

# Recent Progress and Future Directions: The Nano-Drug Delivery System for the Treatment of Vitiligo

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**Abstract:** Vitiligo is a depigmentation disease that seriously affects the physical health, mental health and quality of life of a patient. Therapeutic aim at control immunoreaction by relieving oxidative stress. Unfortunately, the cuticle barrier function and lack of specific accumulation lead to unsatisfactory therapeutic outcomes and side effects. The introduction and innovation of nanotechnology offers inspiration and clues for the development of new strategies to treat vitiligo. However, not many studies have been done to interrogate how nanotechnology can be used for vitiligo treatment. In this review, we summarize and analyze recent studies involving nano-drug delivery systems for the treatment of vitiligo, with a special emphasis on liposomes, niosomes, nanohydrogel and nanoparticles. These studies made significant progress by either increasing drug loading efficiency or enhancing penetration. Based on these studies, there are three proposed principles for topical nano-drug delivery systems treatment of vitiligo including the promotion of transdermal penetration, enhancement of drug retention and facilitation of melanin regeneration. The presentation of these ideas may provide inspirations for the future development of topical drug delivery systems that will conquer vitiligo.

**Keywords:** vitiligo, nano-drug delivery system, transdermal penetration, liposomes, skin

## Introduction

Vitiligo is an acquired idiopathic dermatological disorder characterized by the appearance and development of white macules related to the apoptosis or selective damage of melanocytes. Approximately 0.5–1% of the individuals are diagnosed with vitiligo.<sup>1</sup> The highest reported prevalence has been recorded in India (up to 8.8%), followed by Mexico (2.6–4%) and then Japan (1.68%<sup>2</sup>). Depigmentation usually occurs on exposed areas of the body including the face, neck and arms. The extreme effects of vitiligo often bring dramatic psychological burden to afflicted patients. Although males and females are equally affected by this disease, women more often openly express and address vitiligo for cosmetic purposes and are more likely to seek treatment.<sup>3</sup> The incidence of vitiligo often presents through distinct familial clustering with reports that 20% of the vitiligo patients also have relatives diagnosed with the disorder.<sup>4</sup> Individuals with a positive family history usually have an earlier age of onset as well as a longer duration<sup>5</sup> compared to individuals that do not have a family history of the disease.

Thus far, the etiology of vitiligo has been found to result from multiple factors and has not yet fully been elucidated. In the 1950s, Lerner investigated 600 vitiligo

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patients and found that most patients with segmental vitiligo suffered from emotional imbalances or hyperhidrosis, eventually giving rise to the neural theory.<sup>6</sup> As research progressed, factors including stress, autoimmune diseases, melanocytterhagy and autoinflammation have been identified as important factors contributing to vitiligo.<sup>7</sup> Of all theories, autoimmune disease or autoinflammation<sup>8–11</sup> and oxidative stress<sup>12</sup> as well as interactions among and between them have been accepted as some of the most important factors contributing to the disease.<sup>13</sup> In the disease, antigen-presenting cells activate T cells through the presentation of melanocyte antigens where T cells then directly kill the melanocytes. It has been reported that endogenous killer and inflammatory dendritic cells are in a hyperactive state in patients with vitiligo.<sup>9,10</sup> Various cytokines including INF- $\gamma$ ,<sup>14–16</sup> CXCL10,<sup>14,17,18</sup> TNF- $\alpha$ , IL-6 and IL-17<sup>19–22</sup> are also secreted by innate cells through an autoimmune response.

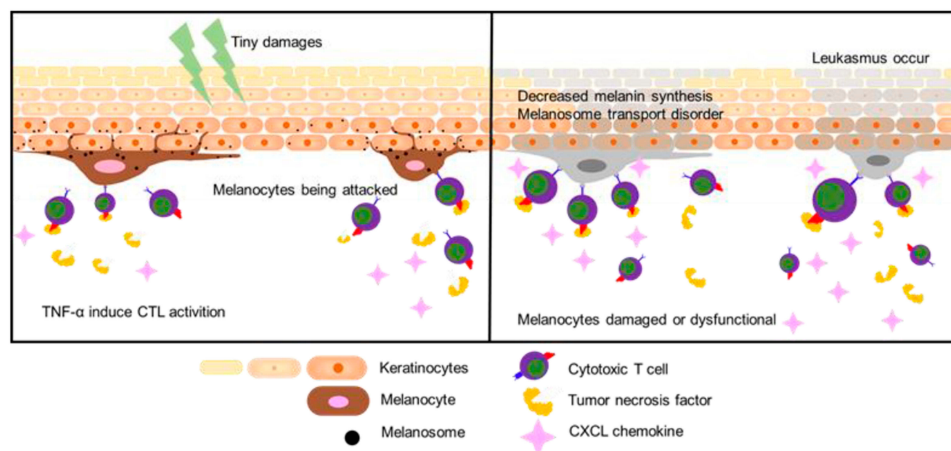
Separate from the autoinflammation theory, oxidative stress is also an important risk factor for vitiligo. Melanin synthesized by melanocytes is toxic which stimulates the cell stress signaling pathway in these cells. Moreover, active energy metabolism in the mitochondria leads to an excessive accumulation of reactive oxygen species (ROS). This also gives rise to the development of vitiligo.<sup>23,24</sup>

