

Microneedling-enhanced dermal delivery of catechin and curcuminoid polyphenols from gel, patch and ointment formulations

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ABSTRACT

Polyphenols exhibit a wide range of biological and pharmacological activities, yet their dermal delivery is often limited by skin permeability and formulation-dependent constraints. In this work, the potential of roller microneedling as a physical strategy to improve dermal delivery of representative polyphenols—epicatechin, epigallocatechin gallate (EGCG), and curcumin—was investigated using common topical dosage forms, namely polymeric gel, patch, and ointment. Standardized skin permeation parameters were first obtained using excised porcine skin under conditions of stratum corneum saturation. The microneedling procedure was characterized, and its effect on polyphenol transdermal permeation from each formulation type was evaluated using Franz diffusion cells. Maximum steady-state permeation fluxes varied from 13.2 $\mu\text{g}/\text{cm}^2/\text{h}$ (curcumin) to 708 $\mu\text{g}/\text{cm}^2/\text{h}$ (epicatechin). Roller microneedling produced well-defined microchannels across the stratum corneum, with a mean interchannel distance of 2.3 mm, without compromising skin integrity, and significantly enhanced polyphenol permeation from all tested vehicles. Depending on the compound and formulation, microneedling increased permeation fluxes and/or reduced lag times, with particularly relevant effects observed for gel-delivered curcumin and ointment-delivered EGCG. Moreover, the polyphenols exhibited a robust antioxidant activity (equivalent to micromolar ascorbic acid) after transdermal permeation, including following microneedle-assisted delivery, supporting the compatibility of microneedling and the formulation vehicles with polyphenol functionality. Overall enhancement factors were consistent, yielding approximately twofold increases in both permeation flux and antioxidant activity. These findings demonstrate that roller microneedling is a practical, low-invasive approach to enhance the dermal delivery of polyphenols from conventional topical formulations, offering a versatile strategy that complements formulation-based optimization for therapeutic and cosmetic applications.

1. Introduction

There is great interest in the delivery of polyphenols by alternative administration routes. Polyphenols like flavonoids, curcumin and resveratrol exhibit a diverse array of biological activities and important pharmacological effects, evidenced by studies conducted in both humans and animal models, thereby presenting significant potential for the prevention and treatment of various diseases and health disorders [1–7]. However, the therapeutic efficacy of orally administered polyphenols seems limited by their low solubility, poor absorption, and metabolism in the gut and liver [1,8,9].

Administration via the skin offers distinct advantages for polyphenol-based therapies, enabling localized delivery, avoidance of first-pass metabolism, controlled delivery to the systemic circulation, and user-friendly application [10–13]. Nevertheless, the effectiveness of dermal administration of polyphenols may be constrained by insufficient skin permeability and chemical stability [1,8,14]. A previous study compared the dermal absorption of epicatechin, resveratrol, quercetin and rutin using excised porcine skin [15]. The polyphenols with intermediate polarity ($\log K_{o/w}$) and molecular weight, specifically epicatechin and quercetin, penetrated the stratum corneum and reached the epidermis, with minimal accumulation in the

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dermis, while the larger and more hydrophilic compound (rutin) was practically not absorbed by the skin. Although the polyphenols were applied on the skin with ethanol (a permeation enhancer), none was found to cross the skin to the receptor fluid in significant quantities [15]. Transdermal permeation of epicatechin was also not detected in a different work using a formulation based on polyacrylic acid [14]. More recently, Nair et al. measured the skin permeability of curcumin and reported transdermal fluxes below $1 \mu\text{g cm}^{-2} \text{h}^{-1}$, even when encapsulated in nanoethosomes [16,17]. Indeed, the necessity of appropriate formulations for the effective dermal administration of polyphenols has been previously pointed [1]. In this line, liposome-derived systems, emulsions, micelles, gels, and lipid nanoparticles, have proven to promote the skin permeation of varied polyphenols [8,16,18–21], but the dermal clinical applications of polyphenols are still scarce. For example, different nano-carriers ensured prolonged and faster flux of curcumin or epigallocatechin-3-gallate (EGCG) through skin, yet not reaching the levels of clinically used anti-inflammatory drugs [8,20,22].

It is important to note that some studies of the dermal permeation of polyphenols do not clearly specify the dose applied on the skin, or utilize doses that may be considered relatively low (e.g. Refs. [15,20]), probably insufficient to saturate the stratum corneum and achieve conditions for maximal penetration of the compounds. In this context, the research literature reveals a gap in providing standardized skin permeation parameters for polyphenols, hindering broader comparisons and the optimization of delivery approaches.

The stratum corneum represents the principal barrier to dermal absorption, restricting the penetration of compounds having high polarity and large molecular size [12,23]. The methods to increase transport across the stratum corneum can be grouped as physical (e.g., microneedling), chemical (solvents and other chemical enhancers), formulation-based carriers (e.g. nanoparticles), and hydration/occlusion methods [23,24]. Microneedling is a technique that, by piercing the skin with micro-sized needles, creates transient, water-filled pathways (microchannels) that disrupt the stratum corneum's lipid "mortar", thereby shortening the otherwise tortuous intercellular path while largely sparing the deeper and innervated dermis [23,25,26]. The technique has been well-tolerated in ex vivo and human studies, provided that insertion forces are controlled and materials meet biocompatibility standards [25,26]. Moreover, microneedle roller devices are commercially available and can potentially be combined with the use of diverse formulations and carrier systems to further enhance the transdermal delivery of therapeutic agents [27–29]. Beyond improving flux, this strategy is attractive because it is fast, scalable to larger surface areas, and can be integrated with conventional topical dosage formulations (gels and others to be assessed) without requiring complex applicators or external power sources. From a regulatory perspective, microneedle roller systems are already covered under existing medical-device frameworks, although their classification and requirements vary with the intended use, as detailed in Note S1 of Supplementary Information (SI).

Despite its versatility, roller-based microneedling has mainly been evaluated with standard drugs and macromolecules, and only a few works with polyphenols are published and still lacking a characterization of the microneedling process and the effect on the kinetics of transdermal permeation [30,31]. Furthermore, the preservation of the antioxidant functionalities of the delivered polyphenols remains to be verified. The objective of the present work was to evaluate the effectiveness of skin microneedling to improve the transdermal administration of antioxidant polyphenols from common dosage forms – gel, patch and ointment. In addition, we aimed to contribute standardized parameters for skin permeation of representative polyphenols and characterize the effects of roller microneedling the skin, thereby strengthening the foundations for more informed experimental and modelling approaches in future studies.

2. Methods

2.1. Materials

Epicatechin (purity $\geq 99\%$), EGCG ($\geq 95\%$), alginate, Pluronic F-127, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and equine heart cytochrome c were purchased from Sigma-Aldrich. Curcumin was obtained from Alfa Aesar (95% total curcuminoid content, from Turmeric rhizome), as well as the diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98%), while caffeine was from Fluka Chemical Co. ($\geq 99\%$). The catechin ointment was based on the standardized extract of green tea (*Camellia sinensis*) leaf that contains approximately 95% polyphenols, 80% catechins including EGCG [32,33]. The ointment formulation contains white petroleum and isopropyl myristate.

Acetonitrile was obtained from Chem-Lab/Honeywell, while ethanol absolute was from Carlo Erba. Sodium azide and methylene blue were from Riedel-de Haen, while sodium chloride and Folin-Ciocalteu reagent were from Biochem Chemopharma. Formaldehyde solution (37% w/w, with 10% methanol) was from Bresfor, Portugal. All other chemicals used were HPLC or analytical-grade reagents.

The cellulose nitrate membrane (0.45 μm porosity) was obtained from SARTORIUS, and the microneedle roller was from NiceStuff (model MRKEAN-37954715320488, needle length 500 μm).

2.2. Skin and microneedling method

Porcine skin was obtained from pig ears purchased from a local butcher 6–24 h after being slaughtered. The skin samples were dermatomed from the external surface of the ears, using a manual dermatome (from Swann-Morton, UK) with the cutting thickness adjusted before starting. The thickness of the excised skin was recorded with a micrometer and only samples with a final thickness of 500–650 μm were used in this work. The skin was frozen (-20°C) wrapped in aluminum foil until utilization on the dermal permeation assays (no longer than 2 months). On the day of assays, the excised skin samples were thawed at room temperature.

For microneedling, the skin samples were placed on a digital scale, and the roller was applied by manually rolling it six times each in four different directions (vertical, horizontal, and both diagonals). During the procedure, the applied load was controlled by monitoring the mass displayed on the scale, with the aim of maintaining a force close to 20 N. The repeatability of the applied load has been estimated from video recordings of the scale display during rolling procedures. Four videos with independent skin samples were examined, and five frames were extracted from each considering only periods in which the roller was in contact with the skin. The displayed mass was then converted to force using $F = m \times 9.81$, resulting in an applied force of $17.7 \pm 0.4 \text{ N}$, a load above the 5–10 N minimum reported to enable roller microneedles' insertion in skin [28,34].

To confirm that the skin barrier remained intact after microneedle application, each sample was mounted onto a Franz cell with the receptor partially filled with 3 mL of solution. The cells were then positioned horizontally to ensure that the solution remained in direct contact with the dermal side of the skin, allowing any leakage through potential perforations to be detected.

