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A thermosensitive morphine-containing hydrogel for the treatment of large-scale skin wounds

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ABSTRACT

Purpose: Topically applied opioids are an option to induce efficient analgesia in patients with severe skin wounds. For ongoing pain reduction, the vehicle should provide sustained drug release in order to increase the intervals during the regular wound dressing changes. In addition, the formulation should not impair wound healing. Hydrogels provide a moist wound environment, which is known to facilitate the healing process.
Methods and results: Investigating poloxamer hydrogels as a carrier system for morphine in terms of release behavior and (per-)cutaneous absorption, poloxamer 407 25 wt.% hydrogel sustained morphine release up to 24 h. The drug release rate decreased with increasing concentration of the gel forming triblock copolymer. Poloxamer 407 25 wt.% hydrogel retarded morphine uptake into reconstructed human skin and percutaneous drug absorption compared to a hydroxyethyl cellulose reference gel.
Conclusions: The results of our in vitro study indicate that the thermosensitive poloxamer 407 25 wt.% hydrogel is an appropriate carrier system for the topical application of morphine with regard to sustained drug release and ongoing analgesia.

1. Introduction

Skin wounds especially due to burns or skin grafting can be very painful and thus require systemically applied opioids for treatment, which bears the risk of severe and encumbering adverse effects like respiratory depression or constipation. Opioid receptors are expressed in several peripheral structures including sensory nerve endings, human keratinocytes, human dermal fibroblasts as well as melanocytes in the human epidermis (Bigliardi et al., 1998, 2009; Salemi et al., 2005; Stein et al., 2001). Opioid receptors are upregulated during inflammation. By forming clusters in the vicinity of peripheral sensory nerve endings and producing the endogenous opioid receptor ligand β-endorphine, keratinocytes can directly influence pain sensation (Bigliardi-Qi et al., 2004). Moreover, the morphine-enhanced keratinocyte and oral epithelia cell migration facilitate reepithelisation in standardized wound healing models of human skin (Küchler et al., 2010; Wolf et al., 2009) and oral mucosa (Charbaji et al., 2012), respectively. Thus, despite of delayed healing in early stage due to suppressed release of pro-inflammatory peptides (Rook and McCarson, 2007), topical opioid treatment is an option to gain local analgesia and to reduce or possibly avoid systemic unwanted adverse effects. The outcome from case studies and pilot clinical studies on local morphine treatment for painful skin ulcers, however, is not fully convincing with respect to efficacy and tolerability (Flock, 2003; Zeppetella et al., 2003; Zeppetella and Ribeiro, 2005).

A critical situation is the required repeated replacement of the wound dressing, which is very painful for the patient and bears the risk of destroying the regenerated epithelia. Therefore, on the one hand, the interval of the changes should be as long as possible. On

Abbreviations: A, exposed surface area; BSA, bovine serum albumin; C_i, initial concentration in the donor compartment; DAB, German pharmacopoeia; dC_A/d_t, increasing concentration of the substance in the receptor medium with increasing time; EFT-400, Epiderm^{FT} reconstructed human full thickness skin; HEC, hydroxyethyl cellulose; HPLC, high-performance liquid chromatography; K, kinetic constant [μg/h$^{1/2}$]; OECD, Organization for Economic Co-Operation and Development; P, poloxamer; P_{app}, apparent permeability coefficient [cm/s]; PBS, phosphate buffered saline pH 7.4; Q_t, cumulative morphine amounts [μg]; RHE, reconstructed human epidermis; RHS, reconstructed human skin; SD, standard deviation; V, volume.

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the other hand, only while changing the dressing, the opioid formulation can be reapplied topically. In consequence, there is a need for a formulation, which provides sustained opioid release and enables ongoing analgesia. There are a number of additional requirements that this formulation has to fulfill (Boateng et al., 2008). Particularly, its spreading on the wound surface has to be efficient and tolerable and microbiological contamination of the wound must be excluded. Furthermore, the topical formulation should be compatible with the tissue exposed at the skin lesion by providing a moist environment, which is why hydrogels based on hydrophilic polymers appear as the most suited carrier due to their high water content. While hydrophilicity of the involved polymers is a precondition for water uptake, the swelling of the matrix has to be limited by a polymer network structure with covalent netpoints, physical netpoints, or other superlattices in order to establish a hydrogel rather than a polymer solution.

