Bioconjugated Plasmonic Nanoparticles for Enhanced Skin Penetration.

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Abstract

Plasmonic nanoparticles (NPs) are one of the most promising and studied inorganic nanomaterials for different biomedical applications. Plasmonic NPs provide excellent biocompatibility, long-term stability against physical and chemical degradation, relevant optical properties, well-known synthesis methods and tuneable surface functionalities. Herein, a review of recently reported bioconjugated plasmonic NPs using different chemical approaches and loading cargoes (such as drug, gene, and proteins) for enhancement of transdermal delivery across biological tissues is presented. The main aim is the understanding of the interaction of the complex skin structure with biomimetic plasmonic NPs. This knowledge not only plays an important role for enhancing transdermal delivery of pharmaceutical formulations but also for controlling undesired skin penetration of industrial products, such as cosmetic, sunscreen formulations and any other mass-usage consumable containing nanoparticles.

Keywords: Plasmonic nanoparticles, transdermal drug delivery, bioconjugated nanomaterials, skin penetration, gold and silver nanoparticles.

1. Introduction.

This review is focused on plasmonic nanoparticles (NPs) throughout the whole range of inorganic NPs. Plasmonic NPs are composed of colloidal particles of noble metals which present a characteristic surface plasmon resonance (SPR) band. The SPR band is attributed to the electric field of incident light, which induces coherent oscillation of conduction band electrons of the positively charged metallic core (**Figure 1**). These optical and electronic properties of the plasmonic NPs can be easily tuned by changing the size, shape, and surface chemistry.[1–3]

Among the different plasmonic NPs, this work is mainly focus on Au and Ag-based NPs, which are considered as non-allergenic compounds that should not induce cytotoxicity, therefore being highly attractive for biomedical applications.[4, 5] In addition to their inherent characteristic properties of nanometric materials, such as small size and high surface area-to-volume, the surface chemistry of the AuNPs can be easily modified through the covalent Au-S bond.[6, 7] These features have made AuNPs one of the most widely used nanomaterials both in technological and in biomedical applications, such as electronics,[8, 9] catalysis,[10, 11] sensory probes,[12, 13] plasmonic photothermal therapy,[14, 15] targeted drug delivery carrier,[16, 17] contrast agents in x-ray imaging and computed tomography for cancer diagnosis,[18, 19] to name only a few. AgNPs display an inherent antimicrobial capacity against bacteria, viruses, and other eukaryotic microorganisms.[20, 21] For this, silver compounds have been historically used in fields to prevent microbial growth, in wound care and in products such as odour-reducing clothing, acne creams, and face masks.[22, 23]

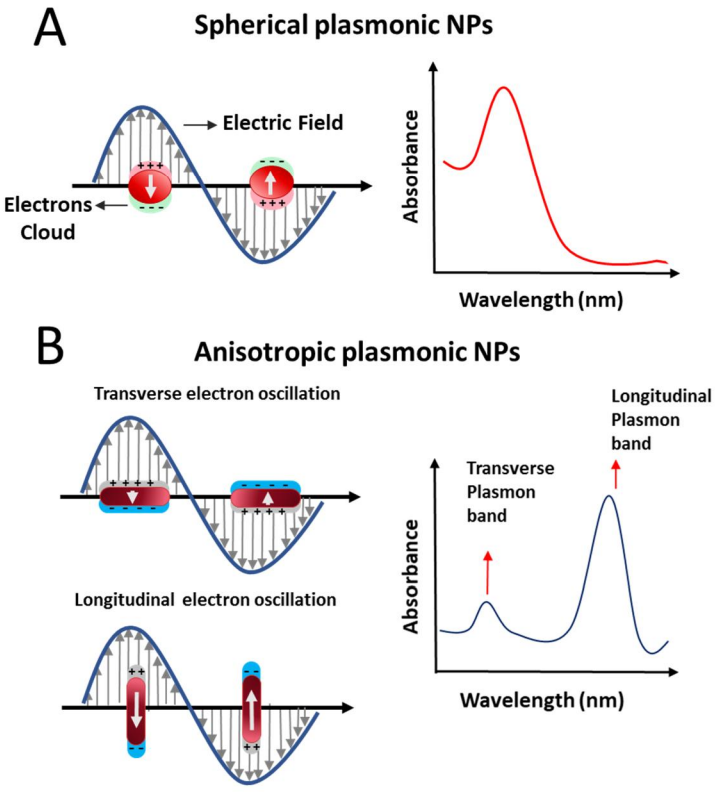

Figure 1. Schematic representation of Surface Plasmon Resonance (SPR) band in spherical (A) and rod-shape (B) plasmonic NPs.

