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An Evaluation of Collagen Peptide for Transdermal Delivery Using Strat-M® Membrane and Excised Mouse Skin

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ABSTRACT

In the fields of medicine and cosmetics, transdermal experiments commonly used to study the transdermal absorption of topical formulations. In this study, we applied Strat-M® membrane to study the in vitro transdermal properties of collagen peptides with different average molecular weights, and compared them with the transdermal absorption through excised mouse skin in vitro. The results here showed that the transdermal rate of collagen peptide through Strat-M® and excised mouse skin increased linearly with time, and the different concentrations of collagen peptide solutions affected the final cumulative transmission. The cumulative transmission per unit area and transdermal rate of CP500 were both the highest among the four CPs. The cumulative transmission per unit area of collagen peptides through Strat-M® has a high correlation with that through excised mouse skin, R2 > 0.98. Strat-M® can replace the excised mouse skin in vitro to carry out the transdermal experiments of collagen peptides. Our results can be used to guide formulators in the selection of vehicles for early development in the pharmaceutical, personal care and cosmetic industries.

INTRODUCTION

Collagen peptide (CP) is the hydrolysis product of collagen, one of the main structural proteins of different connective tissues, such as skin, bone, cartilage and tendons, in mammals (Khatri et al. 2021). CPs play a critical role in the fields of food, medicine and cosmetics due to their natural, safe, and biocompatible characteristics (Song and Li 2018). In recent years, an increasing number of studies have shown that CPs positively impact both the health and the appearance of skin and promote wound healing and hair growth (Hwang et al. 2018; Zhao et al. 2022). Besides oral, topically applied to skin is another route of administration. However, unlike after oral administration, topically applied CPs must pass through the skin, the natural protective barrier of the human body. The penetration of a CP is influenced by factors such as its physical properties, including lipophilicity, solubility and molar mass (Arce et al. 2020; Klebeko et al. 2021). To date, there are few reports on the skin permeability and mechanism of action of topical CPs of different molecular weights.

In the fields of medicine and cosmetics, the parallel artificial membrane permeability assay (PAMPA) is often used for screening active components and studying the penetration of topical preparations (Sinkó et al. 2021). Skin-PAMPA can be a good alternative to skin permeability studies, but membrane selection is imperative because it affects the overall permeation parameters. In vitro permeation experiments using excised human and animal skins are very useful for understanding the skin permeation profiles and skin concentrations of topically applied chemicals, but ethical and economic reasons pose major problems with the availability and use of human skin. In vitro methods that do not use animal tissues have gained increasing attention for evaluating the safety and efficacy of cosmetic ingredients (Kaur et al. 2018). Therefore, skin membrane replacements that use artificial membranes (e.g., Strat-M®, a silicone membrane) designed to mimic human and animal skin offer a competent alternative to estimate the permeation of drugs through the skin (Todo 2017).

In this study, our aims were to investigate the permeability of different molecular weight CPs. Therefore, CPs with different average molecular weights were selected as the experimental subjects. Strat-M® and mouse abdominal skin were used to study CP release and permeation via the skin-PAMPA method. The usefulness and membrane permeation characteristics of Strat-M® were investigated by comparing these parameters with those determined with mouse abdominal skin.

MATERIALS AND METHODS

Materials

Four commercial CPs (m.w.500, m.w.1000, m.w.2000, m.w.3000) were supplied by Beijing SEMNL

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Biotechnology Co., Ltd (Beijing, China). Hydroxyproline (Hyp) and 2,4-dinitrophenylhydrazine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from J.T. Baker (Deventer, Netherlands). All other chemicals and reagents used were analytical pure.

Mice skin preparation

Mice weighted 20-25 g, were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). The mice were shaved on the abdomen and fed overnight to promote the absorption and metabolism of the depilatory creams. Then the mice were killed by detaching their cervical vertebrae. And the abdominal skin of 2.5 cm × 2.5 cm was removed, the subcutaneous adipose tissue and connective tissue were scraped off. At last, the above skins were washed with normal saline and soaked for 30 min.

