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Title: Iontophoresis of minoxidil sulphate loaded microparticles, a strategy for follicular drug targeting?

Article Type: Full Length Article

Keywords: microparticle; iontophoresis; chitosan; minoxidil sulphate; hair follicle; topical drug delivery.

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Abstract: The feasibility of targeting drugs to hair follicles by a combination of microencapsulation and iontophoresis has been evaluated. Minoxidil sulphate (MXS), which is used in the treatment of alopecia, was selected as a relevant drug with respect to follicular penetration. The skin permeation and disposition of MXS encapsulated in chitosan microparticles (MXS-MP) was evaluated in vitro after passive and iontophoretic delivery. Uptake of MXS was quantified at different exposure times in the stratum corneum (SC) and hair follicles. Microencapsulation resulted in increased (6-fold) drug accumulation in the hair follicles relative to delivery from a simple MXS solution. Application of iontophoresis enhanced follicular delivery for both the solution and the microparticle formulations. It appears, therefore, that microencapsulation and iontophoresis can act synergistically to enhance topical drug targeting to hair follicles.

Response to Reviewers: Responses to Reviewer #1:

The paper concerns the application of iontophoresis associated with microparticles to improve follicular delivery of minoxidil. The paper is well organized, however some important details are missing and are of outermost importance to understand and comment the data obtained. Additionally the discussion is very meager and does not try to elucidate the mechanisms that could justify the results obtained.

R: The points raised by the reviewer have been addressed as detailed below and the Discussion has been modified accordingly. The changes made have been highlighted in the revised manuscript.

1. More details on the particles should be given. From the previously published data (ref 2 of the paper) it seems that the particles quickly swell as soon as they are put in contact with EtOH:H₂O. What

is the consequence of the swelling? These details should be given. What about the impact of iontophoresis on these particles?

R: Details of the particles' characteristics have been provided in the manuscript. The particles do indeed swell quickly in contact with the ethanol/water (from 3.0 μm to 5.7 μm) and this permits the sustained release of MXS to be achieved. We envisage that the swollen microparticles remain on skin surface, and that only drug that has been released from the carriers is able to cross the skin. However, the microparticles appear to accumulate in follicular openings, a conclusion supported by the enhanced recovery of MXS from these appendageal structures. The points have been highlighted in the revised manuscript (see Discussion, highlighted in yellow, p. 7-8).

With respect to iontophoresis, it seems likely that the microparticles used are too large to be transported, to any significant extent, through the skin by the applied electric field. However, because the follicles are low resistance pathways for the movement of charge across the skin, we speculate that iontophoresis can quickly and efficiently target MXS-MP to follicular openings, and that MXS is then released from the microparticles directly into the infundibulum (p. 9).

2. In the paper the release of the drug from the MP is completely overlooked. This should be presented (or, if already published, details should be added) and the data discussed also considering this aspect. Is there non encapsulated drug? If yes, what's the contribution of the non encapsulated drug? What's the % of drug released after 3, 6, 12, 24 h?

R: Drug release data are included in the Discussion (p. 8). As stated above, we believe that only drug that has been released from the microparticles is able to cross the skin (with or without iontophoresis); however, because iontophoresis 'targets' the MXS-MP to the follicles, increased levels of drug are made available to be taken up and absorbed at these specific sites (p.9). Our previously published work shows that approximately 30%, 53%, 72% of MXS were released from the microparticles after 3, 6 and 12 h, respectively [2].

3. In Figure 2, when considering the receptor solution (MP and ionto) a 25x enhancement is obtained. The authors should discuss this result. What's the impact of iontophoresis on MPs? Electrorepulsion? Electroosmosis? Effect on drug release? Apparently this strategy could be useful to increase the transdermal delivery?

R: Because the chitosan-based MXS-MP have a positive zeta potential [2], it was hypothesised that an iontophoretic current would enhance follicular localisation when the drug delivery system was associated with the anode (and also permitting advantage to be taken of the anode-to-cathode electroosmotic flow typically established across the net negatively-charged skin membrane). But, as already stated, the swollen, 5.9 μm microparticles are unlikely themselves to penetrate the follicles and only released MXS diffuses into and through the skin. At the pH of the formulation (pH \sim 5.5), MXS is 90% non-ionized suggesting that its enhanced, electrotransport-mediated delivery will be due to a combination of electrorepulsion and electroosmosis [16]. From a practical standpoint, for the topical treatment of alopecia, where a local effect is desired with as little transdermal exposure as possible, the judicious selection of current density and application time in vivo is required for optimal therapy (p. 8).

4. Is there any more general conclusion that could be drawn on the association of MP and iontophoresis? The use of iontophoresis on MP accumulates 3.9 $\mu\text{g}/\text{cm}^2$, while on a simple solution the amount is 2.4 $\mu\text{g}/\text{cm}^2$. Is the difference statistically significant? It's worth it?

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5. Second highlights: please add "after 6 hours" since this is not true for other application times.

R: The information was added.