Topical administration of compounds presents several advantages against other routes, such as oral, nasal, and intravenous administration. Transdermal delivery overcomes the first-pass hepatic metabolism and can reduce harmful side-effects.[24–26] However, skin is a complex multilayer structure (mainly composed of epidermis, dermis, and hypodermis) with highly impermeable barriers to most molecules on the basis of particle size, water-solubility and surface charge.[24] In addition, hair follicles have also been explored as a more permeable transporting channel for transdermal drug delivery.[27–29] An excellent review of the influence of size on the penetration of both metal and non-metal NPs through skin was published by Larese Filon et al.[30]

Bare NPs do not overcome skin barriers efficiently as reported by Núñez-Lozano et al.[31] In other words, non-functionalized NPs constitute poor transdermal drug delivery systems.[32, 33] Therefore, many efforts have been made to enhance the skin penetration of the NPs through purposefully designed chemical functionalization with biomolecules and bioinspired polymers to form biomimetic NPs and providing additional abilities in skin penetration. Herein we aim at compiling the latest advances on the development of different approaches to fabricate bioconjugated plasmonic NPs for enhanced skin penetration. This review is organized as follow: Firstly, the influence of the key parameters on the skin penetration of non-bioconjugated plamonic NPs is discussed and, secondly, some of the more recently reported bioconjugated plasmonic NPs for enhanced skin penetration is presented.

2. Influence of key-parameters on the skin penetration of non-bioconjugated plasmonic NPs.

The ability of plasmonic NPs for skin penetration can be tuned by controlling their core-composition, particle-size and -shape, surface charge, water solubility and the functionalization strategy, including both capping-ligands and delivered cargoes, i.e., drugs, genes, and proteins. Relevant examples of the most prominent studies in each of these parameters are mentioned below:

2.1. Core composition (noble metal type).

The literature indicates a significant difference in terms of skin penetration behaviour between organic and inorganics NPs. There are also significant differences for skin

internalization depending on its chemical composition (i.e. TiO_2, SiO_2, ZnO, FeO, CdSe, Pd, etc.) within the latter group, as previously reported by Larese Filon et el.,.[30] In the case of plasmonic NPs, the in vitro penetration of AgNPs through intact human skin samples has been reported for several authors (with particle size in a narrow range between 19 and 25 nm), suggesting that ions release is the most feasible penetration mechanism.[34, 35] Whilst for AuNPs, its penetration through intact human skin samples has been demonstrated by several authors (size ~12 nm), although the penetration process is unclear, being discarded the ions release mechanism. The absence of Au ions in physiological solutions of AuNPs has been reported, indicating its higher long-term colloidal stability than AgNPs.[30, 36] This high stability could explain the absence or low cytotoxicity of this type of plasmonic NPs.

2.2. Particle size-effect.

Although the literature shows contradictory results in this issue, skin penetration of NPs is considered a size-dependent process.[30] Note that the stability of the NP coating should be taken into account because it plays a key role both in the interaction between skin and NP core (see section 2.4), and between the NPs (i.e. steric stabilization) by avoiding the particle aggregation when they come into contact with the stratum corneum and constituent cells of the skin, mainly keratinocytes.

A representative study showing that AuNPs penetrate through intact skin samples in a size-dependent manner was reported by Sonavane et al., who analysed the penetration of 15, 102 and 198 nm citrate-capped AuNPs, with spherical shapes and surface negative charge, through rat-skin and rat-intestine using Franz diffusion cells.[37] The smallest AuNPs showed higher permeation than the larger particles. In a similar study with rats, Raju and co-workers reported that 22 nm citrate-capped AuNPs showed higher penetration than 105 and 186 nm particles across the thick stratum corneum of the plantar rat skin .[38]

A key aspect on the experimental design for assessing the skin penetration of plasmonic nanoparticles is the in vitro or in vivo model used to perform the analysis. Indeed, the choice of biologically relevant and realistic model for studying the biological effect of nanoparticles is a comparatively unexplored field.[39] Although rat, mouse and rabbits have been extensively used for these penetration studies, pig skin is probably the most similar animal model to the human skin.[40] Pig and human skin are structurally very

similar in thickness and dermal-epidermal thickness ratio. Hair follicles and blood vessel patterns in the skin are also similar. In addition, the thickness of the human skin varies considerably as a function of the body region, gender and age, among other factors. For this reason, the results obtained are quite different depending on the used in vivo model, and even depending on the part used of human body. An interesting study on porcine skin using AgNPs was reported by Samberg and co-workers, who evaluated the in vitro and in vivo toxicity of eight different commercial AgNPs supplied by nanoComposix (San Diego, CA), such as unwashed/uncoated (diameter of 20, 50, and 80 nm), washed/uncoated (20, 50, and 80 nm), and carbon-coated (25 and 35 nm).[22] They observed that the toxicity of AgNPs in HEKs was significantly influenced by the residual contaminants in their supernatant, and that AgNPs themselves may not be responsible for an increase in cell mortality. The degradation of AgNPs within the cell was also considered as a source for reactive oxygen species that would be damaging to cell machinery and DNA.[41]

Obviously, human skin would be ideal to perform in vitro skin penetration analysis. In 2012, Liu et co-workers investigated the in vitro penetration and metabolic effects of 10, 30 and 60 nm citrate-capped AuNPs within viable excised human abdominal skin after 24-hour exposure.[42] Using multiphoton tomograph-fluorescence lifetime imaging microscopy, they only observed penetration into the stratum corneum, without significant penetration into the lower layers. They demonstrated that viable human skin resists permeation of small NPs, which had been previously reported to penetrate deeply in other animal skin models.