In pilot studies, mainly opioid loaded INTRASITE™ gel (2.3% modified carboxymethyl cellulose polymer with 20% propylene glycol) has been tested. This hydrogel provides a moist wound milieu supporting the healing process and facilitates wound debridement, while 50% of the active ingredient is rapidly released within 2 h (Farley, 2011). Alternative hydrogel matrices may be based on thermosensitive triblock copolymers, namely poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) [poloxamers]. Poloxamers can self-assemble into micellar structures and under certain conditions form hydrogels at concentrations above the critical gelation concentration, with the sol-to-gel transition temperature T_{gel} dominated by the polymer concentration as well as polymer composition and block molecular weight [for review see (Dumortier et al., 2006)]. While different models have been proposed to describe the involved processes and morphological changes during sol-to-gel transition of triblock copolymers (Missirlis et al., 2006; Yu et al., 2007), it can be assumed that long distance micellar movements are limited in the gel state and the build lattice acts as a diffusion barrier for incorporated compounds. For instance, poloxamers were used for the percutaneous fentanyl (Liaw and Lin, 2000) and naltrexone (Derakhshandeh et al., 2010) delivery as well as the topical application of many other agents (Liu et al., 2007; Takahashi et al., 2002; Zhang et al., 2002).

The present study describes the development and characterization of a morphine loaded poloxamer 407 25 wt.% hydrogel morphine uptake by and permeation of reconstructed human skin (RHS).

2. Materials and methods

2.1. Materials

EpiDermFT™, reconstructed full thickness human skin (EFT-400, diameter 12 mm, RHS) was delivered by MatTek Corporation (Ashland, MA, USA) along with the maintenance medium. Morphine-HCl, hydromorphon-HCl and hydroxyethylcellulose 250 HX Pharm (= HEC 10000) were purchased from Fagron (Hamburg, Germany). Poloxamer 188 (Lutrol F 68), poloxamer 338 (Lutrol F 108) and poloxamer 407 (Lutrol F 127) were kindly donated by BASF (Ludwigshafen, Germany; Table 1). Bovine serum albumin (BSA) was purchased from Carl Roth, Karlsruhe, Germany. Cellulose nitrate filter (NC 03; pore size 25 nm; diameter 25 mm) and polycarbonate filter (Nucleopure®; pore size 15 nm; diameter 25 mm) were obtained from Whatman (Dassel, Germany).

Stock solutions of the opioids for HPLC analysis were prepared in a mixture of 0.05 M potassium hydrogen phosphate buffer pH 4.9 and acetonitrile (90:10) and remained stable at −20 °C for up to three months.

2.2. Hydrogel preparation and characterization

Poloxamer – hydrogels. Morphine hydrochloride was incorporated into poloxamer – hydrogels using the cold method (Derakhshandeh et al., 2010). Briefly, the opioid was dissolved in pre-cooled (5 °C) bi-distilled water. Bi-distilled water was used since it has to be used for the preparation of hydroxyethylcellulose 10000 hydrogel according to the German pharmacopoeia (DAB). A defined amount of the gel forming agent (see Table 1) was added to the solution and dissolved under stirring for 24 h at 5–10 °C. The morphine-HCl concentrations were 0.5 mg/ml (release and the infinite dose approach of drug absorption studies) and 2.22 mg/ml (finite dose approach of drug absorption studies).

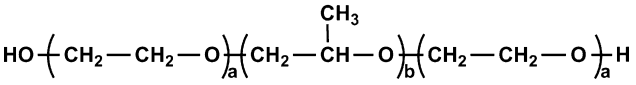
Hydroxyethylcellulose (HEC) 10000 hydrogel. HEC 10000 hydrogel was prepared as described in the German pharmacopoeia (DAB). Briefly, morphine-HCl was dissolved in bi-distilled water. HEC 10000 (2.5 wt.%) was poured in a plastic mortar and homogenized with glycerol 85 wt.% (10 wt.%). Morphine solution (87.5 wt.%) was added in fractions under soft stirring with a pestle. The gel formed within 1 h at room temperature.