Responses to Reviewer #2:

- This is a very well-written and structured research article which contains interesting data for researchers and scientists in the area of dermal and transdermal drug delivery. The investigation of the combination of chitosan microparticles and iontophoresis is an intelligent approach. The experimental design is sophisticated and neatly arranged. Therefore, it is a valuable and excellent contribution to the journal. Since these are interesting results and the main focus of the journal *Colloids and Surfaces B: Biointerfaces* is the impact of colloid and interfacial phenomena on biological systems the authors could discuss the causes and theoretical fundamentals of the significant accumulation of the chitosan microparticles also after the application of iontophoresis in the hair follicles. The physicochemical properties of the chitosan particles and their impact on the preferential follicular penetration might be taken into account for this discussion in more detail.

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May 29th, 2015

To

Prof. Dganit Danino

Editor, Colloids and Surfaces B: Biointerfaces

Re: Manuscript Ref. No.: COLSUB-D-15-00579

Dear Professor Danino,

We are grateful for the Reviewers' comments and suggestions. In response to their comments, we have implemented changes to the manuscript. These changes are highlighted in the manuscript and referenced in the letter of rebuttal, in which we have listed the Reviewers' comments and addressed them one-by-one.

We thank you for the opportunity to submit our revised manuscript and look forward to hearing from you soon.

Yours sincerely,

Renata F. V. Lopez



Reviewer 1

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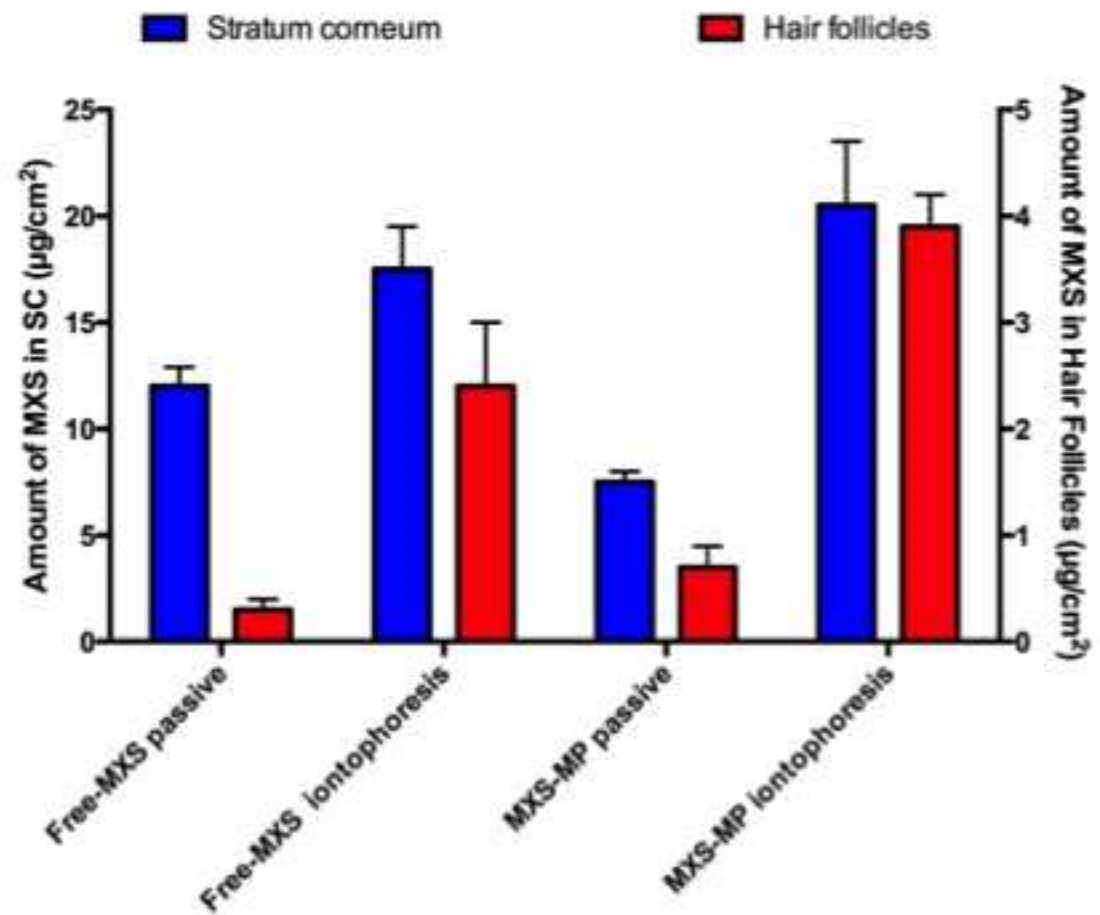
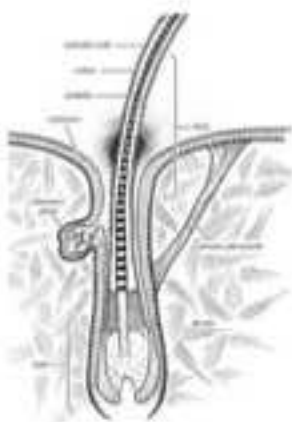
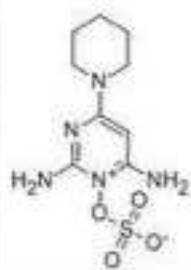
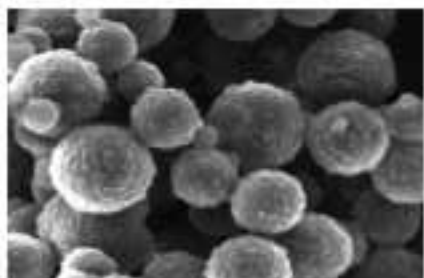
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Highlights

- The feasibility of targeting minoxidil sulphate to hair follicles by a combination of microencapsulation and iontophoresis was evaluated.
- Microencapsulation of MXS resulted in a 6-fold increased drug accumulation in the hair follicles relative to delivery from a simple MXS solution after 6 hours
- Application of iontophoresis enhanced follicular delivery for both the solution and the microparticle formulations
- Microencapsulation and iontophoresis can act synergistically to enhance topical drug targeting to hair follicles

Iontophoresis of minoxidil sulphate loaded microparticles, a strategy for follicular drug targeting?