Note that a remarkable attempt to categorize the size range with its skin penetration ability was carried out by Larese Filon et al.[30] They suggested that: (i) NPs smaller than 4 nm can both penetrate and permeate intact skin, (ii) NPs in the size range between 4 and 20 nm can permeate both intact and damaged skin, (iii) NPs with diameter between 21 and 45 nm can penetrate and permeate damaged skin, and (iv) NPs higher than 45 nm can neither penetrate nor permeate the skin. They also considered the hydrodynamic diameter of the NPs, which is an important parameter of the colloidal particles that can be greatly affected both for the ligand-coating and the electrolyte composition of the colloidal solution.[43]

2.3. Shape-effect.

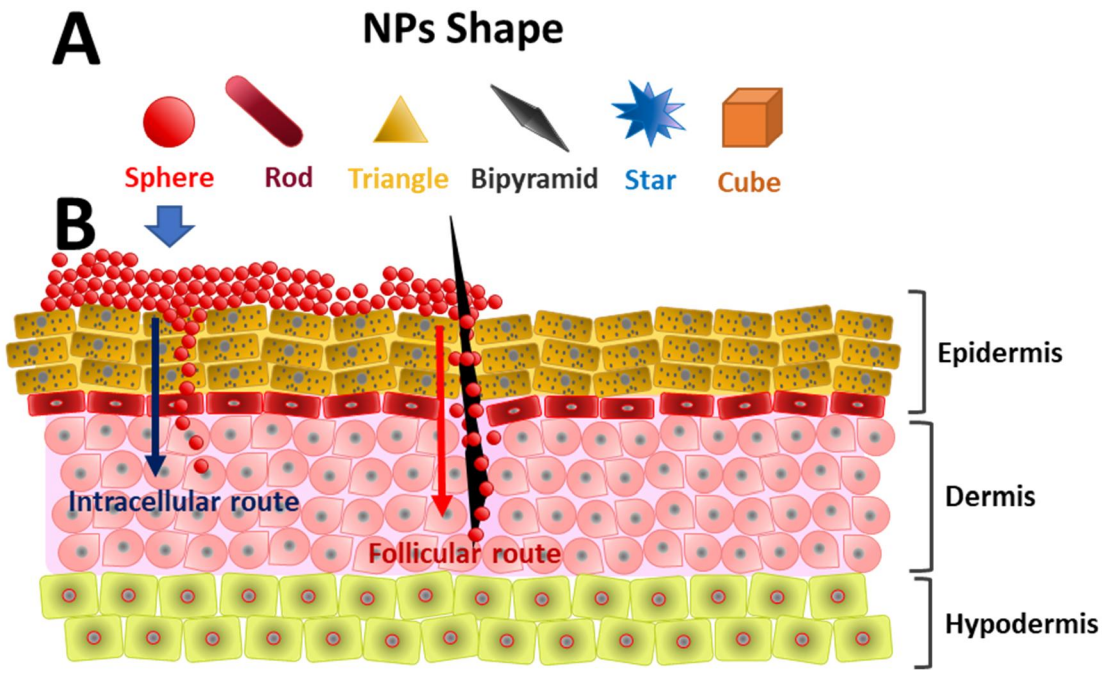

Figure 2. Schematic representation of plasmonic NPs with different geometries (A) and of the human skin layers (B).

Despite the enormous literature reporting protocols for the synthesis of plasmonic NPs with different morphologies, such as rod, triangle, bipyramid, star, cube and others, [44–46] as well as the important effect on the resulting NP flow characteristic that alters cell membrane interactions, macrophage uptake and circulating lifetimes,[47–49] research papers investigating the influence of the NPs shape on skin penetration has been rarely reported (**Figure 2**). This fact could be attributed mainly to the resulting higher size (e. g. > 45 nm) and to the lower resulting long-term stability of the anisotropic NPs (i.e. non-spherical geometries). Only comparative studies between spherical and rod-shape AuNPs can be found in the literature. A relevant study was reported by Fernandes et al.,[50] showing from their culture experiments in mouse and human skin samples than the percentage of PEG-capped Au-nanorods (with an aspect ratio 2.8 ± 0.5) found in all samples was higher than the one obtained for the similar PEG-capped spherical 15 ± 1 nm AuNPs. These results were obtained both for positively and for negatively surface charged NPs, suggesting the great influence of the NP-shape on its penetration capacity. In the case of AgNPs, Tak and co-workers reported a skin penetration study of differently shaped NPs using both in vitro and in vivo models.[51] They used spherical, rod-shape

and triangular AgNPs with similar hydrodynamic diameter (~50 nm) and zeta-potential (+ 30 mV) to perform in vitro analysis on ultra-thin mouse skin section by Franz cell system, and in vivo analysis on hairless mice. In agreement with previous results for AuNPs, they showed that rod-shape AgNPs presented higher permeability index than the spherical and triangular ones. They concluded that different shapes of AgNPs may exhibit diverse antimicrobial activities and skin penetration capabilities depending upon their active metallic facets.

