Introduction

The outermost organ of the human body is the skin, which has a surface area of approximately 1.5 to 2 square meters, providing a protective barrier against the external environment. The stratum corneum of the epidermis is the barrier layer that acts as a defence system in the body by inhibiting the entry of pathogens and protecting from the external environment. The protective function of the stratum corneum limits the permeation of drugs through it. However, highly lipophilic drugs and low molecular weight drugs are passively delivered through the skin, and below the epidermis is a dermal layer that provides support and nourishment to the epidermis [1–3]. The human skin is prone to a disease/ disorder when it is exposed to extreme conditions or environmental or external factors. In skin disorders, various diseases are observed like malignant melanoma skin cancer, non-melanoma skin cancer actinic keratosis (AKs), and others. Skin cancer is a life-threatening disease, the most common sign of cancer in the skin is a change in the appearance of the skin, such as the formation of sores, a large brownish spot with darker speckles, painful lesions that itch or burn, etc. The main two subtypes that cover 95% of all cases are melanoma and non-melanoma skin cancer (NMSC). In skin cancer, the most dangerous are basal cell carcinoma (BCC), squamous cell carcinoma, and malignant melanoma [4]. BCC is the most common type of cancer in the United States. Skin cancer can manifest in various forms, such as melanoma, basal cell, and squamous cell carcinoma. Cutaneous melanoma develops from melanocyte tumor mutation and originates from the lower layers of the epidermis or deeper skin tissues. Pathogenesis of skin cancer is heterogeneous, depending on the location and stage of the malignancy. Surgical intervention may be used to treat primary cutaneous melanoma; however, this is not always practical. Recent therapeutic approaches, including chemotherapy, radiation, immunotherapy, and other treatments, have been reported to have undesirable effects, such as immune response, non-specificity, and drug resistance. The main treatment option for skin cancer is surgery, although there are less invasive treatments available, including topical 5-FU, Imiquimod, and other drugs, such as Diclofenac sodium and Ingenol mebutate. However, the recurrence rate is higher after surgical therapy, requiring the development of more effective treatments. The cure rates associated with topical drugs are much lower compared to surgical therapy. Besides, current topical therapies do not penetrate well and require frequent, prolonged applications. Consequently, patient adherence is inconsistent, and the risk of developing severe inflammation. The conventional preparations (creams, ointments, etc.) exhibit lower efficacy and cause local toxicity (e.g., skin atrophy, skin infections, stretch marks, and redness) due to

rapid loco-regional drug release, poor penetrability, and low bioavailability at the site action resulted in poor efficacy that leads to metastasis. Conventional topical products are associated with various drawbacks such as poor penetrability, low bioavailability at the site action, and the requirement of high dose, causing skin irritation that significantly hinders the drug permeation through the stratum corneum or tumour tissues. Resistance to chemotherapy or other therapies can also occur when cancer cells develop mechanisms to pump out chemotherapy drugs before they can have an effect. This can happen when cancer cells have an increased amount of drug efflux pumps, which pump out anticancer molecules before they can reach their intended target. PDT is a novel and non-invasive treatment approach for skin cancer; however, the photosensitizers low solubility and low stability often limit their application in biological systems. PS are mostly hydrophobic molecules with low solubility in the aqueous phase. 5-Aminolevulenicacid is a highly hydrophilic molecule, and it does not easily permeate from the stratum corneum along with it has limited light penetration ability in tissues or lesions. Cyclopamine is a prototype drug which inhibits the hedgehog pathway in BCC and preclinical studies demonstrated cyclopamine as a potential drug for skin cancer treatment. However, cyclopamine was not effective in skin cancer clinically due to its low solubility and toxicity profile [5]. Monotherapy, or the use of a single treatment approach, in topical skin cancer treatment can be associated with several problems. One major challenge is the limited efficacy of monotherapy, as different types of skin cancer and individuals may respond differently to various treatments. Monotherapy can lead to the development of resistance, where skin cancer cells become less responsive to the treatment over time. This resistance can diminish the effectiveness of the therapy and require alternative treatment options. Additionally, the possibility of incomplete lesion clearance, where specific cancerous lesions may not be completely eradicated. This may result in recurrence or incomplete removal of cancer cells, necessitating further treatment [6].

