorillonite to chitosan enhanced the adhesion of osteoblasts (a) and fibroblasts (b)	(Katti et al.. 2008; Popryadukhin et al., 2012)
	Biocompatible and biodegradable retinal scaffold based on montmorillonite/polyurethane nanocomposite	(Da Silva et al., 2013)
	Biocompatibility and cell proliferation of montmorillonite	(Sandri et al., 2014)
	Montmorillonite-silk fibroin nanocomposite for bone tissue formation	(Mieszawska et al., 2011)
	Composite scaffold based on chitosan–gelatin/nanohydroxyapatite–montmorillonite for tissue engineering	(Olad & Azhar, 2014)
	Montmorillonite -reinforced hydrogels, for the osteo-induction of osteoblast precursor cells	(Mauro et al., 2017)
	Chitosan-montmorillonite-triclosan loaded films compatibility towards human dermal fibroblasts	(Chen et al., 2018)
	Sr^{2+}/chitosan/montmorillonite composite with enhanced properties to be used in bone tissue engineering	(Demir et al., 2018)
	Halloysite nanotubes in tissue engineering	(Fakhrullin & Lvov, 2016)
	Alginate-halloysite composite scaffolds with enhanced fibroblasts attachment and proliferation	(Liu et al., 2015)
	Chitosan–gelatine–agarose doped halloysite scaffolds for tissue engineering	(Naumenko et al., 2016)
	A tri-component hydrogel, based on gellan gum, glycerol, and halloysite nanotubes for soft tissue engineering	(Bonifacio et al., 2017)
	Laponite cross-linked: adhesion and proliferation of HepG2, skin fibroblast, and human endothelial cells	(Haraguchi et al., 2006; Liu et al., 2012)
	Laponite- (a) and attapulgite- (b) poly(lactic-*co*-glycolic acid nanofibers: fibroblast adhesion and proliferation	(Wang et al., 2012; Wang et al., 2015)
	Carboxymethyl chitosan, gelatin, and Laponite biocomposite scaffold with potential use in bone tissue engineering	(Tao et al., 2017)
Natural antibacterial clays	Natural antibacterial clays able to kill human pathogens including antibiotic resistant strains	(Morrison et al., 2016)
	Clays used for the treatment of cutaneous bacterial infections	(Carretero, 2002; Ferrell, 2008; Williams et al., 2004, 2008, 2011; Friedlander et al., 2015)
	Biotic and abiotic actions responsible of antibacterial activity of natural clay minerals	(Otto & Haydel, 2013a)
	Biotic activity of the Jordan red clays	(Falkinham et al., 2009)
	Abiotic processes involved in antibacterial activity of clay minerals	(Otto & Haydel, 2013a)
	Antibacterial activity of some natural clays depend on microbicidal activities of desorbed metal ions	(Otto & Haydel, 2013b)
Antibiotics-loaded nanoclays	Natural zeolite exchanged with inorganic Zn^{2+}-erythromycin	(Cerri et al., 2004, 2006; Bonferoni et al., 2007)

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Table 1 (continued)

Actions	Outcomes	References
	Chlorhexidine/montmorillonite inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*	(Saha et al., 2014)
	Organo-modified bentonite for gentamicin topical application	(Iannuccelli et al., 2018)
	Topical ointment consisting on smectite, illite, rectorite to treat skin infections and skin diseases	(Tuba, 2018).
	Smectite-zwitterion-silver-analgesic system with antimicrobial and pain relieving properties has been patented	(Mukhopadhyay et al., 2018)
	Chitosan-montmorillonite nanocomposite film loaded with triclosan	(Chen et al., 2018)
General cosmetic uses	Clay minerals as dermatological active ingredients and excipients of dermocosmetic products	(López-Galindo et al., 2007; Viseras et al., 2007)
	Dermocosmetic applications of clays are related to their surface, physical and mechanical properties	(Moraes et al., 2017)
Sunscreens	Bentonite and hectorite as sunscreens	(Ghadiri et al., 2015; Mattioli et al., 2015)
	Mineral-based sunscreen containing activated clay with UVB/UVA protection	(Timothy et al., 2015)
	UV shielding of formulations with clay minerals	(Ijiri et al., 2015)
Other clay-based cosmetic products	A wide range of cosmetic products containing clay minerals have been designed and have their patent registered	(Viseras et al., 2007)
	Use of clays as emulgents or emulsifiers in cosmetic products	(Gabriel, 1973; Carter, 1940; Alexander, 1973; Sarfaraz, 2004)
	Optimization of a peel-off facial mask formulation containing green clay and aloe vera	(Beringhs et al., 2013)
	Dry shampoo comprising smectite, natural starches, and a natural oil absorbent	(Perfitt & Carimbocas, 2017)
	An emulsion of bio-minerals more stable, and requiring less energy and time to prepare	(Rochette et al., 2017)