Briefly, when tiny lesions generated through a sunburn, viral infection or physical trauma occur, epidermal damage-associated molecules are released into the body and oxidative stress levels are increased. The adhesion of melanocytes is lost and inflammasomes are activated by the release and induction of molecular and oxidative stress products. After a series of immunoreactions, specific cytotoxic T cells are

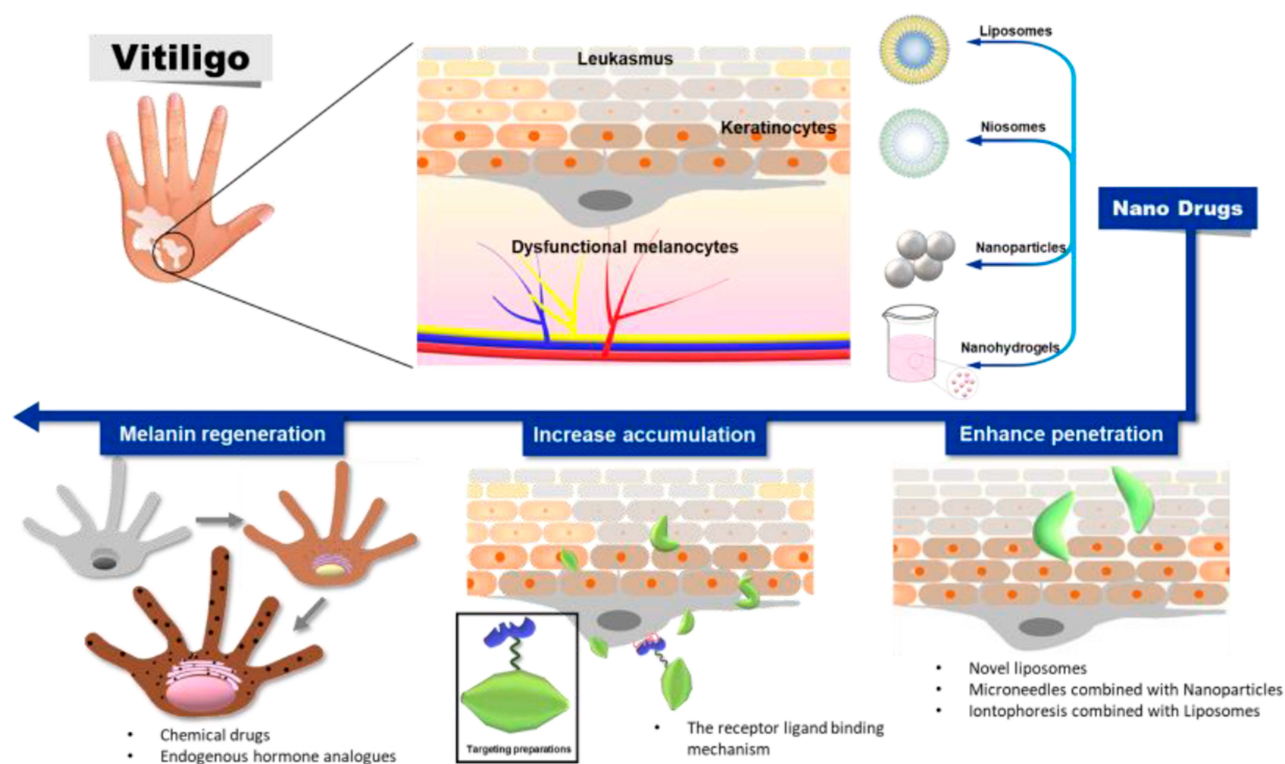
accumulated on the skin, regulatory T cell activity is down-regulated and inflammatory cytokines as well as autoantibodies are produced, ultimately leading to immune-based melanocyte destruction (Figure 1).<sup>25</sup> Likewise, oxidative stress contributes to the onset of depigmentation, and subsequent undesired autoimmune responses lead to the progression of vitiligo.<sup>26–30</sup> Here, we review all nano-drug delivery systems that can be used for the treatment of vitiligo, hoping to offer useful resources and spark inspiration for future research focused on novel vitiligo treatments (Figure 2).

