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Polymeric Nanoparticles to Combat Squamous Cell Carcinomas in Patients with Dystrophic Epidermolysis Bullosa

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Abstract

Skin cancer is the leading cause of malignancy in the United States, with Basal Cell Carcinoma, Squamous Cell Carcinoma, and Melanoma being the three most common diagnoses, respectively. Squamous Cell Carcinoma (SCC) is a particular concern for patients suffering from Dystrophic Epidermolysis Bullosa (DEB), a disease that affects the production and function of collagen VII, a protein that forms the anchoring fibrils which bind the epidermis to the dermis. Patients with DEB suffer from chronic blistering and wounds that have impaired healing capabilities, often leading to the development of SCC and eventual mortality. Nanomedicine is playing an increasing role in the delivery of effective therapeutics to combat a wide range of diseases, including the imaging and treatment of SCC. In this review, we discuss the role of nanoparticles in the treatment of SCC with an emphasis on PLGA nanoparticles and SCCs found in patients suffering from DEB, and address recent patents that are pertinent to the development of novel nanomedical therapeutics.

Keywords

Dystrophic; Epidermolysis Bullosa; Squamous; carcinoma; nanoparticles; nanomedicine; PLGA; EGFR; p53

Introduction

Skin cancer is the leading cause of malignancy in the United States, with over two million people diagnosed annually [1]. Melanoma, which arises from the dendritic cells found within the basal layer of human skin known as melanocytes, is by far the deadliest subtype of skin cancer, accounting for roughly 65% of all skin cancer related deaths. However,

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Conflict of Interest

The authors declare no conflict of interest.

melanoma is relatively rare, accounting for only 3% of all skin cancer diagnosis [2]. Non-melanoma skin cancers (NMSC) such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), alternatively, are much more common. BCCs are the most prevalent form of skin cancer, accounting for 70% - 80% of all diagnosis, and are commonly caused by an upregulation of the Hedgehog Signaling pathway [3-6]. Metastasis of BCCs is extremely rare, usually occurring in instances in which the tumor is left untreated for an extended period of time [6]. SCCs are the second most prevalent skin cancer diagnosis, accounting for 20-25% of all NMSCs, killing 2,500 people in the US each year [7]. Unlike BCC, SCC has a much greater likelihood of metastasis and is associated with a higher mortality rate. SCCs are of particular concern to patients who suffer from chronic non-healing wounds, such as those seen in conjunction with diabetes, vascular disease, and especially Epidermolysis Bullosa [8, 9].

Dystrophic Epidermolysis Bullosa (DEB) is a disease that is closely associated with the formation of lethal SCC. Patients suffering from DEB have complications arising in the function of collagen type VII (C7), leading to widespread blistering and the development of chronic wounds that never heal. The body's constant attempts at re-epithelialization lead to severe malnutrition, failure to thrive, and commonly results in the development of SCC and mortality. Although many methods are currently used in an attempt to cure DEB patients afflicted with SCC, the outlook remains bleak.

In the past few years, an increasing number of researchers have been looking towards nanomedicine in order to develop novel therapeutics to treat a wide range of diseases, including SCC. Nanomedicine involves the use of therapeutics that involve particles and assemblages that are nanoscale in size, and allow for targeted and effective delivery of therapeutics to specific locales within the body. In this review, we will discuss the etiology and pathology of SCC with an emphasis on patients who are concurrently suffering from DEB, and explore the developing field of nanomedicine and its promising use as a therapeutic in combating SCC. A summary of patents that are discussed in this article are listed in Table 1.

Dystrophic Epidermolysis Bullosa

Dystrophic Epidermolysis Bullosa (DEB) is a disease classified by the reduction or absence of C7 in the junction between the patients' dermis and epidermis in an area known as the basement membrane zone (BMZ) (Figure 1). Within the BMZ, C7 forms into anchoring fibrils that interact with collagen I, collagen IV, fibronectin, and laminin 332 to form the extracellular matrix that supports the skin and binds the epidermis to the dermis [10, 11]. C7 also plays a large role in the wound healing process, as it organizes laminin 332 deposition within the BMZ and supports the migration and cytokine production of dermal fibroblasts [12]. Absent or nonfunctional C7 causes dermo-epidermal separation, resulting in blisteration and chronic wounding (Figure 2). DEB usually appears clinically immediately following birth due to the trauma experienced by the baby as it traverses the vaginal canal. This trauma is enough to cause skin separation and severe wounding over large areas of the body. As the patient ages, some wounds may heal and result in scarring. Some wounds, however, may remain for periods of months or even years (Figure 3).