Guilherme M. Gelfuso^{1,2,3}, M. Angélica de Oliveira Barros¹, M. Begoña Delgado-Charro², Richard H. Guy², Renata F. V. Lopez^{1*}

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ABSTRACT

The feasibility of targeting drugs to hair follicles by a combination of microencapsulation and iontophoresis has been evaluated. Minoxidil sulphate (MXS), which is used in the treatment of alopecia, was selected as a relevant drug with respect to follicular penetration. The skin permeation and disposition of MXS encapsulated in chitosan microparticles (MXS-MP) was evaluated *in vitro* after passive and iontophoretic delivery. Uptake of MXS was quantified at different exposure times in the stratum corneum (SC) and hair follicles. Microencapsulation resulted in increased (6-fold) drug accumulation in the hair follicles relative to delivery from a simple MXS solution. Application of iontophoresis enhanced follicular delivery for both the solution and the microparticle formulations. It appears, therefore, that microencapsulation and iontophoresis can act synergistically to enhance topical drug targeting to hair follicles.

Keywords: microparticle; iontophoresis; chitosan; minoxidil sulphate; hair follicle; topical drug delivery.

1. Introduction

Polymeric micro- and nanoparticles have been considered as vehicles for topical drug delivery due, at least in part, to their potential to protect unstable active ingredients and to provide sustained release over prolonged periods of time [1–5]. In addition, the use of such particles to target drugs to the hair follicles has been much debated [4–10], with their accumulation around the openings of hair follicles predicted to permit sustained, localized delivery to these skin appendages. This targeted drug delivery would offer potentially improved treatment of diseases such as acne and alopecia. However, while the association of particulate drug carriers to follicular openings has been clearly visualized in a number of reports in the literature [6–8], there is little quantitative evidence to support the contention that microencapsulation effectively enhances drug targeting to the hair follicles.

The skin disposition of micro- and nanoparticles remains a controversial issue. For example, Campbell et al. [10] used confocal microscopic skin imaging to demonstrate that polystyrene nanoparticles (ranging in size from 20 to 200 nm) did not penetrate beyond the most superficial layer of the skin [the outermost few microns of the stratum corneum (SC)] even when the skin had been modestly compromised. In contrast, Patzelt et al. [8] have claimed that particles of 640 nm diameter penetrated deeper (by about 1000-1200 μm) into hair follicles than those of smaller size (122 nm). In neither of these examples, nor in any other related study, was the delivery of an encapsulated active into the hair follicles, relative to its uptake into the SC, determined as a function of time. Such quantification of the skin disposition of the active, of course, is essential for any insight into the targeting efficiency of these micro/nanoparticle formulations.

With respect to iontophoresis, on the other hand, the significant contribution of the follicular pathway to topical drug delivery is well documented [11-14]. Iontophoresis employs a small electric current ($\leq 0.5 \text{ mA/cm}^2$) to increase and control drug delivery into and through the skin [15] and the lower electrical resistance of the appendageal path explains the increased involvement of this route during electro-transport [11–14].

We have previously described the ability of iontophoresis to target minoxidil sulphate (MXS) to the follicular route *in vitro* and *in vivo*. The anodal iontophoresis of this active, which is used to treat alopecia, increased by 5-fold the amount of drug reaching the follicular infundibula when compared with passive delivery [16]. Although the application of iontophoresis to enhance the overall skin permeation of lipophilic drugs encapsulated in 100 nm nanoparticles was recently examined [17–19], its use to

improve the follicular targeting of drugs encapsulated in microparticles has not yet been investigated.

In this context, the goal of the research described in this paper was to evaluate whether the combination of iontophoresis and microencapsulation would facilitate and enhance MXS delivery specifically to the hair follicles. The positively charged drug was chosen because of its therapeutic relevance and because its follicular targeting is anticipated to result in improved therapeutic efficacy. Chitosan was used to encapsulate MXS because of its low cost, biodegradability and non-immunogenicity. Further, chitosan is a cationic polymer that forms positively charged microparticles [2], a favorable feature for anodal iontophoresis.

2. Materials and Methods

2.1 Materials

MXS (99%) was kindly provided by Galena Química e Farmacêutica Ltda. (Campinas, Brazil). Chitosan (medium molecular weight, 75-85% of deacetylation), silver wire (99.99%, $\varnothing = 1.5$ mm), silver chloride (99.99%) and platinum wire ($\varnothing = 1.0$ mm) were purchased from Sigma-Aldrich (Steinheim, Germany). HEPES and NaCl were from Acros (Berkeley, NJ, USA). HPLC grade methanol, ethanol and acetonitrile were purchased from Fisher Scientific (Leicestershire, UK), and acetic acid was obtained from Fluka (Steinheim, Germany). The water used in the preparations of all solutions was of Milli-Q grade (Millipore, Illkirch-Graffenstaden, France).

