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Nanomedicine Approaches to Negotiate Local Biobarriers for Topical Drug Delivery

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Topical treatments have been widely adopted to address a broad range of conditions across multiple sites thanks to their convenience, versatility, and effectiveness. While bypassing systemic biobarriers and avoiding systemic side effects by delivering directly to the target tissue, topical treatments still face significant local biobarriers that limit their efficacy. The toolset available for nanodelivery systems and their inherent multifunctionality can contribute to simultaneously address otherwise intractable challenges related to barrier function evasion, drug solubility, bioavailability, pharmacokinetics, smart and sustained release, quantitative co-delivery, and local targeting which are key to successful topical treatments. This review summarizes the outstanding challenges associated with the topical treatments of key diseases of the skin, mucosae, eyes, and ears, and highlights how nanodelivery systems are being developed to address them effectively.

1. The Clinical Value of Topical Application

Topical application is a major route for delivering treatments because it is simple, effective, and noninvasive, enabling self-administration and high patient compliance. Topical administration reduces systemic adverse effects, facilitates targeting through direct application at the site of interest, and avoids first-pass metabolism. The topical approach is very effective and useful for delivering small (<500 Da), lipophilic, and potent solutes for local and systemic therapy in humans.[1] The skin is the most explored site for the topical delivery of drugs, where they are applied to produce both local and systemic effects. Beyond the skin, drops for topical application to the eye and ear as well as nasal sprays and inhalers are broadly available on the market.[2,3] Mucosae including the mouth, colon, lungs, and genitals are also very effective sites for topical delivery.[4] Topical skin treatments can address skin infections,[5] inflammatory conditions like atopic dermatitis,[6] eczema,[7] psoriasis,[8] acne,[9] basal cell carcinoma,[10] vitiligo,[11] hair loss,[12] and roughness of the skin.[13] Topical formulations can also treat eye infections,[14] conjunctivitis,[15] as well as hearing loss,[16] and ear infections.[17] Furthermore, several topical drugs are efficient for treating oral cancer,[18] gastrointestinal (GI) diseases,[19] lung,[20] and genital infections.[21] Indeed, due to the broad range of application modalities, delivery sites, and condition treated, topical drugs require a vast array of formulations including tablets, aerosols, drops, gels, creams, solutions, lotions, ointments, foams, and shampoos.[22–24]

Despite the vast use of topical approaches, the effective administration of drugs on the skin, mucosae, and accessible sites (eye, ear, airways) remains a major challenge for contemporary medicine. Critical determinants for effective drug delivery across these various locations are i) the physiochemical nature of the drug, ii) the properties of the formulations used for delivery, and iii) the physical, chemical, and physiological condition of the site of delivery. Being the most exposed organ of the body, the skin undergoes multiple forms of stresses daily. Any dysregulation on the skin surface can lead to hypersensitivity, impaired stratum corneum regeneration, and other skin disorders which in turn can affect the efficiency of topical treatments.[25] Therefore, topical formulations should provide sufficient flexibility to account for this broad range of variability in conditions. Similar to the skin, a wide range of conditions present at other delivery sites of the body, hampering the efficient and targeted delivery of drugs. For example, mucosae can vary the physicochemical composition of the mucus and their pH in response to exogenous and endogenous stimuli, thus altering the parameters of key delivery barriers.[26] Among all these challenges, conventional approaches for topical administration have to strike a balance between delivery efficiency, desired pharmacokinetics, and tolerable local side effects, as their delivery typically requires a range of chemical and physical enhancers which disrupt the integrity of the target tissue. These approaches can induce serious side effects including local irritation, burning or inflammation, systemic distribution

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with associated consequences, drug rejection, and toxicity to off-target cells.[27] In particular, delivery of biologicals, which include nucleic acids, proteins, and peptides is extremely challenging by topical application due to the excellent barrier function of the target tissues. Despite these challenges, the broad adoption of topical delivery is a direct reflection of the several key advantages it provides.

1.1. Direct Application to the Target Area

Topical treatments are directly applied on the area that requires treatment. Thus, their formulation must allow dispensing them to the target area but also remaining in place for as long as necessary. These two requirements need a careful balancing, which, depending on the specific application, leads to a vast range of liquid and semi-solid formulations. In liquid formulations, different types of lotions, liniment, solutions, emulsions, suspensions, and collodion have been used. These formulations are used when requiring readily dispersion or absorption within the target tissue. Lotions containing betamethasone valerate effectively treat scalp psoriasis with high patient acceptability.[28] Using liniment-based formulation, peplomycin delivery to the mucosa effectively treats bladder cancer.[24] Over the past decade, smart biomaterials that can monitor local conditions and release therapeutics when necessary are being used for chronic wound dressings, e.g., in the treatment of patients suffering from diabetic foot ulcer where polymeric and metallic nanoparticles (NPs) and electrospun nanofibers usage can improve regeneration of wounded dermal and epidermal tissues.[29]

1.2. Reduced Side Effects

Systemic treatment through oral, intravenous, or intraperitoneal drug delivery induces off-target side effects to other organs of the body, including those systems receiving the delivery (e.g., gastrointestinal, respiratory, cardiovascular), the organs involved in first-pass metabolism (liver, kidneys) and those particularly susceptible to individual drugs. For a broad range of drugs, these side effects can outweigh the therapeutic benefit to the point of preventing their systemic administration, in order to preserve the overall organism. Often these drugs are potent therapeutics for specific conditions, which can present in exposed sites of the body. In these instances, topical administration, by minimizing systemic distribution, enables their use for effective treatments. The antifungal agent ketoconazole, is highly effective in the topical treatment of fungal infections of the skin, mouth, and vagina, but its oral use is prohibited by the Food and Drug Administration (FDA) and The European Medicines Agency due to its severe liver toxicity.[30] Oral tamoxifen-based systemic therapy is advised for high risk breast cancer patients. During the process, oral tamoxifen is broken down into its active component 4-OHT in the liver which can lead to serious harmful side effects such as the activation of proteins that cause blood clots.[31] A hydroxyl cellulose gel-based application of 4-OHT on the breast skin surface can overcome this problem. This approach results in high drug levels in the breast, low drugs levels in the circulation, reduction of tumor burden, and minimal side effects.[32] Use of nonsteroidal anti-inflammatory drugs (NSAIDs) often leads to dose-dependent effects such as GI disturbances, cardiovascular events, and renal toxicity. Approved topical formulations allow minimizing systemic concentration of the drug while maximizing its delivery at the target site. The FDA has approved diclofenac sodium 1.5% topical solution (containing dimethyl sulfoxide as a penetration enhancer), diclofenac sodium gel 1%, and a diclofenac hydroxyethylpyrrolidine 1.3% patch for the treatment of pain associated with osteoarthritis.[33] These formulations do not show any systemic effects. Dermatologists also often prescribe 3% diclofenac in 2.5% hyaluronic acid ("Solaraze") for treating actinic keratosis.[34] Ibuprofen creams and gels, ketoprofen gel, felbinac gel and cutaneous foam, and piroxicam gel are all approved by the European Union and, based on a meta analyses study, they have demonstrated effective and safe topical delivery.[35]

1.3. Broader Selection of Drugs and Dosage Regulation

The limited systemic biodistribution of topically administered treatments expands the range of usable drugs, enabling access to drugs with higher specific potency for the treatment but strong systemic or organ-specific side effects. Even for drugs that can be tolerated by the organism, the minimal biodistribution associated with topical administration allows using higher doses, and longer treatments leading to more effective response. On the other hand, since drug efficacy is improved and prolonged by the limited loss of therapeutic agent away from the targeting site, it is possible to reduce dosage for the active agent while maintaining the maximum potency.[36]

1.4. Improved Tissue Targeting

Conventional systemic delivery has limited ability for targeting drugs to the intended site, due to the numerous biobarriers opposing delivery. Typically using these approaches, only fractions of a percent of drug reach the intended site; this reduces the ability to control the variability of delivery efficiency across patients and in different conditions for the same patient. This approach is effective when using drugs/formulations with broad therapeutic windows and high therapeutic indices so that this large variability and low efficiency can be offset. Many classes of drugs, including chemotherapeutics for cancer, have notoriously shallow therapeutic windows and therapeutic indices close to 1, making their systemic delivery a significant challenge.[37] Opting for topical delivery of these drugs when possible bypasses these challenges, as the tailored delivery to the intended site of action guarantees a better control over the amount of drug received by the desired target. Fluorouracil (5-FU) is widely used for the treatment of skin cancer. Systemic administration 5-FU causes a broad range of side effects that can lead to interrupting treatment. Topical administration of 5-FU leads to a local accumulation of up to 86% to 92% of the delivered dose and systemic absorption of less than 2% in noncompromised skin, with blood plasma and urine accumulation of only 0.55%, resulting in drastically reduced side effects, which enable the use of topical 5-FU for precancerous and benign conditions.[38,39] Topical delivery can also be beneficial for cellular-level targeting as it

removes the design constrains necessary for efficient systemic biobarrier avoidance, which often conflict with the ability of the delivery system to provide cellular-level targeting.

1.5. Improved Patient Compliance

Chronic diseases require prolonged treatments with associated issues of poor compliance and reduced quality of life for patients, which are closely associated with the invasiveness and complexity of the treatment. Indeed, the most important factors associated with nonadherence to treatment include poor perceived efficacy, inconvenience in drug usage, forgetfulness, and fear of serious side effects. Topical medicines are effective at improving treatment compliance thanks to their simple self-administration, reduced local and systemic side effects, and the ease of adapting formulation to the specificity of delivery site and approach.[40]

2. The Clinical Rationale for using Nanomaterials in Topical Delivery

The inclusion of nanomaterials within topical formulations promises to provide advanced solutions to address the key challenges in the field. Indeed, the inherent multifunctionality of nanodelivery systems is well suited to address the competing needs of improving delivery, reducing local side effects, and modulating pharmacokinetics by negotiating a broad range of local biobarriers (**Figure 1**). Nanoscale materials can interact in a controlled and complex way with their environment and ultimately improve the delivery of drugs, thanks to their emergent physicochemical properties, which include high specific surface area, tunable reactivity, and controllable surface chemistry.[41,42] Nanostructures can easily negotiate access across cell membranes, tissues, organs, and target specific cells or subcellular structures based on their biochemical milieu.[43–45] Drug loading into NPs by physical encapsulation, adsorption, or chemical conjugation can substantially modulate pharmacokinetics and therapeutic index while enhancing availability and prolonging pharmacological activity.[46] Nanodelivery can regulate the solubility of drugs, while allowing the quantitative co-delivery of multiple drugs to the target site.[47] Nanomaterials can modulate the kinetics of drug release by tuning their own erosion or degradation as well as drug diffusion, or by "smart" triggered release in response to environmental cues (temperature, pH, oxidative stress) or external triggers (ultrasound, light, radio-frequency, and magnetic field).[48] This improved capability of nanodelivery systems over conventional approaches is the key to address the outstanding challenges in topical drug administration, as highlighted in **Table 1**.

Many nanodelivery systems have been developed for topical use, employing most types of available 0D nanostructures (i.e., NPs) and some selected 1D and 2D nanostructures (e.g., nanowires, nanofibers, nanoneedles; **Figure 2**). The nature of each nanomaterial, in terms of its composition, dimensionality, physicochemical, and biological properties, bestows them with unique features to address specific challenges for topical delivery.

2.1. NPs (0D Nanomaterials)

Lipid vesicles: Lipid vesicles are unilamellar or multilamellar phospholipid bilayers which are among the first nanodelivery systems developed and have since been extensively used for the treatment of human disease.[67] Key advantages of lipid vesicles include their nontoxic and nonimmunogenic nature, the ability to deliver hydrophobic, hydrophilic, and amphiphilic drugs with sustained release, thus prolonging their activity and an established, effective, and safe profile for in vivo administration known to improve therapeutic index.[67,68] A broad range of commercially available formulations exist, among the most common are niosomes, ethosomes, transfersomes, proliposomes, pharmacosomes, and vesosomes. Liposomes have been widely used in topical applications for delivering antibiotics, peptides, and antitumor agents for skin cancer.[69] For transdermal delivery approaches, niosomes, ethosomes, and transfersomes provide better tissue penetration.[70]

Solid lipid NPs (SLNs): SLNs have been developed as more advanced lipid nanocarriers to address the stability and drug encapsulation issues that can be encountered with lipid vesicles. SLNs have been extensively developed for cosmetic and skin applications including treatment of eczema and acne.[71] Indeed, SLNs have been used as carriers for several drugs relevant to the topical treatment of skin conditions, including clotrimazole, isotretinoin, triptolide, glucocorticoids, and vitamin A.[72] SLNs are biodegradable and provide improved stability, protection of loaded drugs from degradation, and site-specific targeting. They also show minimal toxicity thanks to their derivation from physiological lipids.[72,73] Nanostructured lipid carriers (NLCs) have been developed to improve over the limited solubility and exclusion upon crystallization experienced when formulating drugs with SLNs. Antifungal drugs have been used as a model for NLCs delivery systems.[74] NLCs have also been used for the treatment of psoriasis.[75] Both SLNs and NLCs are also being actively evaluated for use in mucosal drug delivery.[76]

Polymeric NPs: A vast range of synthetic and natural polymer NPs have been proposed for topical delivery.[77] Polymeric particles can assume the same conformations as their lipid counterpart, generating solid particles, micelles, or polymerosomes (liposome equivalent). Due to the broad tunability of the physical properties and chemical functionality of polymers, the key properties of the NPs can be tuned to specific applications through the rational design of the carrier. Modulating polymer chain length, susceptibility to hydrolysis and enzymatic degradation provides broad tunability for the degradation/erosion of the NPs that controls drug release. Modulating polarity and surface charge regulates loading efficiency and the interaction with cell membranes, providing a strategy to optimize loading based on the nature of the payload and promote intracellular delivery. Natural polymeric NPs like chitosan have been used in porcine skin for the topical delivery of acyclovir for the treatment of herpes infection.[78] Synthetic biodegradable polymers such as poly(lactide-*co*-glycolide) copolymers (PLGA) can contribute to the transdermal delivery of indomethacin (analgesic).[79] Co-polymeric PLGA-chitosan NPs are effective for topical delivery of anti-inflammatory drugs such as ketoprofen and spantide II in psoriatic mice model.[80]

Micellar NPs (MNPs): Polymeric micelles are nanostructures made from amphiphilic block copolymers, which associate in aqueous solution. Hydrophobic drugs can be easily loaded in