2.3. Characterization of the microneedling

The microneedling perforation was studied using an insertion test and skin staining. Parafilm® was used for the insertion test following the method described in Ref. [35]. Briefly, a sheet of Parafilm® was folded until obtaining a 1 mm thickness and the manual rolling was done unidirectionally in a single pass. Following this, puncture marks were visualized in each Parafilm® layer corresponding to the targeted depths (0, 130, 260, 390 μm). Puncture diameters were quantified in ImageJ. For each puncture, the equivalent circular diameter was calculated from

the measured puncture area, enabling consistent comparison even when punctures were slightly elliptical. For each depth, puncture diameter is reported as mean $\pm$ SE from 4 punctures across 3 samples.

For skin staining, microneedle-treated skin was immersed in 0.1% methylene blue for 5 min, the excess was washed with distilled water, and the tissue was fixed at 4 °C for 24 h in a 10% (v/v) dilution of the formaldehyde stock reagent in water. After washing with distilled water, the skin piece was cut into transverse sections and observed with an optical microscope. To characterize puncture geometry and spatial distribution, puncture diameters and nearest-neighbor distances were quantified in ImageJ. Punctures were identified by a 10% contrast enhancement and a total of 30 were measured. Nearest-neighbor puncture distance was determined by extracting the centroid of each puncture and computing, for each distance, the Euclidean distance to the closest neighboring centroid. The set of nearest-neighbor distances was then exported for statistical analysis.

The distributions of puncture diameter and nearest-neighbor distance were plotted using OriginPro as histograms overlaid with a normal distribution curve. Bin widths were set to 200 μ m for puncture diameter and 500 μ m for nearest-neighboring distances. Mean and median values are reported to summarize central tendency in the presence of skewed distributions.

2.4. Percutaneous permeation assays

The skin permeation assays were carried out following the OECD guidelines [36], and using Franz-type diffusion cells with a 5-mL receptor compartment and a diffusion area of 0.64 cm² (obtained from PermeGear, Germany). The porcine skin was mounted between the donor and receptor compartments of the Franz cells, with the epidermis facing the donor, and the receptor was filled with a suitable fluid [37]. The skin was conditioned for 30 min with the receptor fluid, which was replaced by a fresh volume before the test compounds were applied in the donor compartment. All assays occurred at 32 °C with a magnetic agitator maintained in the receptor fluid at 600 rpm. The integrity of the skin in each batch was controlled using caffeine as reference compound [36], and skin batches presenting a permeability coefficient 100x higher or lower than the accepted value [10,37], were not used.

Given the distinct solubility of the tested compounds, different receptor media were used to ensure that partition to the receptor fluid does not act as a rate-limiting step in the permeation process [36]. The studies with catechins were carried out with a saline (sodium chloride 0.9% w/v) solution in the receptor compartment of the Franz cells. For curcumin, 30% (v/v) ethanol in saline was used, a concentration that guaranteed sufficient solubility of the permeant in the receptor and avoiding the high percentage ethanolic solutions that can interfere with the diffusivity of hydrophobic compounds [37].

The permeation of epicatechin, EGCG and curcumin in conditions of stratum corneum saturation was followed after application of the compounds as acetone solutions in the donor compartment. A mass of 8 mg of epicatechin, 49 mg of EGCG or 0.8 mg of curcumin was applied dissolved in 50 μ L acetone.

The polyphenol-loaded gels, prepared as described in section 2.5, were studied with the application of 500 μ L of the epicatechin-loaded gel or 142 μ L of the curcumin gel in the donor. The placement of the epicatechin-loaded patch (area 143 mm²) necessitated a different procedure. After being carefully positioned on the epidermal side of the skin, the patch was secured with two strips of medical-grade stretching tape wrapped around the upper part of the Franz cell receptor. This prevented sliding caused by surface humidity and, then, clamping the donor compartment in place on top of the receptor ensured stable contact throughout the permeation assay. In the case of the ointment, a mass of 10 mg was spread on the surface of the skin (positioned in the Franz cell) with the aid of a small spatula to ensure all the visible surface was evenly covered.

Throughout the permeation assays, samples (200 μ L) were collected

from the receptor compartment at defined time points for drug's quantification as described in 2.7, and the sampled volume was immediately replaced by fresh receptor media. In general, samples were collected at intervals of 0.5 or 1 h, for up to 8 h, but the sampling frequency and assay duration were adjusted to the specific study as can be read from the x-axis of the kinetic data plots. The permeation fluxes were calculated from a minimum of 5 time points and a 2-h period during the linear phase of permeation, and the lag time from the interception of the linear phase with the time axis. Skin permeation blank assays were also run with plain skin, an empty donor and receptor fluid equal to the used in the catechins and curcumin permeation assays. The receptor fluid was collected at the end of 8 h and these skin blank samples were used as controls in the antioxidant capacity assays (section 2.8).

2.5. Preparation of polyphenol-loaded gel and patch

The Pluronic F-127 gel was prepared in water at 20% (w/v) concentration, using the cold method [38,39]. For the loading with epicatechin and curcumin, the polyphenols were included in the dissolution step of the polymer at 4 °C. Epicatechin was incorporated during a 24-h dissolution period, while curcumin was incorporated over 48 h. Epicatechin was encapsulated at 0.09% (w/w) and curcumin at 1% (w/w) in the gel having in account the total mass of the formulation.

The alginate patches were prepared by casting followed by gelation [40]. Initially, a solution of sodium alginate at 2% (w/v) was prepared in water combined with glycerol 30% (w/w of alginate), placed in round molds and dried in an oven at 40 °C for 6 days. Just before the drug delivery assays, epicatechin was encapsulated within the alginate matrix during gelation with 200 mM calcium chloride. Epicatechin (8.42 μ mol in 168.4 μ L) was added to the calcium solution, bringing the total volume to 1 mL of solution used to gelate each alginate patch with surface area of 143 mm² overnight at 4 °C. Before skin application, the films were rapidly washed in saline solution to remove surface epicatechin.

2.6. Drug release assays

The release of epicatechin and curcumin by the loaded gels and patches was studied using static diffusion cells, in similarity to the skin permeation assays described in section 2.4. The experimental conditions were the same, including the employed Franz cells, the receptor fluids, the volume of the gels and patch application in the donor. Instead of skin, the release assays were carried with a cellulose nitrate membrane (0.45 μ m porosity) between the donor and receptor compartments. The membrane barrier was conditioned for, at least, 30 min with the receptor fluid before applying the tested delivery systems. Control experiments were also conducted using the polyphenols simply dispersed in water at the same doses applied in the donor compartment of the diffusion cells but not encapsulated in any gel or patch carrier. Identically to the permeation assays, the quantification of the compounds in the receptor's samples was performed by HPLC as described in section 2.7.

The kinetics of drug release was fitted to a first-order model (Eq. (1)):

$$m = m_{\infty} (1 - e^{-kt}) \quad (\text{Eq. 1})$$

Where m is the amount released at time t , m_{∞} is the total releasable amount, and k is the rate constant (h⁻¹). All the drug release data in this work fitted this model quite well, with adjusted correlation coefficients (R_{adj}^2) above 0.97.

2.7. Analysis of the polyphenols

The polyphenols in the receptor samples from the permeation and release assays were analyzed by HPLC or by the Folin-Ciocalteu method. Epicatechin, EGCG and curcumin were quantified by reverse-phase HPLC, using an Agilent 1100 system equipped with a C18 column and UV detection at 268 nm for the catechins and 436 nm for curcumin. The

sample injection volume was 20 μL and the flux of the mobile phase 0.8 mL/min. The mobile phase consisted of acetonitrile:water:triethylammonium phosphate (pH 4.3) in the proportion 16:83:1 for the catechins, and 60:39:1 for curcumin. Representative chromatograms are displayed in Figs. S1 and S2 of SI. The calibration curves of each drug were obtained with standards in the corresponding receptor fluids used in the permeation and release assays. The chromatogram of a curcumin standard in Fig. S2 illustrates the detection of the 3 main curcuminoids typically present in turmeric extracts and integrated for curcumin quantification in the samples.

In addition to HPLC for identification of epicatechin and EGCG, the receptor fluids from the permeation of the catechin ointment were analyzed by the Folin-Ciocalteu method [41]. Briefly, 100 μL of sample was added to 2 mL of Na_2CO_3 (2% w/v), homogenized and, after resting for 2 min, 100 μL of the Folin-Ciocalteu reagent (50% v/v) was added, homogenized and the absorbance at 720 nm measured once incubated for 30 min at room temperature in the dark. The absorbance of blank controls from skin permeation assays (no compounds in the donor) was subtracted from the samples' absorbance and polyphenols were quantified as epicatechin equivalents using calibration curves of epicatechin.

2.8. Assays of antioxidant capacity

The antioxidant capacity of the receptor fluids from dermal permeation assays was evaluated with the ABTS radical decolorization assay [42]. In the study of the ointment, the antioxidant capacity was further assessed by assays of DPPH scavenging [43] and cytochrome c reduction [40,44].

For the ABTS method, the ABTS radical was previously obtained by mixing 10 mL of 7 mM ABTS with 10 mL of 4.9 mM potassium persulfate and leaving the mixture in the dark, at room temperature, for 12–16 h. The decolorization assay was carried out in the spectrophotometer cell and following the absorbance of the ABTS radical at 734 nm. The absorbance autozero was established with saline (epicatechin and ointment assays) or with 30% ethanol (curcumin assays) and then a small volume (20 to 22 μL) of the above ABTS stock solution was added to the cell for an absorbance of 0.7. After a few minutes of registering a stable absorbance, 50 μL of the sample was added to the reaction media, homogenized, and the variation of absorbance was tracked for at least 10 min. The receptor fluids from the ointment study had a higher antioxidant capacity, so the assayed volume of these samples was reduced to 20 μL .

