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Engineering nanomedicines for improved melanoma therapy: progress and promises

Di Bei¹, Jianing Meng¹, and Bi-Botti C Youan^{1,†}

¹Laboratory of Future Nanomedicines & Theoretical Chronopharmaceutics, Division of Pharmaceutical Science, School of Pharmacy, University of Missouri-Kansas City, MO 64110, USA

Abstract

Once metastatic, melanoma remains one of the most aggressive and morbid malignancies. Moreover, in past decades, the overall survival for advanced unresectable melanoma exhibited a constancy of poor prognosis. Low response rates and serious adverse effects have been characteristic of standard therapy based on a combination of chemotherapeutic agents or immunotherapy with IL-2. For example, the chemotherapy including dacarbazine, carmustin, cisplatin and tamoxifen is known as ‘Dartmouth regimen’ while the CVD regimen comprises carmustine, vinblastine and dacarbazine. Thus, there is an urgent and critical need to reformulate these bioactive agents using nanoscience and nanotechnology as alternative strategies. This article overviews current design and evaluation of nanomedicine undertaken to address this unmet medical need. The nanomedicines studied include polymeric nanoparticles, liposomes, polymersomes, dendrimers, cubosomes, niosomes and nanodiamonds. In this preclinical article, nanotechnology provides hope for effective treatment of this aggressive and largely treatment-resistant disease.

Keywords

cancer; engineering; melanoma; nanomedicines; therapy

Melanoma (Figure 1), a malignant tumor that claims the majority of deaths in human skin cancer, is one of the most common cancers in adults. Each year, more than 60,000 people in the USA learn that they have melanoma [1]. Approximately 132,000 cases of malignant melanoma (the most fatal kind of skin cancer) and over 2 million cases of other skin cancers occur worldwide each year. One in every three cancers diagnosed worldwide is a skin cancer [2]. Melanoma is rising at a rate faster than that of all preventable cancers, except lung cancer, in the USA [3].

If melanoma is found and treated in its early stages, the chances of recovery are very good. If it is not found early, melanoma can grow deeper into the skin and spread or undergo

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[†]Author for correspondence: Tel.: +1 816 235 2410, Fax: +1 816 235 5779, youanb@umkc.edu.

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metastasis to other parts of the body. Once it has evolved to malignant melanoma, it is difficult to cure and results in a high death rate.

The current clinical approach and therapy for cutaneous melanoma includes surgery, chemotherapy or immunotherapy, and/or the combination of the two. Surgery remains the best intervention for patients with early-stage melanoma. Many surgeons consider 0.5 cm as the standard for excision [4], but a 0.2 cm margin might be acceptable for margin-controlled surgery. Tumor recurrence or relapse is a common failure in treatment. Mohs micrographic surgery (also known as chemosurgery [5]) has a cure rate between 97 and 99.8% [6]. Unfortunately, attempts to improve survival by surgically removing lymph nodes result in no overall survival benefits [7]. Other than surgery, there are two major alternatives to this disease management: chemotherapy and immunotherapy. Chemotherapy typically includes administration of dacarbazine, cisplatin, temozolomide and paclitaxel. Dacarbazine is administered alone or in combination with other drugs, including carmustine and cisplatin [8,9]. The combination of dacarbazine, carmustine, cisplatin and tamoxifen is termed the 'Dartmouth regimen' [8]. The combination of cisplatin, vinblastine and dacarbazine is another treatment for melanoma known as the 'CVD regimen'.

The continuous search for better patient outcome had led to immunological approaches to the treatment of malignant melanoma. Multiple peptide, dendritic cell, adjuvant, lymphocyte and virus-based strategies have been established and tested. They reveal some degree of success in preclinical and clinical studies. A recent study in melanoma immunotherapy has demonstrated that complex vaccines and the combination of different approaches, including dendritic cell vaccines in conjunction with costimulatory molecules, are superior to conventional immunization protocols in induction of tumor-specific immune responses [4].

Although the current therapies have their advantages, they are either not effective enough or they cause serious side effects and toxicity. The two biotechnology-derived drugs that appear most active against melanoma are IFN- α and IL-2. The response rates for IFN range from 8 to 22%, and long-term administration on a daily or a three-times-per-week basis appears superior to once per week or more intermittent schedules [10]. The response to IL-2 regimens is similar and is in the range of 10 to 20% [11–13]. In the randomized experiments to date, it has been reported that no drug or combination of therapies is superior to dacarbazine [8]. Therefore, an urgent need exists to produce novel drug delivery systems, perhaps using nanoscience and nanotechnology, which offer improved efficacy and fewer side effects, particularly in melanoma therapy [14–17].

