

In Vitro and In Vivo Evaluation of Wound Healing by Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel

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INTRODUCTION

Chronic wounds are a major clinical problem that leads to considerable morbidity and mortality worldwide. It is exacerbated by several factors, including an aging population and comorbidities, such as diabetes. A patent-pending, powder-to-hydrogel, film-forming topical hydrogel base was developed (Figure 1), which possesses tissue-protective and microbiome-supportive properties. The newly-developed wound care compounding base was incorporated phenytoin 2% and misoprostol 0.0024% for *in vitro* and *in vivo* testing. The performance of this formulation was compared to a product of reference: phenytoin 2% and misoprostol 0.0024% in poloxamer gel - commonly used in wound healing pharmaceutical formulations due to its versatility and advantageous properties.

MATERIALS & METHODS

The process of re-epithelialization *in vitro* was evaluated using primary human keratinocytes and the Oris™ cell migration assay kit (Platypus Technologies, Inc.), which consists of a 96-well plate, cell seeding stoppers that inhibit the spread of cells into the migration zone (center of the wells), and a black mask that allows for detection of cell migration. The cells were treated with test formulations for 24 hours and then stained with Calcein AM, a non-fluorescent dye that is converted to green fluorescent calcein by viable cells. The process of wound healing *in vivo* was evaluated using the diabetic mice wound healing model (BKS-db), including 12 male mice distributed in a control group and two test groups (topical hydrogel and poloxamer formulations). Two full-thickness excisional skin wounds were made to the back of each mouse and the test formulations were applied daily on the skin wounds for a total of 9 days. Ethics approval was obtained for this animal study.

RESULTS AND DISCUSSION

Re-epithelialization is a process in wound healing that involves the migration of keratinocytes (cells within the epidermal layer of the skin) from the edge, towards the center of the wound, to form a thin layer of cells over the exposed area. The abilities of the formulations to facilitate migration was quantified based on the green fluorescence detected by the plate reader. Keratinocyte migration showed a mean change of $70.62\% \pm 43.27$ ($p=6.95E-06$) from control for the topical hydrogel formulation, as opposed to $-29.99\% \pm 22.85$ ($p=0.0007$) for the poloxamer gel formulation (Figure 2). Keratinocyte migration is part of the re-epithelialization process in wound healing and, therefore, the topical hydrogel formulation is likely to have greater wound healing abilities than the product of reference.