2.4. Influence of the capping-ligand, including surface-chemistry and -charge.

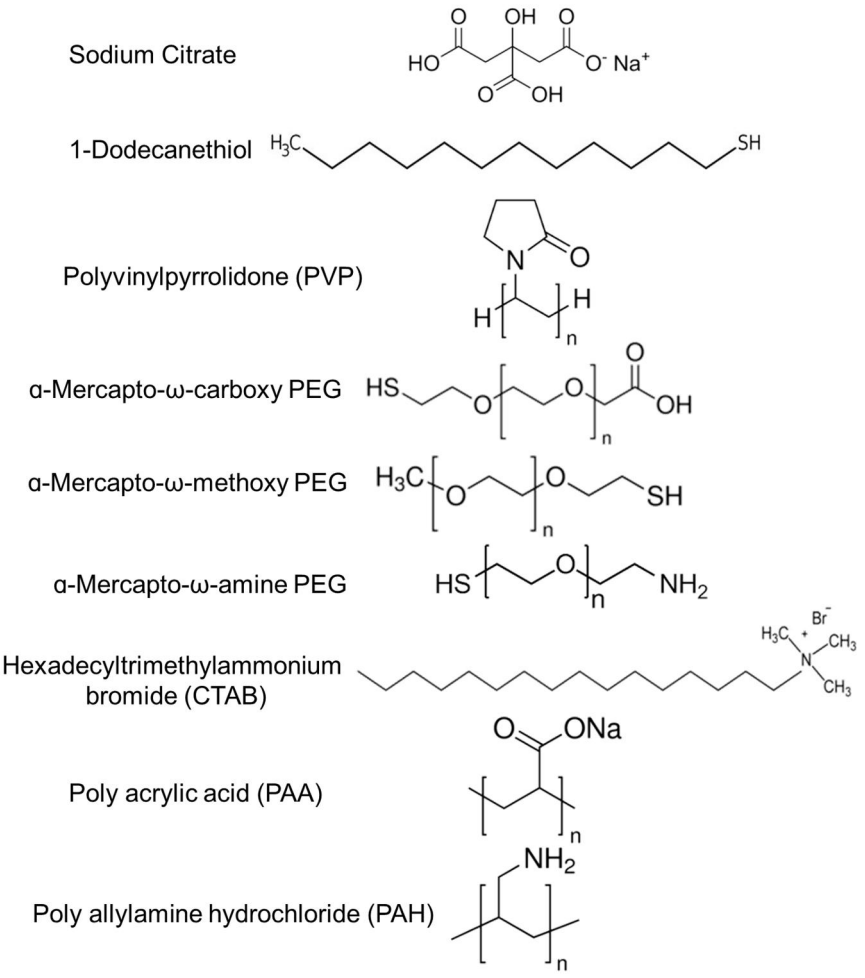

Figure 3. Representative stabilizing ligand for plasmonic NPs.

Another important parameter that greatly affects the skin penetration rate of the NPs is the surface charge and chemistry, which is directly associated with the stabilizing ligand composition and the type of the metal-ligand bonding interaction. **Figure 3** summarizes the most frequently found capping ligands, including the type of interaction with the metallic core. Citrate is the main ligand for the synthesis of plasmonic NPs,[52, 53] and

it is the most widely reported. Despite the resulting water-soluble NPs present a good long-term stability, with a negatively charged surface due to their carboxylate groups, the NPs tended to aggregate easily in contact with the skin (specially for the smallest sizes) due to their relatively weak metal-ligand interaction.[7] Labouta and co-workers showed that water-soluble spherical-shape 15 nm citrate-capped AuNPs tended to aggregate on the superficial stratum corneum.[54] In this study, the penetration rate of hydrophilic 15 nm citrate-capped AuNPs versus hydrophobic 6 nm dodecanethiol-capped AuNPs was also compared, showing that non-water-soluble particles penetrated through the stratum corneum and into viable epidermal layers of human skin. This enhance on the skin penetration using dodecanethiol as capping ligand could be attributed to: (a) The stronger Au-S binding interaction, which avoid aggregation of NPs in contact with the stratum corneum, and (b) to the solubility in organic solvent (i.e. toluene) that could facilitate the interaction with the cell membranes. A related study from the same research group compared the different penetration rate through human skin of two water-soluble AuNPs (15 nm citrate- and 6 nm lecithin-capped) against two toluene-solved ones (6 nm dodecanethiol- and cetrimide-capped).[55] They concluded that the vehicle (toluene-versus-water) had a minimal effect on skin penetration of AuNPs.