2.2 Skin

Porcine skin was obtained less than 1 hour post-sacrifice from a local abattoir. The whole skin was removed from the abdominal region of the animal, separated from the underlying fascia, dermatomed to a nominal thickness of 700 μm , and stored frozen at -20°C for a maximum of six months before use.

2.3 Preparation of MXS loaded chitosan microparticles (MXS-MP)

MXS-MP were prepared as previously described [2]. Briefly, 3 g chitosan and 1.5 g MXS were dissolved in 200 mL of an aqueous solution of 1% (w/v) acetic acid at pH 4.0. This solution was then spray-dried with a 1.0 mm pressurized atomizer at a feed rate of 6 mL/min, and atomizing airflow rate of 6 m^3/min (Labmaq model MSD 0.5 spray

dryer, Ribeirão Preto, Brazil). The inlet and outlet temperatures were maintained at 135°C and 75–80°C, respectively. Post-preparation, the microparticles were kept at room temperature in a closed container until use. The spherical microparticles had a mean (\pm S.D.) diameter of 3.0 (\pm 1.5) μm and a zeta potential of +5.9 (\pm 5.2) mV; the drug loading efficiency of the chitosan microparticles was at least 80%. [2]. The amount of chitosan relative to MXS was 2:1 (w/w); 7.5 g of the MXS-loaded microparticles were suspended in 93 mL of ethanol/water (6:4 v/v), creating a formulation containing 2% w/v of MXS.

2.4 Passive drug delivery

The skin was thawed and mounted in a vertical Franz diffusion cell (area = 0.8 cm²). The donor compartment was filled with 1 mL of either a suspension of MXS-MP in ethanol-water (pH 5.5), or free MXS dissolved in the same vehicle (the control). Both formulations contained 2% w/v MXS. The donor compartment was closed with Parafilm[®] to minimize solvent evaporation. The receptor chamber was filled with a physiological buffer (pH 7.4) containing 25 mM HEPES and 133 mM NaCl and was magnetically stirred throughout the experiment.

Permeation experiments (n = 4-5) were performed with each of the formulations (MXS-MP or free MXS), over periods of 3, 6, 9, 12, 18 and 24 h, so that the kinetics of MXS delivery and skin disposition could be characterized. At the end of the experiments, the amounts of MXS present in the SC, the hair follicles and the receptor solution were quantified. For the SC and follicles, a differential tape stripping technique was employed (see section 2.7).

2.5 Iontophoretic drug delivery

These experiments (n = 3) were conducted following a similar procedure to that described above except that a direct constant current of 0.4 mA (0.5 mA/cm²) was applied (in this case for 3 hours only) and flow-through iontophoretic cells (LG-1088-IC-Laboratory Glass Apparatus Inc., Berkeley, CA, USA) were used. The iontophoretic current was passed between Ag/AgCl electrodes prepared as previously described [20] via a power supply (Kepco APH 500DM Power Supply, Toronto, Canada). At the end of iontophoresis, the amounts of MXS in the SC, hair follicles and receptor solution were again determined (see section 2.7).

2.6 Post-iontophoresis passive drug delivery

In a further series of experiments ($n = 4$), iontophoresis was carried out for 3 hours using the MXS-MP formulation and then stopped. Subsequently, the formulation was left in contact with the skin for an additional 3, 6 or 12 h, following which the amounts of MXS in the SC, hair follicles and receptor solution were determined (see section 2.7).

2.7 Differential tape stripping of MXS

The follicular and SC uptake of MXS was determined using differential tape-stripping. This methodology combines tape-stripping with cyanoacrylate skin surface biopsies [21]. The skin was removed from the diffusion cell and placed onto a flat board with the SC facing up. The residual formulation was cleaned from the SC surface with an isopropanol-soaked gauze pad and the skin was then tape-stripped 15 times, using Scotch Book Tape No. 845 (3M, St Paul, MN). The glistening, exposed viable layers of the epidermis suggested that complete removal of the SC had been achieved by this procedure. The amount of MXS in the tapes was determined by analytically (see section 2.8) after a 12-hour extraction of the drug with methanol.

Subsequent to SC removal, a drop of cyanoacrylate superglue (Henkel Loctite, Dublin, Ireland) was applied to the stripped skin area and covered with a further tape-strip using light pressure. After total polymerization of the glue (~5 minutes), the tape-strip was removed. This provided a biopsy containing follicular casts [21] from which MXS was extracted with methanol and quantified (see section 2.8). The efficiency of recovery of MXS from the SC and the follicular material was $94 \pm 1\%$ and $87 \pm 2\%$ [16], respectively.