The DPPH scavenging assay was carried out by a similar procedure, in a total reaction volume of 1 mL and using a stock ethanolic solution of DPPH with concentration 2.5 mM. The reaction media was composed of ethanol and a small volume (approx. 25 μL) of DPPH stock solution was added to the cell for an absorbance of 0.7 before triggering with 50 μL of sample and following the reaction for 30 min. For the cytochrome c assay, 50 μL of sample was added to 10 mM sodium phosphate buffer pH 8.0 supplemented with the protein at 20 μM concentration. The total reaction volume was 0.5 mL and the kinetics of cytochrome c reduction was followed by absorbance readings at 550 nm for at least 10 min.

The evaluation of the antioxidant capacity by the three methods included in all cases two types of controls: Blank assays with fresh receptor fluids (saline only or 30% ethanol), and Skin blank assays with the 8-h receptors from blank skin permeations (section 2.4). The absorbance changes measured with the skin blanks were subtracted from the absorbance changes measured with the samples of polyphenols' skin permeation. Moreover, the antioxidant capacity methods were also applied to skin blank samples spiked with epicatechin or curcumin at concentrations as determined by HPLC in the dermal permeation samples, or spiked with ascorbic acid at concentrations from 1 to 20 μM .

The response of the ABTS assay to epicatechin and ascorbic acid was studied with the antioxidants added to the reaction volume in concentrations resulting in 0 to 100% radical scavenging. The concentration-

response data were fitted to a Hill equation (Eq. (2)):

$$\text{Scavenging (\%)} = \frac{100 [A]^n}{IC_{50}^n + [A]^n} \quad (\text{Eq. 2})$$

Where $[A]$ is the concentration of the tested antioxidant in the reaction media, IC_{50} is the antioxidant concentration that scavenges 50% of the free radical, and n is the Hill coefficient. The data from both antioxidants fitted this equation quite well, with adjusted correlation coefficients (R_{adj}^2) above 0.98.

2.9. Statistical analysis

All the assays were carried out at least in triplicate experiments for each condition. The significance of the differences between experimental conditions was analyzed using one-way analysis of variance, and statistically significant differences were identified when $p \leq 0.05$.

3. Results

3.1. Permeation parameters of polyphenols

The three polyphenols selected for the work have significant different lipophilicities, as predictable from the logarithm of the octanol/water partition coefficient ($\log K_{o/w}$) – epicatechin (0.36), EGCG (1.17) and curcumin (3.20) – or from their water solubilities, presented in Table S1 of SI. Facing the limited availability of dermal permeation data for these major polyphenols under standardized conditions, we chose to start by studying their percutaneous permeation through porcine skin in steady-state conditions with saturated stratum corneum. Porcine skin is a recognized model for human dermal absorption of chemicals and saturating the stratum corneum allows to measure the maximum fluxes of permeation [36,45,46]. Following an empirical correlation [47], the amounts of epicatechin, EGCG and curcumin estimated to saturate the stratum corneum were 0.8, 47 and 0.04 mg, respectively (Table S1). The use of acetone as a vehicle enabled the application of greater quantities to ensure the rapid stratum corneum saturation, so doses 10–20 times higher were deposited in the donor compartment of the Franz cells in these permeation assays, as described in Methods section 2.4.

Fig. 1 shows the accumulation of the polyphenols in the receptor compartment of the Franz cells after penetration by way of the skin, and the permeation parameters calculated from the kinetic profile. The compounds presented a clear lag time, but then achieved a steady flux of permeation. The lag time of epicatechin was relatively short (0.49 ± 0.10 h), as well as curcumin (0.96 ± 0.09 h), but EGCG took almost 2 h to cross the skin (Fig. 1A). The results also demonstrated that epicatechin can easily reach high dermal permeation fluxes ($708 \pm 161 \mu\text{g cm}^{-2} \text{ h}^{-1}$), while curcumin flux was more moderate ($13.2 \pm 1.0 \mu\text{g cm}^{-2} \text{ h}^{-1}$). Despite the considerably higher molecular weight and the prolonged lag time, the permeation flux of EGCG under the studied conditions was also substantial (Fig. 1). Nevertheless, it must be noted that these maximum fluxes are obtained with stratum corneum saturation, which constitutes a challenge in practice and alternative strategies are necessary to enhance the transdermal penetration of polyphenols.

3.2. Roller microneedling of skin

To implement an accessible strategy to enhance permeation, a commercially available roller device was selected and evaluated for its perforation performance. Commercial microneedle devices of this type have been widely used as low-cost, reproducible tools to transiently disrupt the stratum corneum by producing microchannels [48,49]. A roller with needle's length of 500 μm was selected after a preliminary study of a similar device with 200- μm needles that returned moderate

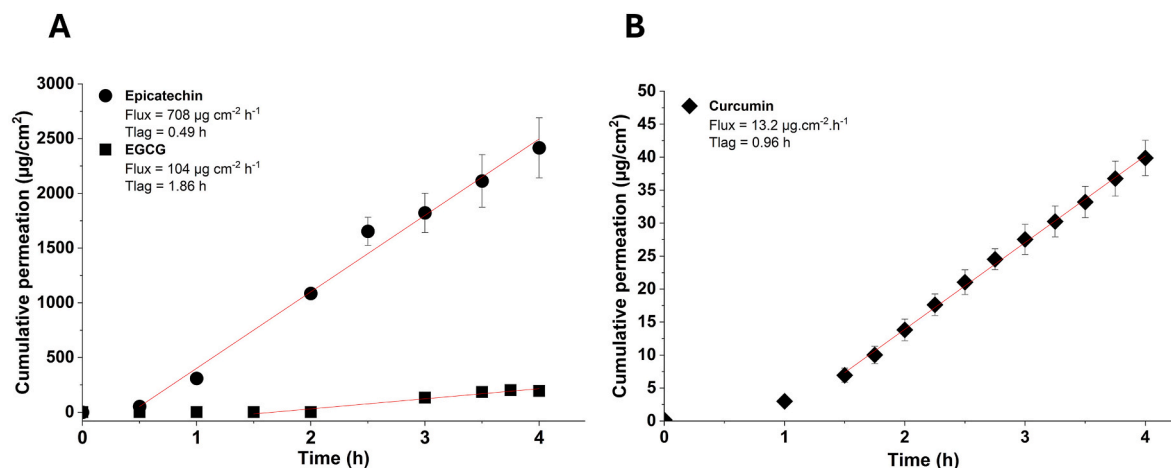


Fig. 1. Skin permeation of epicatechin and EGCG (A) and curcumin (B) under stratum corneum saturation. The lines are the best linear regression fits of the experimental data used to calculate the permeation parameters – flux and lag time (Tlag). The experimental results of epicatechin and curcumin are means $\pm$ SE from triplicate assays for each compound.

(20%) improvements in drug's skin permeation [31], and following other authors' indications of microneedle lengths of 500 to 600 μm as safe and effective for drug delivery [27–29].

In our microscopic analysis (Fig. 2), a pyramidal geometry of the microneedles in the roller was clearly observed. This shape is known to facilitate penetration by concentrating the insertion force at the tip and reducing resistance at the stratum corneum interface [35,50]. But the measured microneedle length (Fig. 2C) exceeded the manufacturer's specified length of 500 μm , so, we decided to characterize the perforation capabilities of the microneedles.

Microneedle penetration was first evaluated using the Parafilm® insertion model (Fig. 3), which has been widely adopted as a simple, reproducible surrogate for insertion profiling and shows good correspondence with insertion outcomes in excised porcine skin when folded to comparable thickness [35]. In this case, puncture marks were evident in layers corresponding to depths up to 390 μm , while deeper layers showed no detectable perforations. In addition, the puncture diameter decreased with increasing depth, consistent with a tapered needle profile and progressive attenuation of penetration due to incomplete insertion. Over the detectable range, a linear fit indicates the puncture diameter decreases by 63 μm for every 100 μm of additional penetration. Importantly, the formation of narrow microchannels at greater depths suggests that the microneedles can reach depths close to their measured length while minimizing lateral damage.

To confirm that these insertion results translated to tissue, methylene blue staining was used to visualize punctures created in skin after

rolling. Fig. 4A shows how the microneedling procedure, that involved rolling in different directions, effectively created perforation punches distributed throughout the skin surface, consistent with the expected advantages of rollers for rapidly creating multiple microchannels over relatively large surface areas. It was also observed that the microneedle perforation is sufficient to open a delivery channel across the outer layers of the skin (Fig. 4B). The two images presented in this panel are representative of the observations of cross sections of microneedled skin in which channels with different depths, from approximately 300 to 500 μm , have been observed. Previous authors using a roller with 0.5 mm microneedles also visualized microchannels with 255 μm [34] and underlined the role of skin elasticity to limit the channels' depth [28,48]. Notably, no evidence of full thickness perforation or structural compromise of the skin was observed, supporting the suitability of the applied microneedling conditions for controlled permeation enhancement. The microneedled skin samples mounted in the Franz cells for the permeation assays did not reveal also an increased incidence of receptor fluid leaking when the cells were oriented horizontally to test the effective sealing of the receptor compartment.