Not only should the bond strength of the metal-ligand interaction be considered but also the coating thickness and the surface coverage degree. Previous studies have demonstrated that polymeric capping ligands as poly(N-vinyl-2-pyrrolidone) (PVP) are well suited to stabilize the surface of AuNPs for skin penetration, despite of the weak metal-ligand interactions via pyrrolidone groups.[56] Huang and co-workers showed that water-soluble 5 nm PVP-capped AuNPs with spherical shape were mice skin permeable. They attributed this effect to the nano-bio interaction with skin lipids and the consequent induction of transient and reversible openings on the stratum corneum. In addition, they highlighted that co-administration of these PVP-capped AuNPs with protein drugs could enhance transdermal drug delivery.[56]

Additionally, the capping ligand confers a given surface charge to the NPs, which greatly affects to the resulting skin penetration rate. For instance, positively charged drug carriers, such as dendrimers and liposomes, are well-known to induce greater drug delivery in the skin.[57, 58] In this direction, Fernandes et al. showed that positively charged PEG-capped AuNPs were found in the skin in 2-6 folds higher amount that their negative counterparts.[50] These results were obtained both for spherical- and for rod-shaped NPs,

and were in agreement with the enhanced skin permeation of cationic liposomes, which was attributed to the "Donnan exclusion effect" and to the better interaction of cationic particles with the negatively charged skin cells.[59] Furthermore, in this study, they also showed that AuNPs functionalized with cell penetrating peptides (CPPs) TAT and R7 were found in the skin in larger quantities than PEGylated AuNPs, demonstrating that bioconjugation greatly enhance the skin penetration rate.[50]

On an alternative strategy, Lee and co-workers investigated the influence of the surface charge of Au-nanorods on skin penetration using layer-by-layer (LbL) polyelectrolyte coating technique.[60] They observed that negatively charged CTAB/PSS-capped Au-nanorods penetrated more rapidly through the skin than the positively ones (CTAB- and CTAB/PSS/PDADMAC-capped). For this, three different multi-layer coated Au-nanorods with particle size of 18 x 40 nm were used: Two positively charged, CTAB- and CTAB/PSS/PDADMAC-capped, and one negatively charged, CTAB/PSS-capped. These surprising results were attributed to both the aggregation of the positively charged Au-nanorods on the stratum corneum and the adsorption of proteins released from the dermis layer to the surface of Au-nanorods. In line with these results, Mahmoud et al. observed that positively charged Au-nanorods aggregated extensively upon exposure to human skin compared to negatively and neutrally charged ones.[61] They attributed these findings to the adsorption of proteins released from the dermis layer to the surface of Au-nanorods. In this study, they prepared 49.5 x 12.0 nm Au-nanorods capped with four different surface ligands: cetyltrimethylammonium (CTAB), poly acrylic acid (PAA), poly(allylamine hydrochloride) (PAH), and methoxy-polyethylene glycol-thiol (m-PEG-SH). Conversely, Hao et al. also investigated the influence of the surface charge on the skin penetration of spherical AuNPs using human reconstructed 3D Episkin model.[62] In this study, three different surfaces charged 5 nm-AuNPs capped with citrate (negative), PVP (neutral), and CTAB (positive) were tested. They observed that, although all AuNPs induced the phase change of lipid lamella and pass through the epidermis, positively charged ones exhibited the most efficient skin penetration through both the paracellular routes and the transcellular pathway, when compared to neutrally or negatively charged NPs.

An interesting alternative for stabilizing NPs surface is the PEGylation, which is a commonly used approach for improving the drug and gene delivery efficiency of NP-based systems to target cells and tissues.[63] Hsiao and co-workers employed this

approach to investigate the positive effects of polyethylene glycol (HS-PEG-COOH) and HS-PEG-oleylamine (OAm) functionalization on the skin permeation of spherical 10 nm AuNPs.[64] Using an in vivo rat model, they showed that PEG- and PEG-OAm-functionalized AuNPs were able to overcome the skin barrier and deposit in the deeper subcutaneous adipose tissue. Moreover, the follicular deposition of AuNPs increased 2-fold after PEG-OAm functionalization, demonstrating a preferential accumulation mediated by the stabilizing ligand. Mahmoud et al. evaluated the preferential accumulation of Au-nanorods into abdominal human skin hair follicles.[65] To this end, they prepared 11.4 x 46.6 nm Au-nanorods with five different surface chemistries (i.e. neutral, anionic, cationic, and hydrophobic), such as CTAB, PAA, methoxy-polyethylene glycol-thiol (m-PEG-SH), PEG-Cystamine, and polystyrene (PS). They observed that the lipophilic properties of sebum-rich hair follicles enhanced the accumulation of hydrophobic PS-Au-nanorods into hair follicles, while neutral m-PEG-S-Au-nanorods were distributed into all skin compartments, especially the dermis, which exhibits hydrophilic characteristics. In addition, both charged Au-nanorods showed a negligible percentage of penetration into any of the skin compartments.

