Perspectives in the use of 5-Fluorouracil microneedle and 3D printing to treat actinic keratosis

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Abstract

Actinic keratosis (AK) is a chronic disease that occurs in fair skin individuals which had extensive exposure to the sun. Clinical and subclinical lesions can coexist across the skin, and when it is a large area there is a field cancerization. As these lesions have the potential to transform into invasive squamous cell carcinoma, treatment and managing are crucial. In Brazil, actinic keratoses constitute the fourth most common dermatological diagnosis, representing the main reason for dermatological consultation for individuals over 65 years. Topical 5-Fluorouracil(5-FU) has been widely used in the treatment of AK lesions for several decades being a pillar of the therapy. However, there are many side effects such as irritation, redness, itching, flaking, pain, and burning, which prevents patients from following the complete treatment. Many strategies are used to improve the therapeutic outcomes of 5-FU and reduce toxicity and side effects. Here is presented the use of 5-FU microneedle for the treatment of AK and give an insight into the 3D printing development in this field. It was found in the literature the use of dissolvable 5-FU microneedle; the combination between 5-FU cream and pre-treatment with microneedle roller. Inkjet printing was used to coating microneedle and stereolithography was used to printing hollow microneedle. It was found that microneedle and 3D printing can improve AK treatment.

Keywords: Microneedle, Combined therapy, Nonmelanoma skin cancer, Skin field cancerization

1. Introduction

Actinic keratosis (AK) occurs more commonly in fair skin individuals which had extensive exposure to the sun. It is most frequent in parts of the body that are often exposed to the sun such as ears, face, neck, and forearms. AK presents as dry, rough, sometimes pigmented lesions of variable thickness and size(SINCLAIR et al., 2020).

The main cause of AK formation is ultraviolet radiation (UVR), wavelength between 290 nm to 320 nm(ROBY et al., 2006). The excessive exposure to UVR starts a cascade of structural damage to cells' DNA and membrane lipids, leading to a generation of overall inflammation(DE OLIVEIRA et al., 2019).

Therefore, the incidence of AK is higher in individuals that living close to the Equator line because there is a relationship between decreasing latitude and higher UVR levels. Closer to the Equator line there is more intense solar UVR with a greater proportion of shorter wavelengths, related to the low angle of incidence of the incoming radiation(ROBY et al., 2006).

In Brazil, AKs constitute the fourth most common dermatological diagnosis, representing the main reason for dermatological consultation in individuals over 65 years (17.2%); in Southern Brazil, this corresponds to 7.4% of the diagnoses and in the North region, to 2.89% of visits(REINEHR et al., 2019).

Males have a three times higher prevalence of extensive actinic damage (defined as ≥10 AKs lesions) compared with females. This is related to differences in sun exposure and protection behaviors between the two groups(WILLENBRINK et al., 2020). However, regardless of gender, individuals who are chronically exposed to solar radiation, due to outdoors occupational or sports, are at high risk of developing AK and subsequently skin cancer(DE OLIVEIRA et al., 2019).

The risk for AKs incidence is significantly increased in immunosuppressed patients, such as solid organ transplant recipients, or patients with chronic lymphocytic leukemia(WILLENBRINK et al., 2020).

Topical 5-FU has been widely used in the treatment of AK lesions for several decades and continues to be a pillar of the therapy. Once inside cells, 5-FU acts preferentially to cause apoptosis in actinic lesions but not in healthy skin(WERSCHLER, 2008).

To treat AK is used topical 5-FU in concentrations ranging from 0.5% to 5%. In Brazil, it is available commercially only at 5% (REINEHR et al., 2019).

High cure rates have been associated with topical 5-FU treatment for AK in patients that persist in following the recommended therapy. However, adverse effects, including irritation, erythema, and erosions, may cause patients to discontinue treatment prematurely, thereby reducing cure rates(WERSCHLER, 2008).

Various 5-FU formulations have been used to reduce side effects associated with treatment, while to maintain therapeutic efficacy, such as microemulsion, liposome, niosome, microsponges, ethosome, transfersomes, chemowraps among others(EWERT DE OLIVEIRA et al., 2020). Here, is focused on the use of microneedles to 5-FU controlled release. It is presented the main research works and additive manufacturing processes used to print microneedle.

1.1 Treating AKs with 5-FU microneedle

Microneedles are micrometer-sized needles that are used to perforate the skin for drug delivery. They are long enough to penetrate the *stratum corneum* but too short to stimulate pain receptors that are located in the dermis(ITA, 2015).

Microneedle could be manufactured with different approaches, shapes, and sizes from a broad range of materials to deliver 5-FU with different concentrations(HAJ-AHMAD et al., 2015).5-FU can be loaded into the microneedle tips and/or base, or coated on the microneedle tips. In the microneedle base approach, the 5-FU can be easily produced, for instance, pre-loading the 5-FU together polymeric base into the negative mold of microneedle during the fabrication process(LIU et al., 2017). The most common categories of microneedles are solid, hollow, coated, hydrogel-forming, and dissolving(ALIMARDANI et al., 2020).