Viscosity characterization. The rheological properties of poloxamer 407 gels (without morphine) were studied using a Haake Mars Modular Advanced Rheometer (Thermo Electron Corporation, Karlsruhe, Germany) with 20 mm plate/plate geometry with an integrated solvent trap in order to avoid water evaporation during the measurement. At a gap size of 1 mm, temperature ramps with a heating rate of 0.5 K/min were applied between 5 and 45 °C in oscillatory measurements with controlled deformation (amplitude 0.03 rad; frequency 0.5 Hz). T_{gel} was determined from the inflection point of the recorded graph of the complex viscosity η^*.

2.3. Morphine release

Morphine release was studied under sink conditions using cellulose nitrate membranes (pore size 25 nm) or polycarbonate membranes (pore size 15 nm) in static Franz diffusion cells (diameter 15 mm, volume 12 ml; PermeGear, Bethlehem, PA, USA) (Lombardi Borgia et al., 2008). Phosphate buffered saline pH 7.4 (PBS) with an addition of 1.5% bovine serum albumin was used as receptor medium which was maintained at 33.5 ± 1 °C and magnetically stirred at 500 rpm. Membranes, which were preincubated in bi-distilled water over night and subsequently for 30 min in

Table 1
Poloxamer properties (technical information, BASF, 2010).

Block copolymer				
	Ethylene oxide repetitive units (a)	Propylene oxide repetitive units (b)	Viscosity [cps]	Pour/melt point [°C]
Poloxamer 188	80	27	1000	52
Poloxamer 338	141	44	2800	57
Poloxamer 407	101	56	3100	56

PBS/BSA, were inserted between the donor and the receptor compartment of the Franz cell. After 30 min of equilibration in the absence of the hydrogel, a sample of the receptor fluid (=0 h sample) was collected. 282.1 μl/cm^2 of the test preparation was applied on the membrane and spread homogeneously using a nylon mesh. The donor compartment was sealed with Nescofilm®. The receptor fluid was collected in fractions of 500 μl and replaced by fresh medium at 0.5–4 h (or 24 h) depending on the tested formulation. Experiments were at least performed in triplicates.

2.4. (Per-)Cutaneous absorption

For the determination of (per-)cutaneous absorption (penetration and permeation, respectively), RHS (EFT-400, three batches; all experiments in duplicate) with both the finite and the infinite dose approach were used (Ackermann et al., 2010; Schäfer-Korting et al., 2006, 2008). Again, the experimental set up included the static Franz diffusion cells and 1.5 wt.% BSA in PBS (receptor medium).

RHS arrived the day before the experiment and were transferred from the delivery plate into a 6-well plate, each plate containing 2.5 ml of pre-warmed maintenance medium. Tissues were pre-incubated (37 °C, 5% CO_2) over night, before being mounted into the Franz cell together with the supporting membrane. The stratum corneum of RHS was facing the donor compartment. Since the size of the EFT-400 with a diameter of 12 mm was too small to be directly mounted onto the Franz cell, special inserts constructed for skin models were used, resulting in a smaller surface area of $A = 0.385$ cm^2 (Netzlaff et al., 2007). The support membrane was in contact with the receptor medium, which was maintained at 33.5 ± 1 °C and magnetically stirred at 500 rpm. After 30 min of equilibration, a sample of receptor fluid (=0 h sample) was collected.

262.3 μl/cm^2 of the test preparation for infinite dose experiments were applied onto RHS, the donor compartment was sealed with Nescofilm and the receptor fluid was collected in fractions of 500 μl for 6 h. For finite dose experiments 25.1 μl/cm^2 of the test preparation were applied for 3 or 6 h, then RHS were removed and the stratum corneum was wiped clean with small cotton tissues soaked in PBS. Subsequently, epidermis, dermis, and the supporting membrane were separated using forceps and kept under −20 °C until subjected to drug analysis for the calculation of the mass balance (Schäfer-Korting et al., 2008).