Determination of molecular weight distribution

According to the method described by Yang et al. (2011), the molecular weight distribution of CPs was determined using an Agilent 1260 high performance liquid chromatography (HPLC) system (Agilent Technologies). The CPs were loaded onto TSK gel G2000 SWXL column (300 × 7.8 mm, Tosoh, Tokyo, Japan) and eluted with 45% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.5 mL/min. The elution was monitored by recording the absorbance at 220 nm. A molecular weight calibration curve was prepared from the average elution volume of the following standards: Cytochrome C (12384 Da), aprotinin (6512 Da), zinc bacitracin (1423 Da), Gly-Gly-Tyr-Arg (451 Da) and Gly-Gly-Gly (189 Da) (Sigma Co., USA).

Determination of hydroxyproline (Hyp) content

According to the method described by Wang et al. (2022), CPs (10 - 20 mg) was mixed with 10 mL HCl (6 mol/L) and hydrolyzed at 110 °C for 22 h. Then the samples were cooled, dried and redissolved with deionized water. The Hyp content of each sample was analyzed by an Amino Acid Analyzer (S-433D, Sykam, Germany).

Transdermal test

The artificial membrane Strat-M® membrane (Merck Millipore, Tullagreen, Carrigtwohill, Ireland) was used directly, and the soaked mouse skin was wrapped in filter paper. The in vitro permeation studies were carried out in a Franz Cell system (Jingtuoyq, Tianjin, China) with a diffusion area of 1.54 cm2 and capacity of 17 mL for the receptor medium, physical saline solution 0.9% (w/v). The Franz Cell system was maintained at a constant temperature of 37 ± 0.5 °C, while the receptor medium was stirred constantly at 300 rpm during the experiment.

For each CP permeating through Strat-M® or the mouse skin, an assay was performed with six diffusion cells. Each membrane was carefully fixed between the sample cell and the receiving cell, with 1 mL of the normal saline solution with 10% (w/v) CPs placed in the sample pool, and saline solution 0.9% (w/v) was used as blank control.

Aliquots of 1 mL were taken from the receptor pool at 2 h, 4 h, 6 h, 8 h and 24 h respectively, and refilled the pool with 1 mL fresh saline. The content of Hyp in each aliquot was determined as follows.

The cumulative amount of the CPs permeated through the membrane (Qn) and the transdermal rate constant per unit area (J) were calculated according to the following formulas:

$$Q_n = \frac{C_n \times 10 \times V + \sum_{i=1}^{n-1} C_i \times 10 \times V_0}{A} \quad (1)$$

Where, Cn is the Hyp concentration measured at the n-th sampling point, in μg/mL; Ci is the Hyp concentration measured at the i-th sampling point, in μg/mL; 10 is the conversion coefficient between Hyp content and CPs content; V is the capacity of the receiving cell, which is 17 mL; V0 is the aliquot volume at each sampling point, which is 1 mL; A is the permeation area, which is 1.54 cm2.

$$J = \frac{dQ}{dt} \quad (2)$$

The slope of the equation is the constant of transdermal rate per unit area (J, μg/cm2/h).

RESULTS

CP molecular weight distributions

The molecular weight distributions of the four CPs were determined, and the results are shown in Table 1.

The average molecular weights of CP500, CP1000, CP2000 and CP3000 were 610.12±4.88 Da, 1078.31±3.73 Da, 2105.58±80.21 Da and 3553.76±26.73 Da, respectively.

CP500 had the highest percentage (54.71%) of the < 500 Da fraction. CP1000 had the highest percentage (34.01%) of the 500-1000 Da fraction. CP2000 had the highest percentage (24.33%) of the 1000-2000 Da fraction. CP3000 had a greater percentage of >5000 Da, 3000-5000 and 2000-3000 fractions than CP2000, CP1000 and CP500 did and lower percentages of the < 500 Da and 500-1000 Da fractions.

Table 1: Molecular weight distribution of CPs.