Solid microneedles act creating micropores in the skin; hollow microneedles provide a channel into the dermis; dissolving microneedles once pierce the skin dissolves releasing the drug; while coated microneedles after insert in the skin, the drug dissolves from the layer, releasing quickly the drug (NAGARKAR et al., 2020).

The use of microneedle is minimally invasive without long-term edema or erythema(HAJ-AHMAD et al., 2015). In the dermatology field, the microneedle rollers are used to stimulate collagen and elastin production(SABRI et al., 2019). Microneedle treatment is attractive because allows self-administration without pain(PARK; KIM, 2020). It is significantly less painful than a 26-gauge hypodermic needle, this performance is achieved by avoiding or minimizing underlying pain nerve stimulation.

Naguib, Kumar and Cui (2014) compared the permeability of commercially available 5-FU topical cream (5%) using pretreatment with microneedle roller and only applying 5-FU topical cream. They

showed that the permeability of 5-FU through the skin was increased by up to 4.5-fold ($p \leq 0.05$) when the skin was pretreated with microneedles (500μm in length, 50μm in base diameter). In their study was used a mouse model with B16-F10 mouse melanoma cells implanted in the subcutaneous space, the antitumor activity of the 5-FU topical cream (5%) was enhanced when the cream was applied on a skin area that was pretreated with microneedles roller, as compared to when the cream was simply applied on a skin area. Although this positive result, the use of microneedle roller associated with 5-FU cream should be cautious because can increase local and systemic toxicity. In addition, it is unknown if perforated the skin layer or the *stratum corneum* using the microneedles will negatively affect the development of the tumors in the application area.

In a different approach, Hao et al. (2020) developed a dissolvable microneedle that can be controlled by near-infrared (NIR) light. They designed a NIR light-responsive 5-FU and indocyanine green (ICG) loaded monomethoxy-poly (ethylene glycol)-polycaprolactone (MPEG-PCL) nanoparticle (5-FU-ICG-MPEG-PCL), and then 5-FU-ICG-MPEG-PCL was integrated with a hyaluronic acid dissolvable microneedle system (HA MN) to get 5-FU-ICG-MPEG-PCL loaded HA MN for treating skin cancers.

They studied the dissolvable microneedle by inserting into the tumor tissue and applying an 808 nm NIR laser. The ICG transformed the light energy into heat energy, which burned the tumor tissue and killed the tumor cells. Using this approach, the A375 tumor-bearing balb/cA-nu mice and A431 tumor-bearing balb/cA-nu mice were cured without recurrence, which demonstrated that this combination therapy could improve the 5-FU anti-tumor ability. Moreover, this microneedle system can achieve a single-dose cure for skin cancer and provide a new possibility for clinical treatment of skin cancer(HAO et al., 2020).

Beyond the use of NIR laser, microneedle can be combined with an electrically micro-pump to improve the controlled release of the drug, which represents an advantage compared to other patches and topical released techniques(GIRI NANDAGOPAL et al., 2014). More advantages listed are decreased microbial penetration during the treatment as compared with a hypodermic needle because the microneedle punctures only the epidermis; and the enhanced of drug efficacy due to permeability on the *stratum corneum* should result in dose reduction(BARIYA et al., 2012).

The microneedle use disadvantage is to treat a widespread area, in such way that is not recommended the use of microneedle in this clinical case(YAMADA; PROW, 2020). Other disadvantages are the influence of the external environment, like hydration of the skin, which could affect delivery; the use of repetitive injection may collapse the veins; the tip of the microneedle may break off and remain within the skin on the removal of the patch; it can occur the block of hollow microneedles due to compressed dermal tissue(BARIYA et al., 2012).

Microneedles are emerging as an efficient 5-FU transdermal delivery system. It allows that 5-FU to be transported into the skin in a minimally invasive way, being a strategy used to increase the skin permeability of 5-FU. The high permeability allows to decrease the dosage necessary to achieve the therapeutic effect and, consequently, in smaller dosages the toxicity decreases.

1.2.3D printed microneedle

Stereolithography and inkjet printing technologies have been used for direct or indirect print microneedle arrays or for coating their surface with anticancer drugs. Compared with traditional fabrication methods, that are mass-produced, these additive manufacturing technologies allow tailoring of dosages according to individual patient needs, answering personalized medicine demand (ECONOMIDOU; LAMPROU; DOUROUMIS, 2018).

The additive manufacturing technologies can contribute also to the customization of the microneedle design, since the geometry of the microneedles can be easily tailored to deliver the anticancer drug in

different intradermal depths, such as release epidermally, dermally, or subdermally(ECONOMIDOU; LAMPROU; DOUROUMIS, 2018). Also solving the disadvantage of the penetration depth variation between patients due to the individual thickness of the *stratum corneum* and other skin layers(BARIYA et al., 2012).