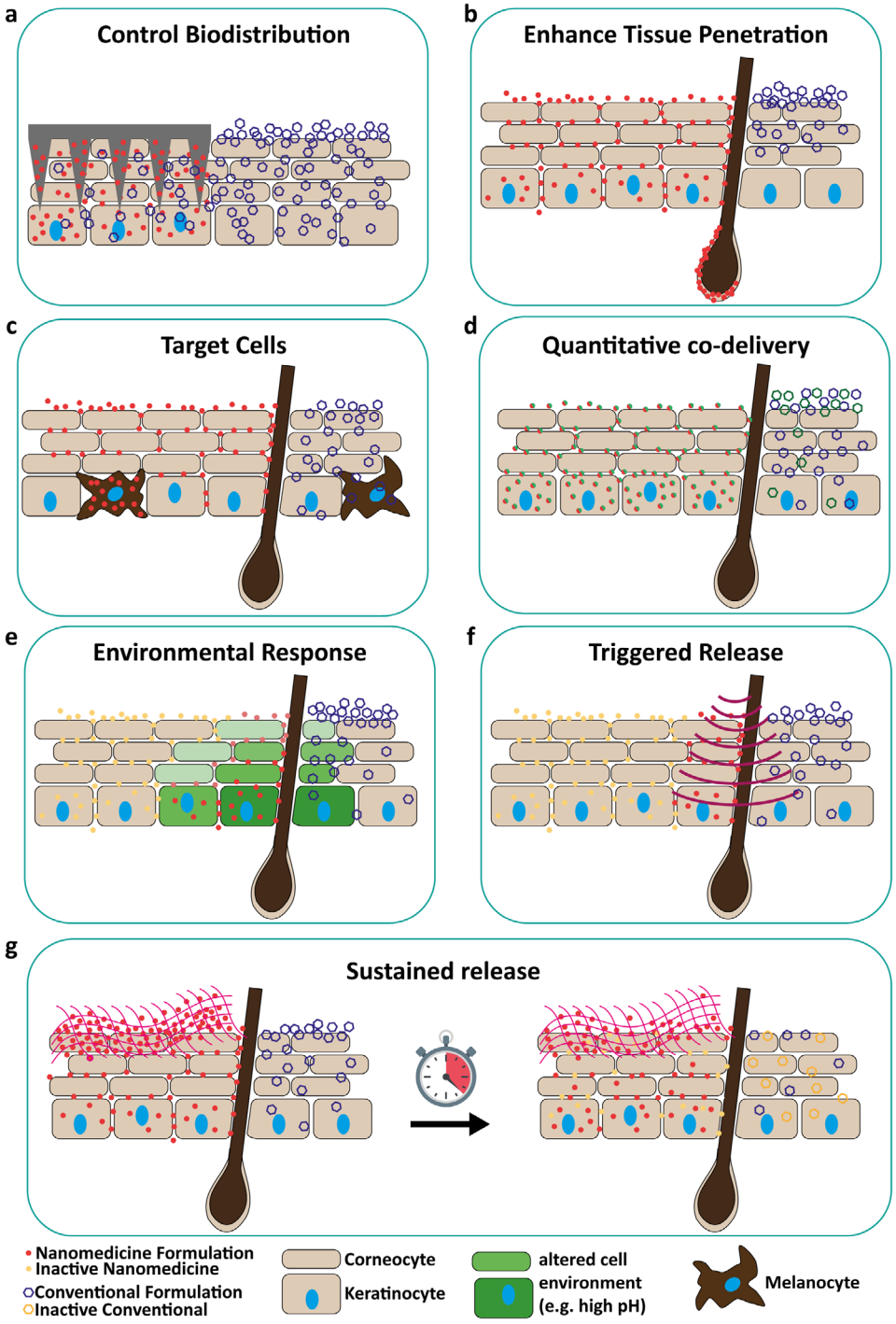

Figure 1. Schematic depicting the contributions of nanomedicine approaches to the modulation of local biobarriers. The skin is used as a prototypical example in the illustration but the approaches depicted are advantageous for topical delivery across all sites. A) Nanodelivery systems such as nanoneedles can deliver payloads intracellularly enhancing the localization of the bioactive agent, thus minimizing its dispersion away from the target area and preventing their inactivation by the resident and recruited immune system present in the extracellular space. B) NPs can penetrate effectively across the superficial barrier layer of epithelia, enhancing delivery to underlying regions of the epithelia. Enhanced penetration occurs by navigating across tight intracellular spaces, by transcytosis and by exploiting natural conduits such as hair shafts. The surface of NPs can be specifically functionalized to further improve penetration across epithelial barriers by either exploiting chemical affinity (hydrophobicity, amphiphilicity) or using bioactive strategies to enhance transcytosis or disrupt intracellular junctions. C) NPs can target specific cells based on their surface markers. This approach can direct treatment toward healthy cells subtypes or diseased cells, overall minimizing side effects to nontargeted cells. D) Nanomaterials can deliver precise ratios of multiple therapeutic in a defined sequence, thus improving the effectiveness of combinatorial therapies. E) Nanosystems can deliver drug in response to a range of environmental conditions including enzymatic activity, pH, and temperature. This responsiveness enables smart targeting of

Table 1. Comparison between conventional and nanodelivery approaches to topical treatment.

Challenge	Conventional approach	Nanodelivery	Application	Comparison	References
Delivery to target	Isotretinoin	Isotretinoin-loaded SLN (solid lipid NPs)	Acne	Increase accumulative uptake and enhanced skin targeting.	[49]
	Doxorubicin (DOX) and paclitaxel (PTX)	Liposome (transferrin/trans-activating transduction-PTX/DOX-LP)	Melanoma	Double-drug liposomal drug delivery systems (DDS) enhanced targeting and increased efficacy.	[50]
Sustained release	Brimonidine tartrate eye drops	Brimonidine-loaded alginate	Glaucoma	Sustained release without initial burst Improved intraocular pressure lowering.	[51]
	Aspirin	Aspirin-loaded albumin	Diabetic retinopathy	Prolonged release in vitro.	[52]
Environmental response	Doxorubicin	DOX-SLN	Skin cancer	Altered partition coefficient to increase stratum corneum lipid matrix accumulation.	[53]
Drug solubility	Coenzyme Q10 (CoQ10) and alpha-lipoic acid (ALA)	Chitosan-coated liposomes loaded with coenzyme Q10 and alpha-lipoic acid (CCAL)	Cosmetics	CCAL improved solubility for sustained drug release and accumulation in vitro	[54]
Pharmacological activity	Quercetin	Quercetin (QU)-loaded TPP-Chitosan NPs (QTCs)	UVB Skin damage	Improved stability, low cytotoxicity, enhanced percutaneous absorption and retention.	[55]
Prolonged retention	Restasis	Cyclosporin A-loaded phenylboronic acid (CsA-PBA)	Dry eye syndrome (DES)	Prolonged ocular surface retention and up to 100-fold dosage reduction.	[56]
	VEGF (vascular endothelial growth factor) plasmid (pVEGF)	pVEGF-loaded nanoneedles	Tissue regeneration	Prolonged VEGF expression-driving neovascularization.	
Improved compliance		Hybrid dendrimer hydrogel/poly(lactic-*co*-glycolic acid) (PLGA) NP platform (HDNP)	Glaucoma	Efficient delivery and sustained release.	[57]
	Silver sulfadiazine & Mupirocin	Fluconazole-loaded eudragit nanofibers	Fungal infections	Ease of application improving compliance	[58]
Accurate dosing	Maxidex	Dexamethasone-loaded PLGA NPs (DX/NP) with media containing both PVA and alginate	Ophthalmic application	2.6-fold increase in ocular drug bioavailability and aseptic accurate dosing.	[59]
Enhanced bioavailability	Eyedrops, ointments	Diclofenac/methoxy-PEG-PCL-chitosan	Ocular disease	Prolonged pre-corneal retention and improved bioavailability.	[60]

the core and their hydrophilic shell makes this system soluble in water and stabilizes the core. MNPs are extensively studied as topical delivery systems thanks to their robust and versatile nature. Micellar nanoemulsions (lotions) offer a promising solution for topical applications. They enable delivery of drugs at higher concentrations and the delivery of large biomolecules including, plasmids, RNA, and proteins.[81] Novavax first developed a transdermal lotion using MNP technology known as Estrasorb. Estrasorb is an estradiol topical emulsion used for short- term estrogen replacement therapy aimed at reducing vasomotor symptoms in menopausal women.[82] Using MNP technology, Novavax is also developing a topical vaccine for the influenza virus.[83] Nanoemulsions are widely used for the topical delivery of several classes of drugs including anesthetics, anticancer agents, antibiotics, and vitamins.[84,85]

Inorganic NPs: Metals, among which gold and silver are extensively used for topical applications, can easily be synthesized in different shapes, including spheres, nanoclusters, nanostars, nanorods, nanocapsules, nanodisks, and nanocuboids.[86–89] The ease of their surface functionalization and their unique, tunable plasmonic properties makes them highly suitable for targeted and "smart" delivery approaches. Metal NPs typically do not biodegrade, making topical application a more desirable delivery strategy for their use in conditions with low morbidity. Indeed,

diseased regions based on their environmental conditions, minimizing off-target delivery. F) Nanomaterials can release drugs upon external stimulation, such as ultrasound, light, radiofrequency, magnetic field. This approach allows precise selection of the timing and localization of drug delivery, enabling programmed, repeated, or sustained drug delivery specifically at the site of interest. G) Nanomaterials can protect the bioactivity of drug in the long term and release them to the tissue with a controlled kinetic, enabling their sustained released and prolonged efficacy.

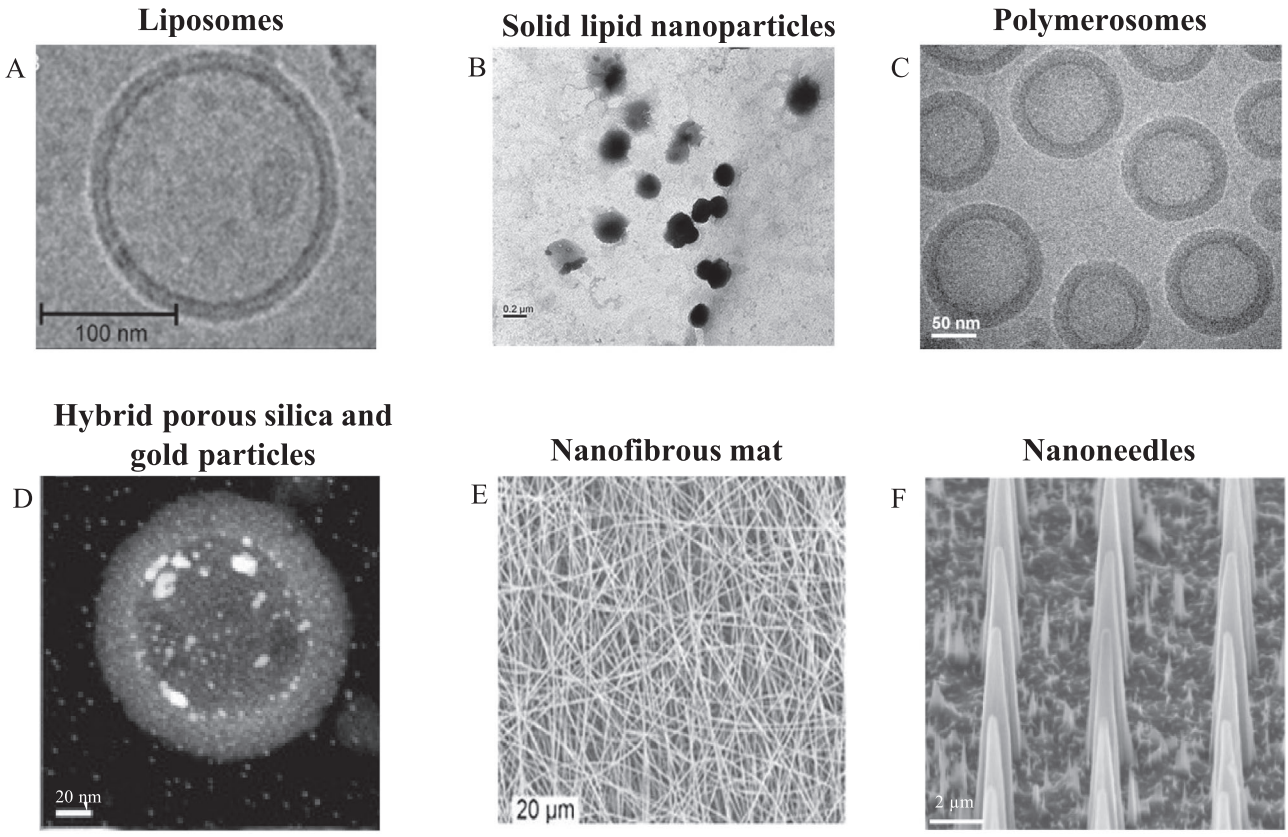

Figure 2. Shape and structures of different nanomaterials discussed in the review. A) Cryo-TEM image of a liposome. B) TEM image of solid lipid NPs. C) Cryo-TEM image of a polymerosome (constructed from PEG-*b*-PBD). PEG, polyethylene glycol; PBD, 1,2 polybutadiene. D) TEM image of gold NP within mesoporous silica shell. E) SEM image of electrospun nanofibers. F) SEM image of porous silicon nanoneedles. Images, A) Reproduced under the terms of Creative Commons CC BY license.[61] Copyright 2020, Springer. B) Reproduced with permission.[62] Copyright 2015, Royal Society of Chemistry. C) Reproduced with permission.[63] Copyright 2014, Royal Society of Chemistry. D) Reproduced with permission.[64] Copyright 2015, National Academy of Sciences. E) Reproduced with permission [65] Copyright 2012, Elsevier. F) Reproduced with permission.[66] Copyright 2015, Springer.

silver (Ag) NPs are very promising antimicrobial systems.[90,91] Due to their antibacterial properties, Ag NPs are added in footwear, wound dressings, cosmetics, and appliances. Gold (Au) NPs are also used as antimicrobial agents, e.g., when added to nanofibers.[92] They are used for the delivery of many drugs; in photodynamic cancer therapy; for biosensing; bioimaging; and for combined drug delivery and imaging in theranostics applications.[93] Ag and Au NPs carrying polyphenols-rich extracts (Cornus mas) are a green technology tool explored for reducing inflammation in a human psoriasis plaque model in vitro.[94] The interaction of inorganic NPs such as Au-NPs with biological fluids leads to the formation of a protein corona which envelops NPs. The nature of the protein corona depends on the composition, size, shape, exposure time of NPs. It also varies with the type of biological fluid, its biophysical properties, and the details of its composition. The corona plays an important role in regulating the interaction between NPs and the surrounding bioenvironment, affecting cell and tissue targeting and thus biodistribution, environmental response, and pharmacokinetics. NPs with engineered protein corona are emerging as advanced drug delivery approaches with improved functionality.[95,96] NPs made from the oxides of titanium and zinc are integral components of several commercially available creams and lotions for the cosmetic industry, as they provide protection from harmful UV radiation.[97] Both can penetrate the epidermis but do not penetrate further or access the vasculature. Mesoporous silica NPs (MSNs) are an important class of inorganic materials for topical drug delivery. MSNs-based formulations can contribute to the topical administration of methotrexate (MTX), a chemotherapeutic agent on the skin surface in animals.[98] This system increases epidermal accumulation of MTX to the deeper layers of the epidermis. The NPs functionalized with a thermoresponsive silane copolymer and loaded with quercetin show potential in dermocosmetic industry.[99] Porous silicon (pSi), obtained from electrochemical processing of crystalline silicon, is broadly employed for drug delivery thanks to its unique combination of properties for an inorganic material. The tunable biodegradability, mesoporous structure, intrinsic photoluminescence, and tailorable photonic properties combine to bestow characteristic advantages when designing trackable, resorbable delivery systems for controlled and sustained delivery.[100,101]