2.5. Quantification of morphine

Aqueous samples were used directly for extraction; bi-distilled water was added to the supporting membrane and cotton tissues before the extraction. Bi-distilled water and one 5 mm stainless steel bead were added to the epidermis and dermis for homogenization (25 Hz, 2.5 min) by the Tissue Lyser II (Qiagen, Hilden, Germany). Following the addition of hydromorphone as internal standard, the samples were adjusted to pH 9.5 using 1 N NaOH and extracted three times with equivalent volumes of ethyl acetate by vortexing the mixture for 1 min. After phase separation by centrifugation (3 min at 14,000 rpm), the organic phases were combined and evaporated (Savant SC 210 A Speed Vac concentrator, Thermo Scientific, Langenselbold, Germany). The residues were dissolved in 500 μl methanol, centrifuged (1 min) and 450 μl of the supernatants were evaporated again. The residues were dissolved in potassium hydrogen phosphate buffer pH 4.9/acetonitrile (90:10, v/v) and 100 μl of the final solution (200 μl for release studies, 120–150 μl for skin absorption studies depending on the matrices) were analyzed by HPLC (column: LiChroCART® 250-4 RP-8, 5 μm, pre-column: LiChroCART® 4-4) with fluorescence detection system in combination with an UV-detector. HPLC conditions were as follows: liquid phase 92 vol.% potassium hydrogen phosphate buffer (pH 4.9) + 8 vol.% acetonitrile, flow rate 1 ml/min, detection: UV (210 nm) plus fluorescence (excitation 235 nm, emission 345 nm), run-time: 30 min. Fluorescence-detection was used for morphine and UV-detection for hydromorphone quantification. The retention time of morphine and hydromorphone was 6.5 min and 10.5 min, respectively. The range of linearity of this method was 0.1–100 μm for morphine and 1–100 μm for hydromorphone ($R^2 \geq 0.99$). Day-to-day variation was tested for both ranges and proven to be suitable ($SD_{rel} \leq 6.5\%$).

2.6. Data evaluation

Drug release. The Higuchi plot was used for the calculation of release kinetics. Mean cumulative morphine amounts found in the receptor compartment were plotted against the square-root of time (Higuchi, 1961; Lombardi Borgia et al., 2008):

$$Q_t = K \cdot t^{(1/2)} \quad (1)$$

In Eq. (1), Q_t represents the cumulative morphine amount found in the receptor medium [μg] and $t^{(1/2)}$ is the square-root of time [$h^{1/2}$]. K (slope of the plot, calculated using linear regression) represents the kinetic constant [$\mu g/h^{1/2}$], which is an indicative for the release rate.

Skin absorption of morphine. In order to characterize the permeation behavior of morphine, the apparent permeability coefficient (P_{app}) and the lag-time (=x-axis intercept) were calculated using an automated method (Niedorf et al., 2008; Schäfer-Korting et al., 2008). The apparent permeability coefficient was calculated using the following Eq. (2), where C_i represents the initial concentration in the donor compartment (0.5 mg/ml), A the exposed surface area (0.385 cm^2), V the volume (12 cm^3), and dC_A/d_t the increasing concentration of the substance in the receptor medium with increasing time.

$$P_{app} \quad (\text{permeability coefficient}) = \left(\frac{V}{A \cdot C_i}\right) \cdot \frac{dC_A}{d_t} \quad (2)$$

2.7. Statistics

Data (mean ± SD) were evaluated by explorative data analysis using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). Release data (K) were subjected to Kruskal–Wallis analysis and in case of a significant difference between the outcome parameters, comparison was performed by Dunn's Multiple Comparison test. Data of (per-)cutaneous absorption (P_{app}, lag-time, amount of morphine in the different matrices) were subjected to Mann–Whitney U test. $p \leq 0.05$ indicates a statistically significant difference.

3. Results and discussion

Morphine loading to hydrogels. Hydrogels and in particular poloxamers were not only used for the topical delivery of various active ingredients (Derakhshandeh et al., 2010; Dumortier et al., 2006; Liaw and Lin, 2000; Liu et al., 2007; Takahashi et al., 2002; Zhang et al., 2002), but were also suggested as "artificial skin" for the treatment of severe burns (Nalbandian et al., 1987; Schmolka, 1972). Thus, poloxamers were chosen as gel forming agents for the development of a morphine loaded hydrogel for topical application on skin wounds. By exploring poloxamers with different block lengths (Table 1), the influence of the gel vehicle on morphine release was derived by the kinetic constants calculated using the Higuchi plot. After an initial lag-time, the slop of the curves was linear (for >4 h; Fig. 1A), which is characteristic for diffusion-controlled release under the given geometric conditions. The corresponding kinetic constants (slope of the regression curve)