3. Bioconjugated plasmonic NPs for transdermal delivery of different cargoes.

A seminal study on the development of bioconjugated plasmonic NPs for enhancing the skin penetration of different cargoes was reported in 2010 by Huang and co-workers.[56] They demonstrated a significant enhance of the transdermal delivery of protein-drugs by co-administration with 5 nm PVP-capped AuNPs. This fact was attributed to the nano-bio interaction with skin lipids, which allowed a reversible openings of the stratum corneum. Thus, this work provided a simple and efficient NPs-mediated method for overcoming the skin barrier for percutaneous protein drug delivery. Labala et al. reported the first bioconjugated plasmonic NPs for iontophoretic transdermal delivery of imatinib mesylate to treat melanoma, using a LbL assembly approach.[66] This LbL polymer capped AuNPs contained PVP and polyethylene imine (PEI), and was subsequentially coated with anionic poly(styrenesulfonate) (PSS) and cationic PEI for the drug loading. The resulting bioconjugated nanosystem showed an average particle size and a zeta-potential of 98 ± 4 nm and + 32 ± 1 mV, respectively, and a shift of the SPR wavelength from 518 to 530 nm. The in vitro skin penetration studies were performed on excised

porcine ear, and they demonstrated that iontophoresis application enhanced the skin penetration of imatinib mesylate loaded AuNP by 6.2-fold compared to passive application.

Bessar et al. prepared a water-soluble sodium 3-mercapto-1-propansulfonate(3MPS)-capped AuNPs, which were loaded with methotrexate (MTX) via electrostatic adsorption. The resulting Au-3MPS@MTX conjugate showed an average size and a zeta-potential of ~5 nm and -32 ± 1 mV, respectively. Then, it was topically administrated on C57BL/6 mouse normal skin in order to assess the absorption behaviour. In vitro and in vivo studies showed that MTX-conjugated AuNPs was much more efficient than MTX alone, suggesting this nanosystem as a potential candidate for topical treatment of psoriasis, see Figure 4A.[67]

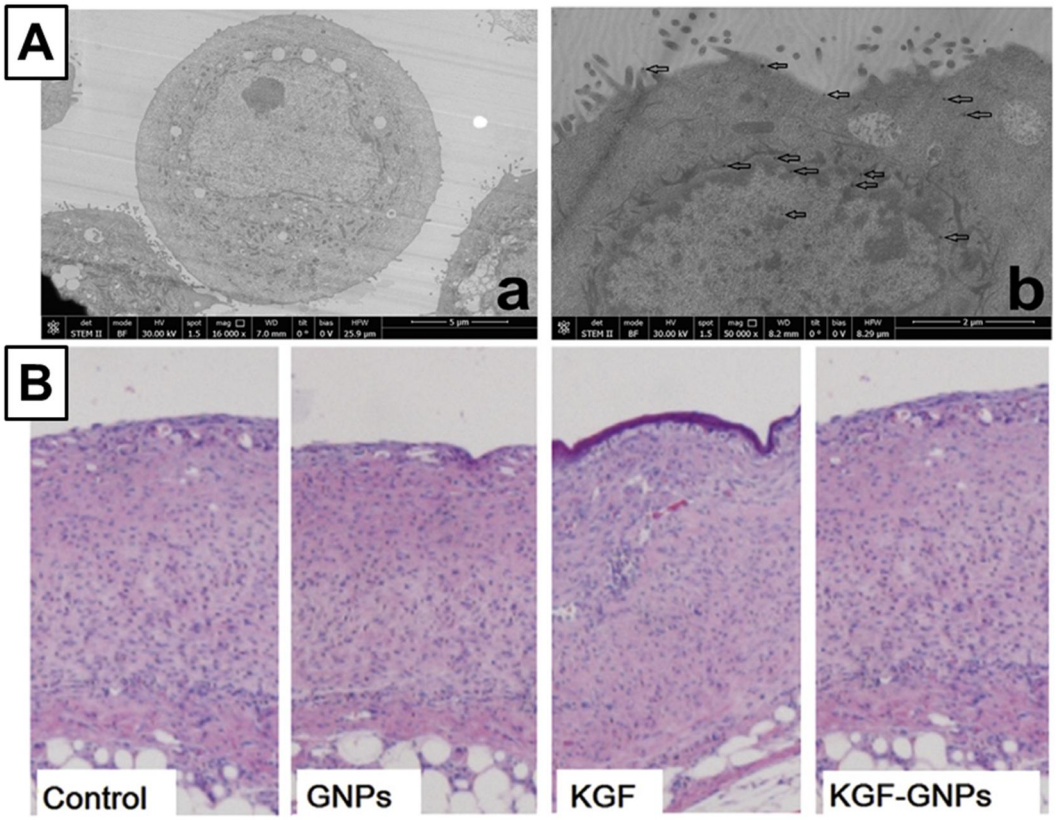

Figure 4. A) Electron microscopy pictures of normal human keratinocytes. a) control b) treated cells with bioconjugated plasmonic NPs.[67] Copyright from Elsevier, 2016. B) Wounds treated with non-coated and bioconjugated plasmonic NPs, showing the absence of granulation tissue.[68] Copyright from Elsevier, 2018.