reached stability after 2 months (Pesciaroli et al., 2016). The potentialities of seven selected kaolinite-rich samples from Egyptian Carboniferous sedimentary deposits were studied in order to evaluate their use in medicinal semisolid formulations as peloids focusing on the effect of particle geometry and kaolinite crystallite size (Awad et al., 2017). Peloids prepared with kaolin and saponite and medicinal mineral waters from Lanjarón Spa (Granada, Spain) were prepared and the optimum maturation time was investigated (Fernández-González et al., 2017).

Wound healing and treatment of skin lesions

The protective functions of the skin are compromised by injury. A wound can be defined as a defect or a break in the skin, resulting from mechanical or thermal damage, or the consequence of an underlying medical or physiological condition.

Wound-healing is a dynamic process in which the collaborative efforts of many different tissues and cell lines are required to recover the integrity of damaged tissue and replace lost tissue. It occurs in four stages: inflammation, migration, proliferation, and maturation. Healing is considered to be complete when the skin surface has reformed and re-established its tensile strength.

(1) Inflammation: The body's initial response to injury and involves both cellular and vascular responses resulting in vasodilation, increased capillary permeation, and stimulation of pain receptors. It occurs within a few minutes to 24 h of injury.
(2) Migration: growth factors in the wound exudate promote the growth and migration of epithelial cells, broblasts, and keratinocytes to the injured area to replace damaged and lost tissue. It lasts for 2–3 d.
(3) Proliferation: This involves the development of new tissue and occurs simultaneously or just after the migration phase. The network is important for developing the tensile strength of the skin. As proliferation continues,

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further epithelial-cell migration takes place across the wound, providing closure and visible wound contraction. During the proliferation stage, the wound is typically beefy red in colour and moist, but not exuding.

(4) Maturation: This final phase of wound healing (also called the 'remodeling phase') involves the diminution of the vasculature and enlargement of collagen fibers, which increase the tensile strength of the repair.

The need for regenerating injured skin rapidly and effectively has stimulated research into advanced therapies for wound care. Advanced wound dressings are designed to control the environment for wound healing. The role of clay minerals in the design of advanced wound dressings has been reviewed thoroughly (Sandri et al., 2016). Previous assessments were that not only was the use of clay minerals as nanocarriers of antimicrobial agents important for treating cutaneous bacterial infections, but also the ability of clays to physically adsorb and remove bacterial cells, toxins, and debris from the wound provided additional benefits aimed at wound healing (Otto & Haydel, 2013a, 2013b).