Nanotechnology & nanoparticles

Nanotechnology is a generalization for techniques, materials and equipment that operate at the nanoscale. It has a revolutionary approach that consists of the design, characterization, preparation and application of structures, devices and systems by controlling shape and size at the nanoscale [18]. Over the past 50 years, nanoparticles and microspheres have been used as carriers of anticancer drugs to increase antitumor potency of the old drugs and reduce toxic side effects [19,20]. There are significant advances in the research of injectable liposome delivery systems. Some of these systems have been marketed to serve the public, making use of their biological compatibility. For example, tumor-targeted immunoliposomes have an enormous advantage for cancer therapy due to their ability to target their drug payload to tumor cells [21,22]. During recent years, cubosomes/hexasomes, exosomes, niosomes, polymersomes, nanodiamonds, dendrimers and nanoemulsions have entered the drug delivery system family as carriers for different routes of drug administration and for the treatment of different cancers and diseases.

It is known that a great deal of modern cosmetics utilize nanosized components. Nanoemulsions are transparent and have unique tactile and texture properties. Small vesicles, which range from 50 to 5000 nm, are contained in nanocapsule, niosome or liposome formulations. These will function to protect cosmetic ingredients from light and/or oxidation [23]. Transdermal delivery and cosmetic research [23,24] have shown the potential of these vesicles to penetrate the stratum corneum of the human skin. Nano-sized drug formulations or nanomedicine may enhance or reduce skin penetration of drug depending on the physicochemical properties of the ingredient and the formulation [25]. Moreover, recent studies suggest the contribution of hair follicle in cutaneous transport [26]. Unlike polymeric nanospheres (Figure 2A, which are matrix systems of nanoparticles), nanocapsules (Figure 2B) are heterogeneous vesicular systems in which the drug is confined to a cavity surrounded by a polymeric membrane. Therefore, nanocapsules are considered as 'reservoir' systems and the core may be aqueous or composed of a lipophilic solvent, usually oil. Nanospheres and nanocapsules have been discussed extensively elsewhere [27–29] and are not the main focus of this discussion. Nanoparticle technology based on human protein albumin exploited natural pathways to selectively deliver larger amounts of drug to tumors while avoiding some of the toxicities of solvent-based formulations. The resulting 130 nm albumin-bound (nabTM) paclitaxel (nab-paclitaxel; Abraxane[®]) was recently approved for use in patients with metastatic breast cancer who have failed combination therapy. Preliminary data also suggested roles for nab-paclitaxel as a single agent and in combination therapy for first-line treatment of metastatic breast cancer as well as in other solid tumors, including malignant melanoma [30]. This article provides an overview of some of the recent nanomedicines with potential for treatment of melanoma.

Liposomes

Liposomes, by definition, are nanoscopic/microscopic structures formed by lipid bilayers. They can be prepared by sonicating lipids in an aqueous phase. Briefly, the preparation process of liposomes involves emulsification [31,32]. In recent years, liposome surfaces were decorated with different ligands with the specific purpose of targeting certain organs and tissues, as well as controlling drug release in specific areas. In that case, the surface of such liposomes was modified as one example [33].

It has been more than 40 years since the first report of the successful preparation of liposome [34]. These systems have been extensively used in drug delivery and targeting [35–41]. The typical liposomes are made of bilayers of phospholipids that are similar to the membrane composition of cells. Since the development of the first liposome, this type of nanomedicine has been used widely in pharmaceutical research. When sequentially extruded down through a 0.2- μ m membrane, the resulting vesicles exhibit a very homogeneous size distribution with a mean diameter of 0.27 μ m, whilst maintaining an acceptable level of encapsulation of the aqueous phase [42]. Their ability to passively target a tumor is known as the enhanced permeability and retention effect. Moreover, they are capable of encapsulating hydrophobic, hydrophilic and amphiphilic drugs. Hydrophobic drugs are entrapped in lipid layers while hydrophilic drugs are entrapped inside the aqueous phase and, thus, they provide a broader choice of drugs for encapsulation, as shown in Figure 3. As a result of extensive research on liposomes, several antitumor liposome delivery systems have entered the market, such as doxorubicin–liposome, camptothecin–liposome and daunorubicin–liposome systems.