An interesting application of bioconjugated plasmonic NPs for enhance transdermal gene delivery was the reported by Niu and co-workers.[69] In order to facilitate the skin penetration of plasmid DNA (i.e. pDNA encoding miRNA-221 inhibitor -Mi221-) deeply into the melanoma tissues, they synthesized an 20-25 nm conjugated AuNPs containing a cell-penetrating TAT peptide and the cationic PEI that could compact the pDNAs into cationic nanocomplexes (i.e. zeta-potential ~ +35 mV). They demonstrated that the resulting plasmonic bioconjugates can penetrate through the intact stratum corneum without any additional enhancement physical method. This study proposed a novel topical gene therapy strategy for skin cancer with great priority to reverse both the progression and metastasis of advanced melanoma. Chen et al. fabricated a biocongugate plasmonic NPs for the transdermal delivery of the vascular endothelial growth factor (VEGF) in wound repair.[70] To that end, they performed the bioconjugation of AuNP-PEG-COOH with VEGF through carbodiimide bonds, obtaining a negative surface charged nanosystem, whose absorption capability was evaluated by a mouse skin model. After the treatment, they observed not only the presence of VEGF into the dermis but also its effect for promoting the angiogenesis, demonstrating that in this case the binding of protein biological factors to AuNPs could preserve the activity of the protein. Other study reported by Safwat el al., who fabricated AuNPs capped with benzalkonium chloride and with PEI for enhanced loading and skin permeability of 5-fluorouracil (i.e. 5-FU/BC-AuNPs and 5-FU/PEI-AuNPs, respectively).[71] They performed ex vivo permeability studies of different 5-FU preparations using mice skin, demonstrating that the permeability of 5-FU was significantly higher for drug-loaded AuNPs compared with the other tested 5-FU samples. This same research group also prepared 5-FU loaded through ionic interactions onto CTAB capped AuNPs, and the resulting nanocomposite was incorporated into gel and cream bases to evaluate its permeability both ex vivo in mice dorsal skin and in vivo A431 tumor-bearing mice.[72] They observed that the nano-formulation provided around 2-fold higher permeability through mice skin compared with free 5-FU gel and cream formulations, and achieved 6.8- and 18.4-fold lower tumour volume compared with the untreated control with the gel- and the cream-based nano-formulation, respectively. On the other hand, Boca et al. performed the first preliminar study to evaluate the potential use under dermatological conditions of Ruxolitinib-conjugated 15 nm AuNPs as alternative for treating alopecia.[73] Using in vitro preclinical setting, they showed that AuNPs@TWEEN-20@Ruxolitinib inhibited the

proliferation of fibroblasts by inhibiting JAK2 protein, suggesting it as a potential strategy to treat alopecia.

Another novel and alternative administration strategy of bioconjugated plasmonic NPs was proposed by Anirudhan et al., who fabricated a nanocomposite film containing methacrylate-stitched β-cyclodextrin embedded with AuNPs and hydrophobic titanium nanotube (TNT) and tested the transdermal delivery of ibuprofen through in vitro rat skin.[74] They showed that the resulting film exhibited an improved drug-delivery performance, which was attributed to synergistic action of AuNP and hydrophobic TNT. They proposed this nanocomposite film as an alternative skin permeation strategy for transdermal drug delivery. Similarly, this same research group proposed a polyelectrolyte membrane fabricated with guar gum, poly(vinyl alcohol) and a nanogold-nanocellulose composite for the topical administration of diltiazem hydrochloride. In vivo analysis on human skin of this film were performed, suggesting its potential use for transdermal drug delivery.[75]

Pan et al. explored the effects on wound healing of keratinocyte growth factor (KGF) cross-linked to AuNPs.[68] Using an animal full-thickness wound model, they showed that KGF-AuNPs were more favourable to wound healing than bare AuNPs or KGF, thus being proposed as a promising wound healing drug for clinical application, see Figure 4B. Crisan et al. evaluated the impact on psoriatic inflammation of AgNPs and AuNPs complexed with Cornus mas (i.e. polyphenols-rich extracts) by using an in vitro model based on pro-inflammatory macrophages.[76] The results obtained from all the performed in vitro analysis suggested that these bioconjugated plasmonic NPs provided an efficient tool for modern psoriasis therapy, circumventing immunosuppression-related side effects of biologicals. In another study, Wang et al. fabricated an antimicrobial peptide (LL37) grafted ultra-small AuNPs (AuNPs@LL37, ~7 nm), which was combined with pro-angiogenic (VEGF) plasmids to analyse its potential use for the topical treatment of diabetic wounds with or without bacterial infection.[77] The resulting bioconjugate (AuNPs@LL37/pDNAs) combined the advantages of cationic surface charged NPs that condense DNA with those of antibacterial peptides and enhance the cellular and nucleus entry to achieve high gene delivery efficiency. AuNPs@LL37/pDNAs were shown to greatly improve the gene transfection efficiency in keratinocytes compared with pristine AuNPs/pDNAs, exhibiting a similar expression to Lipo2000/pDNAs (a well-known highly efficient gene transfection agent), whilst displaying higher antibacterial ability.