A functionalized montmorillonite with epidermal growth factor (EGF) demonstrated that EGF immobilized on montmorillonite can stimulate cell growth and migration in vitro, as is required in the proliferation step of the wound-healing process (Vaiana et al., 2011). A nanocomposite based on montmorillonite and chitosan loaded with silver sulfadiazine has been developed with the ability of not only protecting fibroblasts from the cytotoxic action of the drug but also improving its bacteriostatic and bactericidal properties, especially against *Pseudomonas aeruginosa*. This composite was assessed successfully for use as an advanced wound dressing (Sandri et al., 2014). A comprehensive and detailed study of the structure of the above-mentioned montmorilllonite-chitosan-silver sulfadiazine nanocomposite and the interactions involved was also reported by (Aguzzi et al., 2014)). Dário et al. (2014) observed that the treatment made with a Brazilian clay allowed greater formation of collagen fibers and consequent regeneration of the deep dermis and re-epithelialization and continuous formation of granulation tissue when tested on rat models. Functionalized layered clays with amino acids (arginine, lysine, and leucine) promoted fibroblast proliferation and can be applied potentially as wound dressings to promote the wound-healing process (Ghadiri et al., 2014). Antibacterial activity of clay–ciprofloxacin composites against the common skin bacteria *Staphylococcus epidermidis* and *Propionibacterium acnes* was demonstrated to be a potential delivery system for ciprofloxacin molecules aimed at designing novel wound dressings (Hamilton et al., 2014). A methyl cellulose–sodium alginate–montmorillonite bionanocomposite film possesses interesting wound-healing properties based on both its ability to inhibit the growth of *Enterococcus faecium* and *Pseudomonas aeruginosa* and its potential wound-closure activities (Mishra et al., 2014). A detailed review of the possibilities offered by various natural polymer/clay mineral composite scaffolds used for skin tissue engineering due to their enhanced wound-healing properties has been published (Ninan et al., 2015). The potential use of montmorillonite-chitosan films loaded with chlorhexidine as a potential wound-dressing material to prevent microbial colonization in wounds was assayed and all the prepared films showed good antimicrobial activity (Ambrogi et al., 2017). A silicate (tourmaline)/chitosan composite film for wound-healing applications was obtained with improved cell adhesion and proliferation, larger numbers of newly formed and mature blood vessels, as well as faster regeneration of dermis when tested on porcine burn wounds (Zou et al., 2017). A nanocomposite made of chitosan oligosaccharide/halloysite was prepared and characterized successfully using advanced electron microscopy techniques. It was biocompatible in vitro towards normal human dermal fibroblasts; the results of an in vitro wound-healing test showed that it enhanced in vitro cell proliferation (cells in S-phase) rather than simple fibroblast migration. In vivo wound-healing murine model results were in agreement with the previous in vitro results, providing an early re-epithelialization process and an advanced degree of hemostasis and angiogenesis (Sandri et al., 2017). Polymer films loaded with a carvacrol/clay hybrid for skin ulcer treatment were investigated. Different clays were considered: montmorrilonite, halloysite, and palygorskite; finally, a pharmaceutical-grade palygorskite was selected due to its ability to reduce carvacrol volatility and preservation of its antioxidant properties. The hybrid system provided improved antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli* and cytocompatibility towards human fibroblasts (Tenci et al., 2017). A new clay-based dermal patch system based on montmorillonite-betaine hydrochloride silver nitrate was evaluated for its potential use in first-degree burns and its anti-nociceptive activity (Rangappa et al., 2017). A novel responsive nanocomposite hydrogel based on poly(vinyl alcohol)/chitosan/honey/clay was designed and successfully evaluated for use as a novel wound dressing (Noori et al., 2018). The method of preparation of a burn ointment including montmorillonite aimed at promoting tissue regeneration and skin growth has been patented recently (Zhang et al., 2018).

Cell adhesion, proliferation, and differentiation: Skin engineering and regenerative medicine

Adhesion and proliferation of cells on biomaterials are crucial points in tissue engineering and biotechnology. Studies endeavoring to assess cell proliferation and adhesion to clay minerals are currently a matter of interest. The most studied clay minerals are Laponite, montmorillonite, cloisite, and halloysite (Sandri et al., 2016). Mousa and coworkers recently reviewed and compiled the beneficial effects of clay nanoparticles on cellular adhesion, proliferation, and differentiation. In addition, their attractive mechanical or rheological properties highlight the striking potential of clays for the creation and development of new bioactive scaffolds that may be used in skin-regenerative

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medicine (Mousa et al., 2018). Early studies on sepiolite-collagen complexes observed normal fibroblast proliferation and outgrowth of skin fibroblasts from explants (Lizarbe et al., 1987; Olmo et al., 1987). Fibroblast attachment and spreading was improved by montmorillonite and halloysite, and cells maintained their phenoptype (Kommireddy et al., 2005). The addition of montmorillonite to chitosan enhanced the adhesion of osteoblasts (Katti et al., 2008) and fibroblasts (Popryadukhin et al., 2012). Da Silva et al. (2013) developed a biocompatible and biodegradable retinal scaffold based on a montmorillonite/polyurethane nanocomposite. The biocompatibility and cell proliferation of montmorillonite have been evaluated in cultured normal human dermal fibroblasts (Sandri et al., 2014). A nanocomposite based on montmorillonite and silk fibroin has been developed as biomaterial for bone tissue formation (Mieszawska et al., 2011). A novel composite scaffold based on chitosan-gelatin/nanohydroxyapatite-montmorillonite with improved properties for use in tissue engineering applications was accurately prepared (Olad & Azhar, 2014). Montmorillonite-reinforced hydrogels, based on a peptidomimetic polyamidoamine carrying guanidine pendants were successfully used as substrates for the osteoinduction of osteoblast precursor cells (Mauro et al., 2017). Cell viability tests showed that newly developed chitosan-montmorillonite triclosan loaded films are compatible with human dermal fibroblasts (Chen et al., 2018). A strontium (Sr^{2+}) modified chitosan/montmorillonite composite scaffold has been developed recently with enhanced properties for use in bone tissue engineering (Demir et al., 2018). A full and comprehensive study of the features provided by halloysite nanotubes in tissue engineering was reported (Fakhrullin & Lvov, 2016). Alginate-halloysite composite scaffolds were prepared with enhanced fibroblast attachment and proliferation attributed to the increase in the surface roughness due to the incorporation of halloysite (Liu et al., 2015). Chitosan-gelatine-agarose doped halloysite scaffolds prepared were promising candidates for tissue engineering applications due to their in vitro and in vivo biocompatibility; their ability to enable neovascularization in newly formed connective tissue placed near the scaffold permitted the complete restoration of blood flow (Naumenko et al., 2016). A tri-component hydrogel, based on gellan gum, glycerol, and halloysite nanotubes, was designed for soft tissue engineering applications (Bonifacio et al., 2017). Cross-linked Laponite was able to maintain both the adhesion and proliferation of HepG2, skin fibroblast, and human umbilical vein endothelial in a manner strongly associated with the concentration of clay in the hydrogel (Haraguchi et al., 2006; Liu et al., 2012). Wang et al. (2012) used Laponite to develop poly(lactic-*co*-glycolic acid; PLGA) nanofibers with promoted fibroblast adhesion and proliferation. Similarly, attapulgite was included in PLGA nanofibers as a scaffolding material for osteogenic differentiation of stem cells (Wang et al., 2015). A biocomposite scaffold composed of carboxymethyl chitosan, gelatin, and laponite nanoparticles via freeze drying was prepared with potential use in bone tissue engineering (Tao et al., 2017).