## Therapeutic Approaches

Since the mechanics and dynamics of vitiligo are not fully understood, it has been challenging to develop an effective treatment for vitiligo. The ultimate goals for vitiligo treatment are to prevent the expansion of existing white spots on the skin, promote repigmentation and reduce psychological burden. Currently, strategies that are typically used for vitiligo treatment mainly include drug therapy and phototherapy (Table 1). Corticosteroids, such as beta-methasone dipropionate, clobetasol dipropionate and mometasone furoate, have been first-line options for vitiligo treatment due to their anti-inflammatory and immunosuppressive effects.<sup>31</sup> In recently published guidelines for vitiligo treatment, calcineurin inhibitors such as tacrolimus and pimecrolimus were also recommended as first-line approaches for the management of vitiligo.<sup>32–34</sup> Some clinical trials have shown that calcineurin inhibitors have a similar effect as glucocorticoids, especially for facial leukomas.<sup>35,36</sup> In addition, the accumulation of oxidative products in the skin is also an important factor



**Figure 1** Illustration of the pathogenesis of vitiligo.



**Figure 2** A scheme of the nano-drug delivery systems for vitiligo therapy.

contributing to melanocyte dysfunction. Hence, various antioxidants that remove excess ROS and hydrogen peroxide from the epidermis of vitiliginous skin have been used to restore the oxidation-antioxidant system of the skin.<sup>37</sup> Oral antioxidants such as polypodium leucotomos, vitamin E, vitamin C and minocycline are used for an antioxidation treatment strategy against vitiligo. Separate from drug therapy, phototherapy including narrow-band

ultraviolet (NB-UVA) and psoralen ultraviolet A (PUVA) are relatively safe physical-based approaches.<sup>38–40</sup> Since phototherapy alone and in combination with the drugs mentioned are highly successful in inducing repigmentation, these strategies have been extensively adopted in clinical practice.<sup>41</sup>

The onset, progression and management of vitiligo is usually a long process ranging from months to years. Thus,

**Table I** Treatment Approaches for Vitiligo

Treatment Approaches		Indications	Defects/Side Effects
Topical corticosteroids	Betamethasone dipropionate, clobetasol dipropionate, mometasone furoate	Localized vitiligo, both on adults and children.	Skin atrophy, telangectasias, folliculitis, acneic lesions, hypertrichosis and striae distensae.
Topical immunomodulators	Tacrolimus, pimecrolimus	Adult patients, as a substitution option of corticosteroids.	Photosensitivity, burning sensation, erythema, pruritus, flushing; increased risk of cutaneous and noncutaneous lymphomas.
Antioxidants	Vitamin C, vitamin E, superoxide dismutase, polypodium leucotomos	In association with all kinds of treatment options.	No obvious side effects reported while treatment effect is not obvious.
Phototherapy	NB-UVB, PUVA	Generalized vitiligo both in adults and children. Usually combine with additional drugs.	Hyperpigmentation, erythema, burning and blistering, increased risk of skin cancer.
Other treatments	Surgical therapies, depigmentation, cosmetic approaches, etc.		

long-term use of certain drugs or treatment approaches will inevitably result in a variety of side effects. For example, the side effects of the long-term use of glucocorticoids include skin atrophy, acne and folliculitis.<sup>42</sup> The large-scale use of corticosteroids is not feasible and may lead to a high number of side effects. Thus, corticosteroids are not considered appropriate for the treatment of generalized vitiligo. Although glucocorticoids and calcineurin are clinically complementary and have similar therapeutic effects, a recent study emphasized that tacrolimus showed no apparent therapeutic effects in mitigating segmental vitiligo.<sup>32</sup> The most common side effect of calcineurin inhibitors is a burning sensation during the first two weeks of treatment. In addition, the financial costs of calcineurin inhibitors are much higher than the costs of corticosteroids, which can be a significant financial burden for patients.<sup>37,43,44</sup> Comparing these different treatments, antioxidant drugs are milder with no obvious side effects. Nonetheless, accepted consensus guidelines do not recommend the use of topical antioxidants as a single therapy for vitiligo since most studies were restricted by a limited number of patients.<sup>45</sup> In terms of a non-drug treatment, phototherapy for vitiligo requires frequent treatment (two or three times weekly), which brings inconvenience to patients. Despite the higher success rates that these strategies have for repigmentation, recurrence rates still remain high. Over 50% of the patients are prone to recurrent white spots on repigmented skin within the first year after therapy is discontinued.<sup>25,46</sup> In addition, side effects such as headaches, nausea and ocular as well as renal toxicity may occur as a result of phototherapy treatment.<sup>47,48</sup> In addition, these therapies are also associated with a risk of skin cancer. Thus, phototherapy is recommended only when major treatments are found to be ineffective by the NHS.<sup>49</sup>

With the development of nanotechnology, voluminous nano-drug delivery systems have emerged and been applied to enhance drug penetration through the skin. These systems include microemulsions, nanoemulsions, nanoparticles, lipid carriers and many other nanovesicles and exhibit prominent advantages over conventional methods. In the field of nanomedicine, the number of existing literature focused on the treatment of vitiligo is relatively small.