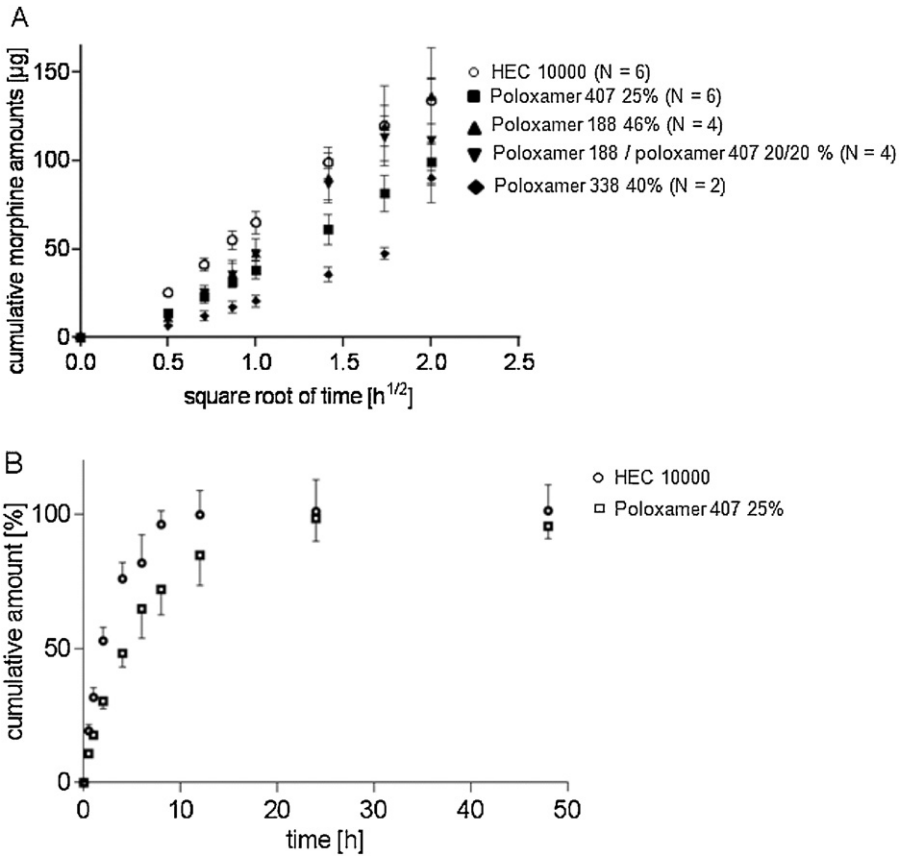


Fig. 1. Cumulative morphine release (mean values ± SD; 4 h) from poloxamer and HEC gels. (A) Higuchi plot and (B) linear plot over a period of 48 h ($n = 6$). Cellulose nitrate membranes with a pore size of 25 nm (A) and polycarbonate membranes (B) with a pore size of 15 nm were used. In all cases, phosphate buffered saline pH 7.4 with an addition of 1.5% BSA as acceptor medium, and bi-distilled water for dissolving the polymers were used.

are depicted in Table 2. Compared to HEC 10000 gel used as reference because of its use in clinical studies, the poloxamer 338 40 wt.% gel and the poloxamer 407 25 wt.% gel resulted in the smallest kinetic constants and therefore provide slowest morphine release. Both poloxamer gels showed a thermosensitive sol–gel transition. At temperatures of about 4–8 °C they can be easily handled as a viscous liquid, but if they are warmed up to room temperature, they undergo a sol-to-gel transition (Derakhshandeh et al., 2010; Dumortier et al., 2006; Escobar-Chavez et al., 2006). This is advantageous for the application on skin wounds, since sols can be applied easily using a syringe and then directly form a firm overlay covering the wound upon contact with the skin. Although $K_{P\ 338\ 40\%}$ is smaller than $K_{P\ 407\ 25\%}$, we decided to use poloxamer 407 25 wt.% hydrogel for further experiments since the critical gelation concentrations of poloxamer 338 40 wt.% is higher due to its shorter hydrophobic block. Furthermore, the highly viscous cold poloxamer 338 40 wt.% solution hampered reproducible application.

