Pomen pravilne izbire volumna Franzove difuzijske celice za *in vitro* preizkušanje sproščanja zdravil pri oblogah za rane

Importance of Franz diffusion cell volume for *in vitro* drug release testing of wound dressings

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Izvleček

Namen: *Obloge za oskrbo rane so med najbolj uporabljanimi izdelki na področju zdravljenja ran. V zadnjem času potekajo številne raziskave, v katerih se v obloge za oskrbo ran vključujejo različne aktivne substance, pogosto zdravilne učinkovine iz različnih farmakodinamičnih skupin. Ob upoštevanju spremenljivega in spreminjajočega se okolja v posameznih vrstah ran je nujno, da se pri* in vitro *vrednotenju učinkovitosti in varnosti takih naprednih materialov, simulira pogoje, ki so značilni za posamezno vrsto rane.*

Metode: *Za ta namen je testiranje sproščanja v Francovih difuzijskih celicah metoda izbire, saj ob sočasnem simuliranju pogojev v rani (temperatura, pH), omogoča določeno variabilnost tudi v smislu prilagajanja velikosti rane (velikost Franzevih difuzijskih celic).*

Abstract

Objective: *Wound dressings are one of the most commonly used products in wound care. Various wound dressings have been developed, containing different active ingredients and often drugs from different pharmacodynamic groups. Considering the changing environment in each wound type, it is important that the experimental design for evaluating the* in vitro *drug release of such materials takes into account these varying conditions. Hence, this study aimed to examine the importance of Franz diffusion cell volume for* in vitro *drug release testing of wound dressings.*

Methods: *Franz diffusion cells were used for* in vitro *drug release testing, which not only simulated different conditions during wound healing but also allowed a certain degree of possible changes in the experimental design (e.g.,*

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Rezultati: *V tej raziskavi smo primerjali dve Franzovi difuzijski celici z različnima volumnoma receptorskega medija, pri čemer smo iz alginatne obloge kot modelno učinkovino sproščali naproksen. Z raziskavo smo potrdili, da volumen receptorskega medija ne vpliva le na količino sproščene zdravilne učinkovine, temveč ima tudi signifikanten vpliv na mehanizem sproščanja.*

Zaključek: *Razlika v volumnu receptorskega dela Francovih difuzijskih celic omogoča simuliranje ran tako z večjim kot tudi z manjšim volumnom izločka. Zaključimo lahko, da je pri načrtovanju* in vitro *testiranj sproščanja zdravilnih učinkovin nujno potrebno določiti ciljno vrsto ran, za katere bo izdelek namenjen.*

diffusion cell size).

Results: *In this study, we compared two sets of Franz diffusion cells with different volumes of the receptor medium to evaluate naproxen release from an alginate-based model wound dressing. We confirmed that the volume of the receptor medium not only affected the amount of drug released in the selected time but also significantly influenced the release mechanism.*

Conclusion: *Different volumes of the Franz diffusion cell receptor compartments allowed the simulation of wounds with a higher and a lower degree of exudation. The experimental design for* in vitro *drug release testing based on Franz diffusion cells must thoroughly consider the targeted wound type for which the dressings are being developed.*

INTRODUCTION

Wound healing is an essential part of survival skills and is thus crucial to the existence of life (1). It is often described as a complex process broadly divided into four distinct phases: hemostasis, inflammation, proliferation, and remodeling (2, 3).

Although some processes overlap between phases, different combinations of inflammatory mediators and growth factors are known to orchestrate key processes in each phase (4). Consequently, novel pharmacological therapeutic approaches targeting wound healing are developed based on these specific drug targets (5).

Various wound dressings have been explored considering the aforementioned aspects and a wide range of clinical manifestations and complications associated with the healing of different wound types (6-11). Many recent studies focus on developing wound dressings that allow the controlled release and delivery of drugs to the desired wound sites (12, 13).