2.8 MXS analysis

MXS in the methanolic skin and follicular cast extraction samples was assayed by HPLC with mass spectrophotometric detection (LCMS-2010 EV, Shimadzu, Kyoto, Japan). 5 μL samples were eluted with a mobile phase comprising water - acetic acid - acetonitrile (70:0.1:30 v/v; pH 3.3) pumped a flow rate of 1.0 mL/min in a reverse-phase column (4.0 x 125.0 mm, 5 μm , from Dionex Co., Salt Lake City, UT, USA). Detection was performed in positive ion mode (1.65 kV, $m/z = 210.05$). The calibration curve was linear ($r = 0.99$), over the concentration range 0.05 to 1.00 $\mu\text{g/mL}$. The LOQ and LOD were 0.05 and 0.005 $\mu\text{g/mL}$, respectively.

MXS in the aqueous receptor solution samples contained a considerable amount of salt, which could influence the mass detection, and hence was quantified by reverse-phase HPLC. 10 μL aliquots of the samples were injected into a liquid chromatograph (LC-2010A HT, Shimadzu, Kyoto, Japan) equipped with an automatic sample injector and the same reverse-phase column. The mobile phase was water - acetic acid - acetonitrile (30:0.1:70 v/v; pH 3.3) pumped a flow rate of 1.0 mL/min. MXS was detected by UV absorbance at 285 nm. The calibration curve was linear ($r = 0.99$) again over the concentration range of 0.05 to 1.00 $\mu\text{g/mL}$. The LOQ and LOD were 0.05 and 0.005 $\mu\text{g/mL}$, respectively.

2.9 Statistical analysis of the data

Three to five replicates of each transport experiment were performed. Results are presented as mean \pm standard deviation (SD) and expressed in terms of the quantity of MXS per unit area of skin ($\mu\text{g/cm}^2$). Skin permeation fluxes ($\mu\text{g/cm}^2/\text{h}$) and lag times were determined from the slopes and intersection of the linear section of the plot of cumulative amount of MXS permeated ($\mu\text{g/cm}^2$) as a function of time (h), respectively.

Differences between the passive penetration of MXS ($\mu\text{g/cm}^2$) from the microparticle formulation and the control solution were assessed by a parametric t-test. When comparing the passive and iontophoretic delivery of MXS from the microparticles with the drug's simple diffusion from the control solution, an ANOVA followed by Tukey's test was used. Statistical significance was fixed at $p < 0.05$.

3. Results and Discussion

The skin disposition and percutaneous absorption of MXS following passive drug delivery from either a simple solution or the microparticle suspension is shown in Figure 1 (and in Supplementary Table 1). The amount of drug that penetrated the skin into the receptor solution increased in the normal way as a function of time, with slightly more than double the quantity being absorbed from an aqueous solution as compared to that from the microparticle formulation (Figure 1a); the apparent steady-state fluxes of 2.08 (± 0.11) and 0.87 (± 0.15) $\mu\text{g/cm}^2/\text{h}$, respectively, were significantly different ($p < 0.05$). These results are consistent with the microparticles remaining on skin surface, and that it is only the drug, which has been released in a sustained manner from the carriers, that is able to cross the skin.

The SC and follicular uptakes of MXS, determined by the differential stripping technique, when delivered from the control solution and from the microparticles, are presented in Figure 1b. The levels of drug recovered in the SC tape-strips exhibit no clear trend. At 8, 12 and 18 hours, there is no obvious difference in the uptake from the two formulations; on the other hand, at 3 and 6 hours, and at 24 hours, the MXS recovered in the SC was apparently significantly, though less than 2-fold, higher ($p < 0.05$) from the control solution. In contrast, follicular delivery was consistently [and, again, significantly ($p < 0.05$)] greater from the microparticle suspension, a difference of nearly 5-fold being achieved at 6 hours. As previously reported [2], chitosan MXS-MP particles do indeed swell quickly in contact with ethanol/water (from 3.0 μm to 5.7 μm) and they release MXS efficiently thereafter (approximately 30%, 53%, and 72% after 3, 6 and 12 h, respectively [2]). It is reasonable to assume that these swollen microparticles remain on skin surface, being unable to cross the skin themselves. However, the microparticles appear to accumulate in follicular openings, a conclusion supported by the enhanced recovery of MXS from these appendageal structures.

Notably, the quantities of MXS recovered from the follicles were appreciably smaller than those found in the SC. However, when the differential surface areas available for SC and follicular transport are taken into account (i.e., the follicles are typically estimated to occupy only 0.09-1.28% of the total skin surface [22]), it is clear that the microencapsulation of the drug has dramatically improved the efficiency with which it has reached (or been targeted to) the follicles. Furthermore, it may be anticipated that this finding would be even more significant in human scalp skin (as compared to the porcine abdominal tissue used here) where the follicular density is approximately an order of magnitude higher (240 follicles/ cm^2 compared to about 20 in the pig [23,24]).

Previously, it has been shown [16] both *in vitro* and *in vivo* that the follicular delivery of MXS from an aqueous formulation was increased by the application of iontophoresis. Because the chitosan-based MXS-MP have a positive zeta potential [2], it was hypothesised that an iontophoretic current would enhance follicular localisation when the drug delivery system was associated with the anode (and also permitting advantage to be taken of the anode-to-cathode electroosmotic flow typically established across the net negatively-charged skin membrane [15]). At the pH of the formulation (pH \sim 5.5), MXS is 90% non-ionized suggesting that its enhanced, electrotransport-mediated delivery will be due to a combination of electrorepulsion and electroosmosis [16]. Experiments were performed, therefore, in which the passive data discussed already were compared to results obtained when the formulations (solution control and MXS-MP) were subjected to a 3-hour period of anodal iontophoresis. MXS

permeation to the receptor phase, as well as SC and follicular uptake, were determined both immediately post-application of current, and (for MXS-MP) as a function of time over the next 12 hours with no current applied but with the formulation remaining in contact with the skin.