2.2. Nanostructures (1D and 2D Nanomaterials)

Nanowires: Nanowires are high-aspect ratio nanomaterials with nanoscale diameter and extended length. They can be synthesized through bottom-up (e.g., vapor liquid solid and

solution phase synthesis, laser ablation, and template-based methods) or top down (e.g., electron beam lithography, focused ion beam, plasma etching, and wet etching) approaches.[102] Nanowires can provide robust regulation of cell growth and high biocompatibility.[103] Dispersed nanowires can cross the cell membrane while maintaining its integrity thus enabling the efficient cytosolic delivery of drugs and biomolecules (DNAs, RNAs, peptides, proteins, and siRNA).[104–108] The topical administration of compounds (AP173, AP713, and AP364) using TiO_2-based nanowires improves their therapeutic efficacy and delivery in a rat model of spinal cord injury.[109] Titania-based nanowires can also inhibit the growth of *Staphylococcus aureus* (*S. aureus*) more efficiently than its counterpart NP. The antimicrobial activity of this nanoformulation remains effective against multi drug-resistant pathogenic strains of *S. aureus*, promising clinical potency for the topical treatment of infections.[110]

Nanofibers: Nanofibers have nanometer-scale diameter and intertwine over the long range into mesh structures to form "woven" mats with macroscopic dimensions. Nanofibers can easily be manufactured as bandages which are promising for the topical delivery of biologicals. Electrospinning is the most versatile and most common process for fabricating nanofibrous matrices.[111] Electrospun nanofibers are useful for the delivery of therapeutic agents ranging from nanomedicines to macromolecules including nucleic acids and proteins. Electrospinning is relatively economical, simple, and operator friendly, leading to the production of nanofibers on an industrial scale.[112] Mixing drugs and biologicals in the electrospinning feedstock allows simple and versatile loading and display of bioactive agents. Electrospun nanofiber mats can be manufactured from multiple biocompatible and biodegradable polymers (natural, synthetic, and their combination).[113] They have a high surface-to-volume ratio which enables the efficient delivery of both hydrophobic and hydrophilic drugs, while their flexibility aids conformal application to tissues enhancing delivery.[114] Fiber diameter, drug to polymer ratio, porosity, and morphology can be modulated according to specific clinical requirements.[115] Nanofiber drug delivery system are actively developed for the topical treatment of highly contaminated/infectious wounds, through the incorporation of antimicrobial, antibiotic, and antifungal agents.[116] Cefalozin, a broad-spectrum antibiotic used for treating wounds, can be incorporated within PLGA electrospun nanofibers.[117] Delivery of antibiotics such as cefoxitin sodium from electrospun copolymers of PLGA and polyethylene glycol (PEG) and linezolid from PCL fiber mats, respectively, can inhibit *S. aureus* growth in vitro.[118,119] Poly-urethane-based nanofibers can topically deliver itraconazole, a hydrophobic antifungal agent.[114] Several studies show that the topical application of growth factors (epidermal, platelet derived, vascular endothelial, and basic fibroblast) into electrospun fiber mats can accelerate wound healing.[120–124] Cosmetic skin care takes advantage of the improved delivery using nanofiber mats that have high surface area and conform to the shape of the skin. During oxidative stress, vitamin E-loaded silk fiber can preserve the viability of skin fibroblasts in vitro.[125] Electrospun mats can also help in treating keloid scars resulting from abnormal growth of granulation tissues. Dexamethasone infused into suppresses keloids in vivo. Addition of hydrophilic green tea polyphenols to these mats, further reduces the scars by enhancing dexamethasone release.[126]

Nanoneedles: Nanoneedles are vertical arrays of high-aspect ratio nanostructures for the direct intracellular delivery of cell-impermeable drugs. They can be manufactured from a broad range of inorganic materials including metals, oxides, and semiconductors, using different top down and bottom up approaches.[45,127,128] These approaches yield vertical arrays of nanowires, nanopillars, nanocones, and nanostraws which share a common ability to tightly interface with cells and facilitate the exchange of biomolecules across the cell membrane. This mechanism of action contrasts with microneedles, which are both longer and wider than nanoneedles and act by penetrating and disrupting the surface of a tissue in order to enhance permeation of biological payloads, enabling more effective and faster therapeutic action.[129,130] Microneedles, although painless, induce an immune response making them well suited for the delivery of antigens such as DNA vaccines, but less amenable to the delivery of long-acting biologicals which can be rapidly degraded by immune cells recruited to the inflamed area.[131,132] In contrast, when nanoneedles interface with cells, their smaller size and high aspect ratio maintain tissue integrity and cell viability while inducing strong deformations of the plasma membrane which lead to enhanced intracellular uptake of the payload presented from the needles, thus improving intracellular delivery. This approach significantly enhances the delivery of many classes of hard-to-deliver biologicals including DNAs, RNAs, proteins, and peptides.[66,106,133] Solid nanoneedles load drugs on their outer surface and typically rely on electrostatic or hydrophobic interaction to achieve drug loading, thus limiting their release efficiency.[134] Hollow nanoneedles have an inner conduit that connects a drug reservoir to the delivery site, enabling significant amounts of payloads to be delivered over long times.[133] This improved control over payload loading and delivery is counterbalanced by significantly more complex fabrication processes necessary to incorporate an accessible reservoir. Porous nanoneedles allow loading drugs within their porous structure, thus increasing overall payload over solid nanoneedles and controlling its release through diffusion from the porous structure, without requiring additional processing for the development of a conduit and reservoir [66,128,135] Nanoneedles are aptly suited for topical delivery as they minimize tissue damage and prolong drug retention,[66] enhance intracellular uptake,[136,137] and can be manufactured on flexible substrates.[138] Indeed, nanoneedles can mediate topical gene therapy inducing sustained gene expression and formation of new vasculature following the topical application of plasmids coding for the VEGF protein.[66]

3. Topical Drug Delivery with Nanomaterials

3.1. Skin

The skin is composed of heterogenous multilayer tissues and is the largest organ of the human body with a surface area of 2 m^2. It protects the body from the exogenous environment by serving as the first line of defense against the ingress of foreign bodies including molecules, microorganisms, and particulate. It helps in regulating body temperature and protects it from the loss of salts and fluids. Human skin is generally 1.5 mm thick and is composed of an epidermis and a dermis layer.[143,144] The epidermis includes the viable epidermis underlying the nonviable

epidermis (stratum corneum (SC)) which forms the outermost layer of the skin.[145] The dermis supports blood and lymphatic vessels, sebaceous and sweat glands, connective tissues, hair follicles, and nerve endings. Underneath the dermis, the subcutaneous tissue or hypodermis comprises adipose cells, fibroblasts, macrophages, and fibrous connective tissues and provides cushioning and support to the dermis and epidermis.[143,146]

The skin is a highly exploited route for drug delivery because of its large surface area and simple access. Drug delivery to the epidermis can be topical to induce local effects or transdermal: intended to achieve systemic effects by diffusing through the circulation.[116] A major challenge associated with topical delivery is to surpass the resistance posed by the SC. The SC comprises 15–20 layers of corneocytes—anucleated, keratin filled, dense, and functionally dead cells—surrounded by several lipid bilayers including cholesterol, fatty acids, and ceramides. This peculiar "bricks and mortar" arrangement of the SC with multiple layers of corneocytes as bricks and the extracellular lipids as mortar is key to the skin effectiveness as a barrier.[143,147–150] This structure confers water resistance and severely limits the diffusion of pharmaceutically active molecules. Indeed, the SC only allows the passage of small lipophilic and hydrophilic molecules, typically of less than 500 Da.[151,152] On the opposite front, topical skin delivery also needs to confine the action of drug to the desired site, without reaching the dermis from where it can be absorbed from the capillaries and delivered systemically through the circulation. Thanks to their peculiar characteristics, nanomaterials provide key advantages to negotiate skin biobarriers and enhance the treatment of a broad range of skin diseases.[116] Indeed, nanomaterials can effectively penetrate the brick and mortar structure to enhance delivery to the stratum corneum[153] or migrate through the hair shaft accumulating at hair follicles.[154]

Atopic dermatitis (eczema) (AD): AD is a multifactorial disorder resulting in a chronic relapsing inflammatory skin disorder. It commonly associates with other atopic manifestations including asthma, allergic rhinitis, and peanut allergies.[155] The complex etiology of AD involves multiple genetic, immunological, and environmental factors contributing to skin barrier abnormalities and immune dysfunctions. Skin barrier abnormalities in AD are primarily caused by a decreased expression of filaggrin, ceramides, antimicrobial peptides (AMPs), and increased expression of serine protease which ultimately lead to weakening of tight junctions, reducing the effectiveness of the SC barrier. Indeed, the increase in lipid packing and the reduction in ceramide content lead to an irregular SC layer. This irregularity increases transdermal water loss and facilitates the penetration of microbes, irritants, and allergens.[156–158] Deficiencies in skin barrier function results in AD-associated *Staphylococcus aureus* infection. Filaggrin (FLG) is an important target in AD treatment. Its loss-of-function is an important genetic cause of AD since FLG is a structural protein which participates in keratinization, moisturization and antimicrobial activity.[159] In addition to FLG abnormalities, alterations in FLG-like proteins, hornerin, and FLG family member 2 are associated with lesional and nonlesional skin barrier symptoms of AD.[160] AD patients not harboring FLG gene mutations can show reduced FLG expression due to Th2 immune skewing.[161]

Topical application of functional FLG linked to the cell-penetrating peptide motif RMR can treat AD.[162] The FLG-RMR drug conjugate localizes to the SC, the skin layer where FLG deficiency has pathological implications. FLG-RMR restores the healthy phenotype in AD murine models following the internalization and processing of the FLG recombinant protein. Topical application of water-soluble C_{60} fullerenes can regulate the immune landscape in AD to modulate FLG expression. The treatment downregulates IgE and Th2 cytokines while upregulating Th1 cytokines. The resulting foxp3$^+$ and filaggrin upregulation improves skin barrier function.[163] Combining these intrinsic immunomodulatory potentials of nanomaterials with their ability to enhance and control the sustained local delivery of proteins[164] and to improve the effectiveness of cell-penetrating strategies[165] shows potential for improvement in AD treatments by combining synergistic approaches and enhancing their individual effectiveness.

NPs are potential carriers for antibiotics, corticosteroids, and calcineurin inhibitors to improve the treatment and resolution of AD. Cyclosporin A-loaded SLN (CsA-SLN) prepared by hot homogenization method was used in murine models of AD. Topical treatment of CsA-SLN shows twofold higher skin permeation and less secretion of Th2 cytokines (IL-4 and IL-5) ultimately leading to reduced skin inflammation compared to free CsA delivery.[166] The formulation of Tacrolimus (FK506) and nicotinamide (NIC) within a chitosan NP (CS-NP) for topical treatment, known as FK506–NIC–CS–NP yields synergistic effects.[139] NIC increases FK506 entrapment (92.2%) within CS–NP. The NIC–CS–NPs significantly enhance drug permeation and deposition onto the skin compared to free FK506 (**Figure 3**A–C). Moreover, NIC–CS–NPs reduce dose requirements by 2/3, based on clinical symptoms and skin tissue analysis of AD mice. Triptolide (TPL) nanoemulsion gels have significant therapeutic effects compared to TPL-gels reducing the expression of inflammatory cytokines in mice. TPL nanoemulsion gels are a promising percutaneous nanocarrier with low toxicity and improved drug retention capacity for the clinical treatment of AD.[167] Core–multishell nanocarriers (CMS) composed of dendritic hPG-amid-C18-mPEG are being explored as unimolecular micelles for improved AD treatment. CMS accumulate in the SC where they can act as a drug depot.[168]

Tofacitinib is a small molecule inhibitor JAK-STAT that downregulates production and release of Th2 cytokines such IL-4 reducing inflammation.[169] Topical delivery of tofacitinib 2% is a promising therapy for AD that succeeded in a phase IIa trial. Tofactinib-loaded NPs can improve immune regulation in the treatment of rheumatoid arthritis and this approach could translate to improve efficacy in its topical application for AD.[170]

Emerging systemic therapies for AD include monoclonal antibodies targeting Th2 cytokines such as IL-4 and IL-13 (Dupilumab), IL-13 (Lebrikizumab/Tralokinumab), IL-31 (Nemolizumab).[169] These approaches appear highly effective but carry important immunomodulatory systemic side effects that are hampering their clinical adoption and can be obliviated by topical delivery. Owing to the success at improving skin penetration for large biologicals including antibodies,[171] nanoapproaches can contribute to convert these therapies from systemic to topical.

Acne vulgaris: Acne vulgaris is one of the most common skin disorders caused by multiple factors including increased sebum production, microbial infection by *Propionibacterium acnes* and