Antibacterial Purposes

As mentioned above, skin acts as a physical barrier to avoid the invasion of external pathogens. The desiccated, nutrient-poor, acidic environment, contributes to the adversity that microorganisms must deal with to colonize human skin (Byrd, Belkaid, & Segre, 2018). Besides, topical antimicrobial therapy emerges as an attractive route for the treatment of infectious diseases due to the increased resistance to oral-administered systemic antimicrobial therapy (Lam et al., 2018).

Natural Antibacterial Clays Natural antibacterial clays when hydrated and applied topically are able to kill human pathogens, including the antibiotic-resistant strains proliferating worldwide. Only certain clays are bactericidal ((Morrison et al., 2016); Williams, 2019, this issue). Examples of clays and soils being used for the treatment of cutaneous bacterial infections are well known (Carretero, 2002; Ferrell, 2008; Friedlander et al., 2015; Williams et al., 2004; Williams et al., 2008; Williams et al., 2011). Antibacterial activity of natural clay minerals is the result of two types of actions: biotic and abiotic (Otto, 2014; Otto & Haydel, 2013a). A good example of biotic activity is the Jordan red clays, the antimicrobial activity of which is explained by the proliferation of bacteria naturally present within the clays and their concomitant production of antimicrobial compounds (Falkinham et al., 2009). Other biotic influences, including protozoan or mycobacterial predation, lytic microorganisms, and bacteriophages, may also be responsible for controlling bacterial growth. Additionally, abiotic processes are also responsible for the antimicrobial activity of some clays (Otto & Haydel, 2013a). Clays bind toxic metals to their surface due to their net negative charge and then release those exchangeable metal ions from the clay surface. The antibacterial activity of these natural clays thus depends on microbiocidal activities of the desorbed metal ions (Otto & Haydel, 2013b; Otto et al., 2014, 2016).

Antibiotics-loaded Nanoclays Clays act as topical delivery agents for various antimicrobial products. A natural zeolite was exchanged with inorganic Zn^{2+}. The micronized composite was subsequently charged with erythromycin to investigate the antimicrobial efficacy against erythromycin-resistant *Propionibacterium* strains. A 99.5% reduction in *P. acnes* viability was observed (Bonferoni et al., 2007; Cerri et al., 2004, 2006). Furthermore, chlorhexidine intercalated into a montmorillonite had the aim of being useful in skin pathologies due to its successful inhibition of the growth of a wide range of microorganisms including both *Staphylococcus aureus* and *Escherichia coli* (Saha et al., 2014). An organo-modified bentonite for gentamicin topical application was developed with sustained antibacterial activity and enhanced drug permeation rate (Iannuccelli et al., 2018). A topical ointment consisting of the clay minerals

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smectite, illite, and rectorite alone or in combination has been patented recently aimed at treating bacterially caused skin infections and skin diseases (Tuba, 2018). A multifunctional smectite-zwitterion-silver-analgesic system with both antimicrobial and pain relieving properties has been patented (Mukhopadhyay et al., 2018). A chitosan-montmorillonite nanocomposite film was loaded with the antibiotic triclosan and an intelligent pH responsive long-term release was obtained. High sterilization efficiency of the films was found against *Staphylococcus aureus, Escherichia coli*, and *Staphylococcus epidermidis*. Furthermore, cell biocompatibility measurements toward L929 fibroblasts and human lens epithelial cells showed no adverse effects of the multilayer film (Chen et al., 2018).