Figure 2 (and Supplementary Table 1) shows the disposition of MXS at the end of 3 hours of iontophoresis following application of the solution control and microparticle formulations, and includes the corresponding passive data for reference. Clearly, iontophoresis increased MXS delivery from both the control solution and the microparticles both into the SC and, even more dramatically, into the follicles and across the skin. However, when comparing the two formulations with iontophoresis, there was no significant difference ($p > 0.05$) in either SC uptake or permeation of MXS; only for follicular targeting was MXS delivery significantly better ($p < 0.05$) with iontophoresis from the microparticle vehicle ($3.9 \pm 0.3 \mu\text{g}/\text{cm}^2$ compared to $2.4 \pm 0.6 \mu\text{g}/\text{cm}^2$ from a simple solution). For both formulations, though, the relative enhancement in follicular uptake achieved by the application of current was 6- to 8-fold, with the improved bioavailability being achieved more rapidly as well.

Even with iontophoresis, the microparticles used are too large to be transported, to any significant extent, through the skin by the applied electric field. However, because the follicles are low resistance pathways for the movement of charge across the skin [11-14], we speculate that iontophoresis can quickly and efficiently target MXS-MP to follicular openings, and that MXS is then released from the microparticles directly into the infundibulum. As stated above, we believe that only drug that has been released from the microparticles is able to cross the skin (with or without iontophoresis); nevertheless, because iontophoresis 'targets' the MXS-MP to the follicles, increased levels of drug are made available to be taken up and absorbed at these specific sites. From a practical standpoint, for the topical treatment of alopecia, where a local effect is desired with as little transdermal exposure as possible, the judicious selection of current density and application time *in vivo* is required for optimal therapy.

MXS disposition following 3 hours of iontophoresis of the microparticle formulation was then assessed over the next 12 hours (while leaving the formulation in contact with the skin). These experiments were able to shed light on whether the apparently enhanced deposition of microparticles into hair follicle openings could sustain MXS delivery post-iontophoresis. The results are shown in Figure 3. While the permeation of MXS to the receptor phase continued to increase with time (a finding not unexpected as the MXS-MP were left in contact with the skin once the current had been turned off), the levels of

drug in the SC and, crucially, in the follicles remained essentially unchanged, supporting the continued availability of the drug close to the therapeutic target.

4. Conclusion

The encapsulation of MXS in chitosan microparticles (MXS-MP) *in vitro* after passive delivery resulted in increased (6-fold) drug accumulation in the hair follicles relative to delivery from a simple MXS solution. Application of iontophoresis further enhanced follicular delivery from both the solution and the microparticle formulations. **The amount of MXS taken up into follicles after iontophoresis of microparticles was significantly greater ($p < 0.05$) compared to that from a simple MXS solution. We consider that this important enhancement in MXS follicular retention together with the sustained release of the drug when iontophoresis is combined with microparticles represents a potentially useful strategy for the targeted treatment of alopecia.**

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Figures Legends

Figure 1. Percutaneous absorption and skin disposition of MXS as a function of time after passive delivery from either an aqueous solution (control) or from a microparticle suspension. (a) Skin permeation of MXS into the receptor phase. (b) SC and follicular uptakes of MXS (left and right hand y-axes, respectively).

Figure 2. Percutaneous absorption and skin disposition of MXS as a function of time after 3 hours of iontophoresis or passive delivery from either an aqueous solution (control) or from a microparticle suspension (mean \pm SD of 3 replicates).

Figure 3. MXS levels permeated to the receptor phase, and localized in the SC and hair follicles as a function of time post 3 hours of iontophoresis following application of MXS-MP (values are mean \pm SD of 4 replicates).