The reduction or absence in C7 is due to a mutation in the COL7A1 gene [11]. The COL7A1 gene is especially large with 118 exons, and there are over 100 distinct mutations that are known to result in the DEB phenotype [13]. DEB patients may have a less severe dominant form called Dominant Dystrophic Epidermolysis Bullosa (DDEB), or a more severe recessive form called Recessive Dystrophic Epidermolysis Bullosa (RDEB) [14]. DDEB is characterized by chronic painful wounding that occurs after mild trauma. Unlike the wounds found in RDEB, wounding in DDEB patients often heals [15]. RDEB is often much worse, and is characterized by extensive blistering and chronic wounding of the skin and mucosa, leading to pseudosyndactyly, chronic blood loss, inflammation, infection, anemia, delayed puberty, and failure to thrive [15]. Additionally, anal erosions can lead to constipation, corneal erosions can lead to scarring and a loss of vision, and amyloidosis of the lungs and kidneys can lead to problems in pulmonary and renal function [16].

Multiple approaches have been explored in recent articles as well as patents to treat patients with DEB, including viral and non-viral gene therapies [11], intradermal injection of C7 producing fibroblasts [17], intravenous injection of C7 [18], microneedle application of C7 protein [19], injection of human umbilical cord derived unrestricted somatic stem cells [20], bone marrow and blood transfusions [21], and grafting of autologous skin that has reverted back to wild type gene expression [22, 23]. However, all attempts to establish a reliable and effective therapy have thus far had only limited success.

Squamous Cell Carcinoma in Patients with DEB

Ultimately, chronic wounding leads to increased instances of SCC, with RDEB patients showing a 70% chance of mortality before the age of 45 and >90% mortality by age 55 (Figure 4) [11, 24]. The weakened structure of the extracellular matrix found in DEB patients is much more permissive to tumor development, leading to increased rates of growth, metastasis, and eventual death [24]. Patients who have a compromised immune system due to immunosuppressants are at an additional risk for development of SCC, adding further complications to DEB patients who are considering bone marrow transplant as a therapeutic option [25, 26].

The recommended treatment for DEB patients with SCC is wide surgical excision of the tumor, with surgical amputations being commonplace [27]. As such, many patients are, crudely speaking, whittled away before finally succumbing to the disease. Due to the physiology of their skin, excision surgeries and amputations are much more traumatic for DEB patients than for patients with normal skin pathologies. Patients afflicted with DEB already have severely diminished skin strength and a reduced ability to repair wounding. Therefore, surgical excisions and amputations could themselves become chronic wounds that never heal and lead to further SCC malignancies. Additionally, patients may choose to forgo amputation for psychosocial reasons, i.e. reasons of independence and quality of life. Chemotherapy and radiation therapy have been used in some patients, both alone and in combination with surgery, however no significant benefit has been observed [27]. It is imperative that alternate methods of intervention be investigated as treatment options for DEB patients suffering from SCC so as to mitigate the necessity of surgical excision and

amputation, and allow for effective alternative therapies that do not require traumatic surgeries.

Molecularly speaking, SCCs found in DEB patients are extremely similar to those caused by ultraviolet (UV) radiation [24]. SCC can be caused by an inactivation of TO53 gene, which encodes for tumor suppressor protein 53 (p53). P53 halts cell cycle progression in the event that the DNA is damaged, and will induce apoptosis if the DNA damage is not capable of repair [5]. Inactivation of the gene can cause the cells to rapidly undertake the cell cycle, leading to uncontrollable proliferation and tumorigenesis. Multiple types of therapies have been developed that specifically target the p53 gene, including viral delivery of wildtype p53, viruses designed to specifically target and kill cells without functional p53, small molecule interventions designed to restore wild type function in mutated p53, and small molecules designed to prevent inactivation of wild type p53 [28]. A recent patent utilizing certain small molecule compounds prevents p53 downregulation by inhibiting its interaction with the MDM2 protein [29]. MDM2 is a known suppressor of p53, and by selectively inhibiting its ability to function, the patent aims to restore normal p53 function [30].

Additionally, overexpression of epidermal growth factor receptor (EGFR) has been implicated in many epithelial cancers, including SCC [5]. EGFR is expressed in a majority of cutaneous SCCs, and anti-EGFR therapeutics such as cetuximab have been used clinically to treat SCC in both DEB and non-DEB related instances [31, 32]. Though cetuximab tends to show positive initial results in locally recurrent SCC, RDEB patients with distant metastasis suffered further progression and eventual mortality at the hands of SCC [33]. This serves to emphasize the importance of early detection and treatment of SCC prior to metastasis, as well as the need for superior targeting and delivery mechanisms that localize to areas of SCC involvement.