Remarkably, the puncture diameters measured from Parafilm and from stained skin images (Fig. 4C) were significantly larger than the estimated microneedle base diameter of 100 μm . A hypothesis for this mismatch is that the measured value represents the surface opening produced in a deformable substrate (Parafilm® or skin) rather than the local shaft diameter of the microneedle. Viscoelastic stretching and local tearing around the entry point may enlarge the surface puncture and,

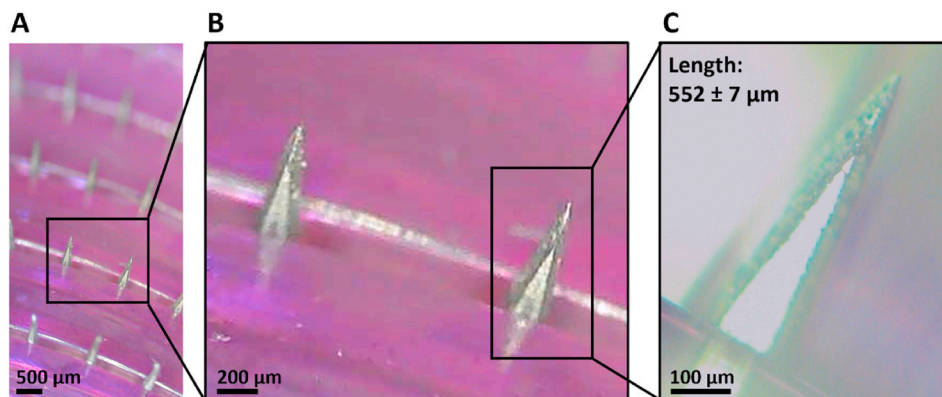


Fig. 2. Microscopic analysis of the microneedles in the roller used in this study. (A) Perspective of the roller's microneedle array. (B) Magnified visualization of individual microneedles. (C) Detail of a microneedle in the array and result from measurements ($n = 10$) of the length of needles in the roller.

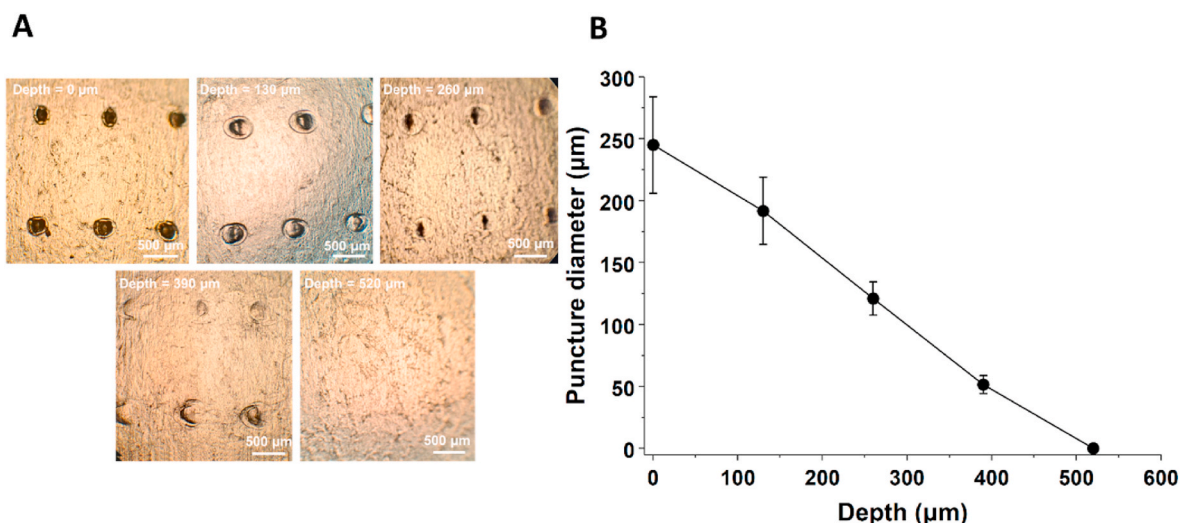


Fig. 3. Parafilm insertion test of the microneedles. (A) Microscopic image of Parafilm® sheets after perforation with the microneedle roller. (B) Graphical representation of the punch diameter in relation to the perforation depth.

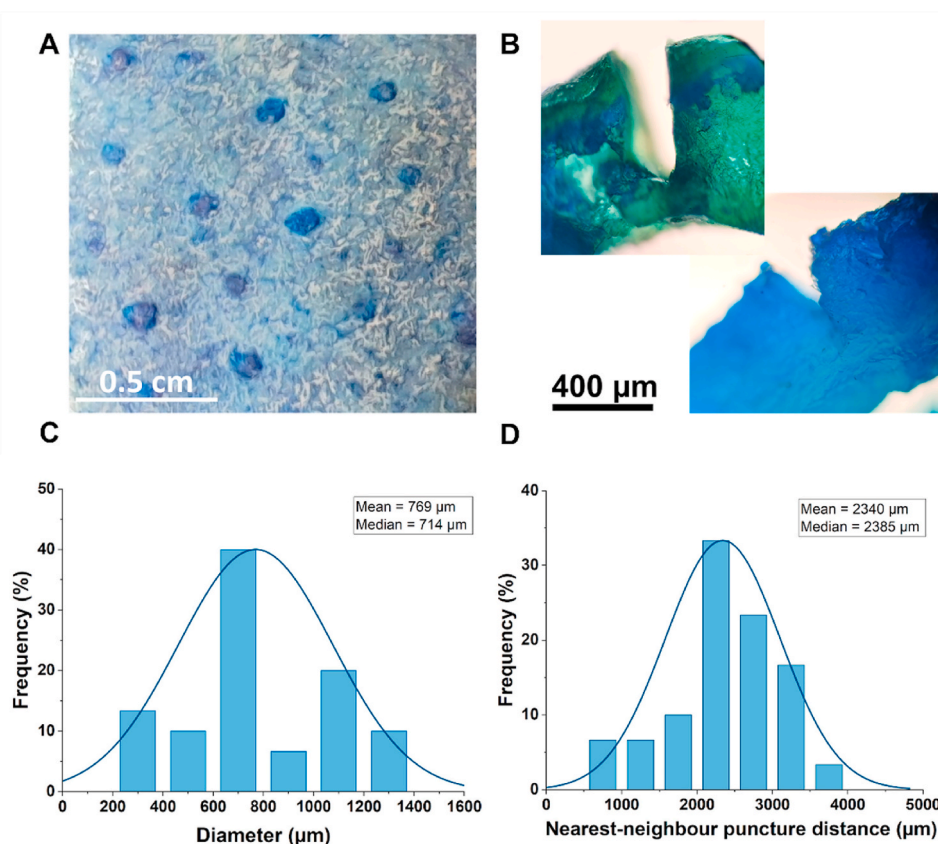


Fig. 4. Porcine skin after microneedling and stained with methylene blue. (A) Image of skin punches stained blue. (B) Cross sections of skin punctures. (C) Distribution of puncture diameter (n = 30). (D) Distribution of nearest-neighbor puncture distance measured (n = 30). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

besides this, roller application adds a tangential force that promotes elliptical opening and microtearing. Likewise, the deformation caused by the rolling is time-dependent and may suffer partial elastic recovery after removal, so the pore opening measured immediately after perforation may progressively contract over time [51–53]. In addition to puncture size, the spatial distribution of micropores was also analyzed, as it determines the density of entry points available for formulation access. The nearest-neighbor analysis (Fig. 4D) shows a distribution

centered at 2.3 mm. The breadth of the distribution is in accordance with the inherently non-periodic nature of roller application and supports interpreting roller microneedling as generating a distributed field of microchannels rather than a regular array.

3.3. Microneedling enhancement of the dermal permeation of epicatechin delivered by a gel and patch

The potential of the microneedling method for improving the dermal delivery of polyphenols was investigated using common dosage forms, specifically hydrogel and patch. The well-accepted Pluronic F-127 (also known as poloxamer 407) was used to formulate epicatechin in a hydrogel suitable for dermal application [38,54]. The epicatechin dose was $640 \mu\text{g}/\text{cm}^2$ and was guided by a polyphenol (8% capsaicin) patch clinically approved for peripheral neuropathic pain relief [55].

The kinetics of epicatechin release by the gel was evaluated in release assays conducted in Franz cells with a mounted porous membrane, a method that approximates to the conditions of dermal delivery. In addition to the details given in Methods section 2.6, a schematic representation of the Franz diffusion cell and an overview of its use in drug release studies can be found in a previous publication [10]. The results in Fig. 5A showed that encapsulation in the Pluronic hydrogel successfully controlled the release of epicatechin. The gel-encapsulated epicatechin was gradually released during several hours, with a first-order kinetic constant of 0.146 h^{-1} , much slower than when simple water donors were assayed.

When the epicatechin gel was applied on skin (Fig. 5B), the penetration of epicatechin to the receptor compartment of the Franz cells was slower than observed before in the assays of stratum corneum saturation. More precisely, the permeation flux of gel-delivered epicatechin through the porcine skin was $7.02 \pm 1.21 \mu\text{g cm}^{-2} \text{ h}^{-1}$, two orders of magnitude below the previously observed (Fig. 1A). This difference confirms the importance of stratum corneum saturation to obtain maximum skin permeation fluxes – the amount of epicatechin gradually released by the gel over time (Fig. 5A) is far below the estimated necessary to saturate the stratum corneum, 0.8 mg (Table S1 of SI).

Very important for the objectives of the present work, microneedling the skin before application of the epicatechin gel clearly accelerated the penetration of the polyphenol through skin (Fig. 5B). Compared to untreated (plain) skin, the amount of epicatechin permeating microneedled skin in 8 h was approximately double – 77.2 ± 13.3 versus $36.4 \pm 5.3 \mu\text{g}$. Normalized to the diffusion area (0.64 cm^2), these 8-h permeated amounts are 120.6 ± 20.8 versus $56.9 \pm 8.3 \mu\text{g cm}^{-2}$. The skin permeation parameters measured in the different conditions are shown in Table 1 and, indeed, the microneedling technique significantly increased the flux of gel-formulated epicatechin. Under these

conditions, the permeation lag time was short and microneedling did not have a noticeable effect.