Figure 1
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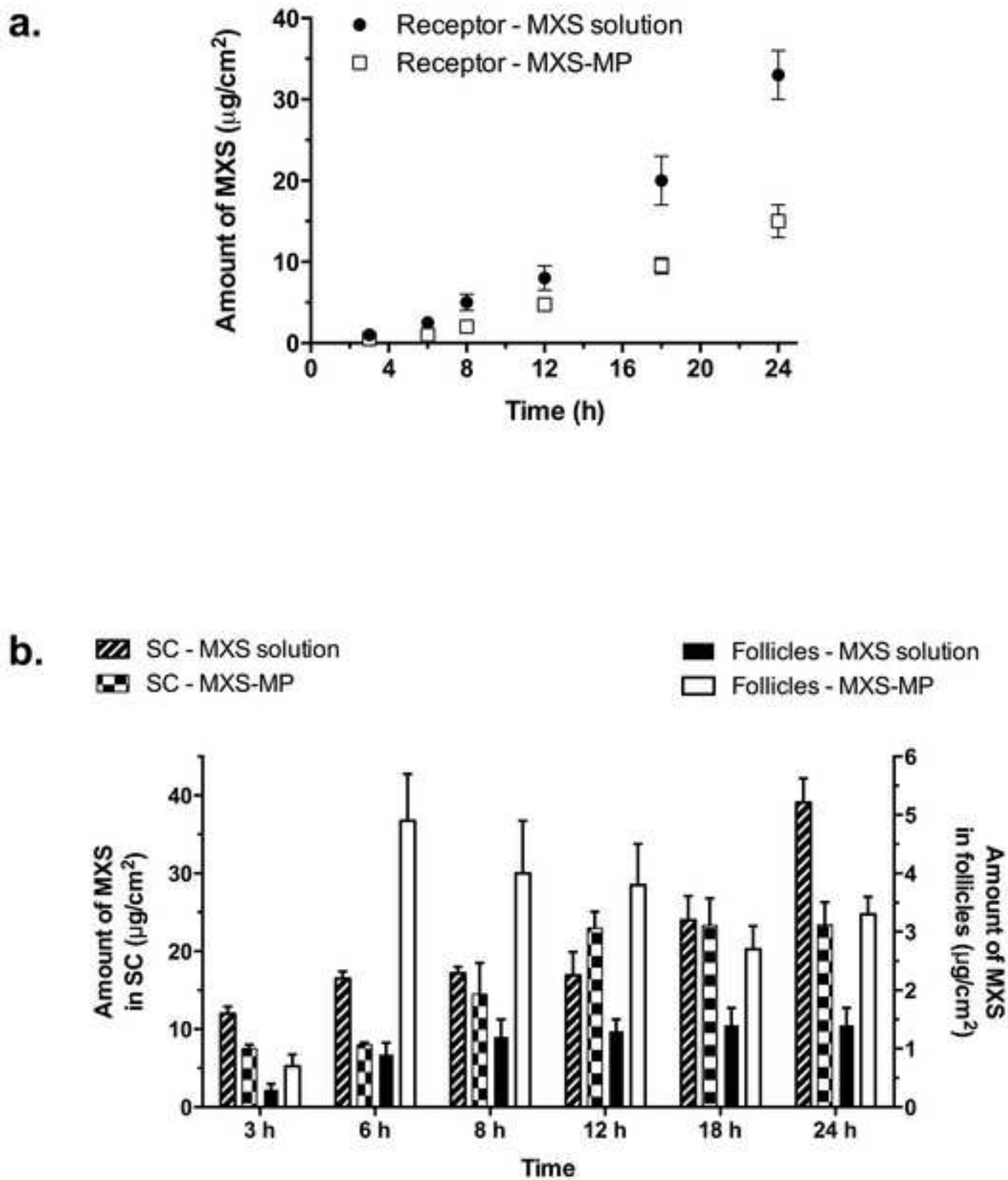