Samples	<500 Da	500-1000 Da	1000-2000 Da	2000-3000 Da	3000-5000 Da	>5000 Da	Average
CP500	54.71±0.21	30.47±0.63	11.5±0.37	2.26±0.12	0.87±0.09	0.00	610.12±4.88
CP1000	28.67±0.04	34.01±0.72	23.83±0.32	7.56±0.10	4.84±0.15	0.00	1078.31±3.73
CP2000	17.17±1.00	22.39±0.79	24.33±0.64	13.56±0.36	13.57±0.55	1.33±0.16	2105.58±80.21
CP3000	6.79±0.08	13.52±0.18	20.32±0.45	14.7±0.17	18.76±0.14	4.6±0.04	3553.76±26.73

Table 2: The cumulative transmission per unit area of the CPs of different concentrations

Samples	C (%)	Q_n (μg/cm2)		
		4 h	8 h	24 h
CP500	1	1688	2889	4353
	5	2896	4782	6240
	10	3469	5575	7504
CP1000	1	829	1601	2468
	5	1308	2406	3966
	10	1523	2659	4454
CP2000	1	468	839	1758
	5	758	1259	2572
	10	921	1497	3079
CP3000	1	208	766	1205
	5	267	783	1302
	10	292	863	1518

In vitro permeation of CPs with different molecular weight distributions using Strat-M®

To study the effects of different concentrations of CPs on the Strat-M® penetration rate, CPs with different concentrations (1%, 5% and 10%) were prepared for transdermal experiments. The results are shown in Table 2.

As shown in Table 2, the cumulative transmissions per unit area of the four CPs increased with increasing concentration. Moreover, the cumulative transmission per unit area of each concentration of the four CPs increased over time. CP500 had the highest cumulative transmission per unit area among the four CPs, while CP3000 had the lowest.

In vitro CP transdermal rate using Strat-M®

To compare the transdermal rates of the four CPs, CP solutions prepared with 10% normal saline were applied to Strat-M®. The cumulative transmission per unit area data is shown in Fig. 1.

As shown in Fig. 1, the cumulative transmission per unit area of the four CPs increased with time, and the transdermal rates of the CPs varied greatly according to the average molecular weight. The transdermal rate of CP500 was the highest among the four CPs, and its cumulative transmission per unit area reached 7504 ± 1584 μg/cm2 at 24 h. The transdermal rate of CP3000 was the lowest.

Ex vivo transdermal rates of the CPs using excised mouse skin

To compare the transdermal rates of the four CPs, 10% normal saline solutions of the CPs were prepared to assess their penetration through mouse abdominal skin. The cumulative transmission per unit area data is shown

in Fig. 2.

When using excised mouse skin, the cumulative transmission per unit area of the four CPs increased over time. Among them, the transmission rate of CP500 was the highest, and its cumulative penetration per unit area (Qn) was 3773 ± 275 μg/cm2 at 8 h. Moreover, the Qn of CP3000 was the lowest, at 693 ± 132 μg/cm2 at 8 h. Thus, the same trend was observed when using the Strat-M® membrane and excised mouse abdominal skin for the CPs of different molecular weights.

Figure 1: The cumulative transmissions of the CPs through Strat-M®.