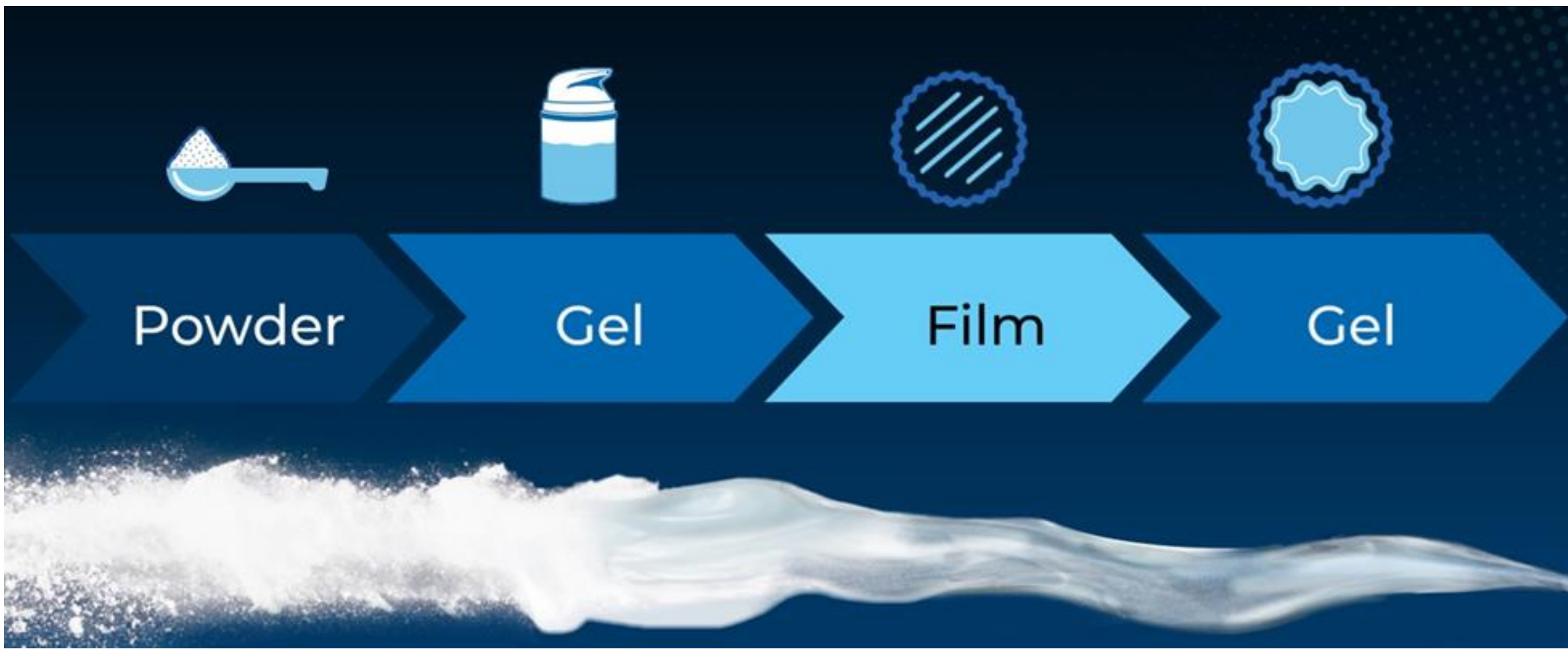


Figure 1. Sequential transformation scheme of the topical hydrogel base (powder into hydrogel, hydrogel into film, film into hydrogel).

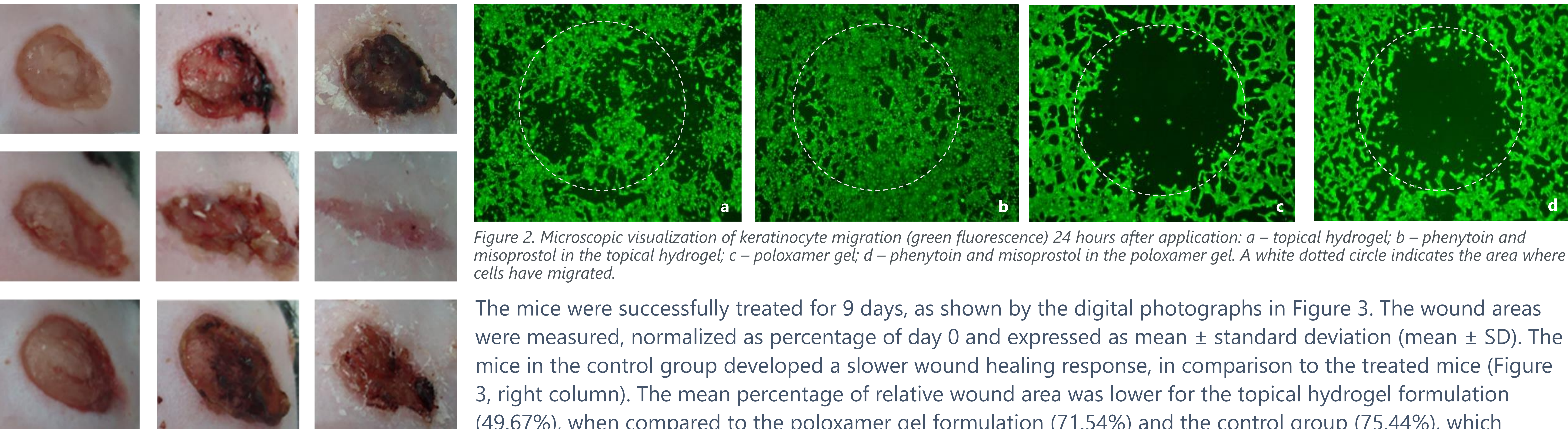


Figure 2. Microscopic visualization of keratinocyte migration (green fluorescence) 24 hours after application: a – topical hydrogel; b – phenytoin and misoprostol in the topical hydrogel; c – poloxamer gel; d – phenytoin and misoprostol in the poloxamer gel. A white dotted circle indicates the area where cells have migrated.