Figure 2
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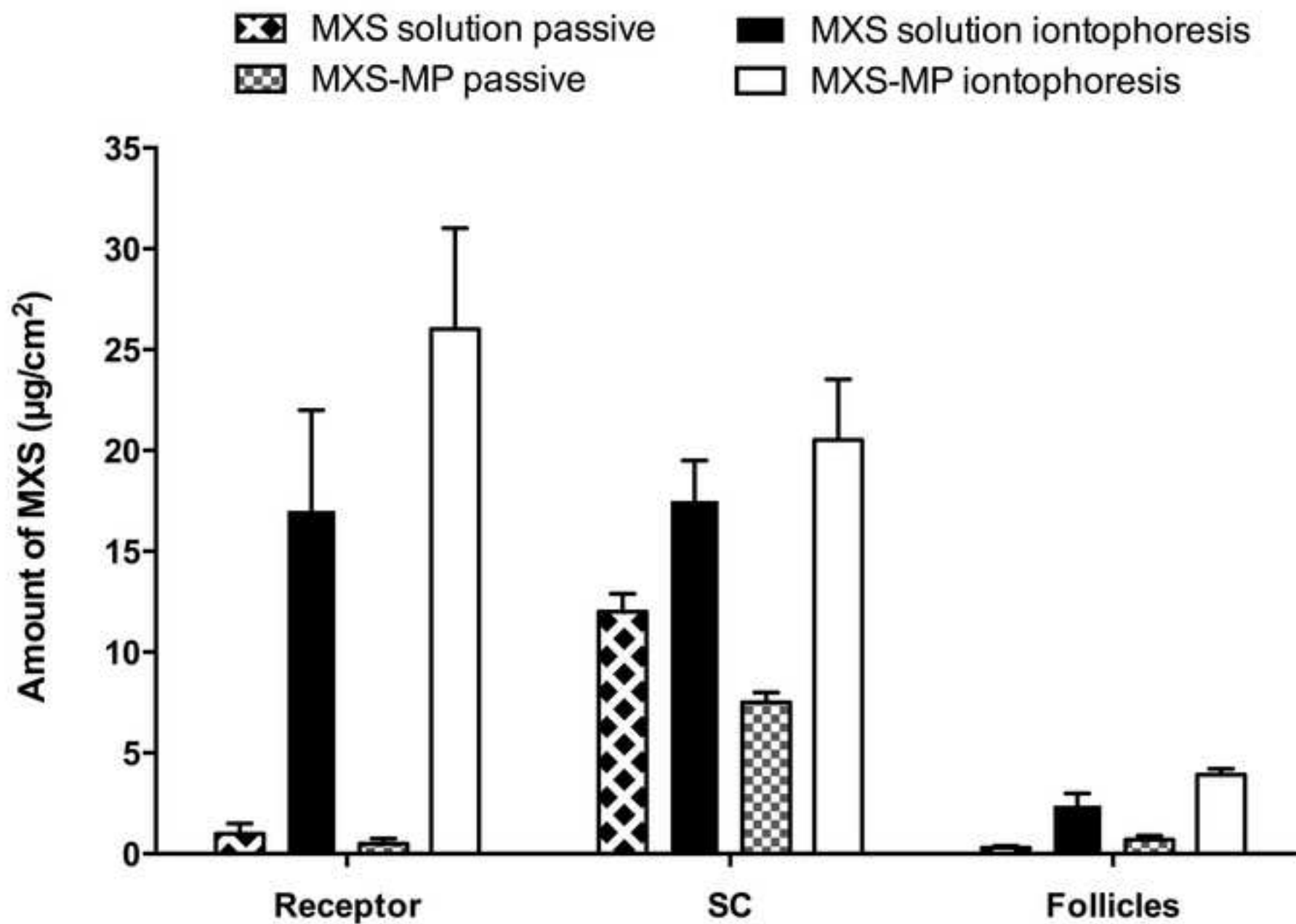
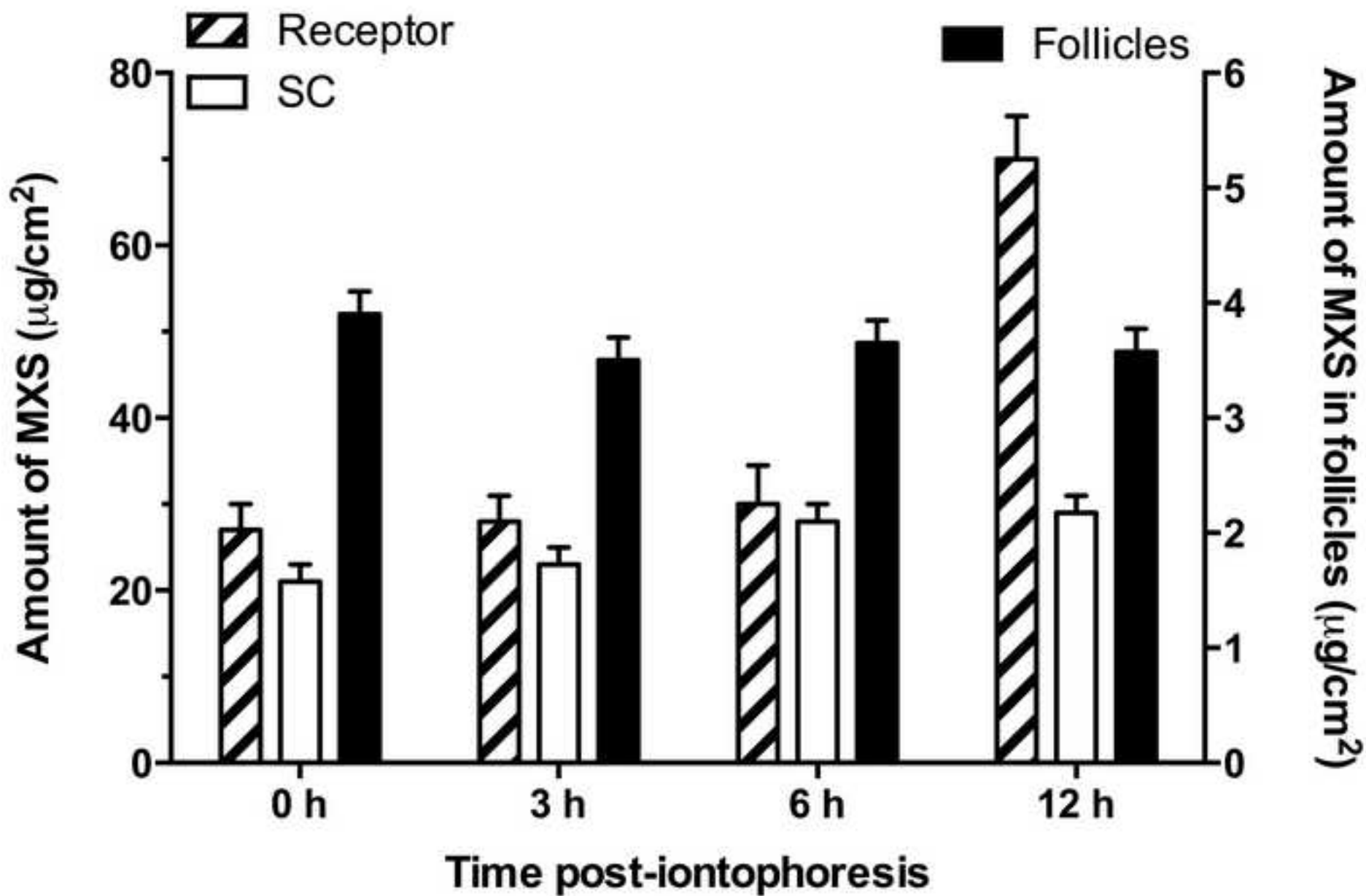


Figure 3
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Supplementary Table 1

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