Nanoparticles

A highly promising route of drug administration that can combat both locally recurrent and metastasized SCCs is the use of nanoparticles. Nanoparticles are defined as solid and spherical structures with sizes of around 100 nm or less (Figure 5) [34]. Many different materials are used to construct nanoparticles, ranging from metals such as gold, silicon, iron, and cobalt, to magnetic oxides such as iron oxide, to liposomes, micelles, and finally polymers [35, 36]. A recent patent even elucidates how to create nanoparticle carriers out of glass microparticles that contain 10 nm pores [37]. In the current development of imaging and drug delivery systems, nanoparticles play a crucial role due to their sub-cellular and sub-micron size which allows them to efficiently travel throughout the bloodstream and penetrate deeply into organs and other tissues [38]. Once at the intended sight of action, the small diameter of nanoparticles allows them to enter target cells via either diffusion or phagocytosis [36]. This ability to efficiently traverse throughout all parts of the body and target specific cell types is particularly important for DEB patients who suffer from SCCs that have metastasized throughout the body, and allows for effective treatment without the need for direct, site-specific application. Nanoparticles can then deliver a variety of drugs, vaccines, small molecules, biological macromolecules (i.e. peptides and proteins), nucleic acids, DNA, siRNA, oligonucleotides, viruses, and a range of different cells [34, 39]. While

intravenous injection still remains the most preferred route of administration a wide variety of other, less invasive options such as oral, mucosal or transdermal delivery are currently being explored [40].

Advantages of Nanoparticles

Nanoparticles offer a full spectrum of advantages. They improve the therapeutic value of various water soluble and insoluble drugs by enhancing bioavailability, solubility, and retention time. Additionally, nanoparticles protect the drugs that they transport from degradation and interaction with the biological environment. Peptides and proteins, for example, are easily destroyed by metabolic pathways and enzymatic activities in the human body, and thus often show a short half-life *in vivo* [41]. By protecting these proteins and peptides from destruction, nanoparticles allow them to survive long enough to reach their target tissues.

Another advantage of nanoparticles is their ability to interact on the tissular or cellular level. Due to their small size, the particles can be endocytosed or phagocytosed into cells and macrophages [35]. The underlying mechanism of this rapid internalization is the destabilization of vesicular membranes and the absorption into the cytosol caused by interactions between the membranes and the nanoparticles [34].

Targeted drug delivery with the help of nanoparticles also increases patients' compliance, extends product life cycles, provides product differentiation, and helps reduce health costs [41]. Due to these promising advantages, many different clinical trials have been conducted using nanoparticle technology, and many more are being conducted currently. Successful outcomes of these trails can be seen in drug-loaded biodegradable nanoparticles that are commercially available for treating major diseases [42].

Nanoparticle Targeting Strategies for Squamous Cell Carcinomas

Ensuring that therapeutics arrive at their necessary destinations is essential to increase drug efficacy while also reducing undesired toxicity. Features inherent in nanoparticles greatly assist in the targeted delivery of therapeutics to their desired locations, mitigating their effects on non-target organ systems [43]. This ability is especially important when nanoparticles are carrying a drug designed to induce apoptosis in malignant cells, as is often done when treating SCC and other types of cancer. An example of the benefits of a nanoparticle targeting system can be seen in a recent patent that utilizes gold nanoparticles to assist in the delivery of epigallocatechin-3-gallate (E3G), a molecule that induces apoptosis when delivered to tumorigenic cells [44]. When distributed systemically, E3G can cause unwanted consequences, such as hepatitis and severe liver failure [45]. Thus, the cost to benefit ratio of a direct injection of E3G into the blood stream is not always favorable. Nanoparticles allow E3G to be specifically targeted to its point of action in cancerous cells, thereby preventing the occurrence of unwanted side effects that may result from systemic application. The gold nanoparticles used in this patent prevent E3G interaction with the liver, and delay activation of the molecule until it reaches its target site within the tumor [44]. This targeting turns an otherwise toxic agent into an effective therapeutic.

