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DEVELOPMENT OF A TOPICAL GEL CONTAINING A DIPEPTIDYL PEPTIDASE-4 INHIBITOR FOR WOUND HEALING APPLICATIONS

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Abstract

Chronic wounds are challenging for healthcare because they are difficult to treat and cannot heal by themselves. Active compounds that can accelerate wound healing are, therefore, necessary. Dipeptidyl peptidase (DPP)-4 inhibitors are antihyperglycemic drugs widely used in patients with type 2 diabetes that not only maintain the homeostasis of blood sugar levels but have also been shown to promote chronic wound healing. In this study, we formulated a topical gel containing, sitagliptin, a commonly used DPP-4 inhibitor drug to treat diabetes, using Carbopol 940 as a base due to its high viscosity and biocompatibility. The characteristics of the sitagliptin gel, including its physical appearance, viscoelastic properties, swelling and degradation, and stability, were investigated. The gel appeared to be transparent with a uniform distribution of drug molecules and was stable at 4 °C for more than 1 month. Moreover, the gel was shown to exhibit shear thinning pseudoplastic behavior, which is desirable for topical gels. The gel could absorb up to 250% of liquid within 2 days but later degraded in aqueous solution following zeroth-order kinetics. In the in vitro release study, the cumulative release data were best fitted with the first order kinetic model, in which the release rate depended on the concentration. To further demonstrate the use of the DPP-4 inhibitor gel, the gel was applied directly onto subcutaneous wounds on experimental pigs. The topical gel was shown to exhibit the desired spread ability without causing any inflammation around the wound area which was comparable to IntraSite® gel and commercial silver nanoparticle cream.

Keywords

Carbopol 940, Chronic Wounds, DPP-4 Inhibitor, Kinetic Model, Sitagliptin

1. Introduction

A definition of wound is disruption of the continuity of skin, mucous membrane, or organ tissue (Kujath & Michelsen, 2008). The wound is classified as acute or chronic according to the duration and nature of the healing process. Acute wounds result from unexpected accidents or surgical injuries and heal at a predictable time. Nevertheless, chronic wounds, which mainly result from burns, diabetic ulcers, and leg ulcers, do not heal in a fixed period of time (Dhivya et al., 2015). Chronic wounds remain a major challenge in healthcare around the world and have significant financial implications (Frykberg & Banks, 2015). Therefore, the development of wound management devices has been studied.

Hydrogels are commonly used formulations that combine with drugs to increase viscosity and residence time during cutaneous treatment. Hydrogel bases therefore offer great

potential, as they can easily be combined with various substances that help wound healing and/or have anti-inflammatory properties to promote chronic wound healing (Rüther & Voss, 2021). Carbopol is a hydrogel that has been extensively utilized as a primary medication carrier for transdermal usage. It has the benefits of high viscosity, high compatibility with other drugs, good thermal stability, and excellent tissue compatibility (Hayati et al., 2018).

Gliptins or dipeptidyl peptidase (DPP)-4 inhibitors are widely used in patients with type 2 diabetes due to their effectiveness in controlling blood sugar levels and the minimum risk of hypoglycemia (Long et al., 2018). DPP-4 inhibitors not only maintain the homoeostasis of blood sugar levels but also have wound healing effects. Sitagliptin is a DPP-4 inhibitor that decreases proinflammatory cytokines, leading to less inflammation around the wound area and the initiation of wound closure. Furthermore, these two drugs also increase iNOS enzyme and hydroxyproline levels, which are important in angiogenesis and collagen synthesis, respectively (Chandrashekar et. al., 2017). Although DPP-4 inhibitors have therapeutic efficacy in diabetic wound healing, their effect has always been indirect through oral administration for the treatment of diabetes. To the best of our knowledge, there have never been any studies that apply DPP-4 inhibitors directly to wounds to study their effect on wound healing or formulate them into topical gels.