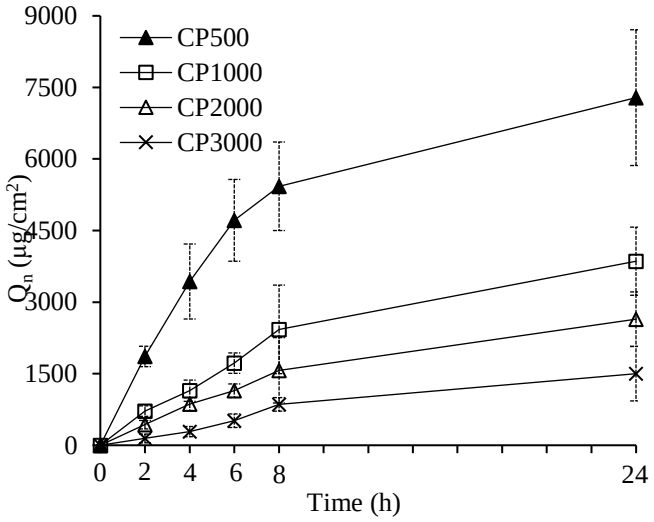
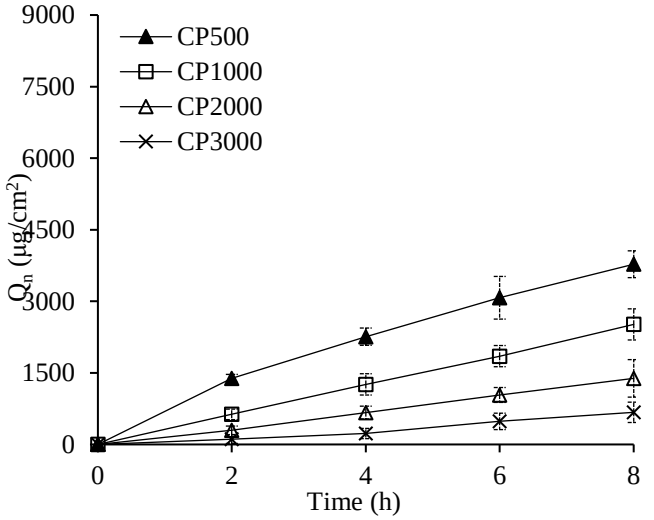


Figure 2: The cumulative transmission of the CPs through excised mice skin



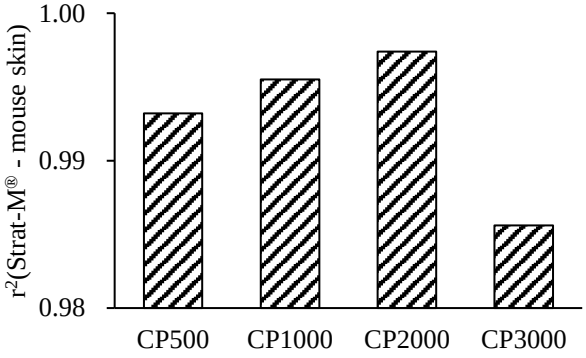
Comparison of the transdermal properties of CPs using Strat-M® and excised mouse skin

The kinetic parameters of CP penetration through Strat-M® and excised mouse skin are shown in Table 3 and Table 4.

By comparing the data in Table 3 and Table 4, the kinetic parameters of CP penetration through Strat-M® and excised mouse skin increased linearly with transdermal time and followed zero-order kinetics. The transdermal absorption coefficient (J), or the transdermal rate constant per unit area, through both membranes, followed the same order of J (CP500) > J (CP1000) > J (CP2000) > J (CP3000).

A comparison of the cumulative amount of CP penetrated per unit area through Strat-M® and excised mouse skin are shown in Fig. 3. All of the correlation coefficients (r2) were greater than 0.98, indicating that the two models have a high correlation with the percutaneous permeability of the collagen peptides. Strat-M® can thus be used as a substitute for excised mouse skin in transdermal experiments.

Figure 3: Correlation between penetration of CPs through Strat-M® and excised mouse skin



DISCUSSION

In vitro permeation studies are meant to evaluate the transdermal diffusion of compounds across the skin layers. Strat-M®, which was specifically designed for skin penetration studies, consists of two layers of polyethersulfone in the outer layer and a more diffusive polyolefin layer in the bottom layer (Arce et al. 2020; Klebeko et al. 2021). In addition, Strat-M® contains simulated skin lipids similar to those found in the human stratum corneum (Mijaljica et al. 2024; Uchida et al. 2015). Strat-M® can be applied as a simple, inexpensive, and stable membrane for diffusion studies of active ingredients, excipients, and finished products for both topical and transdermal formulations. The advantages of this technique include ease of use, no pretreatment requirements, consistency between lots, and no need for special storage (Simon et al. 2016). In this experiment, the cumulative transmission per unit area of the CPs with average molecular weights of 500 Da, 1000 Da, 2000 Da and 3000 Da increased with time, following zero-order kinetics. The CPs with different average molecular weights penetrated through Strat-M® at different rates. These results were consistent with those obtained using