In this article, we summarized liposome delivery systems for traditional chemotherapy drugs, such as cisplatin [31], vincristine [32], doxorubicin [43] and glucocorticoids [44]. For immunotherapy, for example IFN- β -loaded liposomes, showed a promising effect [45]. Novel bioactive agents, including Toll-like receptor (TLR) or TLR3/TLR9 agonists [46] and

gene therapy drugs, such as polyinosinic–polycytidylic acid [47] and protease-activated receptor-1 siRNA [48], are also discussed. For imaging application, functionalized quantum dot liposomes exhibited relatively long retention time [33]. Table 1 is a comparison of liposomal systems for diverse therapies regarding their preparation process, size, drug payload (encapsulation efficiency) and stability, as well as toxicity, and *in vitro* and *in vivo* effect.

With respect to the use of liposome as a drug delivery template for melanoma treatment, many specific *in vitro* and *in vivo* data are available in the literature. It was reported that phosphatidylethanolamine liposomal cisplatin had a higher cytotoxicity than classic liposomes or free cisplatin, a high level of intratumoral drug concentration for 72 h and efficiently delivered approximately 3.6-times more drug than the free drug [31]. Traditional chemotherapy drugs, such as combretastatin and doxorubicin, were also coencapsulated into liposomes to form a potent therapy to greatly inhibit melanoma tumor growth [43]. Liposomes containing glucocorticoids were found to be highly potent to suppress tumor angiogenesis and inflammation at the same time [44]. Liposomal prednisolone phosphate was able to strongly inhibit endothelial cell proliferation and reduce proangiogenic protein (such as bFGF) levels, which were related to tumor angiogenesis [49]. Liposomal IFN- β led to direct cell death and stimulated natural killer cells that could be effective during antimelanoma therapy [45]. Cationic liposome containing polyinosinic–polycytidylic acid significantly increased tyrosinase-related protein (TRP)-2-specific IFN-producing cells and resulted in an augmentation of the antitumor immune response [47]. This showed another possibility in immunotherapy for melanoma by peritumoral injection. Functionalized quantum dot–liposome hybrid offered great potential for melanoma imaging due to its rapid accumulation and retention within the tumor [33]. Liposomal siRNA could decrease melanoma growth and metastasis *in vivo* [48]. TLR agonists, such as TLR3/TLR9, produce uniquely effective vaccine adjuvants that elicited strong T-cell responses against melanoma tumor [46]. In addition, there was a clinical pharmacological report on liposomal vincristine sulfate shown to enlighten melanoma therapy [32]. Due to its long history in drug delivery research, this nanomedicine template has been extensively used in melanoma research, as recently reviewed [50]. For example, doxorubicin [51,52], human leukocyte antigen-B7 (HLA-B7) microglobulin or Allovectin-7 [53,54], thymidine kinase [55] and an allogeneic vaccine from human melanoma cell line [56] have all been clinically tested, demonstrating good tolerance but failure to show any beneficial activity.

Dendrimers

The word dendrimer originates from the Greek dendron, meaning ‘tree’. Ideally, they are perfectly monodisperse macromolecules with a regular and highly branched 3D architecture [57]. Dendrimers are originally planar molecules that can assume tridimensional characteristics by progressive increase of their branching complexity and flexibility. This produces the modification of their geometry towards spheroid, progressively closed structures [57,58]. The spheroid nanostructures are capable of encapsulating bioactive molecules by either trapping them inside the inner void space or covalently linking them on their surfaces. The size, morphology and bioactivity are determined by the generation/shells, usually G0 to G4, chemical composition of the core, branches and functional groups on the surfaces (Figure 4). Generally, the synthesis involves relatively easy preparation from the molecular level to nanoscale. The advantages of dendrimers include the controllable size (usually 1 to over 10 nm), morphology and functional groups on the surface, making them one of the most promising nanotechnologies commercially available. Dendrimers’ diameter increases with the generation linear progression. The size can be as small as that of the hemoglobin.