In this study, characterizations of a topical gel were demonstrated. The concentration of sitagliptin, the selected DPP-4 inhibitor, in Carbopol 940 was determined. After that, the kinetic release of sitagliptin from Carbopol 940 was determined by fitting the experimental data with commonly used kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The stability of sitagliptin in Carbopol 940 has been demonstrated. Finally, application of the gel onto subcutaneous wounds on experimental pigs was studied. Note that the bioactivity of DDP-4 inhibitors was not studied because drug release and stability are the aim of this study.

2. Literature Review

(Chandrashekar et. al., 2017) studied the wound healing potential of linagliptin, saxagliptin, sitagliptin, and vildagliptin in nondiabetic Wistar albino rats using a nasogastric tube on excised wounds. The scar area at 4, 8, 12, and 16 days, as well as the time taken for complete scar formation, were measured. After that, the rats were sacrificed, granulation tissue was evaluated by wound tissue biopsy, and serum was tested for TNF-1α, IL-6, iNOS, hydroxyproline, and glucose estimation on the 16th day. The results showed that the percentage of wound closure

in the saxagliptin- and vildagliptin-treated groups was comparable to that in the control group. The results of time for complete epithelization and scar area showed that saxagliptin and vildagliptin had insignificant differences from the control group, whereas the linagliptin and sitagliptin groups had early and small scar areas, indicating better collagenesis. On the 16th day, serum parameters on tissue remodeling revealed that the linagliptin and sitagliptin groups had significant serum hydroxyproline and iNOS concentrations, indicating increased collagen synthesis and vascularity of the wound tissue, respectively. They also aided wound healing by preventing the ongoing inflammatory process, as evidenced by a significant decrease in serum TNF-1. Blood glucose levels (mg/dl) showed no significant differences after 8 or 16 days of drug treatment in all treatment groups, indicating antihyperglycemic action. In conclusion, only linagliptin and sitagliptin improved wound healing potential in rat excised wounds.

(Shih et al., 2018) studied the effect of the DPP-4i sitagliptin on wound healing in healthy C57BL/6 mice. C57BL/6 mice were given 0 and 20 mg/kg/d sitagliptin orally and then captured at 7 and 14 days after wounding. The results showed that the wound was clearly visible after 7 days in the untreated group. The wound decreased after 14 days, but skin trauma was still observed. Wound healing was significantly improved after 7 and 14 days of treatment with 20 mg/kg/d sitagliptin, as shown in Figure 1. After that, the recovery rate in different doses of 0, 2.5, 10, and 20 mg/kg/d sitagliptin was investigated at 3, 7, and 14 days, as shown in Figure 2. At 7 days after wounding, wound healing improved with sitagliptin doses. There was no difference between 0, 2.5, and 10 mg/kg/d sitagliptin at 14 days. Treatment with 20 mg/kg/d sitagliptin improved wound healing more than the other treatments at 7 and 14 days. Therefore, sitagliptin promotes wound healing in C57BL/6 mice.

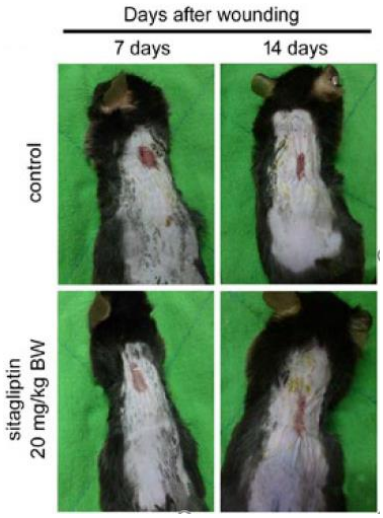

Figure 1: *Photographs of Mice in Each Treatment Group. Images of Each Mouse Depict The Same Wound as Healing Progressed for 7 and 14 D*
(Source: Shih et al., 2018)