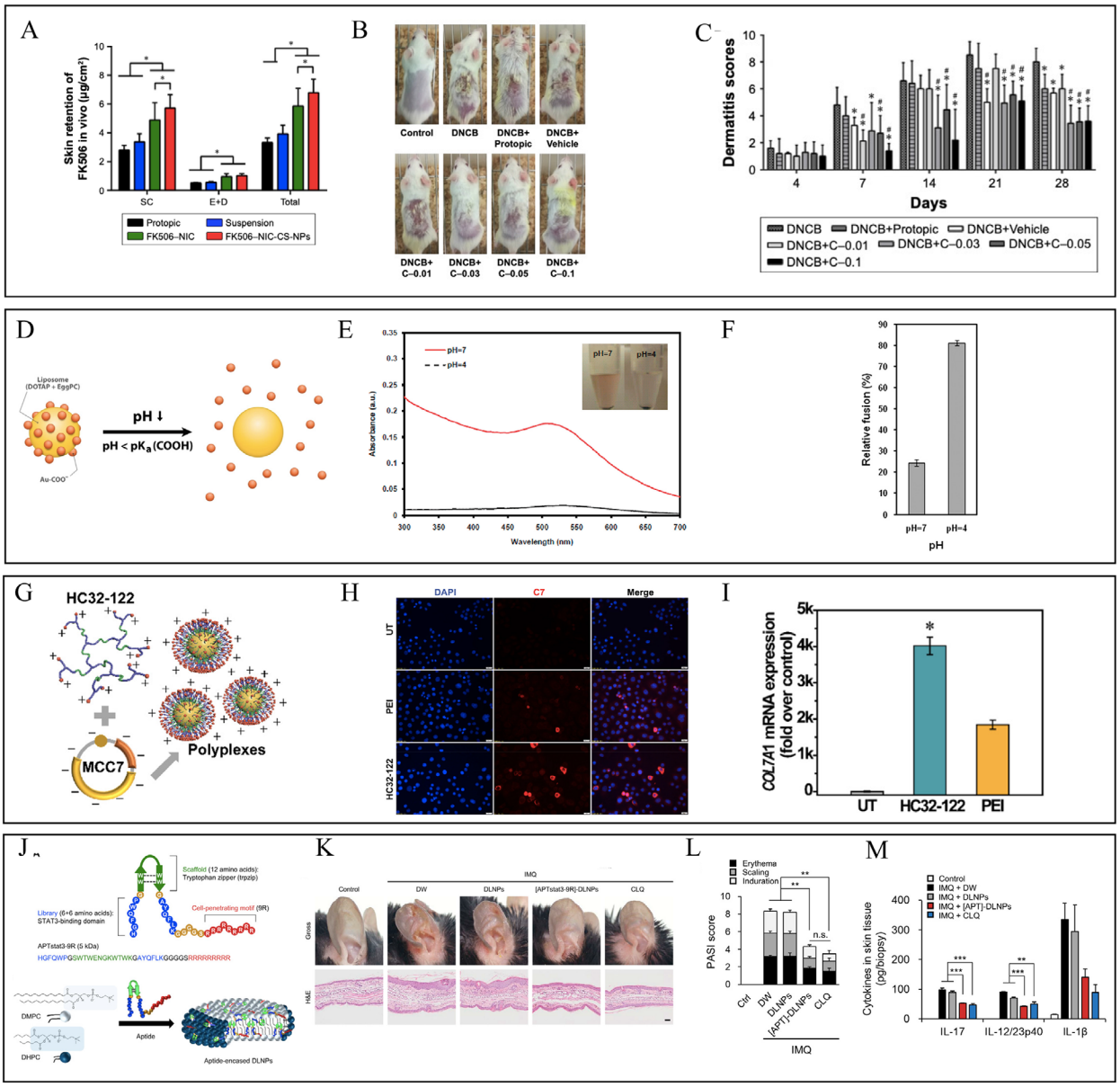

Figure 3. Nanomaterials for drug delivery to the skin. A) In vivo skin retention of FK506 across the stratum corneum (SC), the epidermis, and dermis (E+D) and overall as a function of formulation. B) Morphological clinical features of AD in mice. C) Dermatitis score shown in the adjacent graph following FK506 treatment with different formulations. **p* 0.05 in comparison with the DNCB group; #*p* 0.05 in comparison with the DNCB+Protopic group. FK506 = tacrolimus; FK506–NIC = FK506 dissolved in 20% nicotinamide aqueous solution; FK506–NIC-CS-NPs = tacrolimus-loaded chitosan NPs containing nicotinamide; E+D, epidermis and dermis; SC = stratum corneum; C–0.01 = FK506–NIC-CS-NPs containing 20%, w/v NIC and 0.01%, w/v FK506; C–0.03 = FK506–NIC-CS-NPs containing 20%, w/v NIC and 0.03%, w/v FK506; C–0.05 = FK506–NIC-CS-NPs containing 20%, w/v NIC and 0.05%, w/v FK506; C–0.1 = FK506–NIC-CS-NPs containing 20%, w/v NIC and 0.1%, w/v FK506; DNCB = 1-chloro-2,4-dinitrobenzene. D) Schematic showing the carboxyl-modified gold NP (AuC)-stabilized liposome and its destabilization at acidic pH. Deprotonated AuC stabilizes liposomes at neutral pH, whereas at acidic pH AuC NPs become protonated and is detached from liposome and resulting in the formation of free liposome showing fusion activity. E) UV-visible spectra of AuC-liposome at pH 7 (red line) and pH 4 (black dashed line) after unbound AuC is removed. A clear UV spectrum at pH 7 indicates strong binding of deprotonated AuC on the surface of liposome whereas absence of spectra at pH 4 indicates detachment of protonated AuC. FRET technique used to study AuC-mediated liposome fusion at pH 7 and 4. In the experiment, a fluorescent donor (C6NBD) and a fluorescent quencher (DMPE-RhB) were incorporated in the anionic liposomes and then the FRET-labeled complex was mixed with AuC-stabilized cationic liposomes. F) Graph showing relative fusion potential of AuC cationic liposomes with anionic liposomes compared to AuB particles at pH 7 and 4. G) Schematic showing the formation of HC32-122/MCC7 polymeric NPs. Highly branched multifunctional poly (*β*-amino ester) HPAE = HC32−122; mincircle DNA encoding COL7A1 = MCC7. H) Immunofluorescence images of transfected RDEBK cells with polymeric NPs indicating high amount of collagen 7 (C7) expression around nucleus. I) qRT-PCR gene expression analysis of COL7A1 gene showing >4000-fold upregulation in NPs treated cells. J) Schematic showing the structure of APTstat3-9R and its formulation with DLNPs composed of long-chain phospholipid (DMPC) and short-chain phospholipid (DHPC). K) Morphological representation and histological examination of mouse ear skin post 6 days of transcutaneous delivery of DW, DLNPs, [APT]-DLNPs, or CLQ. Ctrl = Vaseline-applied mice; IMQ = imiquimod-applied mice; APT = APTstat3-9R; CLQ = clobetasol propionate cream. L) Graph showing

Staphylococcus epidermidis leading to excess inflammation of sebaceous glands.[172] Common topical medicines for the treatment include direct administration of antibiotic and retinoids, but these treatments lead to bacterial resistance and skin irritation. Oral administration of therapeutics is also not considered safe due to their severe systemic side effects. Retinoids reduce dyskeratosis and inflammation. Among retinoids, all *trans* retinoic acid or tretinoin are effective for mild to moderate acne treatment. The topical use of retinoic acid has frequent side effects which include erythema, light sensitivity, and skin irritation. NP preparations can reduce side effects, improve patient compliance and treatment outcome. In animal models, retinoic acid-loaded SLNs significantly reduce drug-induced irritation without compromising therapeutic efficiency in comparison to conventional formulations.[173] Furthermore, co-loading within LSN can combine the effects of anti-inflammatory tretinoin with antimicrobial chitosan. These NPs have high drug retention efficiency, high physical stability up to 1 year, cytocompatibility with keratinocytes, and effective bactericidal activity toward *S. Aureus* and *P. acnes* in vitro.[174] Tretinoin-loaded electrospun polycaprolactone nanofibers are a potential biodegradable anti-acne patch showing prolonged release of tretinoin and remarkable antimicrobial activity against *S. aureus*.[175] Adsorbing carboxyl-modified gold NPs to the outer surface of liposome regulates their fusion activity. The gold NPs prevent liposomes from fusing with each other at neutral pH. However, at acidic pH < 5 (pH of skin) the gold NPs detach from the liposomes and promote fusion. Fusion efficiency of gold NPs-stabilized cationic liposomes with anionic liposomes is 25% at neutral pH and interestingly 80% at pH 4 (Figure 3D–F),[140] illustrating a smart delivery approach for skin disease where pH is dysregulated.

Recessive dystrophic epidermolysis bullosa (RDEB): RDEB is an inherited skin disorder caused by the deficiency of functional type VII collagen at the basement membrane zone of the stratified epithelium, owing to mutations in the COL7A1 gene.[176] Patients with RDEB suffer from severe blistering and chronic wounds which may eventually lead to infection and development of lethal squamous cell carcinomas. At present, there is no cure for RDEB but palliative treatment can help and control symptoms. Treatments include surgery, medications (antibiotics like tetracycline, gentamicin, phenytoin and anti-inflammatory agents like H1 antihistamine, tacrolimus, morphine, codeine) and rehabilitation. Gene and cell therapies alongside protein replacement approaches are being investigated as curative treatments. One major hurdle for RDEB gene therapy is the complex delivery of the large COL7A1 gene encoding for type VII collagen. Preclinical studies addressed this challenge through intradermal injection of C7 expressing lentiviral vector and direct C7 protein delivery.[177] The development of the Moloney murine leukemia virus pLZRS harboring C7 gene represented a significant breakthrough as it allowed transducing keratinocytes in patients.[176] Cell therapies using autologous COL7A1 fibroblasts and keratinocytes have been developed. More advanced in vivo topical gene therapies aimed at developing safer approaches and preventing immune reaction are undergoing early phase clinical trials. These mainly include the use of retroviral, SIN-retro, SIN-lenti, and HSV vectors—the latter in cream formulation (Krystal biotech).[176,177] To this end, researchers are taking advantage of the unique properties of nanoscale therapeutics to negotiate the conventional barriers for gene therapy, including the instability of oligonucleotides in biological systems and the inefficient delivery of genetic material across the skin barrier. Recently, an efficient NP consisting of highly branched poly(β-amino ester)/*COL7A1* was used for gene delivery in RDEB keratinocytes. This method has high efficiency, biocompatibility, ease of manipulation, and long-term stability and is being regarded as a promising tool for RDEB treatment (Figure 3G–I).[141] The high efficiency of nanofibers and nanoneedles for biological delivery to the skin and their capacity to induce topical gene expression, represents a promise for the development of facile, and stable bandages capable of long-term topical gene therapy for RDEB.[66,114,115]

Psoriasis: Psoriasis is a persistent, recurring autoimmune disorder of the skin resulting in chronic inflammation where morphologically highly inflamed red erythematous plaques and scales present on the skin surface. Psoriasis induces epidermal hyperplasia and hyperkeratosis which make the skin very tough and hard.[178] Furthermore, increase of keratinocyte proliferation and change in the composition of lipid ceramides disrupt the formation of the SC. These changes further limit the penetration of drugs to the skin. Indeed, conventional therapies show low drug penetration, significant toxicity, and require high dosing frequency.[179] Phototherapy is a potential treatment which is not broadly adopted as it requires multiple sessions with associated low patient compliance and high costs.[179] For an efficient and improved treatment, it is important to design formulations with reduced cutaneous side effects, increased permeation through the skin surface, and minimal systemic side effects on kidney and liver, such as those induced by oral and injectable therapies. NPs provide a promising approach for its treatment. Corticosteroids are commonly used to treat psoriasis, and NPs can improve their delivery. Nanoemulsions loaded with combination clobetasol propionate (CP) and calcipotriol (CT) incorporated in Carbopol hydrogel can yield a drug concentration of 0.05% w/w (CP) and 0.005% w/w (CT). These particles reduce inflammation, erythema, and skin thickness in BALB/c mice model compared to a CP–CT gel.[180] Retinoids like tazarotene (topical) and acitretin (oral) are FDA approved drugs for psoriasis which inhibit inflammation and keratinocyte proliferation.[179] An acitretin-niosome-loaded gel improves the induction of orthokeratosis and reduces epithelial thickness compared to a commercial gel (Zarotex) and free acitretin in ex vivo skin models.[142,181] The niosome-loaded gel does not cause any skin irritation and dose requirement is substantially reduced. Owing to these advantages, noisome-based delivery is regarded as safe

clinical score of skin parameters including erythema, scaling, and induration at day 7. [APT]-DLNPs-treated mice shows comparable recovery in terms of skin inflammation with commercial CLQ treatment. M) Graph showing relative amounts of cytokines in the skin homogenates of mice. [APT]-DLNPs-treated mice shows substantial reduction in the secretion of pro-inflammatory cytokines. (A–C) Reproduced with permission.[139] Copyright 2018, Dove Medical Press; (D–F) Reproduced with permission.[140] Copyright 2010, American Chemical Society; (G–I) Reproduced with permission.[141] Copyright 2019, American Chemical Society; and (J–M) Reproduced with permission.[142] Copyright 2018, American Chemical Society.

dermatological practice for treating psoriasis. Small molecule, STAT3 inhibitor can treat psoriatic skin inflammation. Topical delivery of the high-affinity peptide specific for STAT3 (APTstat3) tagged with a 9-arginine cell-penetrating peptide (APTstat3-9R) reduces inflammation-driven disease-progression by modulating inhibition of STAT3 signaling in vivo. Furthermore, APTstat3-9R with specific lipid formulations known as discoidal lipid NPs (DLNPs), show efficient skin permeation of the peptide after topical delivery and thereby inhibiting psoriatic skin inflammation (Figure 3J–M).[142] NPs can also deliver gene therapy treatments for psoriasis. siRNA against DEFB4 gene complexed within ethosomes known as SECosomes can be applied on psoriatic skin, showing improvement in a mouse model.[182] Anti-EGFR siRNA conjugated to gold NP can treat psoriasis, resulting in a decreased T cell response at the site of administration.[183]

3.2. Mucosae

The total surface area of the human mucosae is nearly 400 m^2. Mucosae are distributed across the body where they mainly line the GI tract, the airways, and the genitals.[26,184] Although the morphology of mucous membranes at different sites varies, they all consist of a surface epithelial layer beneath which lies a deeper layer of connective tissues. Generally, the epithelial layer of the membrane comprises either a simple columnar epithelium or a stratified squamous epithelium with many goblet cells. Underneath the epithelium lies the basement membrane and a layer of loose connective tissue called the lamina propria, which vascularizes the epithelium. Often, a thin layer of smooth muscle, muscularis mucosa is also present to assist in the local movement and peristalsis of the mucosa. The mucosa is supported by the submucosa which is a dense, irregular layer of connective tissue consisting of many vascular capillaries. The mucosa is also a crucial interface between the organs and the microbiome and a new layer of mucous is formed daily to provide protection to the body.[185] The pH, ecology of microbes, thickness, viscoelastic properties, and composition of the mucus layer, and the distribution of blood vessels are specific to individual mucosae and can vary with age, sex, and disease condition.[186,187] Goblet cells within the mucosa are the source of the mucus, which is a gel-like substance with adhesive and elastic properties, developed to protect organs from ingress of foreign agents.[188] The thickness, clearance rate, and pH at different mucosal sites tightly govern the physical and chemical nature of the mucus. Mucus is rich in carbohydrates, lipids, proteins, salts, cellular debris, and bacteria. The main component of mucus is mucin which is a large mucopolysaccharide molecule with 3–10 nm diameter. Mucin fibers form when mucin monomers link by reversible disulfide bonds. This reversible crosslinking and interweaving of mucin fibers ultimately form the mucus layer.

Mucosal sites represent an effective drug delivery route due to their anatomy comprising a thin epithelium, a complex network of loose connective tissue and a rich blood supply.[189,190] Rapid drug action, high patient compliance, and reduced treatment time are key distinctive benefits of mucosal drug delivery. Yet, as described above, mucosae display a broad range physiological conditions which represent key limiting factors for successful drug delivery. The mucus layer is a phenomenally effective physiological barrier to drug delivery, capable of inactivating drugs, rapidly recycling them, and altogether preventing them from reaching the underlying tissues. Physiological barriers like differences in pH of fluids, thickness of mucosal layers, differences in blood connectivity, mucosal clearance rate, microflora, enzymes, changes in the volume and surface area of dissolution present within the mucosal routes pose obstacles to the efficient drug delivery to mucosal sites. *Mucoadhesion* and *Mucopenetration* are two central strategies used to negotiate mucosal barriers and enhance drug absorption. Indeed, nanoscale drug delivery systems can enhance these strategies to negotiate the mucus layer and improve the efficiency of treatments.