Since the early 1980s, the dendrimer products in commercial use include Starburst[®] dendrimers, Combburst[®] and Priostar[®], among others. Table 2 is a comparison of dendrimer systems for diverse therapies and their preparation process, size, drug payload (encapsulation efficiency), stability, toxicity, and *in vitro* and *in vivo* effect. It has been reported that multivalent neoglycoconjugates (such as polyamidoamine [PAMAM]-GlcNAc8) could stimulate an immune response against melanoma [59]. The modification of the surface charge of polycationic PAMAMs would change their deposition into tissues [60]. In an early report, boronated Starburst dendrimer–monoclonal antibody immunoconjugates were used in neutron capture therapy for melanoma and hepatic and splenic concentration could be reduced [61]. The schematic diagram and the structure of this Starburst dendrimer were shown [61]. There are also more recent reports related to dendrimer application to melanoma. For example, it was observed that Au-dendrimer's size and charge could greatly affect their biodistribution, and the organ selectivity made it possible to target specific tissues and organs [62,63]. In another study, $\alpha_v\beta_3$ -targeting peptide exhibited selective concentration of the dendrimer complex in the tumor in comparison to drug level in blood, although the mechanism was not clear [64]. The *in vitro* toxicity of synthesized PAMAM dendrimers in physiologic concentration range suggest promise towards the future use of this nanomedicine [65]. Overall, dendrimers have great potential in drug nanoformulations for melanoma therapy owing to their advantages, such as thermodynamic stability, high solubility in water and uniform size distribution, but advances in synthetic methods are needed to produce biocompatible and biodegradable dendrimers.

Cubosomes

Cubosomes (Figure 5) consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. Self-assembled cubosomes as active drug delivery systems are receiving more and more attention and interest after the first discovery and nomination [66,67].

The preparation mostly involves simple emulsification of monoglyceride and a polymer, accompanied by sonication and homogenization. The preparation methods fall into two categories, including top-down and bottom-up techniques [68]. In the top-down technique, a coarse dispersion of cubosome is usually formed first, which is then tailored into more uniform and finer particle dispersions with the help of high energy input devices (e.g., homogenization and sonication). However, in the bottom-up technique, cubosomes are formed instead by assembling nanomaterials into the final cubosome dispersion.

Several studies have been carried out on the physical and chemical properties of cubosomes. The preparation of cubosomes based on different materials has been reported [68–71]. It has also been reported that cubosomes transform into hexasomes, exhibiting a time-resolved behavior due to pH-induced lipid hydrolysis [72]. The internal and structural changes of cubosomes could be controlled by adjustment in lipid composition [73]. The specific type of cubosomes was researched to identify their detailed structure [74,75]. The cubic symmetry and ionexchange properties [76], the bilayer phase transition [77], the effect of vitamin E and polymer on cubosome structure [71], the instability of cubosomes in plasma due to interactions with lipoproteins (high-density lipoprotein and low-density lipoprotein) and albumin [78] were also reported.

At present, most of the research related to cubosomes has been focused on the preparation process, structural characteristics, characterization and stability. For example, recently, few anticancer drugs have been successfully encapsulated in cubosomes [79–81] and characterized physicochemically [82].

Although the *in vitro* and *in vivo* pharmacological studies of cubosomes have not been widely conducted, the unique structure of this promising nanocarrier suggests its application in melanoma treatment. There are several publications concerning the release profile of cubosomes [83], application as a percutaneous delivery system [84] and application as a formulation for skin hydration [85].

Overall, cubosome have great potential in drug nanoformulations for melanoma therapy owing to their potential advantages, including high drug payloads due to high internal surface area and cubic crystalline structures [86], relatively simple preparation method, biodegradability of lipids, the ability of encapsulating hydrophobic, hydrophilic and amphiphilic substances, targeting and controlled release of bioactive agents.

Polymersomes

Polymersomes (Figure 6) are nanostructures composed of amphiphilic block copolymers that have a size range from 50 nm to 5 μ m and encapsulate drugs inside the vesicle membrane [87]. They are capable of encapsulating hydrophobic and hydrophilic drugs [88] and they can be surface functionalized. Their polymeric membrane potentially offers a protective barrier to proteins, peptides, DNA and RNA fragments against deleterious factors that may be present in the biological environment. Polymersomes share many similarities with liposomes, but are more stable and less permeable to small water-soluble molecules than liposomes [89]. Furthermore, the flexibility of polymersome membrane makes them capable of targeting and controlling drug release. In previous literature, one example of modified polymersome was studied [90]. Another example of polyester of either polylactic acid or poly(ϵ -caprolactone) polymersomes showing controlled release properties was also reported [91].

The polymersomes were able to conjugate biologically active ligands, such as avidin, antibodies and biotin, to their surface and, thus, provide targeted therapy and imaging strategy [88]. It was reported that polymersome could be used in controlled release of multiple drugs against a tumor due to its enhanced permeability and retention effect and relatively higher drug loadings into polymersome compared with liposomal formulation [92]. Polymersome encapsulating doxorubicin and/or paclitaxel was widely researched as a treatment for tumor [88,93]. Biotin functionalized leuko-polymersome [90] to monitor or treat inflammation, cancer and cardiovascular disease, and Tat-conjugated polymersomes [94], an excellent agent for cellular trafficking, were also investigated as having great potential against cancer. The comparison between polymersome systems stated above and their detailed *in vitro* and *in vivo* information are shown in Table 3.