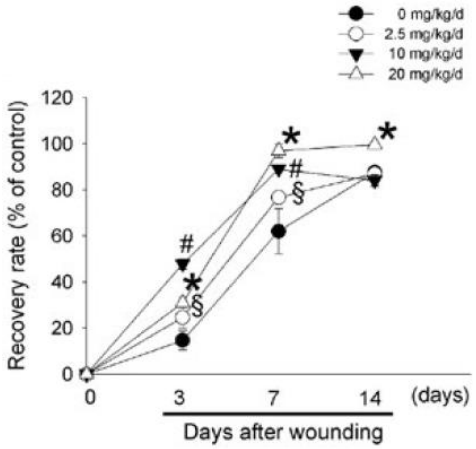

Figure 2: *The Results of a Quantitative Analysis of Wound Healing Over Time in Mice from Different Groups Treated with Or Without Sitagliptin*
(Source: Shih et al., 2018)

3. Materials and Methods

3.1. Materials

Carbopol 940 was obtained from Corel Pharma Chem, India. A DPP-4 inhibitor drug (sitagliptin phosphate monohydrate) was purchased from the European Pharmacopoeia, France. Triethanolamine and dialysis tubing with an MWCO of 10 kDa were purchased from Thermo Fisher Scientific, USA.

3.2. Formulation of Topical Gel

Sitagliptin at 100 µg/ml, 150 µg/ml, and 200 µg/ml and 0.5% w/w Carbopol 940 were dissolved in deionized water at room temperature. This mixture was stirred until a homogenized gel formulation was obtained. After that, triethanolamine was added to neutralize the pH to 7.2-7.4.

3.3. Characterization of Gel Formulations

3.3.1. Visual Observation: One milliliter of topical gel without sitagliptin was placed on a plate with 10 ml of water around the gel and incubated at 37 °C for 5 days to observe the characteristics and change of the gel with water and without water.

3.3.2. Viscoelastic Properties: The viscosity and shear stress of the gel without sitagliptin before and after neutralization of the pH using triethanolamine were determined to investigate viscoelastic properties by HAAKE™ MARS™ Rheometers (Thermo Fisher Scientific, USA).

3.4. Swelling and Degradation Test

Two milliliters of topical gel without sitagliptin were added to a glass vial. Two milliliters of water were carefully layered over the gel surface and then incubated at 37 °C. The surface medium was discarded, and the weight of the remaining gel was recorded after 1 h, 2 h, 3 h, 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h. Fresh deionized water was added to the vial before placing it back into the incubator. The swelling index percentage was calculated using the following equation:

$$\% \text{ Swelling} = \frac{W_t - W_i}{W_i} \times 100 \qquad (1)$$

where W_t and W_i are the weight of the gel at a specific time interval and beginning, respectively.

3.5. In Vitro Release of Sitagliptin

A topical gel containing sitagliptin was incubated with phosphate-buffered saline (PBS) at pH 7.4 and 37 °C to simulate wounds using dialysis tubing. First, 10 ml of phosphate-buffered saline was added to a glass vial. One milliliter of the gel was placed in the dialysis tubing. Afterwards, the dialysis tubing was placed in a glass vial. The samples were collected every 30 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 24 hours, 48 hours, and 72 hours. The concentration of sitagliptin was varied at 100, 150, and 200 µg/ml. The absorbance of the collected samples was measured using a UV spectrophotometer at a wavelength of 267 nm. The absorbance was later converted to the drug concentration using a previously established standard curve.

3.6. Kinetics of the Drug Release

The cumulative release data of sitagliptin from the topical gel at various time points were fitted to 4 commonly used kinetic models, including zero-order, first-order, Higuchi models,

and Korsmeyer-Peppas. The coefficient of determination (R^2) was used to assess the fit of the experimental data to the kinetic model equations.

3.7. Stability Study

Topical gels with and without sitagliptin at 150 µg/ml were transferred to glass vials, in which the glass vials were closed with and without parafilm to simulate well and poor storage conditions, respectively, and kept at 4 °C. The stability test was carried out for 1 week, 2 weeks, and 1 month after formulation. At specific time intervals, the weight, % drug content, and pH of the gel were recorded. Characterizations are also conducted to investigate the change in the gel formulations.

3.8. Application

A topical gel containing sitagliptin at 100 and 150 µg/ml was directly applied to subcutaneous wounds on experimental pigs to investigate the characteristics and effect of the gel on the skin. This experiment was conducted in collaboration with the Faculty of Medicine Siriraj Hospital.