Polymeric patches are another promising approach for the dermal administration of polyphenols and we decided to test the microneedling technique with patch-delivered epicatechin [40]. As shown in Fig. 6A, the encapsulation in the alginate patch prepared as described in Methods section 2.5 also allowed to control the presentation of epicatechin, although the first-order kinetic constant (2.684 h^{-1}) indicates a release faster than from the Pluronic gel. Moreover, the quantity of epicatechin freed by the patch into the media was lower than the gel (Fig. 5A), so it can be anticipated to be even further from enabling the saturation of the skin stratum corneum. In concordance, the skin permeation fluxes of patch-delivered epicatechin (Fig. 6B) were inferior, although the lag times were similar to the gel-delivered epicatechin (Table 1). Nevertheless, skin microneedling also promoted the transdermal penetration of patch-released epicatechin, as indicated by the 8-h permeated amount of 9.0 ± 0.7 versus $5.1 \pm 1.9 \mu\text{g}$ through plain skin (Fig. 6B) and the significantly higher permeation flux (Table 1).

The results in this section support the potential use of microneedle rollers as an effective strategy to enhance the dermal absorption of polyphenols, even when using modest drug reservoirs and the surface available compound is limited.

3.4. Microneedling enhancement of the dermal permeation of curcumin delivered by a gel

Following the positive results with epicatechin, it was important to assess the effect of skin microneedling on the penetration of a more lipophilic polyphenol like curcumin. Curcumin was also encapsulated in Pluronic F-127 hydrogel for dermal delivery, and the initial target concentration was informed by a 2% (w/w) curcumin gel formulation that returned beneficial outcomes in a recent clinical trial with knee osteoarthritis patients [56]. However, this concentration exceeded the loading capacity of the Pluronic F-127 gel system that resulted in precipitation of curcumin, therefore a reduced concentration of 1% (w/w) was selected.

Similar to epicatechin, the gel formulation effectively prolonged the release of curcumin (Fig. 7A) and facilitated the transdermal delivery of the polyphenol for several hours (Fig. 7B). The gel released sizable amounts of curcumin, larger than observed with epicatechin, which can be explained by the greater load of curcumin in the gel. Since the

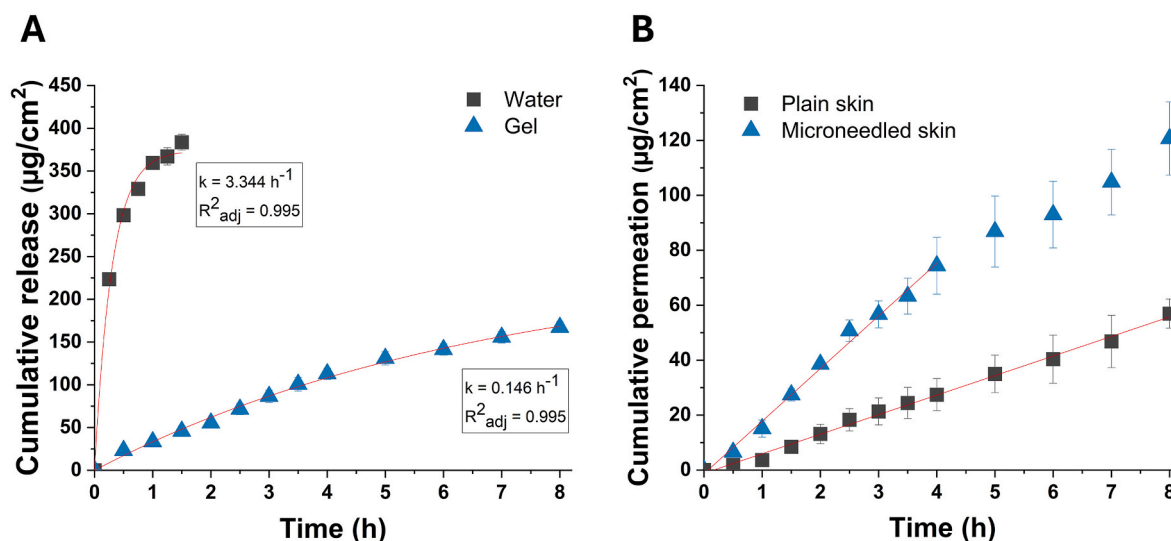


Fig. 5. Kinetics of gel-loaded epicatechin release and skin permeation. (A) Release profile of epicatechin from water dispersions and Pluronic F-127 gels. The lines are the best nonlinear regression fits of the experimental data to the first-order model in Eq. (1) (kinetic constants and adjusted correlation coefficients indicated). (B) Permeation profiles of gel-delivered epicatechin through skin without treatment or after microneedle perforation. The lines are the best linear regression fits of the experimental data used to calculate permeation parameters. All the experimental results shown are means $\pm$ SE from triplicate assays for each condition.

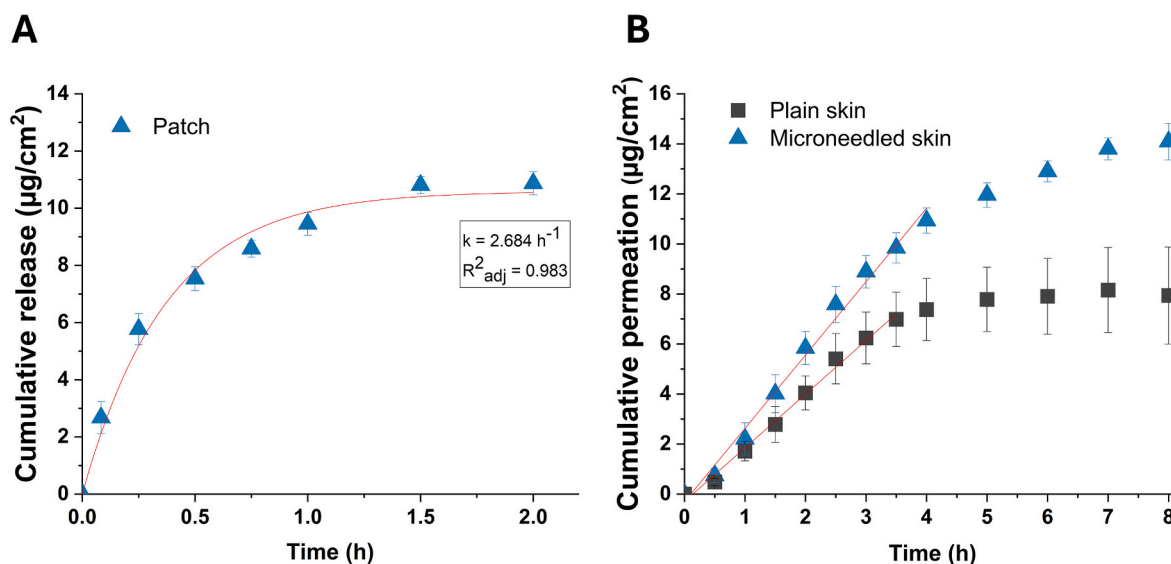


Fig. 6. Kinetics of patch-loaded epicatechin release and skin permeation. (A) Release profile of epicatechin from alginate patch. The line is the best nonlinear regression fit of the experimental data to the first-order model in Eq. (1) (kinetic constant and adjusted correlation coefficient indicated). (B) Permeation profiles of patch-delivered epicatechin through skin without treatment or after microneedle perforation. The lines are the best linear regression fits of the experimental data used to calculate permeation parameters. All the experimental results shown are means $\pm$ SE from triplicate assays for each condition.

Table 1

Permeation parameters of the gel- and patch-delivered polyphenols through skin without treatment or after microneedle perforation. The results shown were calculated from the kinetic data in Figs. 5–7 and are means $\pm$ SE from triplicate assays for each condition. The asterisks (*) indicate that the results obtained with treated skin are significantly different from those with plain skin ($p < 0.05$).

	Flux ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)	Lag time (h)
Gel-delivered epicatechin		
Plain skin	7.02 ± 1.21	0.24 ± 0.15
Microneedled skin	19.21 ± 6.23 *	0.23 ± 0.17
Patch-delivered epicatechin		
Plain skin	2.16 ± 0.47	0.19 ± 0.11
Microneedled skin	3.09 ± 0.17 *	0.21 ± 0.14
Gel-delivered curcumin		
Plain skin	1.32 ± 0.43	1.86 ± 0.23
Microneedled skin	3.47 ± 1.11 *	1.18 ± 0.19 *

amount of curcumin required to saturate the stratum corneum is estimated to be only 0.04 mg (Table S1), the skin permeation assays with the curcumin-loaded gel would be in conditions close to stratum corneum saturation. Accordingly, the permeation parameters obtained from the assays and presented in Table 1 are close to those in Fig. 1B. However, also in these conditions of relatively high availability of a slowly permeating drug on the skin surface, microneedling demonstrated significant advantages as shown by the kinetic profiles in Fig. 7B. At 8h of assay, $15.0 \pm 5.2 \mu\text{g}$ of curcumin permeated the microneedled skin in comparison to $5.5 \pm 1.9 \mu\text{g}$ through plain skin (23.4 ± 8.1 to $8.6 \pm 3.0 \mu\text{g cm}^{-2}$). The permeation flux approximately doubled and a significant reduction in the lag time was also achieved with microneedle rolling of the skin (Table 1). The observed doubling of curcumin flux is consistent with the reported increase achieved for amantadine ($\log K_o/w = 2.4$) also with a 0.5 mm long microneedle roller [34]. Nevertheless, it should be noted that 14- to 21-fold enhancements have been