As seen in the aforementioned patent, the targeting capabilities of nanoparticles are key in their role as effective therapeutics. The three main targeting strategies involving nanoparticles are passive targeting, active targeting, and magnet assisted targeting. Passive targeting relies on the physicochemical properties of nanoparticles and the anatomical differences between diseased and healthy tissues which allow the nanoparticles to migrate through the body to the target region. Rapid growth of blood vessels in tumorigenic regions results in vasculature that is poorly organized and full of leaky fenestrations that are hyperpermeable [35, 46]. The minimal size of nanoparticles allows them to seep out of these fenestrations and accumulate inside the cell through the process of “enhanced permeability”. Additionally, the particles experience an “enhanced retention” because of the ineffective drainage owing to absent or damaged lymphatic vessels [34]. The combination of these two effects is called the “enhanced permeation and retention” effect (EPR) [47]. The EPR effect is an extremely important phenomena in regards to nanoparticles, and can assist in the targeted delivery of molecules that would be toxic if they were to have a prolonged systemic presence. Due to EPR, nanoparticles carrying anti-SCC therapeutics that are injected into the blood stream will circulate throughout the body and accumulate in metastasized tumorigenic sites where they will degrade and release their cargo. Because the nanoparticles are retained within the SCC tumor sites, they will remain there and release their cargo. However, the EPR occurrence is often misinterpreted since it is highly heterogeneous and varies between each individual tumor model as well as between individual patients [34]. Additionally, due to their chronic wounding, the skin of DEB patients is especially leaky. This could lead to leakage of injected nanoparticles out of the wound site, lowering the effectiveness of the therapeutics.

Alternatively, active targeting is preferable for SCCs in DEB patients. For active targeting, receptor specific ligands are bound to the surface of the nanoparticles (Figure 6). These low molecular weight molecules, which include peptides, small organic molecules, antibodies, proteins, and aptamers, are complementary to unique receptors that are overexpressed in the target cells, such as folic acid or thiamine [41, 46]. A recent patent, for example, conjugates ligands that target prostate specific membrane antigen to its nanoparticles in order to specifically delivery therapeutics to designated areas [48]. SCCs in DEB patients can be specifically targeted by increasing the nanoparticles affinity for EGFR. It should be noted that the incorporation of targeting ligands on the surface of nanoparticles can induce an increase in immunogenicity and protein adsorption. However, it has been shown that these nanoparticles are better internalized by cancer cells than they are by healthy cells [34].

Another approach is targeting the therapeutic site with the help of an external magnet. For this magnetic targeting, the polymeric nanoparticles are additionally loaded with magnetic nanoparticles and can therefore follow high field, high gradient, or rare earth magnets that are focused on the target tissue. A recent patent combines low levels of magnetic nanoparticles alongside biodegradable nanoparticles to assist in the targeted delivery of anticancer therapeutics to SCC [49]. However, there are still many remaining challenges to overcome. For example, this technique is limited to tissues close to the body's surface since the magnetic field loses its strength the further it is placed away from the magnetic source. Though this is not a concern for superficial SCCs found on the skin of DEB patients, it is a limiting factor when trying to treat metastasised SCCs found within the body.

Nanoparticles for Imaging Squamous Cell Carcinomas

Nanoparticles are playing an increasing role in diagnostic imaging and tracking, whether they are used to assist in magnetic resonance imaging, near-infrared fluorescent imaging, or other similar techniques [50, 51]. Imaging of SCCs in DEB patients will allow physicians to determine the depth and breadth of tumors, allowing them to monitor tumor growth over time and assist in determining the margins if wide local excision is deemed necessary. Imaging nanoparticles can simply be allowed to accumulate in SCCs via the EPR effect, or else they can be conjugated to peptides, antibodies, polymers, carbohydrates, or aptamers in order to enhance localization to specific sites [52]. A recent patent utilizes the encapsulation of surface enhanced raman scattering nanoparticles, preferably gold, into lipid nanoparticles [53]. These lipid nanoparticles can then be conjugated to anti-EGFR antibodies, allowing for the localization in SCC and possibility for Raman characterization. Another technique uses gold nanorods that are conjugated to anti-EGFR antibodies, allowing for realtime tumor imaging and margin demarcation using narrow band imaging techniques [54]. This process would greatly assist physicians in monitoring SCCs in DEB patients, and tracking their responsiveness to various therapeutics. If surgical excision is deemed necessary, realtime imaging can assist surgeons in determining the margins of SCC encroachment, preventing unnecessary amputations.