## Nano-Drug Delivery System in the Treatment of Vitiligo

Human skin is the largest of the body's organ and helps to carry out many essential functions including such as acting

as a physical barrier, aiding in immune defense, temperature maintenance, UV protection, and moisture retention.<sup>50</sup> Three layers comprise the skin: stratum including the corneum, dermis, and hypodermis. The exposed layer and the stratum corneum contain 70–90% protein and 5–15% lipids, and aid in the process of influencing the role of protecting the body from external and environmental stressors and pollutants.<sup>51</sup> One of the most significant challenges of transdermal delivery such as to treat afflictions such as vitiligo is that only a few therapeutic compounds possess ideal penetration behaviors and characteristics.<sup>52</sup> The best existing technology merely allows drugs with a molecular mass of 100 Da and good lipophilicity to permeate the skin successfully.<sup>53–56</sup> In addition, another obstacle in achieving the desired therapeutic effects is the incompetence of the current dosage forms including creams, ointments, lotions, gels, and other vectors.<sup>57</sup> Generally, all the problems associated with conventional topical preparations suggest that reform and innovation of transdermal drug delivery for the treatment of vitiligo are urgently needed to improve the understanding of this affliction and to increase the possibility of better patient outcomes.

Recent advancements in cutting-edge nanotechnology provide an unprecedented opportunity to overcome shortcomings related to conventional methodologies (Figure 3). In particular, by taking advantage of some features of the stratum corneum, there is potential for the use of a nano-drug delivery system that can enhance the transdermal penetration of drugs and increase therapeutic effects.<sup>58</sup> A large number of novel nano-drug delivery systems carrying therapeutic agents are being developed, such as

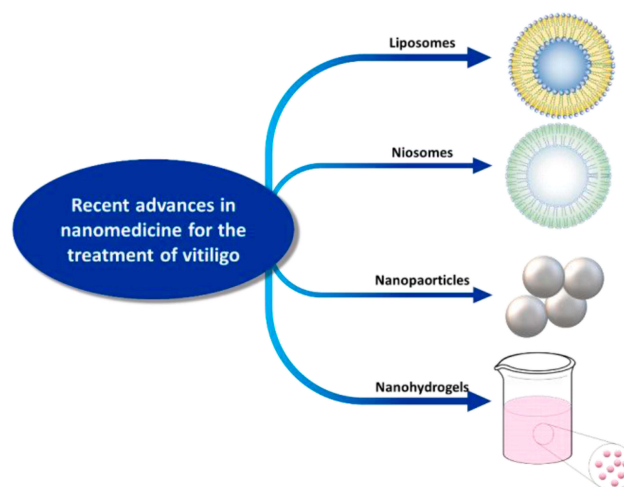


Figure 3 Existing nano-drug delivery system for vitiligo therapy.

liposomes, polymeric nanoparticles, microspheres, solid lipid nanoparticles and nanofibrous structures (Table 2)

## Liposomes and Niosomes

Liposomes are particles formed by lipids that consist of a double layer similar to the natural cell membrane enclosing an aqueous core. Containing non-toxic, biodegradable properties, liposomes are regarded as the most promising nanocarrier for topical drug delivery.<sup>69</sup> The apparent success of liposomes may be the result of lipid vesicles fusing into the stratum corneum, allowing the therapeutic to permeate into deeper skin layers.<sup>70</sup> Liposomes also increase the dissolution of the applied drugs and augment drug solubility on skin surfaces. Furthermore, liposomes are seen as drug repositories that allow for sustained rates of drug release and help reduce the frequency of treatments.<sup>71</sup> One group<sup>59</sup> prepared an 8-methoxypsoralen-loaded liposomal treatment through a film hydration method. After a series of purges, it was found that the diameter of the final vehicle ranged from 100 to 500 nm and showed excellent penetration behavior when compared to controls treated with hydroalcoholic solution. All nano vehicles displayed high capacities for sustaining the levels of 8-MOP release and its permeation—accumulation into skin layers.

Another group<sup>61</sup> reported the effects of both psoralen and resveratrol that were co-loaded with ultradeformable liposomes (UDL) for the treatment of vitiligo. Psoralen is a natural furanocoumarin derivative used in combination with UV or PUVA for the treatment of various skin diseases.<sup>72,73</sup> Due to the presence of a surfactant, the vesicle changes its shape to respond to conditions from the external environment.<sup>74</sup> Resveratrol stimulates mitogen-activated protein kinase signaling and has been demonstrated to have antioxidant activity.<sup>75</sup> Other groups<sup>76</sup> have conducted optimization studies and the most appropriate formula was selected based on particle size, PDI and zeta potential. Co-loaded liposomes were prepared through modified film hydration and liposome particle size was 120 nm with drug loading efficiencies between 2.5% and 5%. In vitro studies and kinetics showed that the carrier can sustain the release of both drugs over prolonged periods of time. In vitro cell studies showed that the carrier significantly stimulated melanin and tyrosinase activity without affecting the antioxidant capacity of psoralen and resveratrol.