Mucoadhesion: Mucoadhesion is the attractive force between the surface of a mucus layer and a biomaterial. Mucoadhesive formulations can increase the residence time of drugs leading to reduced doses, improved bioavailability, sustained drug delivery, and ultimately enhanced therapeutic efficiency. The stability of the drug formulation is also very important as it diffuses through a thick mucus layer in order to reach the underlying cells. Nanofibers made from mucoadhesive polymers and nanoneedles are considered an ideal choice for transmucosal drug delivery due to their surface porosity, topology, and large surface area. NPs instead can be designed to exploit multiple mucoadhesive interactions. Positively charged particles interact with the negatively charged carboxyl and sulfate group of mucin while concentration gradients induce the diffusion of mucoadhesive polymers through the mucin fibers and noncovalent interactions (van der Waals, hydrogen, and hydrophobic bonding) can reinforce the adhesion. Chitosan exploits mucoadhesion through electrostatic interactions and can deliver drugs at several mucosal sites.[195,196] Particles synthesized from other commonly used polymers, such as PEG, carbopol (polyacrylic acid derived), poly(methacrylates), and poly(sebacic acid) can exploit mucoadhesion via hydrogen bonding, polymer entanglements with mucins, and hydrophobic interactions. In vitro PEGylation enhances chemical stability ninefold in simulated gastric fluid compared to naked polylactide particles.[197] "Hairy" silica microparticles, decorated with a mesh of silicon nanowires display prolonged retention at mucosal sites, by enhanced mucus adhesion and reduced sensitivity to peristaltic movements. They enhance the permeability of intestinal Caco-2 cell monolayers for fluorescein isothiocyanate (FITC) delivery. The increased permeability results from changes in the localization of ZO-1 and f-actin, the decrease of ZO-1 and claudin-1 at tight junctions, and an increase in PKC expression. The increase in permeability for cell monolayers is higher than that obtained with PEGylated particles.[198] Two important mucoadhesive nanomaterials currently under clinical investigation are Restasis (Carbopol-containing nanoemulsion) and Emend (nanocrystal particles with hydroxypropyl cellulose).[199] One key issue with mucoadhesion is that the constant clearance and renewal of the mucus ultimately reduces the retention time of the mucoadhesive substance, and biologicals like proteins and peptides are not able to reach the adherent cells in sufficient quantity. Mucopenetration is an attempt to overcome this limitation.

Mucopenetration: Mucus penetrating particles offer the advantage of reaching the underlying cells by penetrating the negatively charged mucus. Several agents can disrupt the mucus layer and decrease its viscosity. These mucolytic agents include pulmozyme, *N*-acetly-L-cysteine (NAC), nacystelyn, and methyl

6-thio-6-deoxy-a-D-galactopyranoside.[200,201] PEG can be covalently conjugated to polymers such as poly(lactic-*co*-glycolic acid) (PLGA-PEG) and polysebacic acid (PSA-PEG) to form a block copolymer, where PEGylation offers stronger mucus penetrating ability to the overall formulation. PLGA-PEG NPs provide excellent stability and release kinetics for several therapeutic agents including peptides and proteins.[202,203] PSA-PEG particles also show rapid penetration in samples of sputum from cystic fibrosis patients. Efficient partitioning of PEG on the surface of PSA helps the rapid transport of the complex through the mucus.[204] Indeed, mucopenetrating NPs are being developed for pulmonary administration of Ivacaftor in patients with cystic fibrosis. Ivacaftor can be loaded within PEGylated fluorescent NPs functionalized with the cell-penetrating Tat peptide which show high cytocompatibility toward human bronchial epithelial cells. The particles enhance the delivery of Ivacaftor to airway epithelial cells, owing to facile diffusion through the mucus and increase Ivacaftor cellular uptake. The Tat peptide strongly enhances uptake by lung epithelial cells.[205] A mucopenetrating biomimetic NP with charge reversal ability (P-R8-Pho NPs) is obtained by densely coating PLGA NPs with cationic octa-arginine (R8) peptide and specific anionic phosphoserine (Pho). The neutral surface (−2.39 mV) of this biomimetic NP shows rapid penetration through the mucus equivalent to mucopenetrating PEGylated NPs. Hydrolysis of anionic Pho induced by intestinal alkaline phosphatase exposes the R8 anionic peptide yielding positively charged NPs (+7.37 mV). This charge reversal promotes efficient cellular uptake mediated by cell-penetrating peptide (CPP) and transepithelial transport in vitro. P-R8-Pho NPs also show excellent stability in simulated gastrointestinal conditions and enhanced absorption in the intestine in vivo. In line with this, P-R8-Pho NPs enable the oral administration of insulin yielding a hypoglycemic effect and a 1.9-fold higher oral bioavailability in diabetic rats compared to single CPP-modified P-R8 NPs.[206]

Recurrent aphthous stomatitis (RAS): RAS is a common mouth disease characterized by the formation of ulcers, severe pain, and erythema.[207,208] T cell-mediated dysfunction is one of the crucial reasons for the destruction of the oral epithelium.[208–210] Due to a limited understanding of its etiology, treatment of RAS is palliative. Though definite prophylactic remedy is lacking, several antibacterial, antiacidic, and anti-inflammatory agents are available for its treatment.[208,211–214] Cyclosporine A (CsA) is a potent immunosuppressor used for treating RAS which exhibits a wide range of side effects including neuropathy, high blood pressure, and nephrotoxicity. Due to the low solubility of the drug in water, few topical-based delivery methods exist.[208,215] Complexing CsA with SLNs enhances its bioavailability and reduces side effect for oral RAS treatment. Carbopol 974 P gels containing SLNs loaded with CsA applied on ulcers of the oral mucosa in vivo show good adhesion, a significant acceleration of wound healing, and a quick reduction of the size of ulcers.[208] Niosomes can enhance targeted delivery, improve drug therapeutic indices and oral bioavailability.[216–218] A clinical study of RAS treatment with propolis extract in niosomal oromucoadhesive films shows satisfactory clearance of mouth ulcers, prolonged retention of the drug accompanied with increased patient acceptance and compliance compared to other conventional dosage of propolis in the form of suspensions, pastes, tinctures, and powder.[219]

Periodontitis: Periodontitis is a very common oral disease characterized by the inflammation of the gums. Uncontrolled bacterial infection is responsible for aberrant inflammatory response that ultimately leads to disruption of the periodontal ligament and loosening/loss of teeth.[220,221] One main line of treatment for periodontitis is mechanical root debridement and reduction of subgingival bacterial colonies.[222] Yet, this mechanical method does not completely remove bacterial colonies from the site of treatment, and antibacterial agents like minocycline and immune modulators are used to improve treatment outcomes.[223,224] The topical delivery of minocycline within the periodontal tissues relies on several formulations including ointments,[225] gels, liposomes,[226] and microspheres.[227] Chitosan-based NPs can sustain the delivery of minocycline yielding high drug concentrations for a prolonged period of time. This approach improves access to intracellular bacterial reservoirs while maintaining low plasma concentrations of the drug.[228] NPs obtained by doping cerium (Ce) into zeolitic imidazolate framework-8 (ZIF-8) show both antibacterial and anti-inflammatory effects in vitro. The anti-inflammatory activity of ZIF-8:Ce is proportional to Ce doping concentration (**Figure 4**A–D).[191] AuNPs exhibit significant anti-inflammatory effects in the periodontal microenvironment of a ligature-induced periodontitis rat model. They also induce M2 polarization of macrophages and affect the differentiation of human periodontal ligament cells (hPDLCs). The treatment significantly promotes the growth of new bone and cementum, and restores periodontal attachment in the lesion area while limiting further tissue destruction and slowing disease progression (Figure 4E–I).[192] Nanohydroxyapatite (nHA) is a biocompatible, osteoinductive, and resorbable material broadly used for periodontal tissue regeneration.[229,230] Composite materials including nHA alongside synthetic polymers, such as polylactic acid (PLA), PLGA, polyamide, chitosan, and PCL retain the bioactivity of nHA while providing superior mechanical properties.[231–234] Mouthwash solutions containing NPs like TiO_2 show superior antibacterial response.[235] NPs of sodium fluoride and fluoride-based varnishes also have antibacterial effects[236] while silver-based NPs are included in toothbrushes to control periodontal pathogens.[236,237]

Leukoplakia: Leukoplakia is a precancerous lesion characterized by white patches of the oral mucosa. Risk factors of the disease include over use of chewing tobacco and association with human papillomavirus (HPV).[238] Currently, the attention is highly focused on identifying and targeting agents that indicate specific steps in the molecular progression of oral leukoplakia. Two key molecular targets in clinical trials are cyclooxygenase (COX)-2 inhibitors and epidermal growth factor receptor (EGFR) inhibitors.[239,240] Chitosan nanogels can deliver celecoxib, a COX-2 inhibitor, for oral cancer chemoprevention in vitro.[241] Addition of the nonionic surfactant laurocapram (Azone) to the nanoformulation enhances penetration through the buccal mucosa.[242] This strategy increases the uptake of the drug and prolongs its retention. A mucoadhesive gel made from Gelucire 50/13 and PX407 loaded with curcumin formulated with SLNs (CuSLNs) is being developed for leukoplakia. This semi-solid delivery system helps in maintaining drug stability and increases the uptake of curcumin compared to CuSLNs alone. It also improves efficacy and compliance for the treatment of precancerous oral lesions in a clinical study.[243]

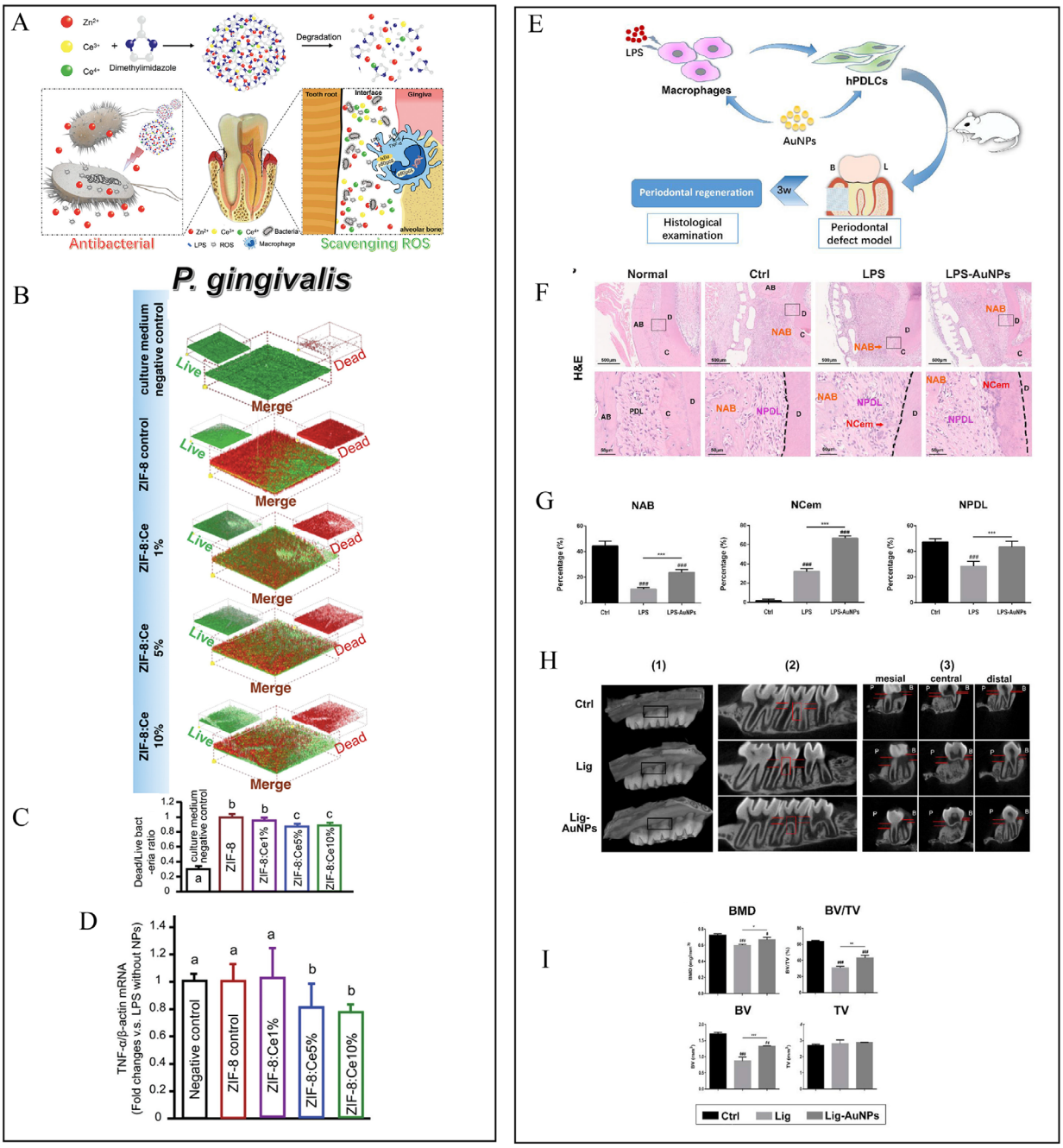

Figure 4. Nanomaterials for drug delivery to the oral mucosa. A) Schematic diagram of the use of ZIF-8:Ce NPs and their antibacterial and anti-inflammatory properties. Antibacterial effect of NPs depends on the release of ROS and its anti-inflammatory effect is due to inactivation inhibition of NF-kB/p65 subunit translocation and thereby promoting M2 phenotype in macrophages. B) Antibacterial assay using ZIF-8 and ZIF-8:Ce NPs. 3D live/dead images of biofilms for *P. gingivalis* with 30 mg mL^{-1} ZIF-8 or ZIF-8:Ce NPs. Green color represents live bacteria, red color represents dead bacteria. C) Graph showing dead/live ratios of *P. gingivalis*. ZIF-8 and ZIF-8:Ce1% groups show mainly dead bacteria. D) qRT-PCR-based gene expression analysis showing downregulation of the key pro-inflammatory gene TNF using ZIF-8:Ce NPs. Use of NPs was seen to promote M2 polarization of macrophages. Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$). E) Schematic diagram of rat periodontal fenestration experiment. hPDLCs in AuNPs-modulated inflammatory macrophage-hPDLCs were used for the experiment. B and L is represented as buccal side and lingual side, respectively. F) H&E staining of rat teeth after 3 weeks of treatment. AB = alveolar bone; C = cementum; D = Dentin; PDL = periodontal ligament; NPDL = newly formed periodontal ligament; NAB = newly formed alveolar bone; NCem = newly formed cementum. G) Graph showing the quantitative analysis of newly regenerated alveolar bone, cementum, and periodontal ligament. ###$p < 0.001$, compared with the Ctrl group. ***$p < 0.001$. H) 3D images taken by Micro-CT of mesio-distal section and bucco-palatal section of maxillary second molars. I) Graph showing different bone-related parameters. BMD, bone mineral density; BV/TV, bone volume/tissue volume. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared with the Ctrl group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. B/P is the central site of buccal palatal side. A–D) Reproduced with permission.[191] Copyright 2019, RSC. E–I) Reproduced under the terms of the CC-BY license.[192] Copyright 2019, Elsevier.