4. Results & Discussion

4.1. Characterization of Gel Formulations

4.1.1. Visual Observation: The physical appearance, homogeneity, texture, and stability of the 0.5% w/w Carbopol 940 gel formulations were studied by visual observations. From Figure 3a, the appearance of the gel is clear, transparent, and steady. After 24 hours, the diameter of the gel slightly decreased from the starting point but greatly decreased at 120 hours because the gel would be dried. For Figure 3b, the gel clearly dissolved in water after 24 hours, as shown by the increased diameter. However, the gel was still visible. At 120 hours, the gel completely dissolved in water.

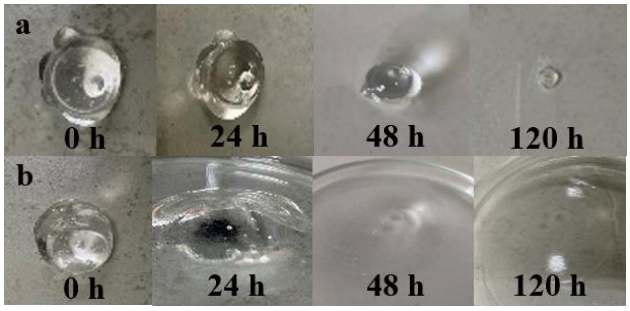

Figure 3: *Gel Formulation with And Without Water at Start and The End of Time Point (A) Gel Without Water (B) Gel with Water*

(Source: Authors' Own Illustration)

4.1.2. Viscoelastic Properties: The shear stresses of the 0.5% w/w Carbopol 940 gel before and after neutralization using triethanolamine at various shear rates were determined to investigate the viscoelastic properties.

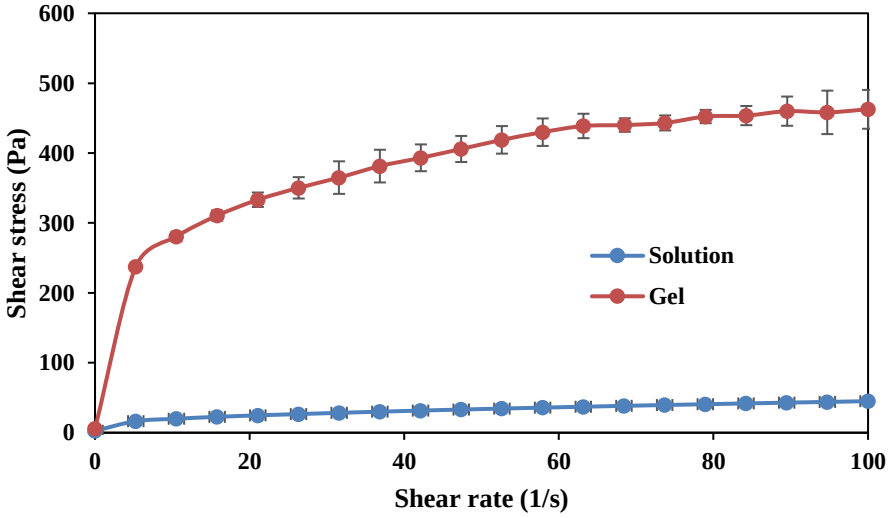

Figure 4: *Rheology Profile of Carbopol 940 Before and After Forming the Gel*

(Source: Authors' Own Illustration)

From Figure 4, an increase in pH caused an increase in the shear stress of the gel. The shear rate increased as the shear stress increased, indicating shear-thinning pseudoplastic behavior. Which is similar to the study of Vishal & Manish, 2013. Therefore, the gel could flow easily when the shear force was applied to the gel on the wound but remained firm when at rest, allowing the gel to stay on the wound for longer periods. The gel could hold its shape when absorbed rather than dissolved into the wound exudate.