Table 2
Type and composition of poloxamer × hydrogels and the corresponding kinetic constants ($n = 2$–6) derived by Higuchi plot from morphine permeation of cellulose nitrate membranes (25 nm) in Franz cells in comparison to hydroxyethylcellulose (HEC 10000) gel (Fig. 1A). All gels were used at a morphine concentration of 0.5 mg/ml.

Gel forming agent	Concentration of the gel forming agent [%, w/w]	Kinetic constant [µg/h$^{1/2}$]
Poloxamer 188	46	87.8
Poloxamer 188 + poloxamer 407	20 + 20	81.9
Poloxamer 407	25	57.4
Poloxamer 338	40	33.5
HEC 10000	–	73.6

Influence of poloxamer 407 concentration. As the concentration of the polymer affects the volume of the matrix occupied by micelles and thus can be expected to have a major influence on the effective diffusion length of drug molecules through the formed gel until being released, we investigated whether this holds true for poloxamer 407 25 wt.% concentration and morphine release, too. When the polymer concentration of morphine loaded poloxamer 407 25 wt.% hydrogels was increased (20 wt.%, 25 wt.% and 27.5 wt.%), the kinetic constants (Table 3) reveal a prolonged morphine release for higher concentrations of poloxamer 407. This is well in accordance with decreasing fentanyl, ceftiofur, or hirudin release rates with increasing concentration of poloxamer 407 (Derakhshandeh et al., 2010; Liu et al., 2007; Zhang et al., 2002) and poloxamer 188 (Liaw and Lin, 2000).

In the micelle-based poloxamer gels, hydrophobic poly(propylene oxide) blocks are aggregated in the core of the micelles and form rather water-poor domains with an expected low diffusivity for water soluble drugs. Therefore, the diffusion controlled drug release will occur through the continuous water rich phase that surrounds the individual hydrophobic domains. This water rich phase should (mostly) be occupied by the micelles hydrophilic poly(ethylene oxide) shell. In contrast to hydrogel networks with defined netpoints (e.g., covalent bonds, crystallites) and known polymer segment lengths in between the netpoints, the mesh or pore sizes can hardly be assessed for such colloidal gels. In the micellar system above T_{gel}, the restricted mobility and deformability of the micelles and their potential interaction limits drug diffusion and, at the same time, defines the macroscopic

Table 3
Dependency of the kinetic constants derived by Higuchi plot from morphine permeation from the pore sizes of membranes used in the Franz cell experiment (25 nm cellulose nitrate membrane vs. 15 nm polycarbonate membranes) and the concentrations. *A significant difference ($p \leq 0.05$) compared to HEC 10000 gel ($n = 4$–6, mean ± SD).

Hydrogel	Kinetic constant [μg/h$^{1/2}$]		η^* at 32 °C [Pa s]	T_{gel} [°C]
	Pore size 25 nm	Pore size 15 nm	-	
Poloxamer 407 20%	73.7 ± 4.8	56.7 ± 7.0	252 ± 3	24.8 ± 0.1
Poloxamer 407 25%	57.4 ± 5.2*	47.5 ± 6.2	359 ± 1	21.8 ± 0.1
Poloxamer 407 27.5%	57.4 ± 8.3*	41.9 ± 2.9*	446 ± 2	19.8 ± 0.1

Complex viscosity η^* and sol–gel transition temperature T_{gel} of morphine-free poloxamer 407 hydrogels with varying concentrations of the gel forming agent ($n = 3$, mean ± SD). Bi-distilled water was used for dissolving the polymers.

mechanical properties such as viscosity of the gel. Therefore, in order to further characterize the gels, rheological characterization was performed under the rough assumption that the hydrogels viscosity at a given temperature may be useful as a surrogate for the micellar packing that effects drug diffusion. As summarized in Table 3, the gels complex viscosity increased with increasing poloxamer concentration and was highest for the poloxamer 407 27.5 wt.% hydrogel. Additionally, the slowest morphine release was obtained for this hydrogel composition (Table 3).

Nevertheless, we decided to use the poloxamer 407 25 wt.% hydrogel for the following experiments because of its superior applicability and only minor differences in release compared to higher concentration (27.5%) of the gel forming agent (Table 3). We also determined T_{gel} of the different poloxamer 407 gels (Table 3), which illustrate the dependency of the thermosensitive gelation from the polymer concentration.