Despite the immense potential of the aforementioned materials for more effective wound treatment, the data on the technical aspects of *in vitro* drug release of such controlled drug delivery systems are limited (9). Studies considering the actual wound environment, including its changing conditions over time, are particularly rare (9). The latter is an even more important feature of wound treatment, considering that various polymeric materials used in dressings (e.g., alginate, viscose, and carboxymethylcellulose) can be significantly (and differentially) affected by these changing conditions in the wound bed (14, 15). This requires careful planning of the *in vitro* evaluation of drug release from novel wound dressings, taking into account possible changes in the particular wound bed type (pH, T value, exudate volume, and so on) (16) (Fig. 1).

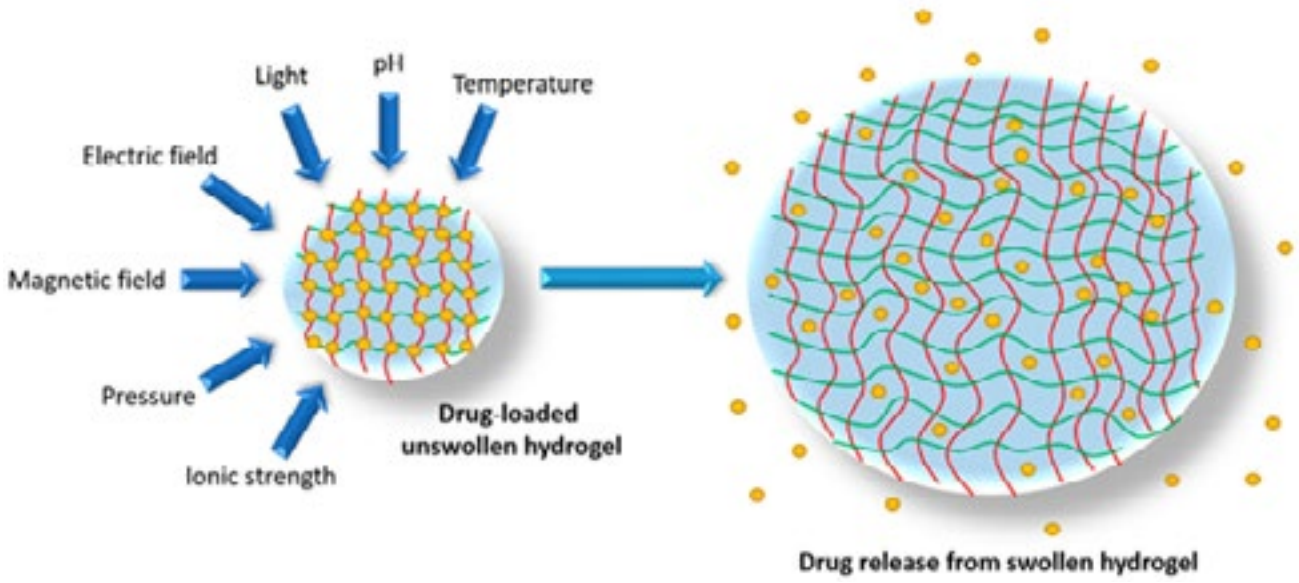

Figure 1. Overview of chemical and physical stimuli that influence drug release from wound dressing (17).

The drug release from polymer-based wound dressings is often controlled by one or more physical processes, including (a) hydration of the polymer, (b) swelling and gelation, (c) diffusion of the drug through the matrix, and (d) eventual erosion of the matrix (18, 19). Wounds exhibit varying degrees of exudation (with variable composition for different wounds). Hence, it is expected that targeted wound healing can be achieved by combining swelling, erosion, and subsequent drug diffusion kinetics as part of the controlled drug release mechanism (9, 20). This is also evident in some of the recently published studies, in which the reported drug release from the prepared wound dressings is explained by a combined mechanism of either two or three of the aforementioned principles (21, 22).