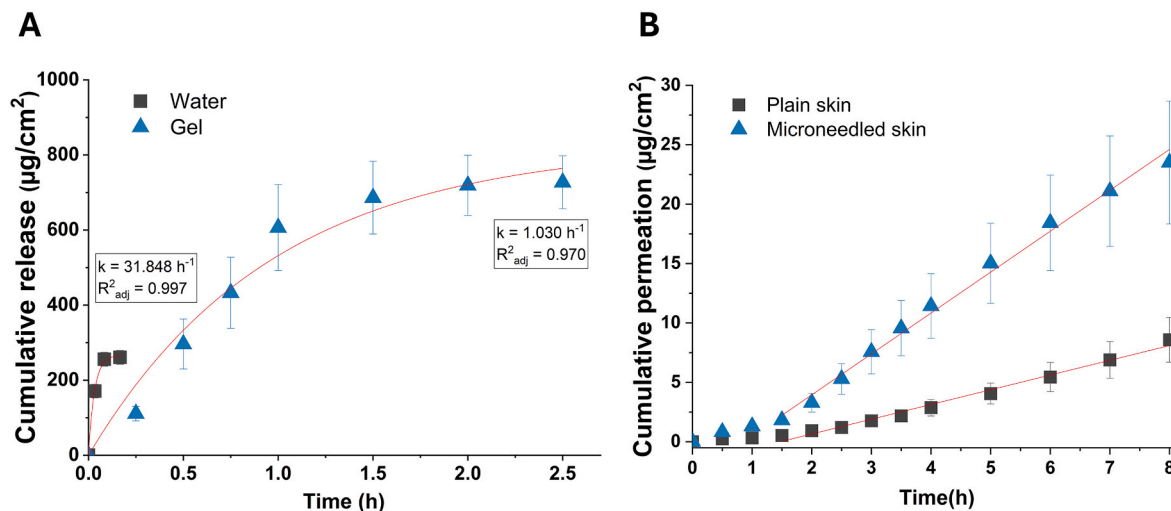


Fig. 7. Kinetics of gel-loaded curcumin release and skin permeation. (A) Release profile of curcumin from water dispersions and Pluronic F-127 gel. The lines are the best nonlinear regression fits of the experimental data to the first-order model in Eq. (1) (kinetic constants and adjusted correlation coefficients indicated). (B) Permeation profiles of gel-delivered curcumin through skin without treatment or after microneedle perforation. The lines are the best linear regression fits of the experimental data used to calculate permeation parameters. All the experimental results shown are means $\pm$ SE from triplicate assays for each condition.

calculated from 2-h dermal permeation experiments with the more hydrophilic drug acetylsalicylic acid and by using a specially fabricated (600 μm) microneedle roller [28].

Since the curcumin incorporated into the gel comprised the typical three curcuminoids found in turmeric extracts, each in a specific proportion (Fig. S2), we analyzed the chromatograms of the receptor samples from the release and skin permeation assays to determine any alteration in the proportions. The results in Table S2 of SI show that the relative peak area for each curcuminoid in the samples had no significant variations in the course of the assays, and remained close to the proportions in the initial mixture (Fig. S2), indicating that none of curcuminoids was preferentially retained within the polymeric gel or did any had an advantage in permeating the skin.

3.5. Antioxidant capacity of the skin-penetrated polyphenols

In addition to promoting the dermal penetration of the compounds, it is crucial that the microneedling technique does not compromise the bioactivity properties of the polyphenols. Given that antioxidant abilities are among the key properties of polyphenols, we examined the radical scavenging capacity of the receptors collected after the dermal permeation. The antioxidant capacity was assessed using the ABTS radical decolorization assay described in section 2.8 of Methods, and the studies aimed to detect any interference from components of the drug carriers or microneedles penetrating through the skin into the polyphenol-receiving fluids.

The experimental traces from the decolorization assays, like those displayed in Fig. 8 panels A-C, show that both epicatechin and curcumin absorbed by skin are able to neutralize the ABTS radical. The decolorization rate decreases with time due to the gradual consumption of the

antioxidant forms of the polyphenols, but the reaction prolongs for several minutes. Independently of the polyphenol and the gel or patch carriers employed, the receptor fluids of microneedled skin revealed a radical scavenging rate and extension superior to the receptors of plain skin, as expected from the polyphenols concentrations measured by HPLC in the receptors (panels B of Figs. 5–7).

Additional assays were carried out with receptor fluids from blank skin permeations (skin blanks, section 2.4) spiked with the polyphenols at the concentrations measured in the receptors from permeation through microneedled skin. The results obtained with these spiked samples closely resembled the permeation receptors from microneedled skin, indicating that neither the roller's microneedling nor the gel/patch carriers impede the antioxidant activity of the transdermally delivered epicatechin and curcumin.

The antioxidant capacity of the receptor fluids, quantified by the decrease in absorbance after 10 min reaction, is represented in Fig. 8D. The data indicates that the receptor fluids from microneedled skin had a radical scavenging capacity approximately double that of the plain skin receptors, which is in good agreement with the observed differences in polyphenol concentrations between the groups of receptors. The results overall show that the polyphenols, even after the topical application and 8-h dermal permeation, and also in the case of microneedle-assisted penetration, maintain their antioxidant capacity and the microneedling process generates no interferences affecting this property.

3.6. Application to a clinically relevant polyphenol ointment

Further expanding the potential applications of the microneedling technique, we also examined the dermal penetration of polyphenols from a catechin ointment. The ointment contained a standardized green

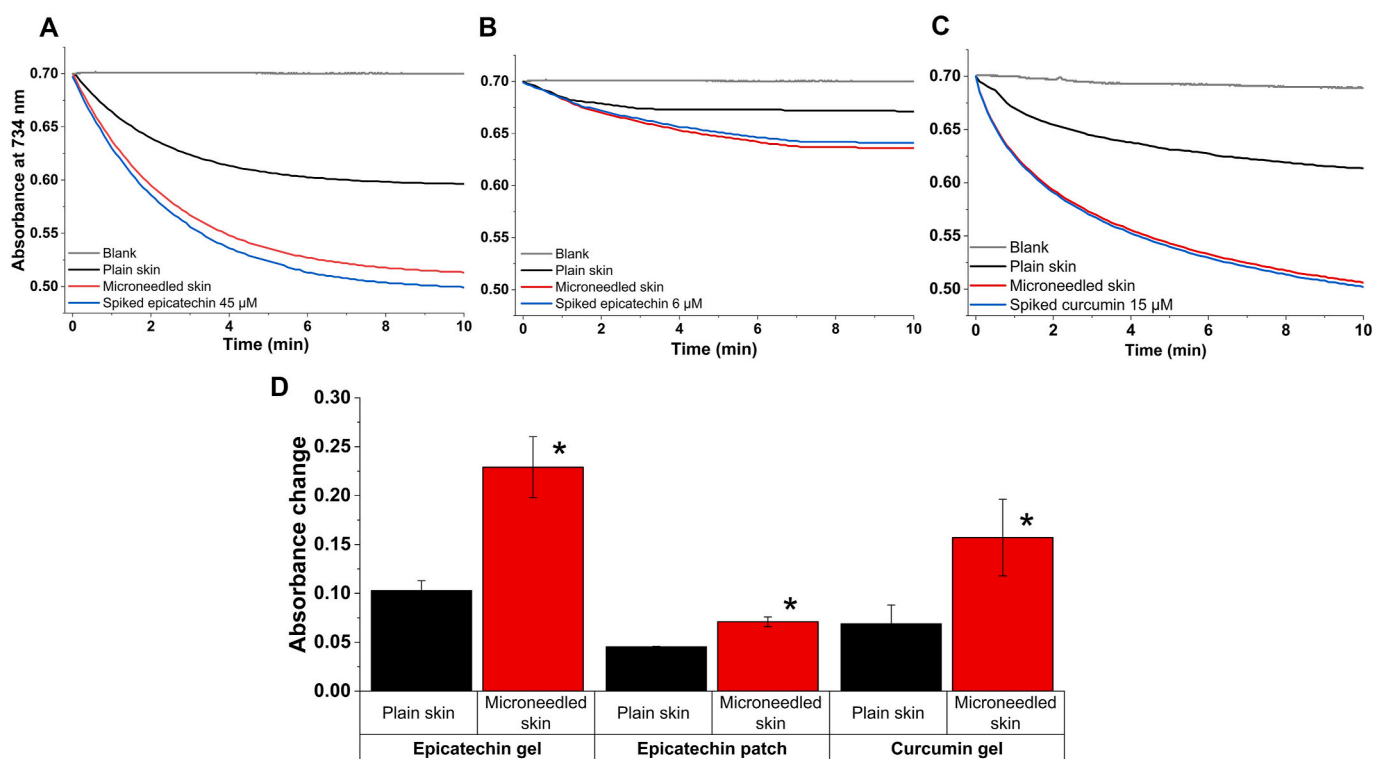


Fig. 8. Antioxidant capacity of gel- and patch-delivered polyphenols after permeation through plain and microneedle-treated skin. The scavenging of the radical of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by the receptor fluids from 8h-permeation assays was monitored by the decrease of the absorbance at 734 nm. Kinetics of ABTS radical scavenging by the permeation receptors of epicatechin gel (A), epicatechin patch (B), and curcumin gel (C). Blank samples of fresh receptor fluids without polyphenols (no permeation) were also tested, as well as skin blanks spiked with the corresponding polyphenol at the concentration expected in the samples from microneedled skin. Panel D displays the quantification of radical scavenging capacities as the absorbance decrease achieved after 10 min reaction. The results shown are representative experimental traces or the mean $\pm$ SE from triplicate permeation samples. The asterisks (*) in panel D indicate that the results obtained with treated skin are significantly different from those with plain skin ($p < 0.05$).

tea extract, including epicatechin and EGCG, that is approved for clinical use and being studied for cancer treatment [32,33,57,58]. In contrast to the aqueous base of the Pluronic gel tested before, the catechin ointment has an oil (white petroleum) base that interacts quite differently with skin. While the hydrogel is smooth and easily spreadable, ointments are greasy, thick and slowly absorbed.