As a result of the hydrophilicity of the polyethylene oxide chain segments and due to the colloidal structure of poloxamer gels lacking suitable netpoints as in physical/covalent networks, poloxamers can absorb water from the acceptor compartment. Accordingly, along with the increase of the gels water content and decreasing polymer concentration, eventually falling below the critical gelation concentration, the gels started to liquify, which resulted in a decreased effective drug diffusion length and enhanced morphine release. The larger the pore sizes of the membrane used in the Franz diffusion cell, the easier water can be absorbed. Two different membranes were used for the determination of the influence of poloxamer 407 concentration on morphine release. Release rates decreased with decreasing pore sizes of the membrane (Table 3) and allowed for a more sensitive discrimination of release properties of the different gels.

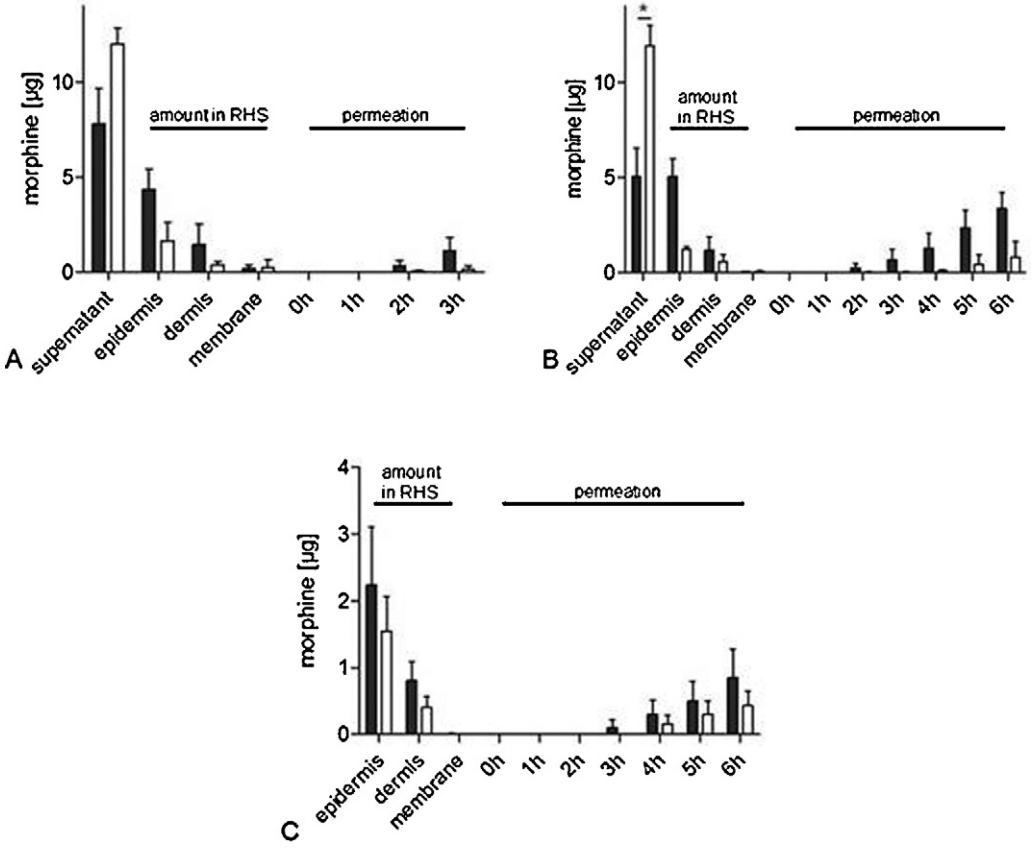


Fig. 2. (Per-)cutaneous morphine absorption using the finite dose approach (application of 15.7 μg morphine) over a period of 3 h (A) and 6 h (B) or the infinite dose approach (C; application of 39.7 μg) over a period of 6 h. Black: hydroxyethylcellulose gel; white: poloxamer 407 25 wt.% hydrogel. *A significant difference was observed ($p \leq 0.05$; $n = 3$).