Thus, this bioconjugated plasmonic NPs were suggested as a suitable strategy for treating chronic diabetic wounds.

More recently, Fratoddi et al. analysed the effects of AuNPs functionalized with 3-mercapto-1-propansulfonate (AuNPs-3MPS) and loading MTX topically administered in vitro on skin model and in vivo on imiquimod-induced psoriasis-like mice model.[78] The showed that the treatment with this system was able to induce a reduction of keratinocytes hyperproliferation, epidermal thickness and also inflammatory infiltrate in the used in vivo model. Hernández-Martínez et al. synthetized and evaluated a nanocomposite of AuNPs functionalized with calreticulin.[79] Using in vitro and in vivo wound healing mice models of diabetes, they assessed the ability of the nanocomposite to promote proliferation and migration. Their results confirmed the utility of this bioconjugated plasmonic NPs (AuNPs-calreticulin) as potential treatment for wound healing of diabetic ulcers.

In the case of AgNPs-based bioconjugates, Mandal and co-workers fabricated a nanocomposite hydrogel comprised of in situ formed Ag nanowires (AgNWs) deposited chemically cross-linked carboxymethyl cellulose (CMC), which demonstrated superior efficacy as transdermal anticancer drug-curcumin carrier.[80] This plasmonic bioconjugate had the capability to encapsulate both hydrophobic/hydrophilic transdermal drugs. The in vitro experiments suggested that the presence of AgNWs on cross-linked CMC enhanced both the penetration power of nanocomposite hydrogel and the drug release in sustained manner. Whilst the ex vivo rat skin permeation analysis confirmed that the drug delivery through the nanocomposite hydrogel was permeable through the rat skin in controlled fashion, killing efficiently the MG 63 cancer cells.

Table 1 summarises the bioconjugated plasmonic NPs cited in this review, indicating both the loaded active molecule and the potential application.

Table 1. Representative cargoes in through bioconjugated plasmonic NPs for skin diseases treatment.

Active molecule	Cargo type	Activity/Application	NPs	Ref.
Imatinib mesylate	Drug	Anticancer	Au	[66]
Methotrexate (MTX)	Drug	Anti-inflammatory (psoriasis)	Au	[67,78]
microRNA mir-221	Gene	Tumour suppressor (melanoma)	Au	[69]
Vascular endothelial growth factor (VEGF)	Protein	Wound repair	Au	[70]
Ibuprofen	Drug	Anti-inflammatory	Au	[74]
Diltiazem hydrochloride	Drug	Vasodilator	Au	[75]
5-fluorouracil (5-FU)	Drug	Anticancer	Au	[71,72]
Ruxolitinib	Drug	Anti-alopecia	Au	[73]
Keratinocyte growth factor (KGF)	Protein	Wound repair	Au	[68]
Polyphenols-rich extracts (Cornus mas)	Natural extract	Anti-inflammatory (psoriasis)	Au & Ag	[76]
Antimicrobial peptide LL-37 & pDNA: Pro-angiogenic (VEGF) plasmids	Protein & Gene	Diabetic wound healing	Au	[77]
Calreticulin	Protein	Diabetic wound healing	Au	[79]
Curcumin	Natural extract	Anticancer	Ag	[80]

Conclusions

Bioconjugated plasmonic NPs are a promising approach for topical administration of different cargos for several diseases. The excellent biocompatibility and the readily adjustable physical and chemical features of the plasmonic NPs are highly attractive options for purposefully designed nanomaterials aimed at biomedical applications. Several examples have been presented herein, illustrating the wide range of cargoes and functionalization strategies that might be included when designing a bioconjugated plasmonic NPs. Different physical and chemical parameters should be taken into account when analysing the effect of plasmonic NPs in human skin. While the chemical routes for

obtaining on-demand plasmonic NPs are relatively well-established and a large number of simple and reproducible experimental protocols are available, the main frontier for mass usage is still a correct assessment of the toxicity of the NPs. Provided a relevant model for human skin, the experimental conditions for studying location and local concentration of plasmonic NPs greatly differs from those found in the synthesis laboratories. Therefore, this fruitful field of research requires more efforts are to fully understand the penetration mechanism of these bioconjugated plasmonic NPs, enabling the decrease of associated toxicity and the potential long-term environmental impacts. In view of the latest contribution to the field, we speculate that a reliable framework will be available in the short term, enabling the second wave of research for directed synthesis and application of plasmonic NPs at in vivo conditions.

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