Since the extract is a mixture of polyphenols, the kinetics of dermal permeation of the compounds was followed using the Folin-Ciocalteu total polyphenol assay (section 2.7 of Methods). As represented in Fig. 9A, the polyphenols from the ointment spread on the skin appeared rapidly in the receptor fluid of the Franz cells and continued to accumulate for several hours. However, the accumulation rate of total polyphenols was significantly higher when the skin had been previously treated by microneedling. At the end of the permeation assay (24 h), the cumulative amount of polyphenols in epicatechin equivalents was $105 \pm 10 \mu\text{g}$ from plain skin and $202 \pm 20 \mu\text{g}$ from treated skin (164 ± 16 and $316 \pm 31 \mu\text{g cm}^{-2}$). The kinetic study of the permeation of ointment polyphenols was extended over a 24-h period, but a significant deceleration became evident around the sixth hour (Fig. 9A). This decrease was noted with both the plain and treated skin, and is likely attributable to the observed drying of the ointment on the skin surface.

The HPLC analysis of the receptor fluids confirmed the permeation of different compounds through the skin, with epicatechin and EGCG being identified, as highlighted in Fig. 9B, based on the residence times in standard chromatograms (Fig. S1). At 2h of permeation, epicatechin was unequivocally detected in all the receptor samples, but in higher

concentrations from microneedled skin assays. The permeation of EGCG was slower, but at 5h it was already detected in the receptors from treated skin, while from plain skin it was necessary to wait 8h and the increases afterwards remained very small (Fig. 9B).

The antioxidant capacity of the receptor fluids was measured by the ABTS radical and DPPH scavenging assays and by the cytochrome *c* reduction method (Fig. 9C and D). The three methods indicated that the receptor fluids from microneedled skin possessed a superior antioxidant capacity, reflecting at least qualitatively the enhanced permeation of polyphenol compounds from the ointment through the treated skin. The testing of samples spiked with epicatechin was less informative in the case of this formulation comprising a mixture of catechins, but assays of skin blanks spiked with micromolar concentrations of epicatechin resulted in absorbance changes similar to the ointment permeation receptors, as illustrated for the cytochrome *c* assay in Fig. 9D.

The antioxidant capacity of skin blanks spiked with ascorbic acid was also assessed by the three methods to compare with the capacity of the ointment permeation receptors. Depending on the antioxidant assay method, ascorbic acid 5 or $20 \mu\text{M}$ supplementations were the final sample concentrations that resulted in absorbance changes closer to the microneedled skin receptors, as exemplified in Fig. 9D. Regarding the results from the cytochrome *c* assay, it is noteworthy that both the kinetics and the 10-min absorbance change monitoring the reduction of this hemoprotein are very similar across ointment permeation receptors from microneedled skin and blanks spiked with micromolar epicatechin and ascorbic acid (Fig. 9D). The results here obtained are also close to

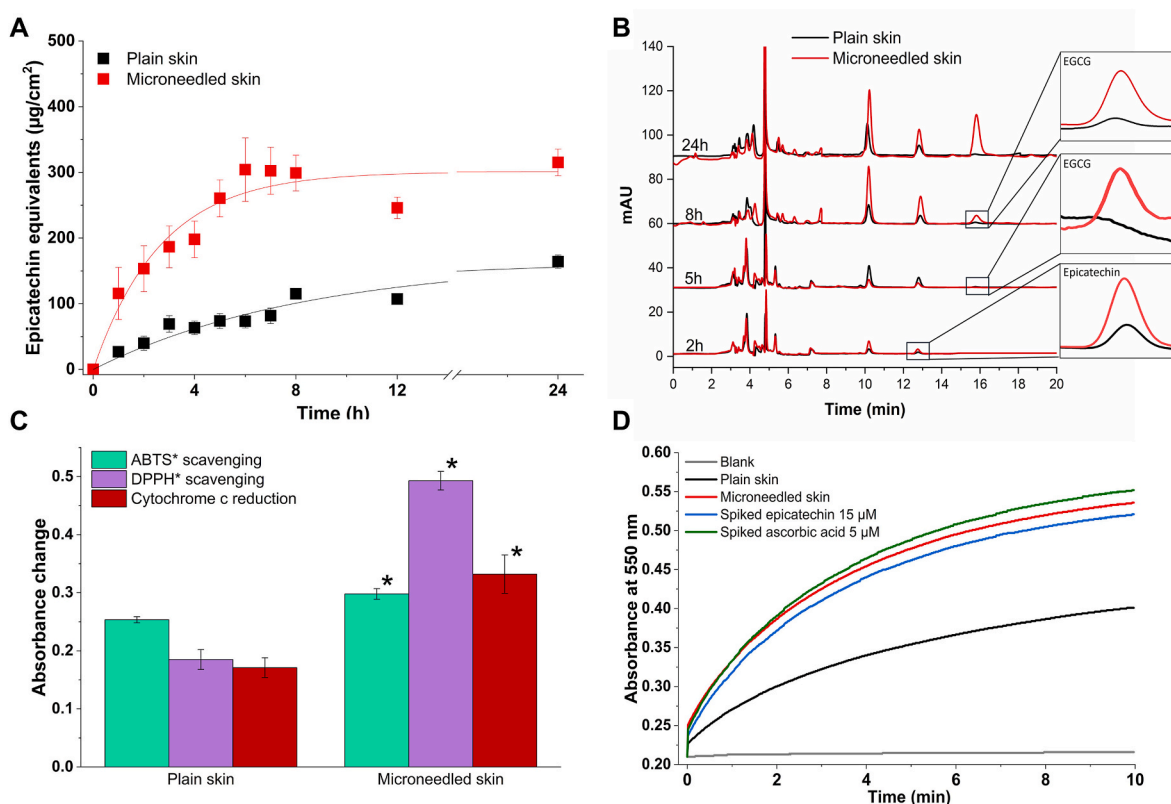


Fig. 9. Permeation of polyphenols from tea catechin ointment through plain and microneedle-treated skin. (A) Kinetics of polyphenol dermal penetration monitored by the Folin-Ciocalteu total polyphenol assay of the permeation receptor fluids. The lines are the best nonlinear regression fits of the experimental data to a first-order model (kinetic constants 0.13 h^{-1} and 0.37 h^{-1} for plain and microneedled skin, respectively). (B) Representative HPLC chromatograms of permeation receptors sampled at 2h, 5h, 8h and 24h, and amplification at the residence times of epicatechin and epigallocatechin-3-gallate (EGCG). (C) Antioxidant capacities of the 8-h permeation receptors quantified as absorbance changes in the assays of scavenging of the radical of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*), scavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH*), and reduction of cytochrome *c*. (D) Kinetics of cytochrome *c* reduction by the permeation receptors, blank samples of fresh saline without polyphenols (no permeation), and skin blanks spiked with epicatechin or ascorbic acid. The chromatograms are presented displaced on the vertical axis for better observation. The results shown are the mean $\pm$ SE or representative experimental traces obtained with samples from triplicate permeation assays. The asterisks (*) in panel C indicate that the results obtained with treated skin are significantly different from those with plain skin ($p < 0.05$).

those in previous publications with the same antioxidants and cytochrome *c* under comparable reaction conditions [40,44].

To further clarify if the antioxidant capacity exhibited by the permeation receptors is the expectable from the polyphenols' concentration, or it is influenced by formulation components or the micro-needling procedure, we decided to perform a concentration-response analysis of the ABTS radical scavenging assay. Fig. 10 shows the assay response to the concentration of epicatechin and ascorbic acid in the reaction media. In the same plot, the complete set of results obtained with the receptors from the permeation assays of epicatechin gel and patch, curcumin gel and ointment are also depicted as a function of the corresponding polyphenol concentration in the ABTS reaction media. The observation of the distribution of these points indicates: (1) the response to the 8 permeation receptors shows a good correlation with the polyphenols' concentration regardless the fact that different polyphenols are present; (2) the response to all the receptors is very close to the response of the method to (pure) epicatechin at the same concentrations, without any apparent divergence of specific polyphenol formulations or of microneedled relative to plain skin. These data reinforce that the catechins and curcuminoids applied by way of the different formulations, including the catechins ointment, and through microneedled skin, retain their antioxidant capacity.

Moreover, the data in Fig. 10 provides additional support for an equivalence of the antioxidant activity of the polyphenol-containing skin permeation receptors and ascorbic acid. Overall, the ABTS radical scavenging degree afforded by the receptors is equivalent to approximately 1 mg/L of ascorbic acid in the total assay volume, that corresponds to low micromolar concentrations in assayed samples like the spiked skin blank in Fig. 9D.