Overall, polymersomes have great potential in drug nanoformulations for melanoma therapy owing to their advantages, such as robust and larger shell enhancing drug loading and stability, and possibility of enhanced drug targeting and relatively longer *in vivo* circulation, especially with worm-like structures [95].

Niosomes

Niosomes (Figure 7) are synthetic analogs of liposomes in structure [96], but have generally more penetrating capability and physicochemical stability, less toxicity and improved therapeutic index for entrapped drugs. In morphology, a niosome is a nanostructure with 100 nm to 2 μ m in diameter, whose center is an aqueous cavity enveloped by layers of nonionic surfactant in lamellar phase.

Current preparation methods include an ether injection method [97,98], thin film hydration method [98], sonication [98], microfluidization [99], multiple membrane extrusion [99],

reverse-phase evaporation technique [100], remote loading [101], bubble method [102] and proniosome preformulation method [103]. The advantages of niosomes include high patient compliance in comparison with conventional oily dosage forms, wide range of solubilities, flexibility and ability to release drugs in a controlled manner. Therefore, they are used often in applications such as targeting vehicles, neoplasia, peptide carriers, hemoglobin carriers and transdermal delivery.

Niosomes were documented to have higher stability without significant toxicity in the skin after topical application of drugs in comparison to liposomes [104]. Although there are not many studies on niosome systems against skin cancer such as melanoma, there is a definite potential for the use of niosomes as an alternative delivery system for melanoma treatment. An *in vitro* study about niosome containing tretinoin exhibited higher cutaneous retention than liposomes and other commercial formulation; moreover, physicochemical properties of a vesicular bilayer component determined the interactions between skin and drug-loaded nanoparticles [105]. Niosome use is preferred by scientists due to the cost-effectiveness and stability. Niosomes containing DNA encoding hepatitis B surface antigen (HBsAg) have been prepared as DNA carriers for effective topical immunization [106]. In topical applications, niosomes demonstrated prolonged circulation, drug release, increased drug retention in skin and enhanced drug permeation across the skin [107]. In a novel nanoformulation composed of bola-surfactant, niosome consisted of α,ω -hexadecyl-bis-(1-aza-18-crown-6). The bolaniosomes promoted the intracellular uptake of the encapsulating drug, significantly improving drug permeation across the skin. In addition, there was a specific report on the niosome targeting skin cancer in which 5-fluorouracil-loaded niosomes, exhibited eight- and four-fold higher drug penetration compared with a free drug control and a mixture of free drug with empty bola-niosome control, respectively [108].

Overall, niosomes have great potential in drug nanoformulations for melanoma therapy owing to their advantages, such as relatively higher chemical stability, improved purity and relatively lower cost in comparison with liposomes.

Nanodiamonds

Nanodiamonds (NDs, Figure 8) belong to the family of carbon nanomaterials, and they are gaining increasing interest in a diverse range of industries [109,110]. NDs are composed of a nanosized tetrahedral network and their structure, which is dependent on their electronic properties, distinguishes them from other carbon nanomaterials.

Nanodiamond particles were reported to be prepared with the detonation technique [111], resulting in particles of approximately 5 nm whose faces are covered with disordered graphitic shells due to a naked surface, without terminating species [112].

The use of NDs in medicine has been motivated by their potentially advantageous properties. First, NDs would potentially protect drugs trapped inside ND agglomerates due to the high surface energy relative to their small size. It is noteworthy that the terms 'aggregation' and 'agglomeration' in this paragraph have different meanings: agglomerates are reversible; aggregates are irreversible. Since agglomerates are reversible, this process would result in drug loading and release, thus reducing cytotoxicity and unwanted drug effects. Second, drugs would not be released until they reach the cancer cells by the enhanced permeability and retention effect or passive targeting after carrying the drug payload to the targeted site due to their strong surface interaction with the ND. However, due to new development in chemical synthesis [113,114], it may now be possible to alternatively functionalize ND surface for active targeting purpose. Third, it is anticipated that after drug delivery and release, the remnant diamonds (carbon-based materials, the main basic element of life) would not induce inflammation in cells owing to their very small size

(escape triggering of inflammation and immunological response). However, these speculations remained to be demonstrated both experimentally and clinically.