4.2. Swelling and Degradation Test

The surface of the topical gel without sitagliptin was incubated with water for 144 hours to observe the change in the gel. At the beginning of the incubation, swelling of the topical gel was observed because water diffused into the matrix of Carbopol 940, a hydrophilic polymer. After that, the polymer network expanded upon relaxation (Nan et al., 2019). The swelling kinetics of the gel follows zeroth-order kinetics with high R-squared values. The highest % swelling of the gel was 250% at 48 hours, which would be sufficient for the hydrogel to absorb wound exudate.

After 72 hours of incubation, the gel gradually degraded and dissolved in water and then completely degraded at 144 hours following zeroth-order kinetics.

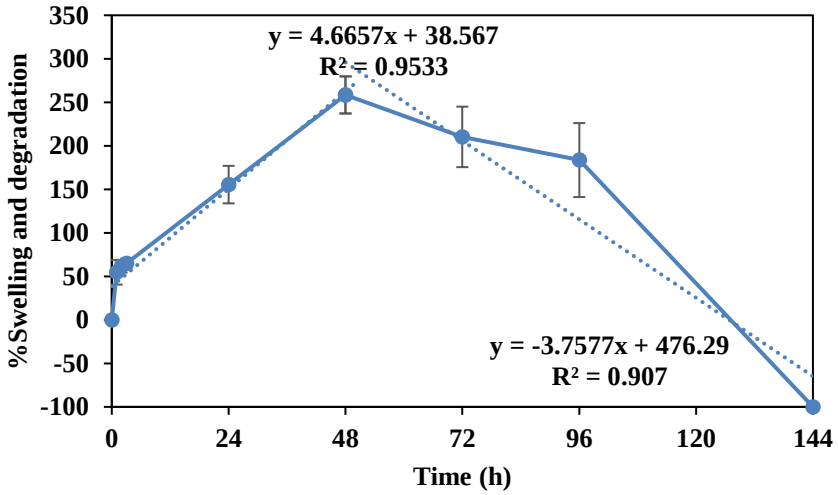

Figure 5: *Swelling and Degradation Profile Of 0.5% w/w Carbopol 940 Gel (Source: Authors' Own Illustration)*

4.3. In Vitro Release of Sitagliptin

The amount of sitagliptin released into the solution was measured to determine the release profile. The cumulative percentage drug release of sitagliptin increased with increasing concentration, as shown in Figure 6, due to a higher concentration gradient between the drug and release medium driving more drug molecules into the receiving medium. The topical gel was able to release more than 80% of the initial concentrations. Furthermore, the cumulative % drug release for all sitagliptin concentrations showed rapid release. Most of the encapsulated drug was released until 8 hours, and then the drug release clearly slowed down after 24 hr. All the drug content was not completely released because the equilibrium had probably been reached, resulting in a significantly small amount of drug release, which could be considered as no drug release. The concentration gradient of the drugs was high between 0 to 8 hours, resulting in high diffusion. The model fitting was conducted using the release data between 0 to 8 hours because the drug was mostly released at these time points, and the difference in the cumulative percentage of sitagliptin release between 8 and 24 hours was only 2 to 7% for all concentrations.

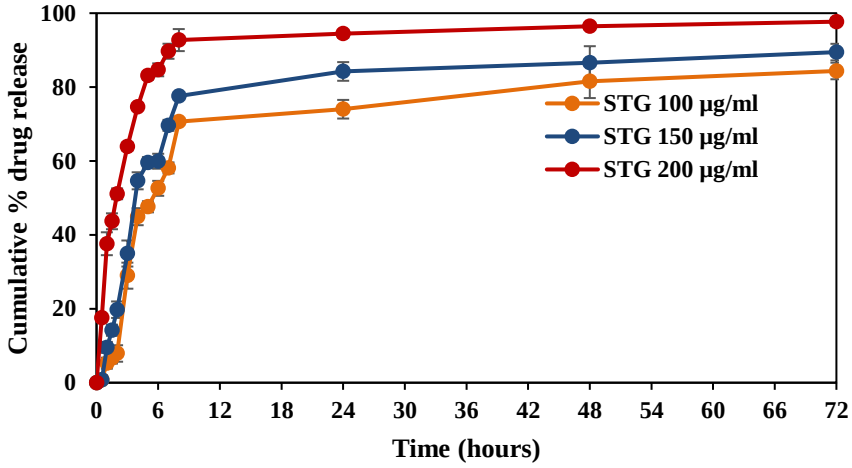

Figure 6: *Cumulative Percentage Drug Release of Sitagliptin*

(Source: Authors' Own Illustration)

Four kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas, which were used to fit the data of sitagliptin at concentrations of 100 µg/ml, 150 µg/ml, and 200 µg/ml, are shown as follows.