One group<sup>60</sup> prepared baicalin and berberine samples that were co-loaded with ultradeformable vesicles and

demonstrated their potential as adjuvants for the treatment of vitiligo. Baicalin and berberine were selected as curative agents due to their antioxidant, anti-inflammatory and proliferative effects.<sup>76–78</sup> In vitro studies showed that preparation enhanced permeation of drugs and antioxidants. Photoprotective effect evaluations showed that co-loaded vesicles increased melanin and tyrosinase activities.

Others<sup>63</sup> constructed an elastic cationic niosome, loading the human tyrosinase plasmid pMEL34. The maximum loading amount of pMEL34 in the elastic vehicle was 150 mg/16 mg of niosomal determined by gel electrophoresis and documentation. Due to the enhancement effects of ethanol on vesicle elasticity, cumulative amounts and flux at 6 h post-treatment with pMEL34 were significantly increased in comparison with pMEL34 loaded into non-elastic cationic niosomes. The level of tyrosinase-associated activities were found to be four times higher than the respective levels for free plasmid and plasmid loaded into nonelastic niosomes, indicating that elastic cationic niosomes have potential as efficient gene topical delivery systems for vitiligo treatment. In 2012, a human tyrosinase plasmid pMEL34 by Tat peptide was described and found to enhance melanin production. The vesicular size and zeta potential were proved that the carrier is still in the range of stable dispersion. In vitro studies have indicated that this type of preparation promotes both tyrosinase gene expression and melanin production with little to no cytotoxic side effects.<sup>62</sup>

Another group<sup>66</sup> prepared 8-MOP ethosomes through a central composite design (CCD). Conventional liposomes deliver drugs to superficial layers of the skin, while ethosomes improve the permeation and retention in skin layers of the drugs.<sup>79</sup> The in vitro skin permeation study showed that this preparation induced enhanced transdermal efficacy, optimized ethosomal formulation and produced significant accumulation of 8-MOP, likely as a result of ethosome deformation. In vivo studies were consistent with in vitro results. Among all the elaborated topical transdermal delivery approaches, lipid nanocarrier is the most optimal one for its high biocompatibility and drug encapsulation efficiency. An increasing number of researchers in this field are paying attention to various novel vesicle delivery systems that possess percutaneous penetration and better therapeutic effects.

## Microemulsions

Microemulsions are thermodynamically stable colloidal systems with a transparent appearance. The conventional

**Table 2** Nano-Drug Delivery System for Vitiligo Therapy

Formulation	Drug/Therapeutic Agent	Outcome	Ref
Liposomes	8-Methoxypsoralen	The release and permeation–accumulation of 8-MOP is increased in vitro	[59]
Deformable liposomes	Baicalin and berberine	Stimulated melanin production and tyrosinase activity. Showed remarkable antioxidant and photoprotective capabilities	[60]
Deformable liposomes	Resveratrol and psoralen	Higher penetration rate, significantly stimulated melanin and tyrosinase activity, potential antioxidant activity, promoted pigmentation and restoration of redox balance by free radical scavenging activity.	[61]
Cationic niosomes	Human tyrosinase plasmid in mouse melanoma cells by Tat peptide	Enhanced the expression of human tyrosinase gene and melanin production with relatively low cytotoxicity; improved the stability of the plasmid loaded in the niosomes.	[62]
Cationic niosomes	Tyrosinase plasmid pMEL34	The expressed tyrosinase activities were four times higher than the free plasmid and the plasmid loaded in nonelastic niosomes. Exhibit potential as a gene topical delivery system for vitiligo.	[63]
Microemulsion-based gel	Clobetasol propionate	Showed better retention in the skin and less irritation potential, permeated and retained into skin layers; significantly enhanced skin permeation and accumulation; patients treated with MBC showed faster repigmentation	[64,65]
Nanosized ethosomes-based hydrogel	8-Methoxypsoralen	Significantly increased skin permeation and accumulation in the epidermal and dermal layers. Showed insignificant phototoxicity and erythema compared with the conventional cream.	[66]
Nanoparticles	Palladium and platinum	Is able to active AHR and NRF2 in human keratinocytes	[67]
Nanoparticles	Polydopamine	The melanin-like nanoparticles were endocytosed by human epidermal keratinocytes and mimicked the behavior of natural melanosomes.	[68]