Inflammatory bowel disease (IBD): IBD is a complex disorder characterized by chronic relapsing inflammation of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the two common clinical forms of IBD.[244] The exact etiology of the disease is not well understood and treatment relies on anti-inflammatory drugs. Targeting oral drugs to the colon for treatment is a major challenge as they have to cross biochemical, physiological and environmental barriers. MSNs coated with ϵ-polylysine can deliver prednisolone (Pred) in a pH-responsive manner to colonic cell lines including LS 174T and Caco-2 and RAW 264.7 macrophages.[245] The NPs preferentially release the drug at the alkaline pH of colon rather than in the acidic environments of the stomach and small intestine. A crosslinked chitosan hydrogel can deliver the anti-inflammatory peptide Lys-Pro-Val (KPV) to the colon of mice. The hydrogel protects the peptide from the physiological conditions of stomach and small intestine and promotes specific targeting to the inflamed colon. The nanogel formulation of KPV reduces intestinal inflammation more than >12 000-fold compared to KPV in solution.[246] Oral administration of poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT) NPs carrying siRNA to silence TNF-α protects from developing a UC-like phenotype in mice. PPADT polymer selectively degrades in response to reactive oxygen species (ROS) and efficiently delivers siRNA to the inflamed intestinal environment with abnormally high levels of ROS.[247] PEGylated rosmarinic acid-derived NPs (RANPs) can contribute to IBD treatment. RA is polyphenol-based compound with anti-inflammatory, anticancer, and antibacterial activities. Localized administration of RANPs to the inflamed colon of colitis mice substantially curbs colonic inflammation, and reduces disease activity compared to only RA treatment. Furthermore, dexamethasone-loaded RANPs enhance the recovery in mice compared to equivalent dose RANPs (**Figure 5**A–F).[193]

Sexually transmitted diseases (STD): STD comprises a wide range of conditions where infection occurs during unprotected sexual contact. A common site of infection is the vaginal mucosa. Common STDs range from viral infections (HIV, HPV, hepatitis, and herpes) to bacterial infections (gonorrhea, syphilis), fungal infection (chlamydia), and parasitic infection (trichomoniasis).[248] The vaginal mucosa has a great potential for the topical treatment of STDs due to its accessibility. Yet, vaginal delivery presents several challenges owing to the variable nature of its epithelium and mucus across the population due to age, menstrual cycle, and sexual activity. The vagina is a S-shaped tube composed of an outer epithelial layer underlain by lamina propria, muscles, and areolar connective tissue.[249,250] Its abundant vascular network offers a distinct advantage for direct drug delivery. Different strategies used for the local treatment of STD include mucus penetrating particles, NP-derived formulations for sustained release of drugs, pH-responsive drug delivery, and targeted intracellular delivery of loaded NPs.[250] Mucus penetrating PEGylated liposomes containing the antiviral agent interferon alpha-2b (IFN α-2b) can contribute to vaginal therapy of HPV. PEGylated liposomes show increased IFN α-2b penetration ex vivo due to improved access to the epithelium.[251] This system is promising in topical delivery of IFN α-2b with enhanced efficacy of local antiviral therapy. Furthermore, zinc oxide tetrapod NPs (ZOTEN) show strong antiviral and immunomodulatory efficacy against herpes simplex virus-2 (HSV-2) in mice. Incubation HSV-2 with ZOTEN protects the mice from viral infection in the vaginal tissue. ZOTEN-neutralized viral particle elicits local immune response comparable to HSV-2 infection but reduces inflammation and severity of the disease highlighting the potential of ZOTEN as a platform to develop live virus vaccines (Figure 5G–J).[194]

Vaginal HIV transmission is a major route to new infections worldwide. To develop an effective preventative treatment, there is a need for long-acting antiretroviral drug (ARV) formulations that can reduce the frequency of application by sustaining the release of the drug and maintaining its stability. One such formulation is topically applied ARV rilpivirine, encapsulated in PLGA NPs and delivered in a thermosensitive pluronic acid gel in the vagina of BLT humanized mice.[252] This formulation offers significant protection to female mice against vaginal high-dose of HIV-1. PLGA NPs assembled within polymeric films can deliver ARV including tenofovir (TFV) and efavirenz (EFV) to the HIV-1-infected vagina of CD-1 mice. The addition of NPs to the film provides an enhanced local pharmacokinetics of EFV. Systemic exposure to both TFV and EFV remains low, thus preventing adverse toxic effects.[253] A pH-responsive polyurethane (PU) electrospun nanofiber can mediate the pH-dependent release of siRNA targeting the HIV-1 co-receptor CCR5 siRNA encapsulated within SLNs. The penetration efficacy of CCR5 siRNA-encapsulating SLNs is >twofold at pH 7.0 (semen-neutralized pH) as compared to pH 4.5 (basal vaginal pH) highlighting a potential for smart delivery upon stimulation. These membranes can also serve as long-term depots for the pH-mediated smart release of NPs.[254]

Protozoan infections caused by *Trichomonas vaginalis* can be locally treated with a thermoresponsive NP-hydrogel composite system. The antirheumatic drug, auranofin (AF), has significant trichomonacidal activity, but its oral delivery induces several adverse effects due to long plasma half-life.[255,256] AF-loaded NPs (PLGA functionalized with PEG) embedded into a chitosan-based hydrogel matrix can be used for intravaginal administration in BALB/c mice. This method has excellent NPs retention in the vaginal tissue (>6 h) and also remarkably increases local AF levels. Importantly, this topical administration reduces plasma and liver levels of AF compared to oral treatment. Overall intravaginal administration of AF-NP outperforms oral delivery when treating vaginal trichomonad and does not induce toxicity at the local or systemic level.[256] *Chlamydia* infection of vaginal epithelial cells can be addressed using poly(ethylenimine)-condensed PDGFR-β siRNA within PLGA-PEG diblock copolymer NPs. This formulation can prevent the acquisition and reoccurrence of the infection by inducing autophagy in the epithelial cells.[257]

3.3. Eye

The eye is a complex and sensitive organ, allowing vision of the external world. Its immunoprivileged nature and anatomical localization make it an easy target for topical treatments, even though its defensive barriers often hamper drug delivery. Based on the drug routes of administration, we can divide the eye in two main sections: the front part, including the cornea, conjunctiva, trabecular meshwork, iris, and ciliary body, often treated by topical drugs or subconjunctival injections and the back of the

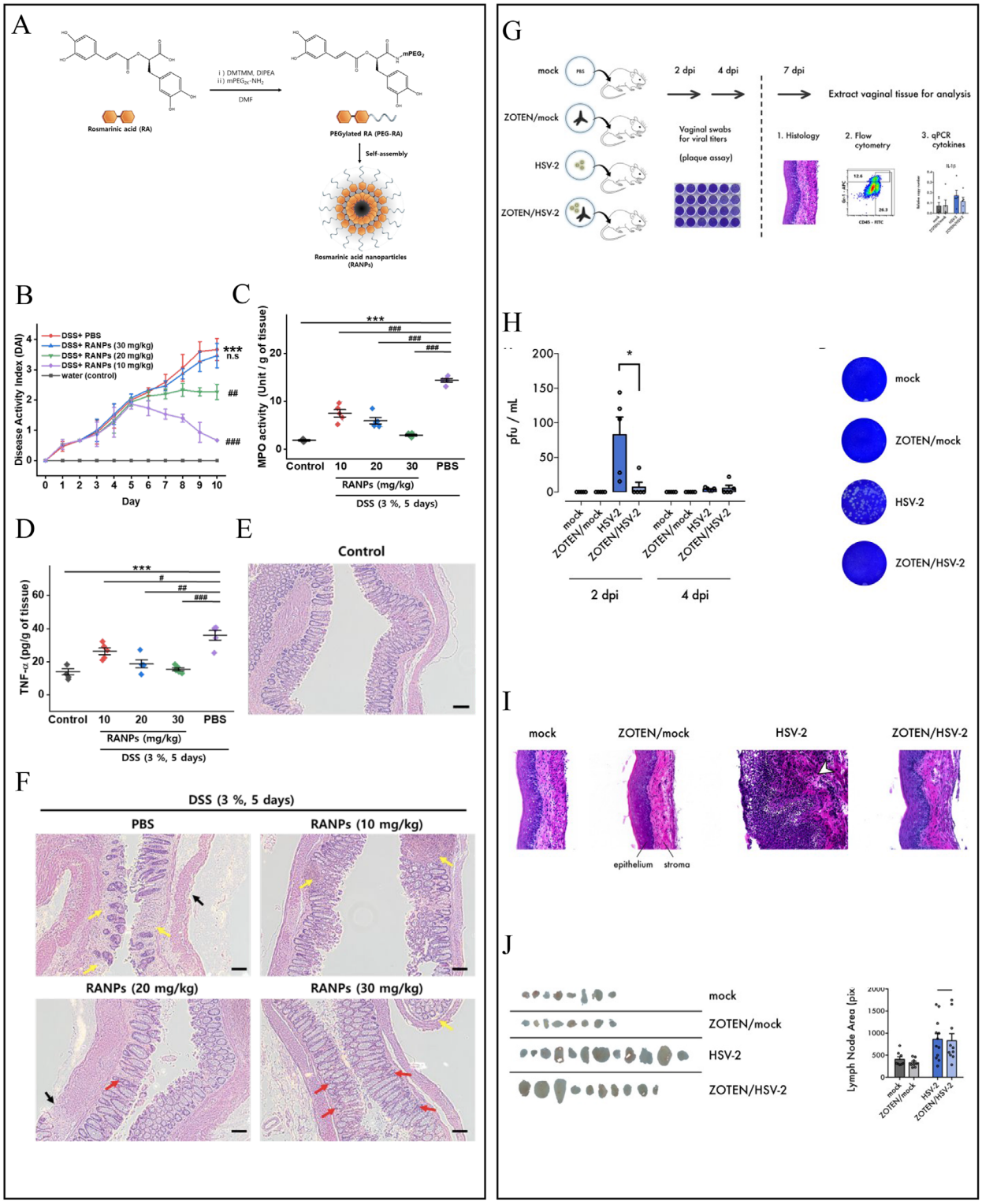

Figure 5. Nanomaterials for drug delivery to intestinal and vaginal mucosa. A) Schematic showing the synthesis of PEG-RA and characterization of RANPs. B–D) Therapeutic and molecular effects of RANPs in DSS-induced colitis mice model with reduced B) DAI, C) MPO activity, and D) TNF-α level in mouse colon tissue. DAI—disease activity index, MPO–myeloperoxidase activity. Values are means ± SD ($n = 5$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs the control group' #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs DSS-induced colitis group). E,F) Representative images of H and E showing reduced inflammation in mice colon treated with RANPs at different concentration. Epithelial ulceration (black arrow), severe edema/inflammation (yellow arrow), and retention/regeneration of crypts (red arrow). G) Schematic showing the experimental design for in vivo infection and analysis. Female BALB/c mice used in the experiment were infected with HSV-2 or mock infected in the presence or absence of ZOTEN. H) ZOTEN treatment

eye, from the lens to the retina, mainly reached by intravitreal injections. All these delivery approaches can benefit from better, less invasive, longer lasting, and patient friendly formulation to improve eye treatments. Key parameters like bioavailability, drug elimination, dosing frequency, and stability of the drug need to be carefully considered when designing ocular drug approaches. Indeed, NP formulations can contribute to address these conflicting requirements.

3.1.1. Front of the Eye

To date, the majority of ocular medications are administered topically (as eye drops, suspensions, or emulsions) to the optical surface, where they encounter and homogenize with the lacrimal fluid, a thin film composed of multiple layers: a lipid, an aqueous, and a gel layer, the latest two containing mucins. The lacrimal film lubricates the cornea and the conjunctiva. The cornea is composed of three overlapping cellular layers: the external epithelium, the stroma, and the inner endothelium. The conjunctiva is composed of a less tightened external epithelium and a connective stroma, it covers the anterior sclera (bulbar conjunctiva) and lines the inside or eyelids (palpebral conjunctiva). Through the corneal and conjunctival route, topical drugs can reach the trabecular meshwork, iris, or ciliary body.

Topical delivery is an easy and minimally invasive procedure to treat diseases affecting the anterior segment of the eye, including dry eye syndrome, conjunctivitis, keratitis, multiple corneal dystrophies, and elevated intraocular pressure (IOP). Among these, fluctuating IOP is regarded as a major risk factor in patients suffering from glaucoma. Better detection and monitoring system are being developed for glaucoma patients. Sensimed Triggerfish contact lens sensor (CLS), is an efficient, safe, and FDA-approved system for detecting intraocular pressure-related changes in the eye over a 24 h period.[261] Topical absorption of ophthalmic formulations is limited by several factors: first, the tight junctions between corneal epithelial cells represent the most important barrier. Second, palpebral and bulbar conjunctiva, differently from the cornea, are permeated by blood and lymphatic vessels, both contributing significantly to the drug clearance from the conjunctiva. Moreover, the blood circulation determines a partial systemic absorption of the drug from the eye, especially when blinking, increasing the risk of side effects.[262,263] Conjunctival circulation and lymphatic vessels are responsible for clearance of either small NPs or macromolecules such as albumin. Finally, metabolic enzymes in lacrimal film can degrade the drug while high tear turnover rate (1 μL mL^{-1}) and reflex tear production let most of the drug drain away from the ocular surface before reaching the target, especially large molecules such as nucleic acids.[264] All those physiological and anatomical barriers reduce conventional nonviscous eye drops retention time into the tear film (volume $\sim$ 7 μL) to 1–3 min, determining a meagre drug bioavailability of 1–3%.[265] Only drugs in their released or dissolved form can be absorbed into the eye through passive diffusion (trans- or para-cellular way) following the concentration gradient of the drug.[262] If the vast majority of drug is drained away before it is released in the eye, a substantial fraction of its bioavailability is lost, potentially reducing the effective dose below therapeutic levels. This rapid drug loss requires increasing the dosing of formulations and frequency of administration, which can lead to a reduced patient compliance.

For those reasons, in recent years drug retention time and bioavailability following topical instillation have been extensively studied and improved with different strategies. One approach consists in formulating the drug as a prodrug that becomes active into the target tissue once cleaved by the cellular enzymes such as esterases, hydrolases, and aminopeptidases, which are abundant in the ocular tissues. The cornea is relatively impermeable due to its cellular barriers, in particular the corneal epithelium and stroma. Those layers limit the absorption of many large lipophilic and hydrophilic drugs. Corneal epithelial tight junctions limit the absorption of hydrophilic drugs through the paracellular route, while the hydrated structure of the stroma represents a barrier to the lipophilic drugs. However, small lipophilic drugs can pass through the transcellular pathway while small hydrophilic drugs are generally absorbed through the paracellular pathway.[266,267] In prodrugs, the physiochemical properties of the drug are modified to enhance its permeability and reach the target starting from a topical administration.[264,268]

Another approach consists in increasing the viscosity of the formulation to prolong drug retention on the ocular surface. Examples are hydrophilic mucoadhesive polymers like PEG or chitosan that can bind mucins of the tear film by hydrogen bonds and/or electrostatic interactions and thus prolong the duration of drug action to the ocular surface, but cannot control drug release. On the contrary, liposomal and suspension formulations, although presenting a slow and controlled drug release, display a shorter preocular contacts, which still limits bioavailability.[262,269] Silicone hydrogel soft contact lens are effective for improving the ocular bioavailability of ophthalmic drugs. Silicon hydrogels show diffusion-limited transport of ophthalmic drugs and, depending upon their composition, can sustain release from 20 days to several months. Their composition can be easily tuned to combine sustained drug release with desirable properties for lens application like ion permeability, degree of hydration, transparency, and wettability.[270] Further methods used to increase drug availability include the insertion of punctum plugs into the lacrimal ducts to block the tear drainage or a mild electric field applied to the front of the eye to create temporary pores in the ocular tissue and allow drug entry (iontophoresis).[264]

Large therapeutic agents like hydrophilic oligonucleotides, often representing the only possibility to treat genetic diseases like a wide spectrum of corneal dystrophies, are the most challenging to deliver into the eye. The negative charge of nucleic acids needs

leads to reduced viral shedding as indicated by plaque assay result and images of crystal violet stained plaque assay. Vaginal swabs were taken after 2 and 4 days of infection. *$p < 0.05$, ns, or unlabeled, not significant. I) H&E staining of vaginal tissue section after 7 days of infection. J) Images of lymph nodes of mice and their size quantification at right showing increase in the size of node after ZOTEN treatment, indicating large number of immune cells activation to control HSV-2. *$p < 0.05$, ns, or unlabeled, not significant. A–F) Reproduced with permission.[193] Copyright 2020, American Chemical Society. G–J) Reproduced under the terms of the Creative Commons Attribution License (CC BY).[194] Copyright 2019, The authors.