4. Discussion

4.1. Standardized permeation parameters

The permeation parameters for representative polyphenols under conditions of stratum corneum saturation are a first important contribution of this work. By applying doses calculated to saturate the stratum corneum, we report for the first time maximum steady-state fluxes and lag times for epicatechin, EGCG, and curcumin. This approach addresses

a recurrent limitation in the existing literature, where low or unspecified applied doses may have led to the conclusion that polyphenols like epicatechin [15,59] are unable to cross the skin. Similarly, the lower transdermal flux of EGCG ($4.7 \mu\text{g cm}^{-2} \text{h}^{-1}$), reported by Avadhani et al. (2017) with a plain solution applied to the skin may also result from the dose (less than 1 mg/cm^2) insufficient to saturate the stratum corneum [20]. Our results demonstrate that catechins can reach transdermal fluxes above $100 \mu\text{g cm}^{-2} \text{h}^{-1}$ when the stratum corneum is saturated, values comparable to those reported for clinical dermally applied drugs [8,60]. However, the experimental data clearly shows that EGCG penetrates the skin at a slower rate than epicatechin, as evidenced by a longer lag time and reduced flux, in coherence with its higher molecular weight that determines a lower skin permeability (Table S1).

Curcumin exhibited substantially lower skin permeation fluxes even under saturation conditions. The results agree with previous studies applying also high doses of curcumin (more than 1 mg/cm^2) with optimized nanocarriers and obtaining fluxes in the order of $10 \mu\text{g cm}^{-2} \text{h}^{-1}$ [22,61], and short lag times $< 1 \text{ h}$ [17,22]. These findings indicate that stratum corneum saturation alone is insufficient to achieve high transdermal absorption of curcumin, highlighting the need for additional enhancement strategies.

4.2. Microneedling as a physical enhancer for polyphenol delivery

The roller microneedling approach evaluated in this study proved to be an effective and reproducible physical enhancement method for transdermal delivery. The microneedles generated microchannels with dimensions sufficient to cross the stratum corneum without compromising overall skin integrity, as confirmed by microscopy, dye staining, and leakage tests. This balance between effective barrier disruption and structural preservation is essential for both experimental reliability and future translational relevance.

Across all tested formulations—Pluronic F-127 hydrogel, alginate patch, and ointment—microneedling consistently improved the skin permeation of the polyphenols, demonstrated by an increase in flux and/or earlier appearance in the receptor fluid of the assay Franz cells. These observations are in line with the widely accepted mechanism of microneedling, whereby the creation of transient, aqueous microchannels partially bypasses the lipid-dominated diffusion pathway of the intact stratum corneum, thereby facilitating faster and more efficient transport of hydrophilic and moderately lipophilic compounds [25,26]. Furthermore, our kinetic permeation data suggest that the diffusion channels generated by the needles remain active for at least 8 h, which agrees with previous work using roller devices that reported the microchannels can be detected in the skin for several hours [48].

The comparison of different polyphenols and dosage forms provides further insight into the role of microneedle-assisted delivery. For epicatechin delivered by hydrogel and patch (and for ointment EGCG), microneedling significantly enhanced the permeation despite the limited amount of drug released on the skin. These results underscore the value of microneedling in situations where drug availability at the skin surface is intrinsically constrained by controlled-release formulations. For the gel-delivered curcumin, predicted to be present at higher surface availability and close to saturation conditions, microneedling not only doubled the permeation flux but also significantly shortened the lag time, demonstrating that physical enhancement can further improve delivery even when chemical driving forces are already maximized.

Regarding the delivery systems employed, Pluronic F-127 confirmed a good efficacy in solubilizing even the more hydrophobic polyphenol curcumin at relatively high concentration. This effectiveness can be attributed to the interaction of the bioactive with the amphiphilic polymer chains that self-assemble into micelles [38,54,62]. Both Pluronic and alginate have established pharmaceutical track records, encompassing dermal and parenteral applications, thereby rendering them valuable systems for evaluating the skin microneedling method.

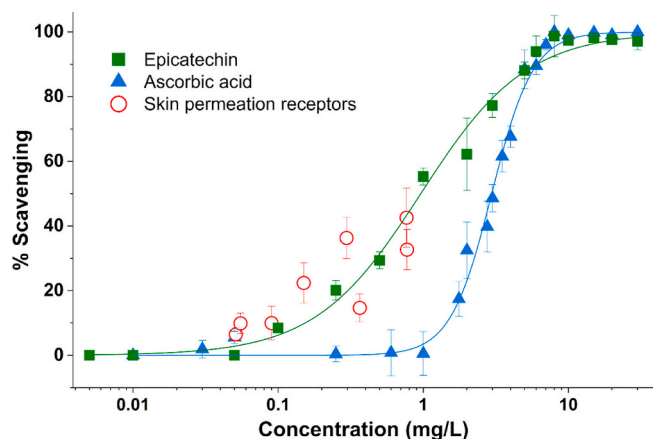


Fig. 10. Response of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay to epicatechin and ascorbic acid, and comparison to the results of the receptor fluids from the permeation assays (epicatechin gel and patch, curcumin gel and ointment). The degree of scavenging is represented as function of the concentration of the antioxidants (ascorbic acid or polyphenols) in the total assay volume. The lines are the best nonlinear regression fits of the experimental data to the Hill equation in Eq. (2) ($\text{IC}_{50} = 0.96 \pm 0.06 \text{ mg/L}$ and $n = 1.20 \pm 0.07$ for epicatechin; and $\text{IC}_{50} = 2.96 \pm 0.06 \text{ mg/L}$ and $n = 3.05 \pm 0.20$ for ascorbic acid). The results shown are the mean $\pm$ SE from triplicate assays for each condition.

The results obtained with the catechin ointment are also particularly noteworthy from an application perspective. Ointments are widely used in clinical and cosmetic practice but are often associated with slow drug release and limited penetration [63,64]. The observation that EGCG was detectable in the receptor compartment at an earlier time with roller-treated skin highlights the ability of microneedling to unlock the dermal delivery potential of otherwise poorly permeating polyphenols from conventional, oil-based formulations.

4.3. Preservation of antioxidant activity after microneedle-assisted permeation

An essential requirement for the success of microneedling-assisted delivery is the preservation of the biological activity of the delivered compounds. The antioxidant assays performed in this study demonstrate that the catechins and curcumin retain their radical scavenging capacity after transdermal permeation, including when delivery is facilitated by roller microneedling the skin. The close correspondence between the activity of permeation receptor fluids and that of spiked control samples and the studied response of the ABTS assay indicates that neither the microneedling process nor the carriers introduce interfering species or promote degradation of the polyphenols.

This finding is particularly important in addressing concerns that mechanical skin disruption or interactions with formulation excipients could affect the stability of sensitive phytochemicals [11,26,40,65]. The maintained antioxidant activity supports the suitability of roller microneedling as a safe enhancement strategy for functional polyphenols intended for therapeutic or cosmetic applications. Nevertheless, it must be underlined that the current study was confined to an *in vitro* assessment, and it is essential to conduct specific studies on the bioactive properties of polyphenols for their intended pharmacological applications.

4.4. Translation considerations and future research directions

The enhancement factors achieved by microneedling in this work—approximately twofold increases in permeation flux and antioxidant activity—are comparable to those reported for some nano-based delivery systems developed for polyphenols [20,21]. However, microneedle rollers offer distinct practical advantages, including simplicity, low invasiveness, and compatibility with a wide range of existing topical vehicles. Importantly, physical enhancement by microneedling does not preclude formulation optimization; rather, it may act synergistically with advanced carriers, including nanoparticles or nanoemulsions, to further improve dermal delivery.

From a translational perspective, the use of pharmaceutically established polymers and a clinically relevant catechin ointment strengthens the significance of the present findings. The enhanced delivery of curcumin and EGCG may have broader implications, given their specific pharmacological status and because their polarity ($\log K_{o/w}$) is similar to other notable polyphenols like resveratrol, kaempferol or baicalein [8,19,57,66].

Nevertheless, the integration of microneedling into therapeutic or cosmetic applications requires careful consideration. Further studies are needed to assess *in vivo* performance, skin barrier recovery following repeated application, and the durability of enhancement under realistic use conditions. The lack of investigation of polyphenol retention in the skin following microneedling is another limitation of the present work. In addition, regulatory classification and standardization of microneedle roller devices remain evolving issues that must be addressed before broader pharmacological implementation. Within these boundaries, the present work provides experimental evidence to support future investigations combining microneedling with formulation-based strategies to expand the scope of dermal polyphenol delivery.

5. Conclusions

This study demonstrates that roller microneedling is an effective physical strategy to enhance the transdermal delivery of polyphenols from conventional topical formulations. Standardized permeation parameters established for epicatechin, EGCG, and curcumin highlight intrinsic differences in skin transport of the compounds. Nonetheless, microneedling consistently increased permeation fluxes and/or reduced lag times across dosage forms without compromising skin integrity. Importantly, substantial antioxidant activity was observed following microneedle-assisted permeation of the polyphenols, supporting the suitability of this approach for functional dermal delivery. Overall, the findings indicate that microneedling can act as a versatile, low-invasive enhancement method to improve the performance of topical polyphenol formulations, providing a solid experimental basis for future translational and regulatory investigations.

CRediT authorship contribution statement

Cristiana Violante: Writing – original draft, Methodology, Investigation. **Dorinda Marques-da-Silva:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Ricardo Lagoa:** Writing – review & editing, Writing – original draft, Supervision, Methodology.

Ethics statement

Animal skin samples were collected from pigs slaughtered for commercial purposes at a licensed slaughterhouse. No animals were sacrificed specifically for research and no *in vivo* experimentation requiring ethical approval was conducted.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the final preparation of this publication, the authors used ChatGPT (OpenAI) to assist with language refinement and improvement of readability of the text, and for drafting part of the graphical abstract. The authors reviewed and edited the content and take full responsibility for the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jddst.2026.108928>.

Data availability

Data will be made available on request.

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