Biodegradable Nanoparticles for Drug Delivery

Biodegradable nanoparticles are of particular interest when prolonged therapies are necessary, such as when combating SCC, due to their physiological safety. Biodegradability is defined as the degradation *in vivo* via enzymatic or non-enzymatic methods, or through a combination of both. This results in biocompatible, toxicologically safe products that can finally be removed from the body via normal metabolic pathways [55]. As non-toxic, nonthrombogenic, nonimmunogenic, and noninflammatory agents, they do not activate neutrophils [42]. In general, biodegradable polymeric nanoparticles in the form of spheres have the drug encapsulated into them when the drug is added to the polymer solution at the time of the nanoparticle production. Alternatively, the drug can be adsorbed onto the surface when the synthesized nanoparticles are incubated in drug solution. This results in the formation of chemical bonds between the drug and the particles [41, 42].

Drug Release

Once biodegradable nanoparticles reach their site of action due to their targeting abilities, they release their cargo in response to external or internal stimuli. Temperature, irradiation with near-IR or UV-vis light, activation with magnetic fields, and the generation of ultrasound can externally trigger the nanoparticles to deliver the medicine into the cells. Differential pH, osmolarity, enzymatic, oxidative, and reductive conditions in the SCC affected cells when compared to healthy cells can also lead to a controlled release [40]. If the drug is attached to the nanoparticles surface, it is dispensed through desorption. If the drug is encapsulated within the nanoparticle, it is dispensed either via diffusion out of the polymer matrix, or due to the erosion of the nanoparticle by hydrolysis or enzymatic degradation. Often all of these conditions apply simultaneously [42].

The release kinetics of nanoparticles can also be altered so as to provide for immediate or sustained drug delivery, an important factor when combating SCCs of different severities. The solubility, loading efficiency, crystallinity, size, and size distribution of the nanoparticles determine the release kinetics [55]. For polymeric nanoparticles, changing the composition of the polymer and the molecular weight can cause degradation to be adjusted to occur over a period of days to months [38]. In general, the rule can be applied that the higher the molecular weight of the polymer, the slower the *in vitro* release [42].

This can be described with a biexponential function:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C represents the concentration of remaining drug in the nanoparticles, while A and B the system characteristic constants at the time t . The system is specified with A for diffusion control and B for degradation control [42].

In principle, drug release is divided into an initial burst phase followed by a delayed diffusion of the remaining drug. The initial drug release is related to drug type, concentration, and nanoparticle hydrophobicity. It is generated by the surface linked fraction of the drug to the nanoparticles, which is often weakly bound. Smaller sizes of nanoparticles result in an increase of this phenomenon [42, 55, 56]. Following the initial burst, an exponentially delayed diffusion rate occurs. During this phase the drug is progressively given off by diffusion while the nanoparticles slowly degrade. In the case of polymeric nanoparticles, entrapped water inside the particles destroys the polymer into soluble oligomeric and monomeric products through which the drug can escape [55, 56]. This initial burst phase followed by a delayed diffusion can be useful when combating SCC tumors, as the initial release phase will kill the affected cells while the delayed diffusion will prevent reoccurrence.

PLGA Nanoparticles

Though there is a multitude of biodegradable polymeric nanoparticles to choose from, one class that has shown particular potential in the treatment of SCC are nanoparticles made from poly (lactide-co-glycolide) (PLGA). PLGA nanoparticles are one of the most common and most successfully developed nanoparticles for drug delivery systems [34]. PLGA nanoparticles exhibit excellent biocompatibility and reabsorbability through natural pathways and cause very minimal systemic toxicity [34, 42]. Thus, PLGA nanoparticles have been accepted by both the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA), and have vast clinical experience. PLGA nanoparticles are also commercially available in a wide variety [34]. They can be processed in almost any desired shape and size, including the fabrication via emulsification-solvent evaporation technique for the encapsulation of hydrophobic drugs, or the double or multiple emulsion technique for the encapsulation of hydrophilic compounds and drugs. Other possible methods are nanoprecipitation, salting out, or spray drying, which are all reviewed in the literature [55]. A recent patent out of Vanderbilt University has developed a method that enables a large amount of control over the size and shape [57]. This allows for enhanced reproducibility and

uniformity of the nanoparticles while also assisting in the fine tuning of drug delivery and drug release mechanisms.

In addition to size, variation in the ratio of lactic acid to glycolic acid results in changes in the degradation rate of the nanoparticles and subsequent the drug release rate. While lactic acid increases the hydrophobicity, glycolic acid increases the hydrophilicity [58]. Therefore, lactic acid absorbs less water, leading to a slower degradation since the degradation occurs by the hydrolysis of its ester linkages. A 50:50 ratio of polylactide to polyglycolide displays the fastest degradation [55]. Other degradation affecting factors are crystallinity, weight average molecular weight, the type of drug, size and shape of the matrix, pH, and enzymes. [55] Thus, a wide range of erosion times can be designated by the controlled fabrication of PLGA nanoparticles [34]. For example, smaller nanoparticles exhibit a higher proportion of surface area to volume which significantly enhances the degradation [55]. As mentioned before, this would allow physicians to tailor their particles to either release large amounts of drug initially to combat invasive tumors, or to release the drug over a protracted period of time to prevent reoccurrence.