At present, there are ongoing studies on the therapeutics application of ND against human diseases [113,114]. Most works have focused on structural and physicochemical analyses [112]. However, there have been some reports of ND hydrogels used for chemotherapeutic delivery in which doxorubicin hydrochloride was applied to functionalized ND to improve the drug efficacy and to achieve a versatile release profile without producing the undesirable effects typically observed with native drugs [115]. ND-embedded microfilm was also successfully used for localized chemotherapeutic elution [116].

Overall, NDs have great potential in drug nanoformulations for melanoma therapy owing to their advantages, such a smaller size compared with other carbon nanomaterials. If the appropriate bioconjugate chemistry could be developed to successfully attach active targeting ligand and bioactive agents while assuring stability and efficacy, NDs could be used to reach a tumor area that could not be reached before with other conventional nanomedicine templates.

Conclusion & future perspective

As nanotechnology makes drug delivery systems much smaller, lighter, stronger, cleaner, more specific and more precise, there is enough evidence to believe that nanomedicine would bring hope for better management of melanoma. Polymeric nanoparticles, liposomes, dendrimers, cubosomes, polymersomes and niosomes are currently leading the research interest in targeting and curing skin cancers. The specific advantages obtained from the described nanocompounds in perspective of melanoma treatments (e.g., more specific targeting) have been given at the end of each section of this article. It is important to underscore that there is a possibility to use new ways of administration, such as an oral or pulmonary route, if the stability problem in the biological microenvironment can be overcome and if we can elucidate strategies to effectively cross the different levels of biological barriers to selectively reach the targeted cancerous cells in the body.

Table 4 shows the potential advantages and disadvantages of each nanosystem. There are still many other types of nanomedicine templates not yet fully tested in melanoma therapy either in the preclinical or clinical setting. These may also be promising area of investigations. These perhaps include vesosomes [117], solid lipid nanoparticles [118], nanogels [119] and the combination of these systems with selective ligands such as bevacizumab antibody [120]. Since many metastases of melanoma are not located in the skin, but rather in deep organs (even in the brain), transdermal therapy is of no use for such metastatic sites. Therefore, specific targeting technology, such as physically enhancing the nanomedicine-specific response by coupling with other triggering elements such as those of magnetoliposomes [121], should be investigated. The future could possibly belong to the design of hybrid biomaterials, such as recently reported Janus dendrimers or dendrimersomes [122], that exhibit stability and mechanical strength inspired from polymersomes with the biological function of stabilized phospholipid liposomes with superior uniformity of size, ease of formation and chemical functionalization. Furthermore, the stability and concerns of some existing and newly designed nanomaterials for topical application remained to be elucidated [123]. If well designed, future nanomedicines intended for improved melanoma therapy would safely penetrate transdermally via various mechanisms (Figure 1) [124], and locally release the required therapeutic dose (in case of melanoma with no metastasis in deep organs), thus avoiding unwanted side effects of current chemotherapy. This overview of the literature uniquely sheds light on future research opportunities and may inspire scholars in the field of pharmaceutical and biomedical

research to develop the ‘magic bullet’ for melanoma and, thus, making better use of both new and old drugs. More studies concerning the thorough biological fate of the newly engineered drug nanocarrier, their safety and clinical advantage and acceptance are still in urgent need before transitioning from laboratory bench to patient beside. As recently suggested, further progress can be accelerated by applying existing melanoma or in general cancer control knowledge across all segments of the population and by supporting new discoveries in cancer prevention, early detection and treatment [125].

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Executive summary

- Some progress has been made experimentally in engineering nanomedicines intended to improve melanoma therapy. These include examples such as:
 - Polymeric nanoparticles that could be used in matrix (nanospheres) or reservoir (nanocapsules) systems containing paclitaxel.
 - Liposomes (lipid vesicles made of phospholipid bilayer) containing cisplatin, doxorubicin, IFN- β or vincristine.
 - Polymersomes (polymer vesicles) containing paclitaxel or doxorubicin.
 - Dendrimers (branched polymer with uniform size distribution) containing doxorubicin and RGD peptides.
 - Cubosomes (lyotropic system with cavernous and cubic structure) containing dacarbazine.
 - Niosomes (analog to liposome but made of synthetic surfactants, such as the new nonionic surfactant, α,ω -hexadecyl-bis-[1-aza-18-crown-6]; Bola-surfactant) enhancing the intracellular uptake of bioactive agents.
 - Nanodiamonds (detonated and ultracrystalline diamond) with high surface area containing bioactive agents.
- Nanotechnology provides many promises for safer and more effective treatment of this aggressive and largely treatment-resistant disease if existing and proven preclinical nanomedicines can be translated clinically and/or if novel nanomedicines built on the advantages/disadvantages of existing system could be discovered.