To overcome these limitations of conventional therapy for skin cancer, advanced drug delivery systems (nanocarriers and combination-based treatment) are explored to deliver the therapeutics. Combining multiple treatment modalities is a safe and effective strategy in cancer management. Combination treatment is the co-delivery of two therapeutic agents or a combination of various therapies, such as chemotherapy with immunotherapy, photothermal therapy (PTT) combined with PDT, radiotherapy, or other therapies. A combinational therapy approach has various benefits, including enhanced therapeutic effects, reduced toxic effects, improved therapeutic outcomes, suppression of drug resistance, and release of the drug in a

controlled and sustained manner [7–9]. Combinational therapy approaches can also have multiple molecular targets in the tumor. In skin cancer, combined therapy helps to avoid adverse effects, such as burning sensations, and also decreases the tumor recurrence rate

1. Objectives of the present research and development endeavor

To achieve the aim of the thesis, the following objectives have been designed. The thesis is divided into chapters focusing on each objective culminating in the research agenda.

1. *Design and characterization of itraconazole and chlorin e6 dual-drug loaded lipidic nanocarriers for skin cancer treatment*

    i. Analytical method development and validation for the analysis of itraconazole (ITZ) and chlorin e6 (Ce6) by RP-HPLC and Spectrofluorometric techniques
    ii. Preparation and characterization of dual drug-loaded lipidic nanoparticles of itraconazole and Chlorin e6 (ITZ/Ce6@LNPs) by quality-by-design approach
    iii. Stability study of ITZ/Ce6@LNPs and ITZ/Ce6@LNPs Gel
    iv. In-vitro Cell Culture Studies
    v. Ex-vivo skin permeation studies
    vi. Ex vivo bioimaging in tumor skin of the C57BL/6 mice
    vii. Topical biodistribution study in C57BL/6 mice skin cancer model
    viii. In vivo antitumor efficacy study in C57BL/6 mice skin cancer model

2. *Design and characterization of curcumin and chlorin e6 dual-drug loaded lipidic nanocarriers for skin cancer treatment*

    i. Simultaneous analytical method development and validation for the analysis of curcumin (CUR) and chlorin e6 (Ce6) by RP-HPLC technique
    ii. Preparation and characterization of dual drug-loaded lipidic nanoparticles (DDLN) of curcumin and chlorin e6 (ITZ/Ce6@LNPs) by quality-by-design approach
    iii. Stability Study of DDLN and DDLN Gel
    iv. In-vitro Cell Culture Studies
    v. Ex-vivo skin permeation studies
    vi. Ex-vivo skin permeation studies by confocal microscopy
    vii. Dermatopharmacokinetic study of DDLN gel formulation

Titled "Design and Characterization of Itraconazole and Chlorin e6 Dual-Drug Loaded Lipidic Nanocarriers for Skin Cancer Treatment," we prepared dual drug-loaded lipidic nanoparticles (ITZ/Ce6@LNPs) using the QbD approach. These nanoparticles were characterized using various techniques, including particle size distribution, zeta potential, scanning electron microscopy, quantification of singlet oxygen generation, and percent cumulative drug release, etc. Moreover, we conducted in-vitro cell culture studies to further evaluate the nanoparticles. These studies involved cytotoxicity assays, cellular uptake studies, combination index analysis, assessment of mitochondrial membrane potential, analysis of nuclear morphology, in-vitro reactive oxygen generation assays, apoptosis assays, and cell cycle analysis, etc. Further, we loaded the developed nanoformulation (ITZ/Ce6@LNPs) into sepineo gel and subjected it to rheological characterization, ex-vivo permeation studies, and ex-vivo bioimaging in the tumor skin of C57BL/6 mice, etc. Additionally, we performed a topical biodistribution study on tumor-bearing mice using an in vivo imaging system (IVIS). The developed formulation was further evaluated through an in-vivo antitumor efficacy study using a B16F10-induced mice skin cancer model.

In this work, chlorine e6 as a photosensitizer and itraconazole as an anticancer agent (Repurposing agent) encapsulated in lipid nanocarriers and embedded in the SEPINEO gel to enhance the skin and tumor penetration. The optimized formulation showed a uniform nano-size range with high entrapment efficiency for both drugs. On the cellular level, the dual-drug-based lipidic nanoparticles exhibited synergistic effects against skin cancer cell lines (B16F10 and A431 cell lines). Moreover, a cellular uptake study with B16F10 and A431 revealed that ITZ/Ce6@LNPs show higher cellular uptake than free drugs. This developed dual-drug formulation gel provided high skin penetration and exhibited higher singlet oxygen generation and stability. The skin retention study showed that ITZ/Ce6@LNPs gel has high skin retention compared to the free drugs (ITZ+Ce6), which was confirmed by the IVIS. In-vivo antitumor efficacy resulted that the tumor weight in the ITZ/Ce6/LNPs gel-treated group being significantly reduced when compared to the control, free Ce6, ITZ, and ITZ+Ce6The combination of ITZ and Ce6 delivery by a lipidic system exhibits more therapeutic efficiency than the individual treatment.