PLGA nanoparticles can be administered via oral, intravenous, or transdermal routes. Oral administration poses the greatest obstacle for PLGA nanoparticles aimed at treating SCCs, as the nanoparticles must traverse the highly acidic stomach and then be absorbed in the small intestine before finally reaching systemic circulation. Conversely, intravenously administered PLGA particles are instantly incorporated into the blood stream, and thus have shown a much higher biodistribution when compared to oral administration [59]. Due to the cutaneous nature of SCCs, transdermal application of nanoparticle therapeutics is especially enticing. Transdermal deliveries are complicated by the stratum corneum, the 10-20 μm thick outer layer of the skin that acts as a physical barrier and hinders foreign compounds from penetrating into the epidermis below [60, 61]. A recent patent shows that hydrophilic compounds can be simply conjugated to the surface of PLGA nanoparticles and still achieve penetration into the stratum corneum, where the nanoparticles then dissolve and release their drug into both the stratum corneum and epidermis [62]. However, this only allows for the superficial delivery of drugs, and cannot assist in combating SCCs that have invaded the dermis. To facilitate greater diffusion, PLGA nanoparticles are usually coated with highly hydrophobic molecules. A recent patent illustrates the effectiveness of hydrophobic compounds by utilizing a glycerol monooleate and cetyl alcohol based cream to deliver itraconazole deep into the dermis to combat BCC [63]. A similar approach can be taken by coating anti-SCC loaded PLGA nanoparticles with glycerol monooleate or cetyl alcohol, thereby allowing delivery of therapeutics to SCC tumors that have invaded into the papillary and reticular dermis. Alternatively, dissolvable needles can be used to physically break the stratum corneum and implant PLGA nanoparticles into the epidermis [64]. However, transdermal delivery does not result in the high levels of bioavailability as does intravenous delivery (which by definition has a bioavailability of 100%), and so would not be as effective at treating metastasized SCCs.

A problem inherent in PLGA nanoparticles is their negative surface charge, which determines their interaction with and uptake by cells. While positive surface charges allow higher internalization rates owing to ionic interactions with negatively charged cell

membranes, they also appear to escape the lysosomes after cell internalization [34]. Therefore, it is useful to modify the surface of PLGA nanoparticles in such a way that they seem to be positively charged and thus hide the hydrophobicity. Usually, this can be achieved by attaching the hydrophilic and non-ionic polyethylene glycol (PEG) onto the nanoparticles. This polymer shows good biocompatibility and increases the blood circulation half-life time by several orders of magnitude, thereby improving the possibility of the nanoparticles reaching their therapeutic site. These efforts lead to the effect that the nanoparticles appear invisible to the body's natural defense system since hydrophilic surfaces are not recognized as foreign materials. Otherwise, the nanoparticles would be eliminated from the blood stream and instead would accumulate in the liver or the spleen [34, 42].

PLGA nanoparticles can be used to assist in the targeted and sustained delivery of a wide array of agents, including small molecules, RNA, and genes to SCCs in DEB patients [65]. In instances of p53 inactivation, nanoparticles could either deliver p53 genes to SCC cells where p53 has been mutated, or introduce small interfering RNAs and/or small molecules that prevent the synthesis and action of p53 inhibitors. Additionally, PLGA nanoparticles can deliver small molecule inhibitors or siRNAs into SCC cells where EGFR is overexpressed. Targeted PLGA nanoparticles could also introduce toxic agents specifically to SCC tumors by targeting p53 inactivation or EGFR overexpression, leading to necrosis and preventing metastasis. A recent patent even utilizes PLGA nanoparticles to deliver temoporfin, a photosensitizer for the use of photodynamic therapy when treating tumorigenic cells [66].

However, there are still remaining challenges with PLGA nanoparticles that have to be met. One major disadvantage yet is the poor loading percentage of only around 1%, as well as the high cost of production. Additionally, the fabrication route is often hard to be scaled-up for commercial syntheses. Regarding the release profile, PLGA nanoparticles show a high initial burst release of the drug from the nanoparticles, which rises the concerns that nanoparticles might not be able to reach the target tissue soon enough. This would lead to a loss of efficacy. Also, studies that specifically address nanotoxicological issues have to be carried out since biological systems are extremely complex. Even though encouraging *in vitro* results have been found, most studies are still far from clinical trials [34].