to be shielded with a positively charged carrier that can interact also with the negatively charged ocular epithelium and mucus. An innovative approach involves using cationic polymers and, among them, cell penetrating peptides (CPPs) represent a versatile option.[271,272]

Actually, an important parameter to facilitate drug penetration is the dimension of the carrier and, since it was found that particle sizes should not exceed 10 μm in diameter, nanotechnological formulation for ocular treatments shows promising increasing efficacy, avoiding eye irritation and reflex tearing.[273]

Nanocarriers including NPs, nanomicelles, nanowafers, and nanoneedles can be loaded with small molecules and biologicals for delivery to the eye. These nanocarriers are obtained from biocompatible and biodegradable material such as chitosan, albumin, PEG but also inorganic materials, thus increasing eye tolerability and allowing a deeper penetration into the ocular tissues. Drug penetration is further increased by positively charged nanocarriers that better interact with the negatively charged ocular surface. The ophthalmic nanomicellar solution of Cyclosporine-A (Cequa) is the first nanotechnological treatment approved by FDA for dry eye syndrome in 2018.[264,274]

However, without mucoadhesion, NPs can be drained away, while gels, contact lenses, and solid inserts can be retained. A combination of drug-loaded NPs and one of these devices can prolong the retention time to the ocular surface.[262]

Gelatin-covered MSNs (p/GM) loaded with pilocarpine administered into the anterior chamber of the rabbit eye are effecting in reducing glaucoma high IOP.[275] Contact lenses soaked or loaded with NPs represent a novel approach for increasing drug bioavailability in the eye. The prolonged wearing of contact lenses increases drug residence time, avoiding drug clearance through the tear fluids and consequently improving bioavailability up to 50%. Timolol-loaded NPs dispersed in contact lenses release the drug for up to 1 month of lens wearing, effectively reducing IOP.[265] This slow and extended release of timolol from contact lenses along the concentration gradient, favored the pharmacodynamics effect in reducing IOP. Solid Implants allow a controlled drug release and can be surgically inserted sub-conjunctivally, in the aqueous humor, in the episcleral region or introduced into the *cul-de-sac* of the eye, a pocket depression at the junction of bulbar and palpebral conjunctiva.[264] Cyclosporine-A-loaded PLGA or PCL (poly(ϵ-caprolactone)) NPs incorporated into a fiber composite system persist in the cornea, sclera, and lens up to 90 days following subconjunctival implantation into dry eye induced mice.[276]

Other approaches to control drug release include NPs designed for controlled delivery of betaxolol hydrochloride (BH) for glaucoma treatment. In this topical ophthalmic approach, BH is intercalated into the interlayer gallery of Na-montmorillonite (Na+Mt) and then encased within chitosan NPs (CS). While the BH solution complete its release in 2 h in vitro, the composite BH-Mt CS shows an initial burst release (4 h) with a following prolonged release (10 h). The overall release of BH is extended by Mt mediated swelling of chitosan NPs in a human corneal cell line, while in an in vivo rabbit model of glaucoma, the interactions between the negative charge of the mucin in the cornea and the positive charge of the nanocarrier sustain precorneal retention of the NPs (**Figure 6**A–C).[258]

Lipid-based particulate systems like SLNs and NLCs loaded with the NSAID drug indomethacin from eye drop solution can release drugs across the cornea as well as sclera-choroid-RPE singularly excised tissues ex vivo. Furthermore, surface modification of SLNs with chitosan substantially increases the penetration of indomethacin in rabbit ocular tissues.[277]

Although several novel strategies have been developed to prolong retention time, subconjunctival injections, used in clinical practice for the delivery of local anesthetics and anti-inflammatory drugs, are still considered the method of choice to circumvent ocular washout. Subconjunctival delivery is an invasive procedure requiring specialized ophthalmologist for administration and is used to treat severe eye conditions requiring high drug concentrations, mainly affecting the anterior segment. Subconjunctival injection of dendrimeric NPs loaded with the anticancer drug carboplatin effectively reduces tumor burden in mice without toxic effects,[278] while subconjunctival delivery of thermosensitive hydrogel PLGA-PEG-PLGA NPs can enhance the efficacy and retention of RNA in the mouse eye.[279]

However, since a minimal dose can reach the posterior eye, this approach has been investigated as a potential alternative therapy to reach the retina bypassing the epithelial barriers. Subconjunctival injection of photoreceptor-binding upconversion NPs (pbUCNPs) increased the vision spectrum beyond 700 nm in mammals. These NPs attach to retinal photoreceptors, where they convert near-infrared (NIR) radiation to visible light, allowing mice with this modification to identify NIR light patterns (Figure 6D–F). This approach can augment the vision spectrum of humans and explore animal-light-dependent behaviors.[259]

3.1.2. Back of the Eye

Topical delivery mainly allows drug to reach the front of the eye but it cannot cross the vitreous and reach the retina because it is limited by the lenticular barrier, the blood flow of the iris–ciliary body, and the humor aqueous turnover.[262]

Preclinical success of topically administered drugs reaching the back of the eye at a therapeutic dose has been mainly obtained in small animal models, using liposomes, permeability enhancers, or CPPs.[280] Topical administration reached the retina using, e.g., neutral, submicronsized liposomes (ssLips), 90–110 nm in size[281] or annexin A5-associated liposomes loaded with bevacizumab.[282]

The posterior eye segment is thus generally reached by intravitreal injections. However, drugs remain modestly bound to the vitreous, an isotonic clear gel composed of type-II collagen, hyaluronic acid, proteoglycans, and some hyalocyte cells. The low drug retention is due to the low concentrations of free proteins into the vitreous. Intravitreal drugs are eliminated through the anterior or posterior routes. The posterior route passes across retinal blood barriers and the iris–ciliary body (elimination within few hours) while the anterior route moves toward the aqueous humor to the trabecular meshwork (within days).

The leading causes of vision loss affecting the posterior segment of the eye are age-related macular degeneration (AMD), diabetic retinopathy, and diabetic macular edema (DME). These pathologies are commonly treated by intravitreal injections of anti-VEGF antibodies (AMD and diabetic retinopathy) or

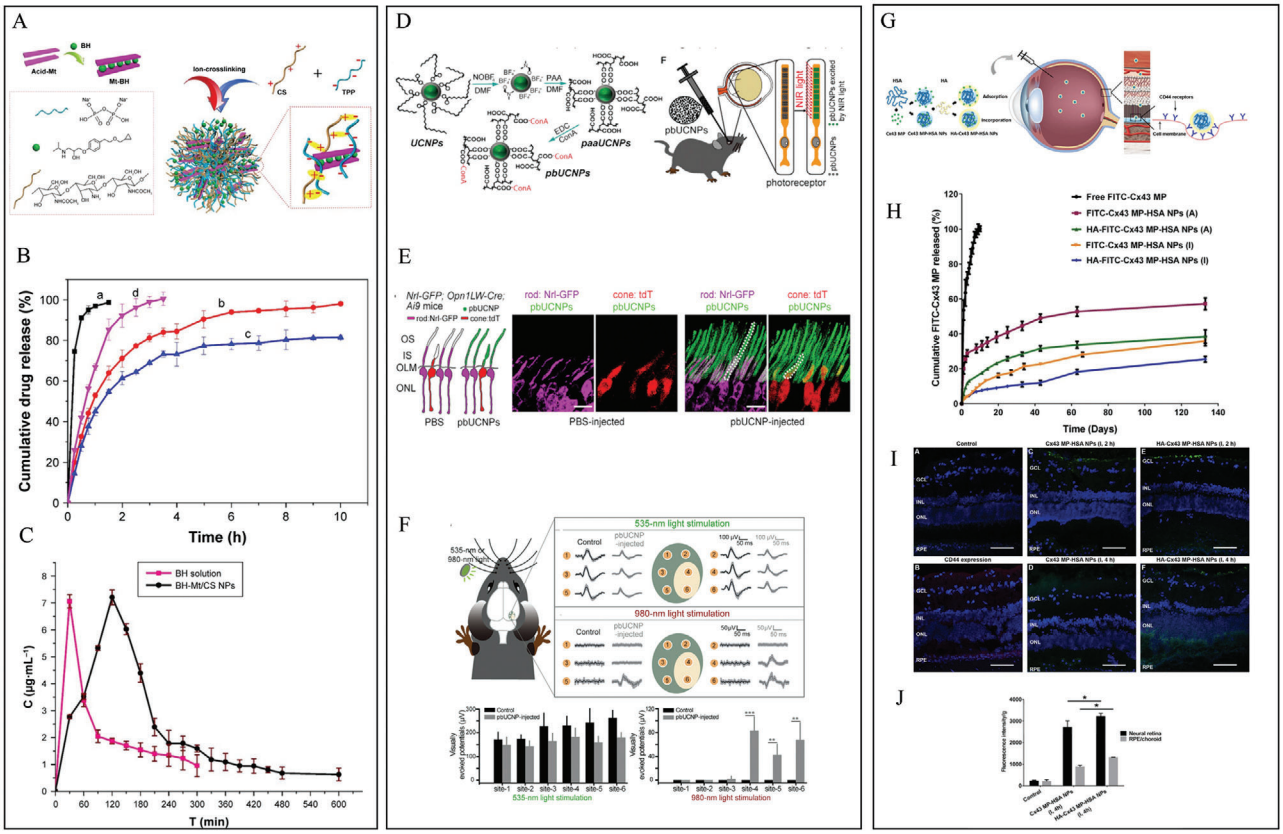

Figure 6. Nanomaterials for drug delivery to the eye. A) Schematic diagram showing the process of formulation of BH-Mt/CS NPs, delivered topically. BH, betaxolol hydrochloride; Mt, montmorillonite; CS, chitosan; TPP, tripolyphosphate. B) Graph showing cumulative in vitro release of BH from various formulations. a) BH solution, b) BH-CS NPs, c) BH-Mt/CS NPs, and d) BH-Mt. Maximum release is seen in BH-Mt/CS NPs (10 h). C) Graph showing increased concentration of BH in rabbit aqueous humor using BH-Mt/CS NPs compared to free BH solution administration. D) Schematic showing the surface modification procedure for ConA-functionalized photoreceptor-binding UCNPs (pbUCNPs) and sub-retinal injection of pbUNCNPs in mice. ConA, concanavalin A protein; UCNPs, core–shell-structured upconversion NPs consisting of 38 ± 2 nm β-$NaYF_4$:20%Yb, 2%Er@β-$NaYF_4$. E) Schematic showing distribution of pbUCNPs (green) in the retina. Rod cells are labeled with Nrl-GFP in pseudo color violet, cone cells are labeled with Opn1LW-Cre; Ai9-lsl-tdTomato in pseudo color red. OS, outer segment of photoreceptors; IS, inner segment of photoreceptors; OLM, outer limiting membrane; ONL, outer nuclear layer. Right panel shows fluorescence images of retina of mice treated with PBS and pbUCNP-injected mice. Rod and cone are shown in dashed contour lines. F) NIR-activated imaging experiment in pbUCNP-injected mice. Upper panel shows six recording sites for visually evoked potentials (VEPs) in mouse visual cortex. VEPs of control mice (black traces) and treated mice (gray traces) are shown under 535 and 980 nm. Lower panel shows peak VEPs triggered by 535 and 980 nm light at each recording site (mean ± SD, $n = 4$ for both, two-sided t-test, $^{**}p < 0.01$, $^{***}p < 0.001$). G) Schematic showing the formation of Cx43 peptide loading into hyaluronic acid (HA)-coated human serum albumin NPs (HSA NPs) and its targeted delivery to the retina. H) Graph showing in vitro drug release of uncoated and HA-coated FITC Cx43 MP HSA by adsorption (A) and incorporation (I). I) Fluorescence images of ex vivo retinal tissue, C,D) of uncoated FITC Cx43 MP-HAS NPs and E,F) of HA-coated FITC Cx43 MP-HAS NPs. NPs are represented in green, CD44 is red, and nucleus is stained with blue (DAPI). Scale bar = 100 μm. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; and RPE, retinal pigment epithelium. J) Graph showing quantitative analysis of green fluorescence per gram of neural retina and RPE/choroid after 4 h incubation. (A–C) Reproduced with permission.[258] Copyright 2018, Dove Medical Press. (D–F) Reproduced with permission.[259] Copyright 2019, Elsevier. (G–J) Reproduced with permission.[260] Copyright 2016, American Chemical Society.

corticosteroids (mainly DME). However, intravitreal injections are highly invasive, expensive, requiring frequent administration (once per month for anti-VEGF) from specialized clinicians and present several risks including subconjunctival hemorrhage (10% of injections), infections,[283] or cataract following intravitreal injections of corticosteroids.[284]

A long-release formulation that would increase drug bioavailability is fundamental to overcome those issues related to a frequent administration into the vitreous. Since drug particle size is important both for retinal penetration and light scattering, which can lead to vision problems, nanomaterials have recently been explored as a promising approach to prolong the retention time and improve drug delivery to the retina. In particular, negatively charged and neutral nanostructures diffuse more rapidly into the vitreous than cationic ones that binds intravitreal hyaluronic acids.[262]

Emerging approaches include hyaluronic acid-coated albumin NPs for the delivery of connexin43 mimetic peptide (Cx43 M), able to block the Cx43 uncontrolled hemichannel opening thus preventing retinal degeneration.[260] In a rat model of retinal ischemia-reperfusion injury, this approach shows a rapid diffusion of the peptide through the vitreous humor alongside its sustained release to the retinal cells compared to free Cx43 MP. Cx43 MP-loaded NPs efficiently lead to reduction in

inflammation and overall improvement of the symptoms of AMD in vivo (Figure 6G–J).

Albumin-coated PLGA NPs, loaded with bevacizumab, an anti-VEGF antibody, and injected into rabbit vitreous, maintain drug activity and concentration above 500 ng mL^{-1} for 2 months as compared with the 2 weeks of bevacizumab solution alone.[285]

Moreover, bevacizumab loaded within PEG (b-PEG) and PLGA (b-PLGA) NPs can contribute to the treatment of choroidal neovascularization (CNV). Laser photocoagulation induces the breaking of Bruch's membrane followed by the intravitreal delivery of NPs in rat eyes. b-PEG and b-PLGA treatments reduce CNV symptoms by 9% and 20.3% compared to free bevacizumab.[286]

Porous silicon (pSi) particles for the dual delivery of daunorubicin (DNR) and dexamethasone (DEX) are being evaluated for the treatment of chorioretinal diseases intravitreally. These agents reduce inflammation and excess proliferation, respectively. Strikingly, pSi delivery produces therapeutic concentration of DNR for up to 100 days and DEX for about 5 months with a single intravitreal injection in a rabbit eye model. Furthermore, the photonic properties of pSi enable noninvasive real-time monitoring of drug release and NP bioresorption.[287]

Long-term delivery of intravitreal nanocarriers can combine into composite systems, hydrogel or intravitreal implants. Composite systems are obtained introducing particles within other particles or gels as, e.g., by filling porous PLGA microparticles with PLA NPs loaded with bevacizumab. This system shows sustained release for 2 months upon intravitreal injection.[288] Silk hydrogels that avoid PLGA hydrogel acidic degradation, loaded with anti-VEGF drugs demonstrate a sustained drug release after intravitreal injection for more than 3 months both in vitro and in vivo (rabbits).[289] Finally, to date, there are two implants for corticosteroid-sustained release approved by FDA: Retisert, a nonbiodegradable implant releasing corticosteroids up to 3 years, although presenting side effects as elevated IOP and cataract, and Ozurdex, a biodegradable PLGA implant delivering dexametasone up to 6 months. This time can be further increased using the novel refillable implants.[290]

Nanocarriers, given their unique characteristics as high tissue penetration potential, low toxicity, biocompatibility, and biodegradability hold great promise for the next-generation therapeutics applied to treat diseases affecting either the front or the back of the eye.