Next, we aimed to investigate morphine release over 48 h from poloxamer 407 25 wt.% hydrogel in comparison to the HEC 10000 gel, the first being superior with respect to the lower kinetic constant (Table 2). As depicted in Fig. 1B, the poloxamer 407 25 wt.% hydrogel provided a prolonged release: 100% morphine release was achieved after 12 h from HEC 10000 gel and after 24 h from poloxamer 407 25 wt.% hydrogel. Thus, both hydrogels provide a more sustained morphine release compared to INTRASITE™ gel which was used in previous clinical studies and case reports (Farley, 2011). An even lower release rate was seen with naltrexone from a similar poloxamer gel, where 80% were released within 48 h (Derakhshandeh et al., 2010).

(Per-)Cutaneous absorption of morphine. Next, we studied the (per-)cutaneous absorption of morphine from poloxamer 407 25 wt.% hydrogel. Again, HEC 10000 gel was used as reference gel. Reconstructed human skin can be used for skin absorption studies, since comparable absorption by RHS, reconstructed human epidermis (RHE), and human skin ex vivo are given (Ackermann et al., 2010; Schäfer-Korting et al., 2008). For the determination of morphine penetration and permeation we decided for the Epiderm^{FT} RHS construct, which was also used for the development of an in vitro 3D wound healing model (Küchler et al., 2010). Morphine penetration into epidermis and dermis and the permeation of morphine through RHS as determined using the finite and infinite dose approach is depicted in Fig. 2. Mass balance calculated following finite dose experiment on HEC 10000 gel being 96.6% proved the very good data quality. A lower mass balance (81.1%) when testing poloxamer 407 25 wt.% gel is due to the higher adhesivity. Adhering to the donor chamber poloxamer 407 25 wt.% gel could not be easily wiped off with cotton wool tissues resulting in a lower mass balance. Accuracy of morphine application and analytical accuracy is not affected.

In comparison to the poloxamer 407 25 wt.% gel, a larger amount of morphine penetrated into epidermis and dermis from HEC 10000 gel. Additionally, larger amounts of morphine could be detected in the receptor compartment illustrating stronger permeation through RHS. We also calculated the apparent permeability coefficient (P_{app}) and the lag-time using the infinite dose approach. The reference gel (P_{app}: $161 \pm 50 \times 10^{-5}$ cm/s) clearly showed a higher apparent permeability coefficient than the poloxamer gel (P_{app}: $87 \pm 30 \times 10^{-5}$ cm/s) and a moderately higher drug penetration after 3 and 6 h (Fig. 2), which indicated faster morphine absorption and correlated with the above reported limited retention of morphine release from the HEC gels. The lag-times of both gels (3.06 ± 0.29 h HEC 10000 gel; 3.17 ± 0.51 h poloxamer 407 25 wt.% gel) did not differ. Thus, morphine release from the gels is of relevance for the uptake rate by the tissue and the delay of systemic exposure. Other than with ester drugs (Lombardi Borgia et al., 2008), major drug biotransformation by keratinocytes is not of relevance with morphine (Heilmann et al., 2012) and this does not superimpose release effects.

Overall, we could show that poloxamer 407 25 wt.% gel is a suitable vehicle for topical morphine administration to human skin, since it provides sustained drug release and also morphine uptake into the RHS. Only 10% of morphine permeated from the poloxamer 407 25 wt.% gel through the RHS into the acceptor chamber during 6 h of exposure. Considering that the barrier function of RHS is weaker compared to human skin because of a reduced thickness and a slightly different lipid composition (Netzlaff et al., 2005; Van Gele et al., 2011), morphine uptake and extent of systemic exposure should be even lower after topical application on human skin, although the influence of a wounded skin surface currently is still open. In fact, preliminary data indicate only marginal morphine permeation after topical application on cutaneous ulcers as studied in this case from INTRASITE™ gel (Ribeiro et al., 2004). A fast hepatic biotransformation will further reduce the risk of systemic adverse reactions.

4. Conclusion

For the treatment of severe skin wounds, topically applied morphine is an option to obtain efficient pain relief. Poloxamer 407 25 wt.% hydrogel appears to be a suitable vehicle for morphine in terms of sustained opioid release and (per-)cutaneous absorption. This formulation providing drug release up to one day may allow to achieve ongoing analgesia and hence extend the interval between the regular changes of the wound dressing and reduce the need for opioids applied systemically, respectively.

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