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Assessment of penetration potential of pH responsive double walled biodegradable nanogels coated with eucalyptus oil for the controlled delivery of 5-fluorouracil: In vitro and ex vivo studies

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ABSTRACT

Penetration enhancers coated biodegradable polymeric nanogels loaded with cytotoxic drugs applied via the topical route, can be a promising strategy for improving the chemotherapeutic efficiency of skin cancers. The major objective of proposed research was to investigate the in vitro and ex vivo chemotherapeutic potential of double walled PLGA-chitosan biodegradable nanogel entrapped with 5-fluororuacil (5-FU) coated with eucalyptus oil, topically applied onto the skin. 5-FU was first entrapped in PLGA core by solvent evaporation technique followed by coating with cationic chitosan for ionic interaction with anionic skin cancer cell membrane. A surface coating of eucalyptus oil (1%) was employed to improve the penetration efficacy of the nanogel into stratum corneum. The surface modified biodegradable double walled nanogel was characterized for particle size, charge and thermal properties followed by pH dependent in vitro analysis. Human keratinocyte (HaCaT) cell line was employed for the bio- and cyto-compatibility testing prior to the hemolysis assay and coagulation assessment. A porcine skin ex vivo screening was performed for assessing the penetration potential of the nanogels. DLS and TEM revealed a particle size about 170 nm for the double walled nanogels. The nanogels also exhibited high thermal stability as analyzed by thermogravimetry (TG) and differential thermal analysis (DTA). The drug entrapment efficacy was about ~40%. The drug release showed sustained release pattern noted up to 24 h. The low hemolysis of 2.39% with short prothrombin time (PT) and activated partial thromboplastin time (APTT) of 14.2 and 35.5 s respectively, revealed high biocompatibility of the nanogels. The cellular uptake and localization was assessed by confocal microscopy. The cytotoxicity (MTT assay) on HaCaT cell line demonstrated high cytocompatibilty of the nanogels. An ex vivo evaluation using porcine skin displayed efficient and steady state flux of 5-FU from the biodegradable nanogles into the skin, while the histology of the porcine skin revealed enhanced penetration potential of eucalyptus oil coated PLGA-chitosan double walled nanogels. Taken together the in vivo and ex vivo results portend promising potential for the utility of the biodegradable nanogels for treating skin cancers.

1. Introduction

Topical or transdermal drug delivery system is regarded as the second most suitable and patient friendly route after the oral route of drug administration. The transdermal route is always been a fascinating route for versatile drug delivery and disease ailments. In this regard, the skin is the largest human organ constituting a total area of 2 m^2 (approximately). However, delivery via the skin is also challenging because skin comprises numerous obstacles for drug penetration [1]. Skin generally has multiple "brick and mortar" type arrangement of keratin rich corneocytes (brick) in an intracellular matrix (mortar) constituting elongated units of triglycerides, fatty acids, cholesterol and ceramides. The two key mechanisms governing the delivery of nanomedicines through this route is the diffusion of hydrophilic molecules intracellularly within the aqueous region at outer surface of intracellular keratin filament and dissemination of hydrophobic (lipophilic) molecules intercellularly at lipid matrix between filaments [2]. Drug delivery to