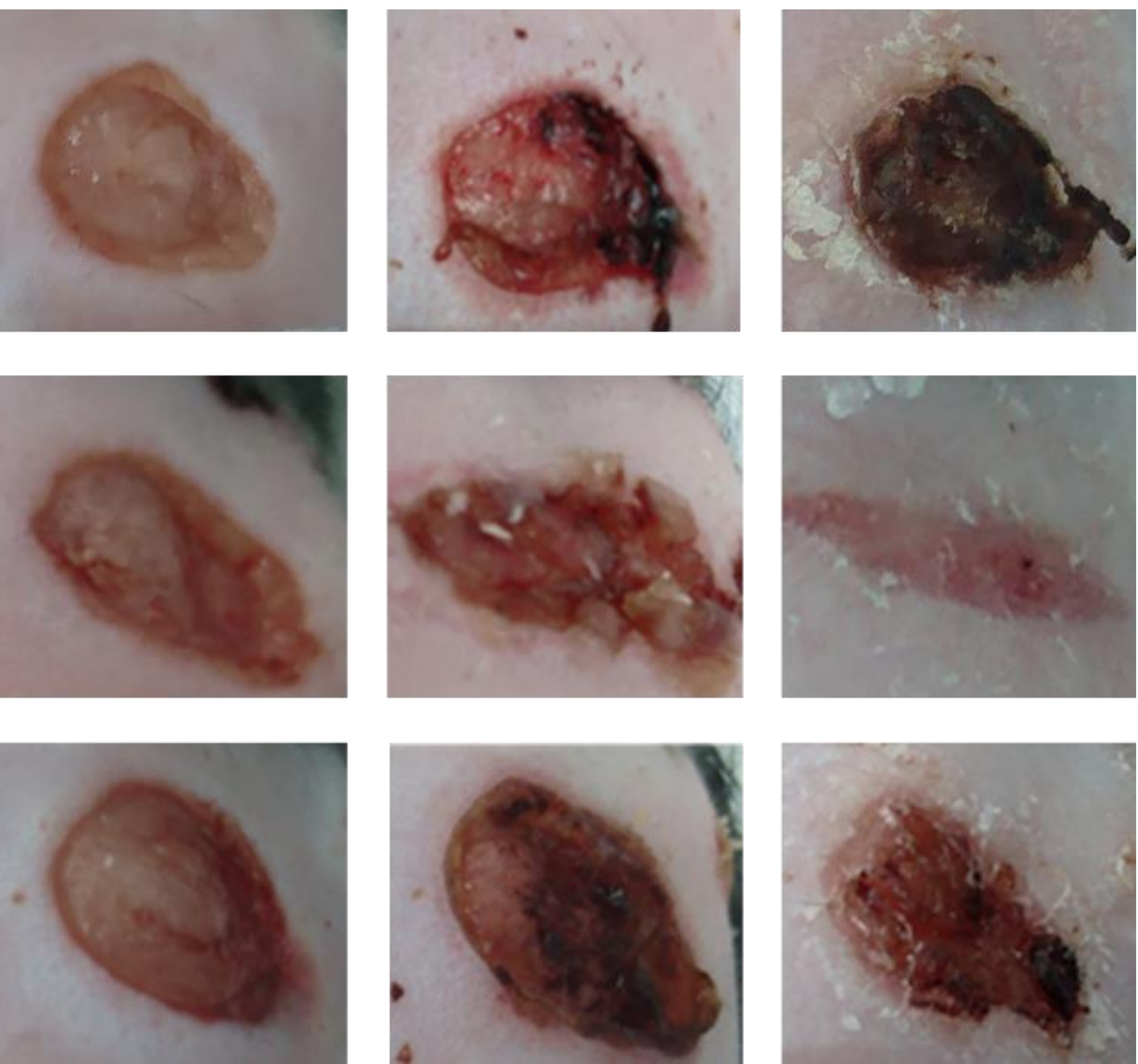


Figure 3. Digital photographs of the wound areas for mice in the control group and test groups, before treatment (left column), day 3 (middle column) and day 9 (right column) of treatment.

The mice were successfully treated for 9 days, as shown by the digital photographs in Figure 3. The wound areas were measured, normalized as percentage of day 0 and expressed as mean $\pm$ standard deviation (mean $\pm$ SD). The mice in the control group developed a slower wound healing response, in comparison to the treated mice (Figure 3, right column). The mean percentage of relative wound area was lower for the topical hydrogel formulation (49.67%), when compared to the poloxamer gel formulation (71.54%) and the control group (75.44%), which reinforces the superior wound healing abilities of the topical hydrogel formulation - its transformative properties and efficacy in drug delivery make it a promising candidate for advanced wound care applications.

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