Table 1: *Constants and R^2 For Each Model*

Concentration of drug (µg/ml)	Zero-order		First-order		Higuchi model		Korsmeyer-Peppas model		
	K	R^2	K	R^2	K	R^2	K	n	R^2
100	9.391	0.9635	0.151	0.9647	28.061	0.8876	7.639	1.087	0.9624
150	10.315	0.9628	0.188	0.9781	31.537	0.9286	11.299	0.997	0.9169
200	10.516	0.8730	0.322	0.9948	31.732	0.9826	33.294	0.660	0.9553

(Source: Authors' Own Illustration)

According to Table 1, all concentrations of sitagliptin follow the first-order model, as indicated by the highest R^2 compared to the other models. Therefore, the rate of release depends on the concentration of the drug in the gel. The higher the drug concentration, the faster the release. Mathematical modeling help to predict the mechanism of drug release and scale up or scale down for further experiments (Shaikh et al., 2015). Thus, the release of sitagliptin from a topical gel could be predicted in the study of experimental pigs using the mathematical model obtained from this study.

4.4. Stability Study

A topical gel and drug-loaded topical gel at 150 µg/ml of sitagliptin were transferred to glass vials, in which the glass vials were closed with and without parafilm and kept at 4 °C. The samples were analyzed 1 week, 2 weeks, and 1 month after the formulation. From Figure 7, the gel shows clear, transparent, no phase change, and steady behavior, and bubbles have been decreasing at specified time intervals for both gels with and without parafilm. Figure 8b and 8c show that the weight and pH of the gel with and without parafilm are comparable, and the results do not change after 1 month, indicating that the environment of the gel is stable at 4 °C. However, the percentage of the drug content from 8a decreased with time, possibly because there was no addition of preservative. Moreover, the gel cannot be kept in a solid form, which leads to drug degradation since solid dosage forms are more stable than liquid dosage forms in the presence of water (Karki et al., 2016).

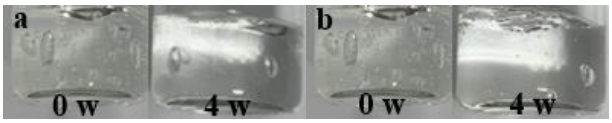

Figure 7: *Stability of The Gel Containing 150 µg/ml Sitagliptin at The Start and End of The Time Points: (A) Gel Closed with Parafilm And (B) Gel Without Parafilm*

(Source: Authors' Own Illustration)