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the desired site is always been a challenging task as it constitutes majority of hurdles like tissue toxicity, early drug degradation, low therapeutic efficiency, repetitive dose intervals and above all, unwanted adverse effects. To eliminate, several of these obstacles, targeted drug delivery system has been introduced which has several advantages such as non-invasive drug therapy, on site drug release and avoiding first pass metabolism [3]. Various approaches and drug delivery techniques deliver bioactives through skin. Penetration technique (passive and active) significantly enhance the topical and transdermal drug delivery system with efficient therapeutic potential at desired site. Topical or transdermal drug delivery therapy is a promising and effective drug delivery techniques for numerous disease and disorders including phthisis, psoriasis, squamous cell carcinoma, basal cell carcinoma etc. [4]. Nano-sized topical formulation has gained noteworthy attention in cancer drug treatment with enhanced cytotoxic potential. Nanogels are characterized as nano-sized three-dimensional system which are chemically or physically cross-linked polymers of natural or synthetic origin. In the current scenario of medical nanotechnology, nanogels are favored candidates in targeted drug delivery with effective therapeutic potentials [5–6]. In terms of size, nanogels are hydrogel particles of size ranging from about 10 nm to 1000 nm and often termed as macromolecular micelles or polymeric microgels system for the enhanced controlled and sustained drug delivery at targeted site able to entrapped large quantity of drug and bioactives. Polymeric nanogel can be engineered to have biodegradable, thermosensitive and pH responsive features which can amplify the therapeutic efficiency of drugs against variety of diseases [7]. Nanotechnology based carriers comprise several architectures such as liposomes, nanoparticles, nanoemulsions and dendrimers. Among them biodegradable nanogels are evolving as one of the promising drug delivery carriers in terms of increasing the stability and loading of the entrapped drugs. Importantly, nanogels are very useful for those drugs whose absorption and solubility are poor [8]. Delivery of nature origin drugs, protein, peptides, DNAs, RNAS and vaccines can also been effectively carried out in a controlled and sustained manner to the targeted sites without degradation and contaminations. The foremost advantage of nanogels system is that it is patient compliant for those having difficulties in oral drug therapy as well as eliminating first pass metabolism and systemic toxicities [9–10]. The favorable particle size of the nanogel systems enable in efficient penetration and diffusion across physical barriers with high absorption in hydrophilic and lipophilic compartments contributing to desired drug release in controlled manner. Topical or transdermal nanogel encounter major obstacles of low drug diffusion across the stratum corneum which delays or retards the drug absorption and release at desired site. In this regard, surface modification of the nanogel system can enhance penetration and drug diffusion across physical barriers resulting in elevated therapeutic transport at targeted site without producing any noteworthy toxicity [11]. Wide varieties of penetration enhancer (natural and synthetic) are utilized in nano drug delivery system making topical delivery more effective and potent. Topical or transdermal drug delivery systems are employed for the local therapeutic actions and for local ailments utilizing cutaneous absorption phenomenon. Local irritation, tissue toxicity, edema and inflammation are the major adverse effects associated with topical drug delivery. Therefore, penetration enhancer should be biocompatible, non- toxic, stable and above all it should be incorporated in small amounts to produce its effects without altering the normal homeostasis of skin or body [12]. One of the other favorable characteristics of penetration enhancer is that they also serve as the depot for the slow and delayed release of drugs administered topically of transdermally. Oleic acid, linoleic acid and maleic acid are the class of fatty acid molecules which shows enhanced solubility [13]. The penetration potential of enhancer moieties is influenced by its structure, long carbon chain and branching. It has been elucidated that the fatty acid with 8–12 carbon chain shows noteworthy penetration and diffusion across stratum corneum facilitating efficient transport of bioactives to the desired site. Essential oils and volatile oils comprising terpenes and terpenoids act as potent penetration enhancers constituting non aromatic carbon, hydrogen and oxygen atoms [14]. The essential oils obtained from eucalyptus oil and chenopodium oil have been reported to clinically enhance the penetration of bioactives. In one study, Eucalyptus oil rich with sesquiterpens showed enhanced absorption of 5-FU and increased the bioactive distribution and partition in the tissue. As the present research is totally focused on the penetration potential of 5-FU against skin cancer, The chief motive behind the selection of eucalyptus oil is to enhance the absorption of 5-FU and increase the bio distribution and partition in the tumor tissue, so the larger amount of bioactive entrapped in nanogel particles reaches to the targeted site for the enhanced antitumor efficacy. Since many researches quoted the small portion of eucalyptus oil can significantly increase the penetration property of nanoformulation, therefore employing 1% v/v eucalyptus oil can enhance the penetration caliber of 5-FU loaded double walled nanogel system against skin cancer [15]. The main disadvantage associated with using essential oils and other penetration enhancers is that they possess skin irritation and edema. The irritation produce by the penetration enhancer is mainly due to the lowering of phase transition temperature of the lipids in the biological membrane. Thus we attempt to fabricate the surface decorated biodegradable polymeric nanogel loaded with antimetabolite anticancer drug 5-FU for the sustained delivery at dermal area against skin cancer evaluated ex-vivo (Fig. 1) [16]. In this research, it is hypothesized that eucalyptus oil may improve the penetration potential of polymeric nanogel particles administered topically resulting in better permeations through stratum corneum due to extraction of lipids or by drug partition phenomenon in tissue with low toxicity, while avoiding local irritation and edema.

2. Material and methods

2.1. Materials

Chitosan with 75% degree of de-acetylation and of medium molecular weight and acetic acid 100% ultra-pure was procured from Hi-media chemical Ltd. Mumbai, India. Sodium tripolyphoshate (TPP), Pluronic F-127, PLGA poly (lactic-co-glycolic acid) of 50:50 strength and PVA (poly vinyl alcohol) 98% hydrolyzed were purchased from sigma Aldrich Bengaluru, India. Poloxamer 108 with molecular weight 102.1 g/mol and Eucalyptus oil 99.9% pure was procured from Merck India Ltd. Mumbai, India. 5-Fluorouracil was procured as a benevolent gift from Taj Pharmaceutical Pvt. Ltd., Hyderabad. Phosphotungestic acid with molecular weight 2880.1 g/mol and NaCl were acquired from Rankem Chemicals Ltd., New Delhi India. Deionized water was produced from Milli-Q Synthesis (18 MΩ, Millipore). All other reagents and chemical were of analytical grade and used as received.

2.2. Preparation of skin permeating double walled nanogel

The double walled nanogel (DWNL) was prepared by the solvent evaporation emulsification technique. Briefly, 10 mg of polylactic-*co*-glycolic acid (PLGA) was dissolved in 2 ml of DCM (dichloromethane); this organic phase was then carefully added to 1% w/v of PVA solution previously containing 0.4% w/v of chitosan and 0.1 ml of triton 100 as surfactant with constant stirring on magnetic stirrer (Remi, India) for 1000 rpm to form a coarse emulsion. The obtained emulsion was broken-down into the nano-droplets by homogenizing at high rate at 40,000 rpm for 10 min. The obtained nanoparticles were then continuous stirred at 750 rpm for about 30 min for the complete evaporation of organic phase [17]. The obtained nanoparticles were then cross-linked by the drop wise addition of 5 ml of 0.4% w/v sodium triployphosphate (TPP) and stirred for about 30 min at 1000 rpm for the complete crosslinking of chitosan. To the above chitosan nano-particles dispersion dissolved mixture of 20% w/v Poloxamer and 3% Pluronic F-127 combination was added and stirred by using magnetic stirrer (REMI)