Figure 1. Melanoma-containing human skin and possible mechanism of nanoparticle transport by paracellular, transfollicular and intracellular routes (left to right).

Figure 2.

The two main types of polymeric nanoparticles known as nanosphere (matrix system) and nanocapsule (reservoir system) with different drug-loading modalities.

Figure 3.
Liposome and its different drug-loading and surface functionalization modalities.

Figure 4.
Dendrimer and its different drug-loading modalities.

Figure 5. Cubosome exhibiting its cavernous internal and cubic structure and its membrane composition with different drug-loading modalities.

Figure 6. Polymersome (polymer vesicle) exhibiting a polymeric shell and different drug-loading possibilities.

Figure 7.
Niosome and its internal synthetic surfactant surrounding drug payload.

Figure 8.
The crystal structure of a nanodiamond.

Table 1

Examples of liposome-based nanomedicines for melanoma.

Bioactive agent	Preparation method	Encapsulation	Stability	Effect <i>in vitro</i>	Animal species; effect <i>in vivo</i>	Ref.
Cisplatin	Emulsification	PE: 50–70% EPC: 0%	PEG reduce stability	NA	Nude mice: PE liposomes stayed in melanoma for over 72h PE liposomes also delivered cisplatin into the tumor ~3.6-times more efficiently than the free drug	[31]
Doxorubicin; combretastatin phosphate, CA4P	NA	NA	NA	NA	C57BL/6 mice: CA4P could help trap doxorubicin inside tumor and, thus, enhance EPR effect	[43]
GCs (PLP and BUP)	Extrusion	NA	NA	No difference in viability and proliferation	C57BL/6 mice: strongest tumor inhibitory effect: LCL–PLP–BUP	[44]
PLP	Extrusion	NA	NA	Toxicity: free drug PLP > liposomal PLP	C57BL/6 mice: liposomal PLP had stronger inhibitory effect on cell proliferation	[49]
IFN- β	Reverse-phase evaporation	NA	NA	NA	C57BL/6 mice: strongest inhibitory effect: liposomal IFN- β ; 5.5-fold reduction in melanoma	[45]
PIC	NA	NA	NA	PIC–liposome reduce melanoma cell	C57BL/6 mice: inhibitory effect is dose dependent	[47]
Functionalized quantum dot–liposome	Emulsification	Proved by CLSM and AFM	Sterically stabilized	Prolong circulation	C57BL/6 mice: longest retention: functionalized quantum dot–liposome	[33]
Thrombin receptor (protease-activated receptor-1) siRNA	NA	NA	Neutral liposome more stable	NA	Athymic nude mice (NCr-nu): inhibition of melanoma growth cell and metastasis <i>in vivo</i>	[48]
TLR3 or TLR9 agonists	Emulsification	NA	NA	NA	C57BL/6 mice: liposome–Ag–nucleic acid complexes; elicited immunization to inhibit melanoma	[46]
Vincristine	NA	>95%	VSLI exhibits greater stability	NA	Human patients: compared with native drug, longer circulation half-life, higher bioavailability, but unchanged route of elimination	[32]

AFM: Atomic force microscope; BUP: Budesonide disodium phosphate; CA4P: Combretastatin A4 phosphate; CLSM: Confocal laser scanning microscopy; EPC: Egg phosphatidylcholine; EPR: Enhanced permeability and retention; GC: Glucocorticoid; LCL: Long-circulating liposomes; NA: Not available; PE: Phosphatidylethanolamine; PEG: Polyethylene glycol; PIC: Polyinosinic-polycytidylic acid; PLP: Prednisolone phosphate; TLR: Toll-like receptor; VSLI: Vincristine

Table 2

Examples of dendrimer-based nanomedicines for melanoma therapy.