formula of microemulsions consists of water and oil stabilized by surfactant and sometimes also contains a -cosurfactant.<sup>80</sup> Based on the physical and chemical properties of surfactants and ingredients of microemulsions, microstructures of microemulsions vary. The advantages of microemulsions used as transdermally delivered drugs show them as being an attractive technological platform for novel pharmaceutical formulations.<sup>81–84</sup> One group<sup>85</sup> reported a clobetasol propionate-loaded microemulsion-based gel that successfully overcame the poor solubility of clobetasol propionate. The effectiveness of the preparation indicated that the stratum corneum swelled due to water retention caused by gel formulation thereby assisting clobetasol propionate penetration into the skin. A clinical study indicated that the extent of repigmentation in patients treated with a clobetasol propionate-loaded microemulsion-based gel was faster and more profound when compared to control groups. Numerous studies and literatures have shown that microemulsion can significantly enhance the percutaneous permeability of drugs though the exact mechanisms that have not been fully understood. It is worth noting that apart from the internal microstructure, the composition or internal phase structure largely determines the permeation of microemulsion. Microemulsions have been a promising approach in the transdermal delivery of anti-inflammatory

drugs (NSAIDs) with a fairly obvious improvement in the permeation rate.

## Nanoparticles

The emerging use of nanoparticles is one of the most impactful components of nanomedicines. Due to large surface-to-volume ratios, nanoparticles not only enhance the contact area between a drug and target tissue but also permits drug release in a controlled manner.<sup>86</sup> In recent years, nanoparticles have been incorporated into common and daily goods. Nanoparticles penetrate the skin based on its size, charge and overall structure.<sup>87</sup> Metal nanoparticles such as palladium and platinum boost enzyme activity and exhibit anti-inflammatory capacity in UV-treated HaCaT keratinocytes. In addition, the rate of apoptosis in cells pretreated with nano-Pt was significantly reduced compared to controls.<sup>88</sup> A mix of Pd and Pt nanoparticles termed PAPANAL has a history as a treatment for chronic diseases such as burns, gastric ulcers and rheumatoid arthritis.<sup>89</sup> PAPANAL also exhibited SOD catalase activity and significantly lowered O<sub>2</sub>- generation levels in mouse skin tissue. Both in vivo and in vitro results showed that PAPANAL effectively suppressed endogenous superoxide levels through SOD and catalase activity via the AHR and NRF2 pathways.<sup>67</sup> Further investigation using

palladium and platinum nanoparticles in the treatment of vitiligo should be performed with caution since metals may possess allergens.<sup>90</sup> By coating or other means of modification, nanoparticles can overcome their inherent shortcomings to achieve efficient transdermal delivery and even release drugs with targeting behavior.

## Principles of Nano-Drug Delivery Systems Designed for Vitiligo Therapy

### Enhancing the Penetrate Capacity of Therapeutic Agents

Similarities between lipid particles and the stratum corneum make liposomes excellent vectors for delivery of therapeutics.<sup>91</sup> Conventional liposomes are thought to be inadequate for transdermal delivery, but more advanced liposomes such as invasomes, transfersomes, ethosomes and niosomes can overcome disadvantages seen with original methods (Table 3).

Invasomes were first proposed as novel modified liposomes in 2003.<sup>92</sup> The presence of soy-phosphatidylcholine (SPC), ethanol and terpenes make significant improvements in percutaneous permeability compared to traditional liposomes.<sup>93,94</sup> One group investigated the transdermal ability of invasomes loaded with ferulic acid. Skin permeation experiments revealed that invasomes possess good permeation capacity and are an ideal carrier for the transdermal delivery of ferulic acid.<sup>95</sup>

Transfersomes were first reported for transdermal delivery in 1992. Surfactants such as sodium cholate, spans and tweens make these carriers more elastic in comparison to traditional liposomes. Another group prepared a transfersome-encapsulating dexamethasone and evaluated its *in vivo* performance in a carrageenan-induced rat paw edema model. After a series of formula optimization studies, Span-80 was selected

as the optimum edge activator that offered maximum deformability. These results demonstrated that drug-loaded transfersomes have better antiedema activity when compared to liposomes, indicating that transfersomes possess better penetration ability.<sup>87</sup>

Microneedles (MN) are micron-sized needle projections with a height of 10–2000  $\mu\text{m}$  and a width of 10–50  $\mu\text{m}$  that painlessly penetrates the skin.<sup>98</sup> In recent years, transdermal drug delivery using microneedles has been widely studied.<sup>99–101</sup> A combination of microneedles and nanocarriers has also been considered for the treatment of diabetes,<sup>102</sup> cancer therapy<sup>103</sup> and immunotherapy.<sup>104</sup> Various nanoparticles with antioxidant or anti-inflammatory properties can be used as therapeutic agents to remove excess ROS and control vitiligo progression. However, poor water dispersibility and weak percutaneous permeability restrict application.<sup>105</sup> The combination of microneedles and nanoparticles brings hope for vitiligo therapy. Kim et al reported gold nanoparticles that were successfully able to be delivered into the hamster cheek at a depth of 100–200 $\mu\text{m}$ .<sup>106</sup> Nevertheless, the main drawback of treating vitiligo with microneedle is that the frequent use of microneedle will inevitably lead to stubborn skin damage and even inflammation. A comprehensive microneedle drug delivery strategy deserves attention and development.