Inkjet printing is a high resolution technology that possibility selective deposition of materials, therefore is a very promising technology for printing microneedle coating with purposes of the development of personalized dosages and complex drug profiles with high reproducibility(ECONOMIDOU; LAMPROU; DOUROUMIS, 2018).

For instance, inkjet printing technology was used in the coating process of metal microneedle arrays with three anticancer drugs, 5-FU, curcumin, and cisplatin for transdermal delivery. The ink jetting processing optimization produced, highly uniform, reproducible, and accurate coatings at various drug polymer ratios. In this configuration, the 5-FU showed a rapid release profile with most of the drug been released within 3h(UDDIN et al., 2015).

Stereolithography which used UV laser beam can print the microneedle in a bottom-up addition fashion by photopolymerization of resin. Using the computer-aided design (CAD) model, the desired microneedle is created and sliced. The virtual microneedle model is sent to equipment and, each layer is written in the photosensitive resin by restoring the path of the laser for the contour to be filled in for each layer. Photo-polymerization of the first layer is followed by separation and realignment after which recoating of the first built layer takes place. The pattern is cured in this second layer. These steps are repeated to construct the microneedle(KRIEGER et al., 2019; NAGARKAR et al., 2020).

Recently, Uddin et al (2020) combined stereolithographic 3D printing and inkjet coating to generates microneedle with desirable anticancer drug amounts and no material losses. 3D printed microneedle with cisplatin anticancer drug was tested using A431-human squamous carcinoma zenografts. The printed microneedle led to complete tumor suppression and elimination with 100% animal survival(UDDIN et al., 2020).

Another additive manufacturing technique that can be used to microneedle print is two-photon polymerization (2PP). It enables the printing of elaborate and complex microneedle in the microscale and nanoscale. In this process, the polymerization is initiated by two-photon absorption which generates an energy that is centered at the laser focal point. After fabrication of the microneedle, the resin is washed with a solvent and cured using ultraviolet light(NAGARKAR et al., 2020).

Hollow microneedle was print using 2PP technology to be used as theranostic. In this hollow microneedle has combined the therapeutic and diagnostic technologies into a single platform. The hollow microneedle enables precise transport of theranostics agents into epidermal, dermal, or subdermal tissues, as well as for the withdrawal of blood and/or interstitial fluid, providing disease imaging and chemotherapy delivery for treatment of cancer at the same time(GITTARD et al., 2011). Additionally, the microneedle devices can be used as a biosensor for transdermal detection of cancer biomarkers(ALIMARDANI et al., 2020).

2PP can directly create the microneedle or printing micro molds that subsequently is cast with biocompatible polymer and drug. For instance, Gittardi *et al* (2009) printed negative micromolds to create an insulin release microneedle. *In vitro* studies demonstrated that the microneedle can penetrate the skin, creating pores that would possibly facilitate the transdermal penetration of insulin and other protein based agents(GITTARD et al., 2009).

Further, a microfluidic-enable hollow microneedle device was printed using stereolithography. The hollow microneedle array was fabricated in a single-piece, multi-inlet, embedding in the 3D microfluidic device. The microfluidic-enable microneedle can facilitate the homogenization of multiple fluids under different flow rates, followed by transdermal delivery of the mixed solution. It is particularly applicable to preclinical investigations that focus on combinational drug therapy, where

the *in-situ* combination of multiple drugs and the adjusting of their physicochemical properties lead to more effective outcomes than the single or premixed drug a long time(YEUNG et al., 2019)

1.3. Biomimetic models for 5-FU screening

The goal of the biomimetic models is to provide the optimal tumor response in different concentrations and therapeutical approaches of 5-FU, allowing personalized clinical prescription and predictive of the optimal treatment.

3D biofabricated cutaneous squamous cell carcinoma (cSCC) tissue model was used to quantify the antitumor effects of 5-FU in tissue. Biomarkers' images indicated that 50% of cancer cells were killed in the tissue after dosing of 1µM 5-FU during 48-hour of treatment. The imaging biomarkers also demonstrated that normal keratinocytes were less affected by 5-FU, showing that 5-FU selectively killed cSCC cells more than keratinocytes(BROWNING et al., 2020).

In the Browning et al., (2020) study was developed and validated a morphologically and genomically accurate 3D skin cSCC tissue model that enables the study of the effect of 5-FU on cancer cells growing in the context of their native tissue microenvironment. They developed a nondestructive, 3D fluorescence confocal imaging assay to test both efficacy and general toxicity of 5-FU. Image biomarkers were derived to quantify the therapeutic effect in the system. Further, pharmacologically validation was performed to demonstrate different effects of 5-FU on A431 SCC cells versus normal keratinocytes in the 3D model system(BROWNING et al., 2020).

2. Conclusions

The decrease in 5-FU dosage, in the duration of treatment, and in complications with systemic toxicity are expected to improve the healing of precancerous skin and to coincide with the reduction in healthcare costs. To achieve this, innovative drug delivered systems, which can enhance the 5-FU bioavailability for topical use, are desired. 5-FU microneedle and 3D printing technologies could make feasible reach these outcomes.

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