TECHNICAL REPORT

PracaSil®-Plus

In Vitro Evaluation of the Safety Profile of PracaSil®-Plus on Human Melanoma Tissue

Abstract: Melanoma is a cancerous skin condition that often leads to surgical removal of the affected tissue, which results in skin scarring. Scars may not only be aesthetically unpleasant but may also have physical consequences such as pain and psychosocial consequences (e.g., anxiety, distress). PracaSil-Plus is a proprietary anhydrous silicone topical base used for incorporation of different APIs indicated in skin regeneration and healing. The purpose of this study was to evaluate the safety profile of PracaSil-Plus on the progression of melanoma, using the MLNM-FT-A375 human melanoma tissue model. Results have demonstrated that PracaSil-Plus did not contribute to the progression of melanoma cells when compared to untreated tissues. Therefore, PracaSil-Plus may be regarded as a safe compounding base to be used by pharmacists in scar management therapy.

Introduction:

Melanoma is a cancerous condition that arises from unregulated growth of melanocytes (pigmented cells), resulting in tumor production on the skin (most common) and mucous membranes [1]. The unregulated growth of melanocytes can be divided into two phases: radial growth phase (RGP), represented by slow proliferation of cells in the epidermis; and vertical growth phase (VGP), a more advanced phase with presence of nodules [2]. Depending on the growth phase of the melanocytes and the aggressiveness of the tumor, melanoma may be classified into one of 5 stages: stage 0 is the least severe while stage 4 is the most severe. With the exception of stage 4, treatment of melanoma involves initial surgical removal of the tumor, which results in skin scarring, the imperfect but normal end point of tissue repair [1]. Unfortunately, scars cannot yet be made to disappear, and may range from a desirable fine line to a variety of abnormal scars, including hypertrophic and keloid scars. Skin scarring is often considered trivial but may also be aesthetically unpleasant and disfiguring, causing distress, anxiety and other psychosocial consequences. Scars may also have physical consequences as tenderness, itching and pain, which is functionally disabling and contributes to diminished quality of life [3-5].

Taking into account that clinical treatments do not entirely eliminate skin scarring, the therapeutic goal is to reduce, as much as possible, the severity of scars. However, not only are scars different but there is also considerable qualitative and quantitative variability in skin scarring between individuals. Consequently, clinical treatments should envisage a personalized approach and be adapted to both the scar and patient specificities [3, 6, 7]. For this reason, plastic surgery and various medication for scar treatment are commonly used to help restore the appearance of the tissue [8]. PracaSil-Plus is a proprietary anhydrous silicone base developed to be applied topically in scar and wound management therapy as a base for incorporation of different active pharmaceutical ingredients (APIs) indicated in skin regeneration and healing.

Therefore, the purpose of this study was to evaluate the safety profile of PracaSil-Plus on the progression of melanoma, using the MLNM-FT-A375 human melanoma tissue model.

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Methodology:

The safety profile of PracaSil-Plus was evaluated *in vitro* using the MLNM-FT-A375 human melanoma tissue model (MatTek Corporation), which comprises of human malignant melanoma cells
(A375), cultured and differentiated to form a multilayered skin model. This model was designed to resemble the progression of melanoma *in vivo* as melanoma cells are found in the tissue at various stages of development [9]. In this study, melanoma tissues were cultured for 7 days prior to sample application. Following tissue preparation, 50 µL of PracaSil-Plus (50 mg/50 µL) were applied topically to a set of melanoma tissues (n=2), every other day, for a period of 14 days. At the same time, another set of tissues (n=2) were left untreated and monitored throughout the study period to serve as negative control. Prior to each re-application with PracaSil-Plus, the surface of the tissues were cleaned with a sterile cotton tip. At days 0, 3, 5, 7, 9, 11, and 14 post-application, tissue cross-sections were obtained and stained using Hematoxylin and Eosin (H&E). Images of tissue cross-sections were captured and analyzed at 10x, 40x, and 100x optical magnifications using an Olympus VS120® slide scanner [10].

Results and Discussion:

In order to assess the effect of PracaSil-Plus on the progression of melanoma, a total of 38 H&E stained tissue slides were analyzed for presence of VGP-like nodes, as well as changes in the size of the nodes and invasion of the melanoma cells into the dermal layer. On day 3 post-application, VGP-like nodes were shown in both non-treated tissues and tissues treated with PracaSil-Plus (Figure 1). Similarly, initial invasion by melanoma cells occurred on day 5 post-treatment while VGP-like nodes increased in size on day 7 for tissues in both groups. By day 9, deeper invasion of melanoma cells in the dermal compartment occurred for non-treated tissues as well as for tissues treated with PracaSil-Plus (images available upon request). Images collected on day 14 post-application revealed deeper invasion into the dermis for both tissue samples, treated and non-treated (Figure 2).