Among the commonest methods for testing the *in vitro* drug release of topical and transdermal formulations is using Franz diffusion cells (23, 24). Such tests are useful for not only the design and development of novel formulations but also toxicity screening and quality control (25, 26). Static diffusion cells are of two types: vertical (Franz cells) and horizontal (side-by-side) cells (27-29). Static diffusion cells are commonly used to test transitions through different membranes (e.g., skin and intestine) and the permeability of tissues and to determine the concentration of molecules in the receptor chamber, which can be used to estimate the time when dynamic equilibrium is reached (8, 9, 30-33). Hence, the purpose of using diffusion cells is to create *in vitro* conditions that resemble the actual physiological environment and then monitor the diffusion of test substances in a desired experimental setup (e.g., skin permeability and drug release) (34-36). The proper choice of basic test equipment is important to reproduce the physiological conditions of the target organs. As our target in this study was the wound environment, we needed to appropriately select the release media and the type and size of Franz diffusion cells used. Our main focus was to show the effect of the size of the selected Franz diffusion cells on the release profile of a drug-loaded alginate-based model wound dressing and, thus, the impact on the interpretation of the release data. The drug used was naproxen (NPX), a common nonsteroidal anti-inflammatory drug.

MATERIALS AND METHODS

Materials

Commercially available alginate wound dressing material was purchased from Tosama, Slovenia. NPX was purchased from Sigma-Aldrich (St. Louis, MO, USA). All materials were used as obtained without further modification before sample preparation or

assay. Ultrapure water (18.2 MΩ cm at 25°C) from an ELGA PureLab water purification system (Veolia Water Technologies, UK) was used to prepare all solutions.

Figure 2. Franz diffusion cell with a volume of 5 mL (left) and 15 mL (right).

Incorporation of the drug into the wound dressing material

The wound dressing samples were cut into squares of size 1 × 1 cm^2 and impregnated with NPX (dissolved in EtOH, 1 mg/mL) for 30 min. The samples were then dried in an oven at 50°C for 5 min, cooled to room temperature, and finally purged with nitrogen gas. The as-prepared samples were immediately used for the *in vitro* release assay and/or other characterization methods.

Optical microscopy

The wound dressing morphology before and after NPX incorporation was observed using a Zeiss Axio T25 upright high definition optical microscope (Zeiss, Germany).

Attenuated total reflectance infrared spectroscopy

The attenuated total reflectance infrared spectroscopy (ATR-IR) spectra of NPX, alginate, and NPX-loaded alginate were acquired using an Agilent Cary 630 Fourier-transform infrared spectroscopy (FTIR) spectrometer (Agilent, Santa Clara, CA, USA) equipped with a diamond crystal (ATR module, measurement range 400–650 cm^{-1}). The scans were performed at three different places in 24 scan repetitions on each sample surface before and after impregnation (37, 38).

In vitro drug release testing

The *in vitro* drug release test was performed using an automated transdermal diffusion cell collection system (Logan System 912-6; Somerset, NJ, USA). The size of the diffusion cells varied (two sizes were used for comparison, such as 5 and 15 mL) (Fig. 2).

The drug-loaded samples were cut into squares of size 1 × 1 cm^2 and placed on top of a rare woven polyethylene terephthalate membrane. The receptor compartment was filled with ultrapure water, and its temperature was maintained at 37°C. The medium was continuously stirred with a magnetic bar at 50 rpm during the dissolution experiment. The samples were collected at various time intervals (1, 5, 10, 20, 30, 60, 120, 180, 240, 300, 360, and 1,440 min) for 24 h. The released/dissolved NPX concentration in the receptor medium was determined by quantifying the absorption band at 232 nm using an ultraviolet–visible (UV-Vis) spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent).

The collected sample volumes were replaced with fresh ultrapure water. The sink conditions were ensured by sampling and subsequent dilution of the samples by replacing the medium. This dilution was considered when calculating concentrations using the Beer–Lambert law. All release studies were performed in triplicate.