Bioactive agent	Preparation method	Encapsulation	Stability	Effect <i>in vitro</i>	Animal species; effect <i>in vivo</i>	Ref.
PAMAM-GlcNAc8 or glycodendrimers	Functionalization conjugation	NA	NA	Enhanced KK cell activity	<i>C57Bl/6</i> mice; dose-dependent survival of animals	[59]
PAMAM dendrimer with ³ H label	NA	NA	NA	NA	Mice: positively surface charged dendrimers had higher tissue deposition compared with neutral surface charged dendrimers	[60]
Gold	NA	33%	Gold has high stability	NA	<i>C57Bl/6J</i> , athymic <i>nu/nu</i> mice; biodistribution is greatly influenced by particle size and surface charge	[63]
RGD peptides	NA	NA	NA	Selectivity of cRGD-dendrimers over the control	Mice: dendrimer concentrated in kidney and reticuloendothelium	[64]
cRGD peptides	NA	NA	NA	Nontoxic with physiological concentration range. Specific and stronger binding to the selected integrin compared with native cRGD	NA	[65]

cRGD: Cyclic arginine-glycine-aspartic acid; GlcNAc8: *N*-acetyl- β -glucosamine; NA: Not available; PAMAM: Polyamidoamine; RGD: Arginine-glycine-aspartic acid.

Table 3

Examples of polymersome-based nanomedicines for melanoma.

Bioactive agent	Preparation method	Encapsulation	Stability	Effect <i>in vitro</i>	Animal species: effect <i>in vivo</i>	Ref.
Biotin-functionalized polymersome	Modular avidin-biotin chemistry	NA	More stable than lipid vesicles	Adhesion was specific	NA	[90]
Cell permeable peptide (Tat) conjugation to near-infrared emissive polymersomes	NA	NA	NA	Significant uptake: 70,000 (10,000 vesicles/cell); no influence on dendritic cell maturation and viability	NA	[94]
Paclitaxel	Membrane extrusion	6.7–13.7% (w/w)	Colloidally stable for 4 months at 4°C	Desirable sustained release profile and long-term stability	NA	[93]
Paclitaxel and doxorubicin	Sonication, freeze-thaw cycles and extrusion	Higher than liposome membrane	NA	NA	Mice: 50% smaller tumors after treatment, twofold higher cell death with polymersome	[92]

NA: Not available; Tat: *Trans*-activator of transcription.

Table 4

Potential advantages and disadvantages of selected nanomedicines for melanoma therapy.

Nanomedicines	Advantages	Disadvantages
Polymeric nanoparticles	Biodegradability, controlled release and ability to target particular organs/tissues while minimizing sides. Ability to deliver proteins, peptides and genes through peroral route of administration. Wide variety of polymeric choice	Possible residual solvent. Complex preparation and functionalization. Thermodynamic instability leading to aggregations. No uniform size distribution
Liposomes	Biodegradable and biocompatible. Easy uptake by cells, capability of encapsulating both hydrophilic and hydrophobic drugs. May be rendered stimuli-responsive (e.g., pH, temperature and magnet)	Complex preparation issues as above. Variable purity and high cost of phospholipids. Instability (to heat, GI tract and storage), low drug encapsulation efficiency and payload. No ability for controlled release for longer time. Not bioadhesive. Not easily transported through skin
Dendrimers	Thermodynamically stable, high solubility in water, high oxygen solubility, efficient rate of oxygen transfer into an aqueous phase. Uniform size distribution	Preparation is complex. Potential toxicity issues related to charges and nature of building blocks
Cubosomes	Use of biodegradable, biocompatible and bioadhesive lipids. High internal surface area may enhance loading with higher solubility parameter in the lipid matrix	Complex preparation. Unstable in plasma. Low encapsulation of water-soluble drug. No demonstrated ability for active targeting using ligands
Polymersomes	Robust and larger shell enhances nanomedicine stability and drug encapsulation if compatible with the polymer. Possibility of drug targeting with block copolymers or longer circulation time with worm-like structures	Complex preparation. Unlike dendrimers, used polymers are still not uniform in size
Niosomes	Higher penetrating capability than conventional preparations of emulsions. Structurally similar to liposomes in having a bilayer, but more stable. In comparison with liposomes, relatively higher stability, improved purity and lower cost. May be used as local depot for sustained release	Complex preparation. Similar to those of liposomes beside the advantages indicated
Nanodiamonds	Biocompatibility, excellent photostability and facile surface functionalizability. Promising nanomaterials for both <i>in vitro</i> and <i>in vivo</i> applications. Small size excellent for cellular targeting via intravenous route	Nonbiodegradable. Possible persistence in organism (liver and spleen). Small size may limit drug payload. Does not provide strong protection against environmental hazard (e.g., pH and enzyme) for sensitive drugs