Dermocosmetics

Clay minerals are part of a large variety of dermocosmetic products, such as facial creams, sunscreen, for skin cleansing, shampoos, and makeup items (liquid and powder foundations, eye shadow, facial masks, lipsticks, etc.) either as dermatological active ingredients or as excipients (López-Galindo et al., 2007; Viseras et al., 2007).

Most of the important properties attributed to clays for dermocosmetic applications are related to their surface properties (surface area, cation exchange capacity, layer charge, among others); rheological properties (thixotrophy, rheopecty, viscosity, plasticity); and other physical and mechanical properties including particle size and shape, color, softness, opacity, reflectance, iridescence, and so on (Moraes et al., 2017).

Sunscreens The detrimental effects of ultraviolet A and B radiations (UVA and UVB) on the skin can lead to the development of malignant carcinomas in cutaneous tissue. Sunscreens are thus dermocosmetic products of great importance to skin health. Thanks to their excellent optical barrier properties, some clay minerals have been included in dermocosmetic formulations of sunscreens, acting as a barrier to block solar radiation and, thus, protect cellular nucleic acids. Clay minerals must have a high index of refraction and optimal light dispersion properties to be used as sunscreens. Bentonite and hectorite meet the required specifications and are already being used as sunscreen (Ghadiri et al., 2015; Mattioli et al., 2015). A mineral-based sunscreen containing activated clay combined with a dispersing agent and one or more inorganic sunscreen actives was patented, resulting in a mineral sunscreen having high UVB/UVA protection and exceptional spreadability that is non-whitening. (Timothy et al., 2015). A composition for cosmetics which has a UV shielding effect and good dispersibility is provided. The composition includes microparticulate titanium dioxide, magnesium and/or calcium hydroxide, and a clay mineral. The clay mineral suitable for the present invention has no limitation imposed upon it, as long as it can be used as a powder to be employed in ordinary cosmetics. Examples are boron nitride, sericite, natural mica, calcined mica, synthetic mica, synthetic sericite, alumina, mica, talc, kaolin, bentonite, and smectite (Ijiri et al., 2015).

Other Clay-Based Cosmetic Products A wide range of cosmetic products containing clay minerals in their composition have been designed throughout time, and most of them have their patent registered (Viseras et al., 2007). The use of clays as emulgents or emulsifiers in cosmetic products is well known. The use of talc as an emulgent in "make-up preparations" because of its large surface area, is notable (Gabriel, 1973). Bentonite was used as an emulsifier in a nail-enamel remover (Carter, 1940), in oil-in-water make-up (Gabriel, 1973), in vanishing low oil-content creams (Alexander, 1973), and in cleansing lotions (Sarfaraz, 2004). The optimization of a peel-off facial mask formulation containing green clay and aloe vera was studied (Beringhs et al., 2013). More recently, a dry shampoo composition comprising a smectite, natural starches, and a natural oil absorbent was developed and was subsequently patented (Perfitt & Carimbocas, 2017). An emulsion of bio-minerals (phyllosilicate, inosilicate, cyclosilicate, tectosilicate, neosilicate, or sorosilicate) was created using a unique process that allows the combination of ingredients to be emulsified in a cold, chemical-free environment to create a product that is more stable and requires less energy and time to prepare and has been registered (Rochette et al., 2017).

SUMMARY AND OUTLOOK

Topical and transdermal products including clay minerals have a long history and remain key formulations for delivering drugs not only onto the skin for local purposes, but also through it for systemic action. Skin is a widely used route of delivery for local and systemic drugs and is potentially a route for their delivery as nanoparticles. Among the wide range of nanoparticles available, clay minerals have been used since ancient times, both as actives and excipients in the treatment of skin illness. The use of nanoclays alone and/or in combination with biopolymers and/or drug in treating local skin and systemic diseases is of interest. In this review, recent work in the field of clay minerals-based nanoparticle delivery to the skin, and future directions currently being explored, is discussed. Once this attempt to summarize and highlight the possibilities offered by clay minerals in advanced skin drug delivery is finished, the final goal is to provide a greater understanding of the countless benefits derived from both this administration path and these types of nanosystems.

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