Alternative Polymeric Nanoparticles

In addition to PLGA, many other biodegradable polymers may be used to create nanoparticles capable of delivering therapeutics to SCCs in patients with DEB. Natural polymers are especially enticing, and include hyaluronic acid, collagen, chitosan, gelatin, lectin, and albumin to name a few [36, 67]. Hyaluronic acid (HA) nanoparticles in particular have shown potential due to their ability to bind to specific receptors in cancer cells, particularly CD44, and have been used for both drug delivery and tumor imaging purposes [68]. CD44 has been shown to interact with EGFR to promote initiation and progression of SCCs [69]. Additionally, CD44 has been shown to internalize and breakdown of HA. Though CD44 is usually expressed at low levels in healthy cells, in tumorigenic cells CD44 is overexpressed in a high-affinity state, leading to an increased interaction and

internalization of HA [70]. This imbues HA with an innate ability to actively target tumorigenic cells, making it a fantastic candidate for the systemic treatment of metastatic SCC in patients with DEB. More importantly, HA has been shown to be capable of traversing the stratum corneum when applied topically [71]. As epithelial SCCs are the greatest concern for DEB patients, the ability of HA materials to efficiently traverse the stratum corneum and deposit into the epidermis and dermis makes them ideal candidates for creation of effective nanoparticle therapeutics.

Chitosan is another natural polymer that has shown promise as a nanocarrier for anti-carcinoma imaging and therapeutic molecules. Chitosan has been shown to be effective at traversing the stratum corneum when applied topically, and can be used for MRI imaging as well as delivery of anti-cancer therapeutics [72-74]. Findings show that HA can be conjugated with chitosan to create HA-chitosan nanoparticle complexes [75, 76]. A recent patent outlines a novel way of creating HA-chitosan complexed nanoparticles [77]. These complexes can be formulated to serve as a dual delivery system capable of simultaneously delivering differentially charged therapeutics, or alternatively, they can be formulated to produce nanoparticles with optimal targeting and pharmacokinetic properties. Thus, HA and chitosan can work synergistically to formulate targeted and effective nanoparticles capable of combating cancerous growths in DEB patients. Depending on the desired delivery site and pharmacokinetic properties, HA-chitosan complexed nanoparticles could provide a useful alternative to PLGA.

Limitations of Polymeric Nanoparticles

Currently, there still exists many limitations and unsolved challenges in the development of polymeric nanoparticles as a whole. The synthesis of polymeric nanoparticles often results in a heterogeneous size distribution within particular sample as well as between every batch. Changing one parameter will inevitably affect another. Such parameters that are interdependent are size, charge, and crystallinity. The size, polydispersity and charge are also highly dependent on measurement techniques. Due to dynamic processes the assembly and disassembly of nanoparticles can change during storage or during circulation in tissue and blood [40].

In vitro assays can only poorly represent the performance of drug-loaded nanoparticles due to their simplicity when compared to the human body. Since every cell line, cell cycle, passage number, and growth medium contributes to the varying efficacy of the drug delivery systems it is difficult to accurately evaluate their therapeutic outcome. Additionally, the cellular processes are dynamic and therefore, an inhibition of one pathway can cause further skewing of the obtained results. *In vivo* studies can better judge the effects of drugs while still represent a simpler model than the human body. Yet, there are remaining limitations in that every animal model and disease status originates different drug behavior. The age, sex, weight, and animal species highly contribute to the outcome of the trial [40].

Current and Future Developments

As methods of production, encapsulation, conjugation, and targeting improve, nanoparticle therapies for patients afflicted with SCC are sure to increase in terms of efficacy while

simultaneously decreasing in terms of cost. Developing the specificity and retention of imaging nanoparticles will assist in the detection and monitoring of SCC, while doing so in nanoparticle carriers of genes, RNAs, small molecules, and toxins will lead to necrosis of SCC tumors. Targeting of nanoparticles is paramount in their use to deliver therapeutics. Without the ability to target nanoparticles to their site of action, unwanted and potentially dangerous side effects could result. Additionally, the development of loading techniques will allow for a smaller volume of nanoparticles to deliver a greater amount of therapeutic. This will decrease the necessity of repeated applications, driving down the cost of nanoparticle production as well as preventing an overload of the body's ability to breakdown the polymeric encasement. By increasing the targeting abilities and loading capacity of nanoparticles, researchers can greatly increase the quality of life in patients with DEB by preventing the need for painful and maiming surgical excisions and amputations, and extend patients' length of life by preventing tumor metastasis. Research in therapeutic nanoparticles for SCC will also benefit non-DEB patients who are afflicted by SCC for other reasons, such as extended periods of UV exposure or immunosuppressants following organ transplant.