By applying a mild electrical current, iontophoresis allows ionized substances to pass through the adjacent skin or tissue without invasion.<sup>107,108</sup> The combination of nano-drug delivery systems and iontophoresis was first reported in 1996. However, unsatisfactory results were obtained using this method.<sup>109</sup> Another group further demonstrated the mechanisms behind the combination strategy. This group observed connexin43 phosphorylation and filamentous actin depolymerization when charged

**Table 3** Brief Introduction of Novel Liposomal Systems

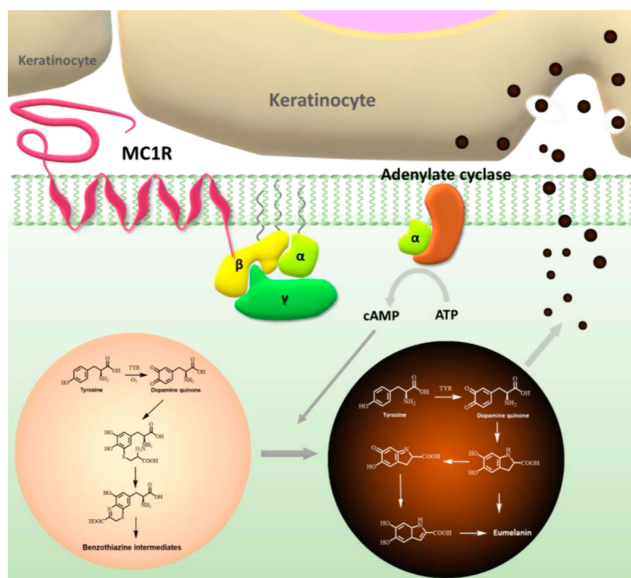
Carriers	First Described	Typical Compositions	Characteristics and Advantages Compared to Conventional Liposomes
Invasomes	A. Fahr et al in 2003. <sup>92</sup>	Phosphatidylcholine, ethanol, terpenes.	Enhance transdermal absorption of both aqueous and lipid-soluble drugs.
Ethosomes	E.Touitou et al in 1996 <sup>96</sup>	Phospholipids, high proportion of ethanol (20–45%)	Smaller particle size; fusion with skin lipids and increase the penetration of ethosomes.
Niosomes	RM.Handjanivila et al in 1979 <sup>96</sup>	Non-ionic surfactants and cholesterol.	A greater bioavailability; more stable than liposomes in oxidizing environment.
Transfersomes	G. Ceve et al in 1992. <sup>97</sup>	Phosphatidylcholine, surfactants (sodium cholate, Tween 80, Tween 60, Span 80, etc.)	Greatly improve the deformability and elasticity of the carriers; migrate into deeper skin layers.

liposomes crossed the intercellular space. The  $\text{Ca}^{2+}$  inflow was also stimulated by the electrical stimulus. All evidence suggested that the electric current changed the physiological properties of the skin and enhanced the permeation of liposomes.<sup>110</sup>

## Increasing Therapeutic Agent Accumulation: The Receptor Ligand Binding Mechanism

Melanocytes are dendritic cells that synthesize and secrete melanin and are located in the basal cell layer of the epidermis. There are plenty of ways therapeutic agents can overcome the stratum corneum barrier and penetrate deeper into the skin. However, driving and accumulating the drug to the target site still remains a challenge. The affinity of the receptor and ligand that encompasses active targeting is widely utilized to exert specific retention. Many receptors on the surface of melanocytes enable the construction of such active targeting vectors.

Melanocortin-1 receptor (MC1R) is a G protein-coupled receptor specifically distributed on the surface of melanocytes (Figure 4). Endogenous ligands of this receptor include  $\alpha$ -MSH,  $\beta$ -MSH,  $\gamma$ -MSH and adrenocorticotrophic hormone (ACTH). Among these ligands,  $\alpha$ -MSH shows the strongest affinity with MC1R.<sup>111</sup> The amino acid sequence of  $\alpha$ -MSH is simple, allowing it to serve as a target molecule enabling nano-drug delivery systems to aggregate in the vicinity of melanocytes.



**Figure 4** Schematic diagram of the role of MC1R in melanin synthesis and secretion.

The endothelin receptor is another receptor located on melanocytes that participates in a variety of physiological activities. During the transformation of melanocytes into melanoma cells, endothelin B receptor expression gradually increases.<sup>112</sup> One group reported that Endothelin-1 (ET-1) promotes melanin synthesis and the formation of dendrites in normal human epidermal melanocytes.<sup>113</sup> Similarly, a seven-transmembrane receptor on keratinocytes termed protease-activated receptor-2 (PAR-2) plays a central role in the transportation of melanosomes.<sup>114</sup> SLIGRL and SLIGKV are two PAR-2-activating peptides that have the same activation capacity as PAR-2 without receptor cleavage.<sup>115,116</sup>

## Promoting Melanin Regeneration

Therapeutic goals for vitiligo treatment consist of inhibiting the inflammatory response and promoting melanin regeneration. Many therapeutic regimens only focus on inhibiting the inflammatory response rather than promoting repigmentation. Thus, there has been a growing demand for drug delivery systems that inhibit the inflammatory response while promoting melanin regeneration.