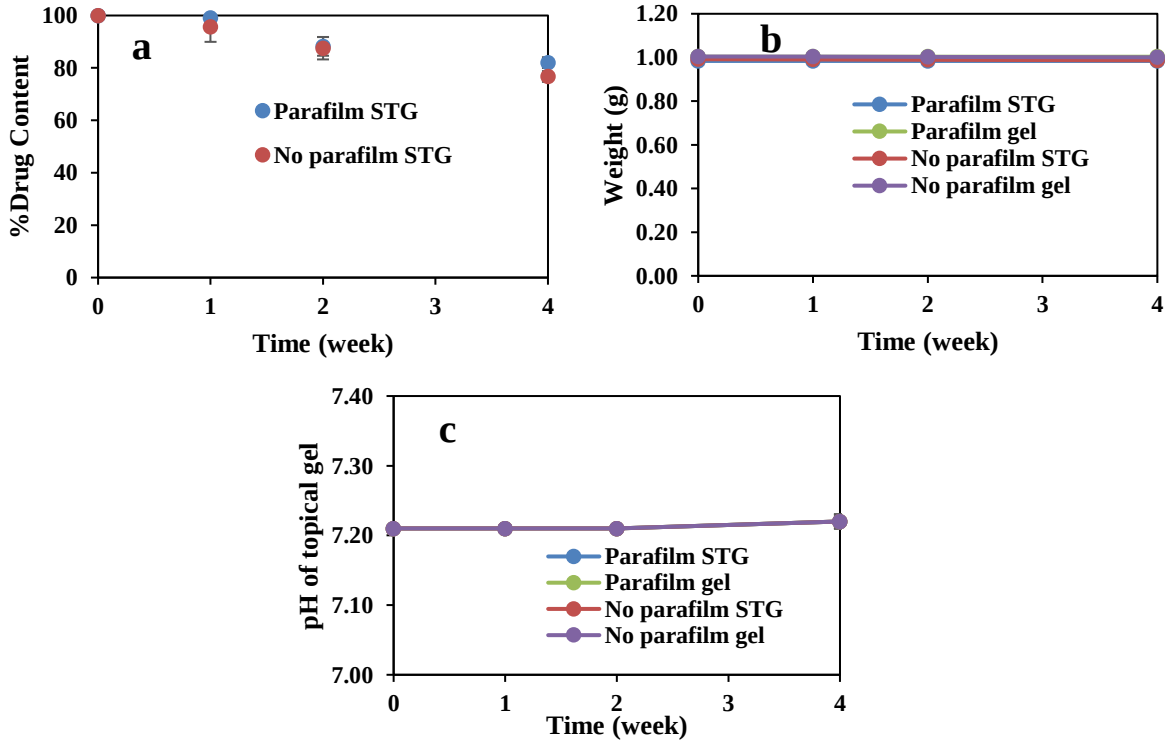

Figure 8: *Stability Test of The Topical Gel Containing 150 µg/ml: (A) % Drug Content, (B) Weight, and (C) pH*

(Source: Authors' Own Illustration)

4.5. Application

Topical gels containing 100 and 150 µg/ml sitagliptin were directly applied to subcutaneous wounds on experimental pigs every other day for 3 weeks to investigate the characteristics and effect of the gel on the skin. This study only focused on the physical properties of the gel applied to the wounds.

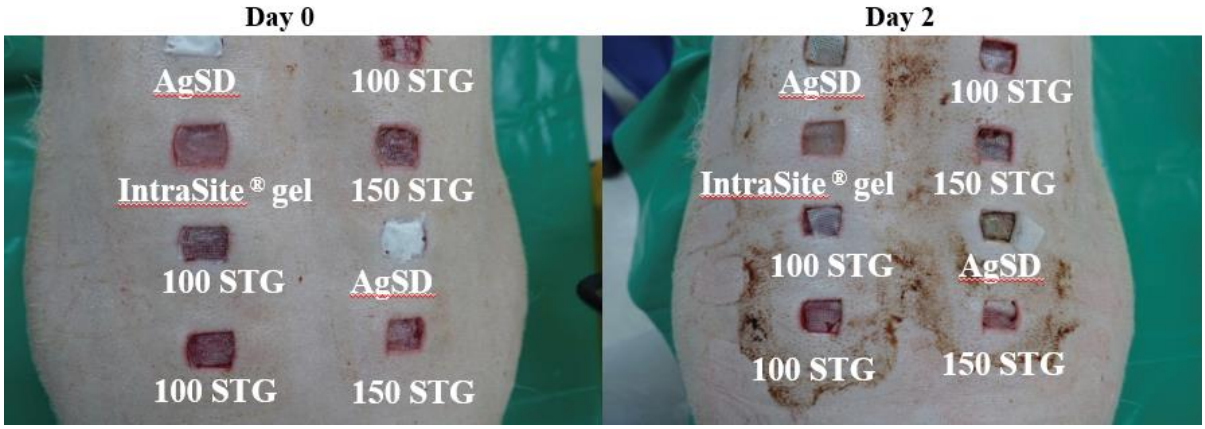

Figure 9: *Topical Gel Containing 100 And 150 µg/ml of Sitagliptin on Wounds of Experimental Pigs*