Titled "Design and Characterization of Curcumin and Chlorin e6 Dual-Drug Loaded Lipidic Nanocarriers for Skin Cancer Treatment," we prepared dual drug-loaded lipidic nanoparticles DDLN using the QbD approach. These nanoparticles were characterized using various techniques, including particle size distribution, zeta potential, scanning electron microscopy, quantification of singlet oxygen generation, and percent cumulative drug release. Moreover, we conducted in-vitro cell culture studies to further evaluate the nanoparticles. These studies involved cytotoxicity assays, cellular uptake studies, combination index analysis, assessment of DNA fragmentation assay, analysis of nuclear morphology, in-vitro reactive oxygen generation assays, apoptosis assays, and cell cycle analysis, etc. Further, we loaded the developed nanoformulation DDLN into sepineo gel and subjected it to rheological characterization, texture analysis, ex-vivo permeation studies quantitatively and qualitatively, etc. Additionally, we performed an ex-vivo permeation study and visualized the depth of penetration using confocal microscopy. Further, The developed formulation was further assisted in the dermatopharmacokinetic study.

In this work, chlorine e6 as a photosensitizer and CUR as an anticancer agent encapsulated in lipid nanocarriers and embedded in the SEPINEO gel to enhance the skin and tumor penetration. The optimized formulation showed a uniform nano-size range with high entrapment efficiency for both drugs. This developed dual-drug formulation gel provided high skin penetration and exhibited higher singlet oxygen generation and stability. The skin retention study showed that DDLN gel has high skin retention compared to the free drugs. On a cellular level, the combined formulation showed an excellent synergetic effect compared to free drugs on the B16F10 and A431 cell lines. Moreover, a cellular uptake study with B16F10 and A431 revealed that DDLN shows higher cellular uptake than free drugs. The combination of Ce6 and CUR delivery by a lipidic system exhibits more therapeutic efficiency than the individual treatment. This lipidic system may provide a unique platform for delivering potent hydrophobic moiety for different ill conditions such as cancer, psoriasis, skin infections, etc. It may also be adequate for clinical testing and commercial application.

References

[1] M.B. Brown, G.P. Martin, S.A. Jones, F.K. Akomeah, Dermal and Transdermal Drug Delivery Systems : Current and Future Prospects, (2006) 175–187. https://doi.org/10.1080/10717540500455975.

[2] D. Singh Malik, N. Mital, G. Kaur, Topical drug delivery systems: A patent review, Expert Opin Ther Pat. 26 (2016) 213–228. https://doi.org/10.1517/13543776.2016.1131267.

[3] M. Gupta, U. Agrawal, S.P. Vyas, Nanocarrier-based topical drug delivery for the treatment of skin diseases, Expert Opin Drug Deliv. 9 (2012) 783–804. https://doi.org/10.1517/17425247.2012.686490.

[4] P. Das, N. Deshmukh, N. Badore, C. Ghulaxe, P. Patel, A Review Article on Melanoma, 8 (2016) 112–117.

[5] R. Jain, S.K. Dubey, G. Singhvi, The Hedgehog pathway and its inhibitors: Emerging therapeutic approaches for basal cell carcinoma, Drug Discov Today. (2021). https://doi.org/10.1016/J.DRUDIS.2021.12.005.

[6] R.B. Mokhtari, T.S. Homayouni, N. Baluch, E. Morgatskaya, S. Kumar, B. Das, H. Yeger, Combination therapy in combating cancer, Oncotarget. 8 (2017) 38022. https://doi.org/10.18632/ONCOTARGET.16723.

[7] S.X. Chen, M. Ma, F. Xue, S. Shen, Q. Chen, Y. Kuang, K. Liang, X. Wang, H. Chen, Construction of microneedle-assisted co-delivery platform and its combining photodynamic/immunotherapy, Journal of Controlled Release. 324 (2020) 218–227. https://doi.org/10.1016/j.jconrel.2020.05.006.

[8] S.R. Lucena, N. Salazar, T. Gracia-Cazaña, A. Zamarrón, S. González, Á. Juarranz, Y. Gilaberte, Combined Treatments with Photodynamic Therapy for Non-Melanoma Skin Cancer, Int J Mol Sci. 16 (2015) 25912–25933. https://doi.org/10.3390/IJMS161025912.

[9] S. Lucena, N. Salazar, T. Gracia-Cazaña, A. Zamarrón, S. González, Á. Juarranz, Y. Gilaberte, Combined Treatments with Photodynamic Therapy for Non-Melanoma Skin Cancer, Int J Mol Sci. 16 (2015) 25912–25933. https://doi.org/10.3390/ijms161025912.

[10] V. Smith, S. Walton, Treatment of Facial Basal Cell Carcinoma : A Review, 2011 (2011). https://doi.org/10.1155/2011/380371.