Tacrolimus is a calcineurin inhibitor that weakens T cell activity and the secretion of proinflammatory cytokines, thus promoting melanocyte migration and repigmentation.<sup>117,118</sup> However, its application is restricted due to the risk of lymphoproliferative disease, narrow therapeutic index and low solubility.<sup>119,120</sup> One group prepared a nanolipid carrier containing lipophilic solubilizers to improve drug solubility. This carrier exhibited a high entrapment efficiency of 96.66% and a significantly higher drug release in vitro and in vivo compared to the commercial ointment. Meanwhile, a skin irritation study revealed less irritation using this carrier when compared to the control group.<sup>121</sup>

As described, many receptors specifically located on the surface of melanocytes and keratinocytes play important roles in controlling melanocyte proliferation, melanin synthesis and melanin migration. On the other hand, their endogenous ligands or the analogues can be used as peptide drugs to activate biochemical reactions associated with melanocytes.  $\alpha$ -Melanocyte-stimulating hormone ( $\alpha$ -MSH), a tridecanoic peptide derived from pro-opiomelanocortin (POMC), plays a significant role in stimulating melanogenic pigmentation in skin tissues.<sup>122</sup> After binding to MC1R, the receptor typically sends signals by increasing cyclic adenosine monophosphate (cAMP) and exhibits powerful anti-inflammatory effects.<sup>123</sup> The short fragments of  $\alpha$ -MSH or its derivatives Lys-Pro-Val (KPV) and (CKPV)<sub>2</sub> are reported



to possess similar or even stronger biological activity.<sup>124</sup> ET-1 can also regulate the synthesis of melanin, promote melanocyte proliferation and help form dendrites. Accordingly, these endogenous molecules or their analogues can be anchored on transdermal delivery systems to ameliorate transdermal efficiency as well as promote melanin regeneration.<sup>125</sup>

## Conclusion

Vitiligo is a common depigmenting disease affecting 1% of the population worldwide. In some regions, the white spotting caused by vitiligo is confounded with leprosy and has devastating effects on patient psychological health and social activities.<sup>126</sup> Treatment and management of vitiligo still shows intractable challenges for both scientists as well as dermatologists. More evidence suggests that the dynamics and pathogenesis of vitiligo are closely related to the immune response of skin and melanocyte response to the immune response. Thus, vitiligo should be treated as a chronic immune disease rather than a problem mainly regarding an approach from a regenerative medicine perspective.<sup>127</sup> Nevertheless, the rapid development of nano-drug delivery systems has brought new insights and ideas for the treatment of vitiligo. These novel methods facilitate the ability for developed drugs to possess sustained or controlled release behavior, enhances the therapeutic efficiency and reduces side effects.

Despite the great potential of the available nano-drug delivery systems, certain limitations exist. Most research regarding novel drug delivery systems for the treatment of vitiligo settle for the minimum control of symptoms rather than optimal outcome to completely cure the disease. Despite these challenges, it is inevitable that scientists will perform and complete the research needed to further optimize nano-drug delivery system efficiency. Various receptors on the surfaces of melanocytes or surrounding keratinocytes including granulocyte colony-stimulating factor (G-CSFR) and melanocortin receptors 1~5 (MC1R~MC5R) increase the feasibility of developing targeted agents. Their endogenous ligands or analogues exhibit great potential for the treatment of vitiligo with minimum side effects. One group proposed ultrashort peptides containing cysteine that spontaneously self-assembled into hydrogels. In vivo studies revealed that one of the formulas exhibits great biocompatibility and limited allergenic potential.<sup>128</sup> In the following studies, self-assembly behavior, nanostructure formation, hydrogelation and phase transition of peptide nanostructures of two selected tripeptides

were investigated by X-ray crystalline diffraction and scanning electron microscopy.<sup>129</sup> A consistent hydrophobic backbone of acetylated Leu-Ile-Val-Ala-Gly plays the role of self-assembly and the C-terminal residue has an obvious impact on the rate and extent in which the peptide fibril interacts with an SMD.<sup>130</sup> By elucidating the crosslink and polymerization mechanisms of small molecule peptide drugs, various peptide-based topical drug delivery systems may be an inevitable trend for vitiligo treatment.

Here, we reviewed the pathogenesis, present therapeutic methods and existing nano-drug delivery systems that have been used and that will be used in the future for the treatment of vitiligo. Vitiligo can eventually be better controlled, managed and even cured considering the significant progress that has been made and the ongoing development of strategies to tackle this malady.

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## Disclosure

The authors report no conflicts of interest in this work.

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