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List of Abbreviations

BCC	Basal Cell Carcinoma
BMZ	Basement Membrane Zone
C7	Collagen Type VII
DEB	Dystrophic Epidermolysis Bullosa
DDEB	Dominant Dystrophic Epidermolysis Bullosa
E3G	Epigallocatechin-3-gallate
EGFR	Epidermal Growth Factor Receptor
EPR	Enhanced Permeation and Retention
FDA	Food and Drug Administration
HA	Hyaluronic Acid
NMSC	Nonmelanoma skin cancer
P53	Tumor Suppressor Protein 53
PLGA	Poly (lactide-co-glycolide)
RDEB	Recessive Dystrophic Epidermolysis Bullosa

SCC	Squamous Cell Carcinoma
UV	Ultraviolet

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Figure 1.
Hemotoxylin and eosin staining showing locations of epidermis, dermis, and basement membrane zone in normal adult human skin. Scale = 100 micrometers.

Figure 2.

(A) In normal skin, collagen type VII anchoring fibrils form in the basement membrane zone, binding the epidermis to the dermis. (B) In patients with Dystrophic Epidermolysis Bullosa, collagen type VII fibrils are either missing or poorly formed. This leads to a weakening of the dermo-epidermal junction, resulting in blister formation.

Figure 3.
A patient with a chronic wound on the chest resulting from Recessive Dystrophic Epidermolysis Bullosa.

Figure 4. Squamous cell carcinoma invading the foot of a patient with Dystrophic Epidermolysis Bullosa.

Figure 5.
Size and morphology of PLGA nanoparticles. Scale = 1 micrometer.

PLGA Nanoparticle

Figure 6.

Nanoparticles can be coated with ligands that specifically bind to proteins found in target cells. For instance, squamous cell carcinoma cells could be targeted with ligands that are attracted to high levels of epidermal growth factor receptor.

Table 1

Table outlining recent patents that could be used in the formulation of nanoparticles designed to characterize and combat squamous cell carcinomas in patients with Dystrophic Epidermolysis Bullosa.

Patent Number	Function	Method
WO 2012149136 A1, 2012	Direct delivery of C7 protein to patients with DEB	Microneedle delivery system
US8618158B2, 2013	Prevents downregulation of p53	Inhibits interaction with MDM2 Protein
US8535725B2, 2012	Novel nanoparticle carrier method	Glass microparticles that contain 10 nanometer pores
US20130190515A1, 2013	Induces apoptosis in tumorigenic cells	Utilizes gold nanoparticles to deliver epigallocatechin-3-gallate (E3G)
EP 2644594 A1, 2013	Targeted delivery of therapeutics to cancer cells	Conjugates ligands that specifically target prostate specific membrane antigen (PSMA). PSMA is expressed in many cancers, including carcinomas.
WO2012051220A1, 2012	Targeted delivery of therapeutics to SCCs	Combines magnetic nanoparticles with biodegradable nanoparticles that are loaded with the therapeutic. Nanoparticles are guided to site of action with an external magnet
US 20130273561 A1, 2013	Raman characterization of SCCs	Encapsulation of surface enhanced Raman scattering nanoparticles, such as gold, into lipid nanoparticles. The lipid nanoparticles are then conjugated to anti-EGFR antibodies to facilitate localization to SCCs.
US8492510B2, 2013	Enhanced control of the shape and size of nanoparticles, allowing greater batch to batch uniformity	Using a novel formulation for the nanoparticle polymer backbone containing pendant epoxide combined with varying amine ratios
EP2667844A2, 2013	Enhanced penetration through the stratum corneum for topically applied PLGA nanoparticles	Conjugation of hydrophilic compounds to the surface of PLGA nanoparticles
US 20130109700 A1, 2013	Enhanced penetration of nanoparticles deep into the dermis	Conjugation of hydrophobic compounds such as glycerol monooleate and cetyl alcohol
EP2509633A2, 2012	Delivery of temoporfin, a photosensitizer, for use in photodynamic therapy of SCCs	PLGA loaded nanoparticle
EP 1652517 B1, 2012	Novel creation of hyaluronic acid nanoparticles	Creates nanoparticles from a hyaluronic acid-sodium salt and positively charged polymer, preferably chitosan