3.4. Ear

Hearing loss is experienced by 5% of the world population making it the most prevalent sensory disorder. Sensorineural hearing loss (SHL) accounts for over 90% of deafness, originates by dysfunction in the inner ear and is addressed using cochlear implants or hearing aids in the absence of effective treatments. While candidate pharmacological approaches for the treatment of SHL exist, physiological and anatomical barriers pose major challenges to delivery to the inner ear. In humans, the inner ear comprises the bony labyrinth which includes two main components; the cochlea responsible for hearing, and the vestibular system responsible for balance. Within the cochlea, the organ of Corti hosts the sensory hair cells, whose degeneration is the leading cause of hearing loss. The imbalance of the three semicircular canals of the vestibular system can cause vertigo or dizziness. Thus, the cochlea and vestibule are the crucial delivery sites to treat hearing loss and balance disorders. Yet, the inner ear is an immuno-privileged site, and drugs face significant challenges when reaching it from circulation, including the blood-inner ear barrier (BLB), limited labyrinthine artery supply, and drug elimination by the cochlea leading to fraction of a percent of systemically administered drugs reaching hair cells in the organ of corti.[295] Direct delivery to the inner ear is hampered by the otic capsule, a dense bone structure covering the cochlea and vestibule.[296] Delivery from the outer and middle ear is hampered by the tympanic membrane, difficulty in accessing the round window membrane (RWM) and variable RWM permeability. The round window membrane is a biobarrier that regulates access of drugs to the cochlea.[296,297] Indeed, intratympanic injections (IT) are the most common treatment for local drug administration to inner ear. Yet IT injection to the middle ear for treating inner ear pathologies suffers from low efficacy due to the poor stability of labile drugs in the degrading middle ear environment and their low biodistribution to the inner ear due to the combined rapid drainage through the Eustachian tube and limited permeability across the RWM. Even if resorting to intracochlear drug delivery, a complex and highly invasive treatment, the tight junctions between cochlear cells hamper drug diffusion and promote its elimination. Drug concentration and residence time in the cochlea is mainly controlled by the rate of fluid exchange and the maintenance of salt concentration. Drainage of drugs through the cochlear aqueduct from the inner ear to the cranial fossa is also responsible for decreasing residence time.[296–298] Chitosan glycerophosphate (CGP) hydrogel matrix can help with ear drug delivery due to its mucoadhesion and ability to load a broad range of drugs. CGP hydrogels can deliver dexamethasone to the inner ear in mouse. They adhere to the RWM and result in up to 92% release of drug in the perilymph after 5 days.[299] Additionally, CGP hydrogels are used for treating Meniere's disease by IT delivery of the ototoxic drug gentamicin which kills vestibular hair cells. With this approach, release of gentamicin is sustained for 7 days compared to 1 day for the liquid form, and the drug accumulates preferentially in vestibular hair cells.[300]

Nanomaterials can help negotiate the key biobarriers hampering drug delivery to the inner ear. In particular, PLGA NPs can deliver drugs across the RWM. Proving the efficacy of delivery, PLGA NPs suspended within hydrogels can mediate the delivery of rhodamine through the RWM to the perilymph of guinea pigs.[301] PLGA NPs also enable the sustained release and enhanced delivery of salvianolic acid B (Sal B), tanshinone IIA (TS IIA), and total panax notoginsenoside (PNS) through the RWM, improving drug accumulation to the inner ear compared to the compounds in solution.[302] PEG-polycaprolactone (PEG-PCL) diblock polymersomes can deliver hydrophobic drugs transtympanically to the organ of Corti.[303] PEGylation enhances the solubility, biocompatibility, and half-life of drugs attached to hydrophilic polymers. Biodegradable BSA NPs increase the residence time of drugs in the middle ear by providing hydrogel-like characteristics. Poly (2-hydroxyethyl aspartamide) (PHEA) amino acid-based NPs show significant uptake in the cochlea attributed to their enhanced permeability and absorption properties.[304] Lipid-based systems and cationic PEG particles are being

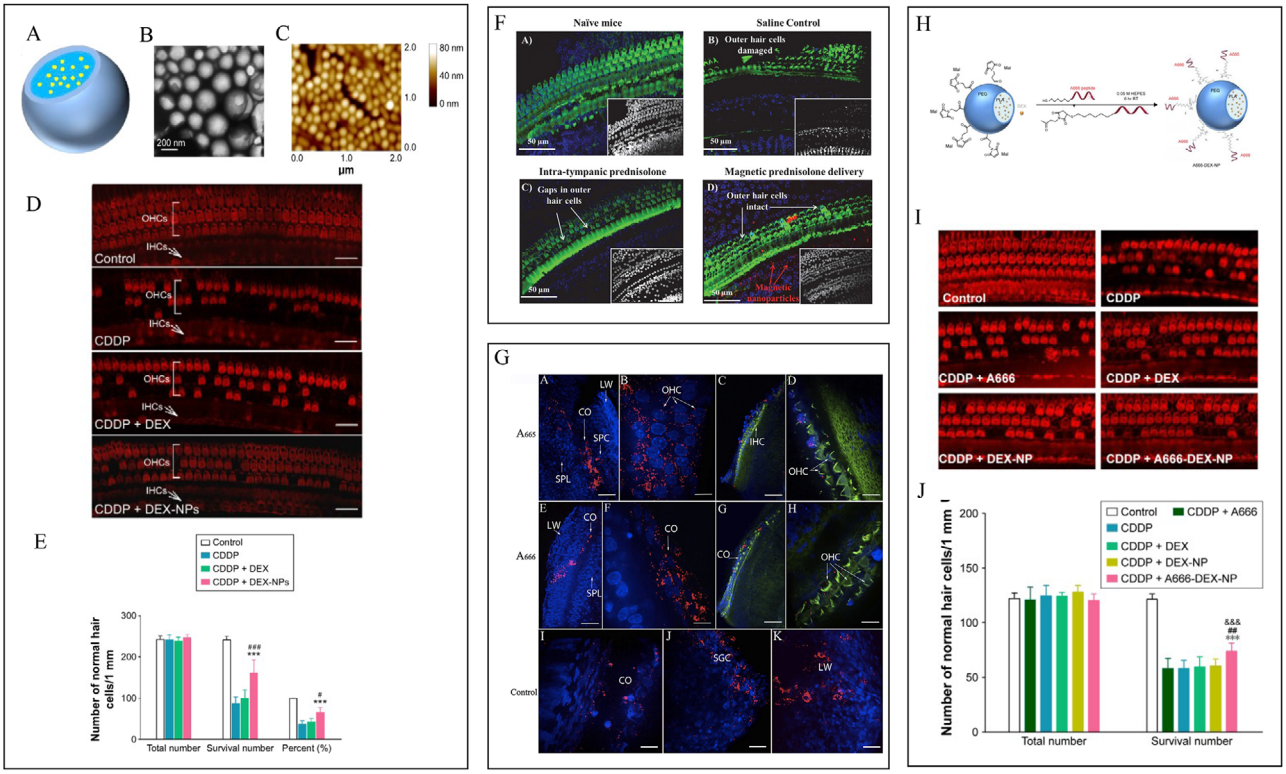

Figure 7. Nanomaterials for drug delivery to the ear. A) Schematic showing the fabrication of DEX-NPs, where yellow represents DEX, light blue sphere is an NPs modified with PEG (PEG-PLA complex). B) TEM image. C) NP image acquired by atomic force microscopy. D) Confocal microscope image of guinea pig's hair cells following treatment. Rhodamine phalloidin staining of the 60% portion from the apex of Corti. Extensive damage to OHCs is seen in CDDP and CDDP + DEX-treated animals compared with CDDP + DEX-NPs. E) Graph representing numbers and percent survival of OHCs in 1 mm length. $^{***}p < 0.001$ versus the CDDP group. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ versus the CDDP + DEX group. F) Confocal images of cytocochleograms of the basal cochlear region of different animal groups. A—animal without cisplatin treatment, B—animal treated with cisplatin in saline, C—animal that received intra-tympanic methyl-prednisolone, and D—animal treated with magnetic NPs delivery of prednisolone. OHCs were stained for actin with Alexa Fluor 488 Phalloidin (green) and DAPI (blue) was used for nuclear staining. G) Confocal microscope image of rat cochlear explants where OHCs are targeted with PEG-b-PCL polymer functionalized with A665 and A666 peptides. After 12 h at a polymer concentration of 6 nmol mL^{-1}, A,B) A665-PEG6K-b-PCL19K and E,F) A666-PEG6K-b-PCL19K PMs were mainly detected in OHCs, although they were also seen in the region of SPC and SPL in lower quantity. C,D,G,H) Increased specific binding of A665-PEG6K-b-PCL19K and A666-PEG6K-b-PCL19K PMs, respectively, after 12 h incubation at lower concentration (3 nmol mL^{-1}). Random distribution of unlabeled PEG5K-b-PCL5K PMs is shown in I) Corti's organ, J) spiral ganglion cells, and K) lateral wall using similar polymer concentration (6 nmol mL^{-1}). Red: PEG-*b*-PCL polymer, blue: DAPI, green: actin stained with FITC-conjugated phalloidin. H) Schematic showing the preparation of A666-DEX-NP. I) Confocal image of the apex of Corti stained for actin using rhodamine-phalloidin for different treatments to show the survival of hair cells. J) Graph depicting the number of survived OHCs in 1 mm length. $^{*}p$·0.05, $^{***}p$·0.001 as compared to CDDP; $^{\#}p$·0.05, $^{\#\#}p$·0.01, $^{\#\#\#}p$·0.001 as compared to CDDP + DEX; &&p·0.01, &&&p·0.001 as compared to CDDP + DEX-NP. Abbreviations: PEG, polyethylene glycol; DEX-NPs, dexamethasone-loaded polyethylene glycol-coated polylactic acid stealth NPs; DEX, dexamethasone; OHCs, outer hair cells; CDDP, *cis*-diamminedichloroplatinumII (use to induce hearing loss in ears); RWM, round window membrane; ST, scala tympani; SPC, supporting cells; SPL, spiral limbus. (A–E) Reproduced with permission.[291] Copyright 2015, Dove Medical Press (F) Reproduced under the terms of the Creative Commons Attribution License (CC BY).[292] Copyright 2017, The authors (G) Reproduced with permission.[293] Copyright 2012, Elsevier (H–J) Reproduced with permission.[294] Copyright 2018, Dove Medical Press.

developed for the middle ear delivery of anti-inflammatory drugs for hair loss treatment.[305] Dex-loaded NPs improve the hearing loss recovery and anti-inflammatory effects more than the clinically used Dex sodium phosphate solution in vivo (**Figure 7**A–E).[291] Cubosomes are biodegradable, phytantriol lipid-based crystalline NPs with a large surface area providing high drug loading capacity. Cubosome-loaded nerve growth factor enhances RWM permeability by 3.28-fold compared to the free drug.[306] Intratympaninc injection of magnetic NPs loaded with prednisolone in cisplatin-treated mice significantly increases outer hair cells viability and reduces cisplatin-mediated ototoxicity (Figure 7F).[292] Silica-based NP can deliver the neuroprotectant BDNF (brain-derived neurotrophic factor) for the treatment of noise-induced hearing loss in guinea pigs.[307]

NPs can also improve targeted drug delivery to specific cells of the cochlea. Conjugating the human nerve growth factor-derived peptide (hNGF_EE) to the surface of polymersomes allows targeting to spiral ganglion neurons. The uptake of the peptide is due to the interaction with NGF receptors, ultimately leading to the NP internalization within spiral ganglion neuronal cells.[308] Exploiting the tyrosine kinase receptors and p75 neurotrophin receptors allows targeting nerve fibers and Schwan cells in cochlear cultures. Two PRESTIN (outer hair cell protein) binding peptides,

A665 and A666 enable PEG-PCL polymersomes to target outer hair cells in cochlear explants and rat models (Figure 7G).[293] Furthermore, following ototoxic damage, the A666 PEG-PLA system enables targeted delivery of Dex to hair cells in vivo thus improving their survival (Figure 7H–J).[294]

4. Conclusion

Topical treatments pose intractable challenges arising from the combination of multiple physiological, physical, and anatomical barriers hampering delivery and retention at the intended site. These combine with the reduction of bioactivity and pharmacokinetics of therapeutic agents induced by their rapid inactivation when exposed to the strongly degrading environment characteristic of exposed tissues. While existing formulations are developed specifically to address these challenges, they often lack the versatility to approach them in the necessary coordinated fashion to maximize efficacy while minimizing dose, administrations, and side effects. Nanodelivery systems with their emergent properties, inherent multifunctionality, and broad tailorability can make significant contributions to coordinated biobarrier avoidance, improving therapeutic efficacy, reducing frequency and amounts of drug administration, and targeted delivery. Yet, at least in the early phases of technology development, these advantages often come with increased complexity and costs, which can reduce the reliability and desirability of the proposed approaches. Nonetheless, nanomedicine has a three decades' strong track record of rapidly reducing costs while increasing efficacy and reliability through the technology translation process. Indeed, currently developed nanomaterials follow gold standard fabrication and validation guidelines to provide highly reproducible properties and effects, thus enabling clear routes toward their rapid and effective upscaling and integration within clinical products.[309] The recent regulatory approvals of Patisiran/ONPATTRO (the first FDA-approved RNAi therapeutic), VYXEOS (a NP capable of delivering synergistic ratios of two drugs), and NBTXR3/Hensify (a radio-enhancing NP that synergizes with standard of care radiation oncology treatments) testify the ongoing success that nanomedicine is achieving in reaching the clinic with viable products.[310] Furthermore, many nanodelivery approaches that show efficacy falling short of making significant impact for intractable, high morbidity diseases such as cancer can be rapidly and effectively repurposed for topical application in more approachable conditions. Indeed, topical drug delivery represents one of the few major commercial successes for the first generation of nanovectors. Among these, vesicles, micelles, and emulsions are currently widely adopted to improve the efficacy of creams, ointments, and eye drops. A key challenge for the broader adoption of the newer generations of NPs discussed here remains the complexity and the higher costs associated with their regulatory approval, as the safety and efficacy of all components must be evaluated from both the medicinal and biomaterial standpoint. These compounds with the need to show compelling benefits over conventional approaches to justify the additional processing required and associated costs, particularly when addressing the treatment of disorders with low morbidity or where existing approaches, although inefficient, are resolutory.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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