As a result, no difference was noted in melanoma cells progression between non-treated tissues and tissues treated with PracaSil-Plus. The presence of VGP-like nodes, as well as increases in nodes size and dermal invasion of melanoma cells, all appear to be synchronized when comparing PracaSil-Plus-treated and non-treated tissues. These results demonstrate that PracaSil-Plus does not contribute to the progression of melanoma and, therefore, it may be used as a topical compounding base following the surgical removal of skin tumors.

In Vitro Evaluation of the Safety Profile of PracaSil®-Plus on Human Melanoma Tissue

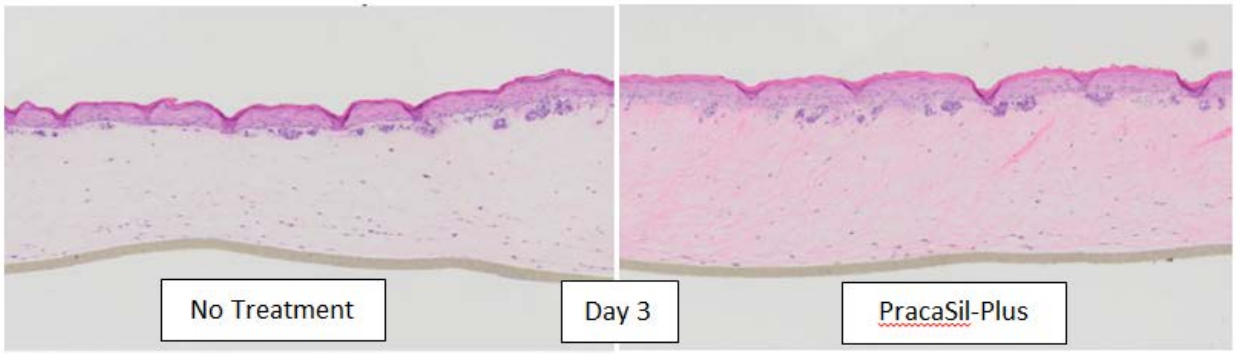

Figure 1. *Melanoma tissue model 3 days post-applicationt (40x magnification).*

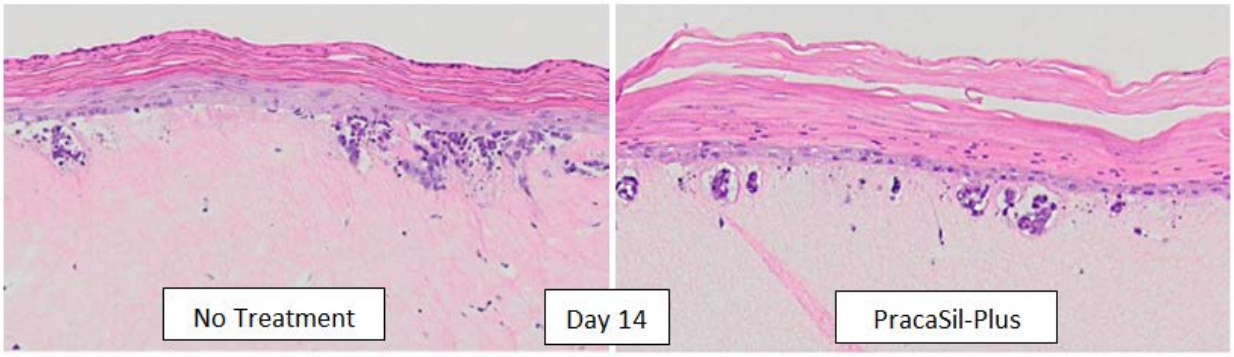

Figure 2. *Melanoma tissue model 14 days post-application (100x magnification).*

Conclusions:

Melanoma is a cancerous skin condition that often leads to surgical removal of the affected tissue, which results in skin scarring. Scar patients are likely to seek clinical treatment, particularly for abnormal scars, as skin scarring is often associated with substantial emotional and financial costs [3-5]. Compounding pharmacists may then have a critical impact in scar and wound management therapy by dispensing personalized topical formulations.

The *in vitro* evaluation of the safety profile of PracaSil-Plus has demonstrated that this topical base did not contribute to the progression of melanoma cells within the human tissue model. Topical application of PracaSil-Plus can thereby be considered safe as there were no significant differences between the progression of melanoma in tissues treated with PracaSil-Plus versus the untreated tissues.

The favorable safety profile demonstrated by PracaSil-Plus is promising data to suggest that this topical base may be safely used by compounding pharmacists for the incorporation of different APIs indicated in scar management therapy.

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