Table 3: The kinetic parameters of the CPs penetrating through Strat-M®

Samples	Q-t (Strat-M®)	r^2 (M)	J (Strat-M®)/(μg/cm²/h)
CP500	Q = 706.0 t + 333.6	0.9689	706
CP1000	Q = 308.0 t – 18.0	0.994	308
CP2000	Q = 204.2 t + 2.4	0.9984	204.2
CP3000	Q = 104.1 t – 55.2	0.9353	104.1

Table 4: The kinetic parameters of the CPs penetrating through excised mouse skin

Samples	Q-t (mouse)	r^2 (mouse)	J (mouse) / (μg/cm²/h)
CP500	Q = 461.9 t + 244.6	0.976	461.9
CP1000	Q = 252.4 t – 13.2	0.9997	252.4
CP2000	Q= 173.1 t - 20.8	0.9986	173.1
CP3000	Q= 87.65 t - 50.8	0.9534	87.65

excised mouse skin in vitro. This study showed that the use of Strat-M® is convenient for subsequent optimization of the receiving medium, compatible materials and dosage form for the preparation of external CPs.

The permeability of CPs plays a very important role in their efficacy and activity when they are topically applied to skin. Therefore, selecting a CP with an appropriate molecular weight may be the key to increasing its penetration and achieving rapid therapeutic effects in tissues under the skin. The skin stratum corneum acts as a barrier through which substances with lower molecular weights are more easily penetrate, and substances with higher molecular weights are more difficult to absorb (Chai et al. 2010; Ossowicz-Rupniewska et al. 2021; Prausnitz and Linger 2008). Our study data showed that the cumulative transmission per unit area of the CP decreased as its molecular weight increased. Strat-M® could differentiate the transdermal rates of these CPs with different molecular weights, possibly because of its porous and multilayer structure with different diffusivities (Neupane et al. 2020). There was a linear relationship between the cumulative amount of CP that penetrated through Strat-M® and the transdermal time, which was significantly different from that reported by Liu (2014), who concluded that the CP transdermal penetration rate within 2 hours was much greater than that after 2 hours. This may be due to the narrow molecular weight distribution of CPs used in the previous study and the wide molecular weight distribution of CPs in this experiment. Thus, the previous results could be regarded as the weighted average rate of the CPs used before, and CPs with different molecular weights penetrated at various speeds. Chai et al. (2010) also reported that the transdermal rates of polypeptides with different molecular weights fractionated by employing 5 kDa, 3 kDa, 1 kDa and 0.5 kDa membranes were significantly greater than that of the mixed tilapia skin collagen peptide before fractionation.

The results here suggested that CP skin permeation may be predicted according to their permeability coefficients determined with Strat-M®. Our results can be used to guide formulators in the selection of vehicles for early development in the pharmaceutical, personal care and cosmetic industries. In vitro permeation studies provide significant insight into the behaviors of formulations in vivo.

CONCLUSION

In this study, we applied Strat-M® membrane to study the in vitro transdermal properties of CPs with different average molecular weights, and compared them with the transdermal absorption through excised mouse skin in vitro. The results showed that the transdermal rate of CPs through Strat-M® and excised mouse skin increased linearly with time, and the different concentrations of CPs solutions affected the final cumulative transmission. The cumulative transmission per unit area of CPs through Strat-M® has a high correlation with that through excised mouse skin, R2 > 0.98. Strat-M® can thus replace the excised mouse skin in vitro to carry out the transdermal experiments of CPs.

DECLARATIONS

Competing Interests

The authors have no competing interest to declare.

Informed Consent

This manuscript does not contain any studies with human subjects performed by any of the authors.

Research Involving Human and Animal Rights

All experiments executed according to the Guide for the Care and Use of Laboratory Animals and approved by the institutional animal ethics committee.

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