(Source: Authors' Own Illustration)

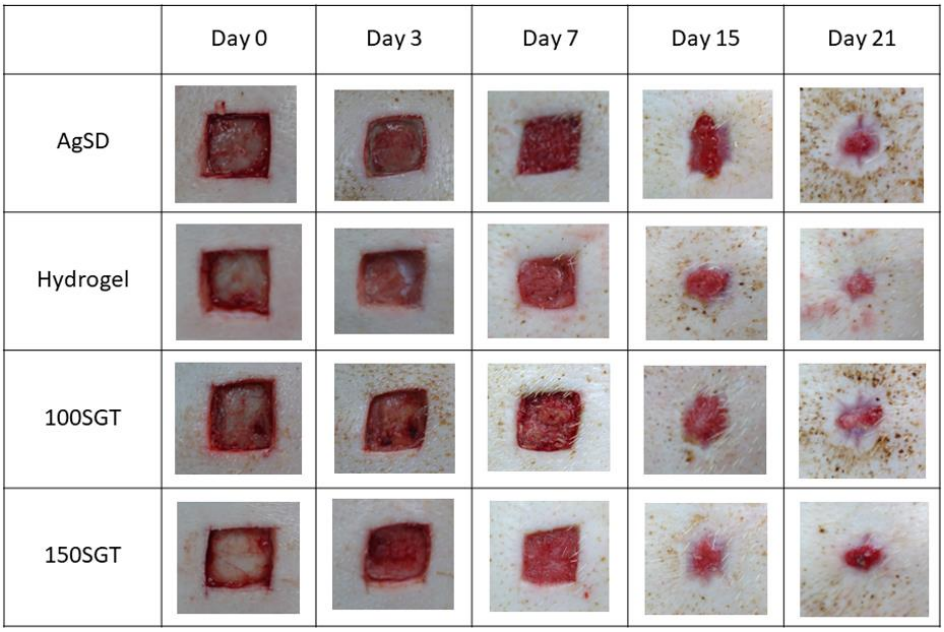

Figure 10: *Wounds of Experimental Pigs After Applying for 21 Days (Source: Authors' Own Illustration)*

From Figure 9, the topical gel containing sitagliptin has been shown to exhibit the desired spread ability and higher moisture around the wound area compared to IntraSite® gel and silver nanoparticle cream. However, the topical gel degraded after 2 days, while others still appeared on the wound. The degradation of the topical gel on an actual wound (*in vivo*) was faster than the degradation result carried out in an *in vitro* setting. The faster gets degradation rate *in vivo* might have been caused by the enzymes present in the wounds, leading to the combination of physical degradation and enzymatic degradation. In addition, there was no inflammation or infection around the wound area after applying the topical gel at both 100 and 150 µg/ml for 21 days, as shown in Figure 10. Therefore, a topical gel with sitagliptin is desirable for chronic wound healing.

5. Conclusion

In conclusion, sitagliptin, a DPP-4 inhibitor, was successfully loaded into Carbopol 940. In terms of characterizations, the topical gel was clear, transparent, uniform, and steady and exhibited shear thinning pseudoplastic behavior. In addition, the gel could absorb up to 250% of the liquid within 2 days, which is desirable for absorption of exudate. After that, the gel degraded following zeroth-order kinetics. In the *in vitro* release assay, the gels were able to release more

than 80% of the initial concentrations after 48 hours at all sitagliptin concentrations. The kinetic release of sitagliptin from Carbopol 940 was best fitted with the first order model, which was dependent on the concentration of the drugs. For the stability test, the weight and pH of the topical gel were stable at 4 °C for more than 1 month, while the drug content slightly decreased, perhaps because of drug degradation. Finally, the gel was applied directly onto subcutaneous wounds on experimental pigs. The topical gel showed the desired spread ability, compared to IntraSite® gel and the commercial silver nanoparticle cream, and degraded after 2 days. Moreover, there was no inflammation around the wound area after 3 weeks.

6. Acknowledgments

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