RESULTS

Morphological evaluation using optical microscopy

First, we used optical microscopy to observe the morphological changes in alginate fibers after adding the drug NPX (Fig. 3).

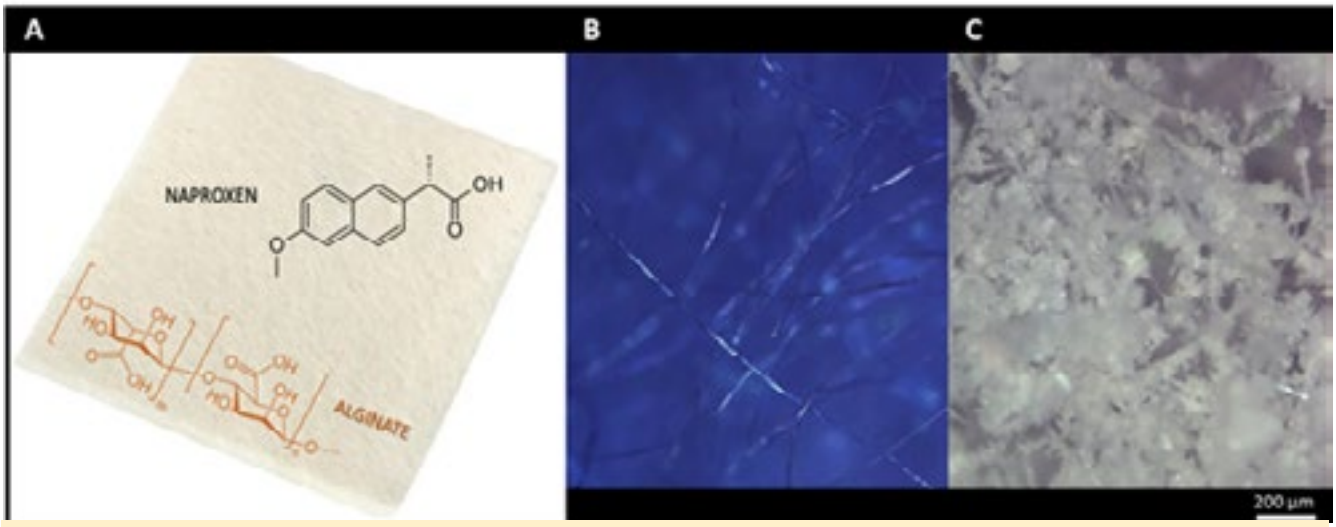

Figure 3. (a) Chemical structures of alginate and NPX. (b) Optical micrograph of alginate fibers before NPX attachment. (c) Optical micrograph of alginate fibers after NPX incorporation.

Conformation of the drug incorporated into the wound dressing material

ATR-IR spectra of the NPX and alginate with and without NPX were acquired to confirm the successful incorporation of the drug into the wound dressing material (Fig. 4). After an initial observation of the spectra, we could confirm that no chemical changes occurred in the drug during its incorporation.

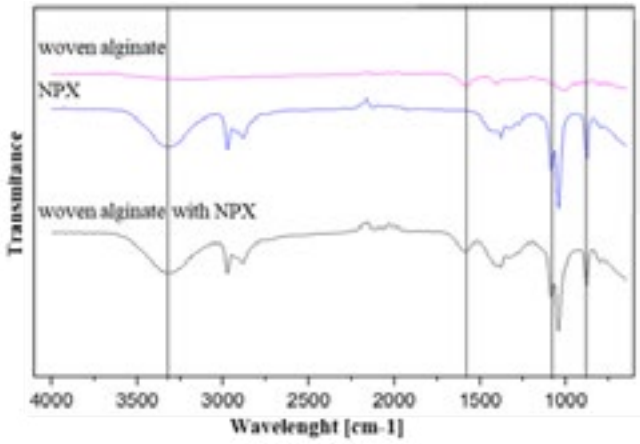

Figure 4. ATR-IR spectra of woven alginate, diluted NPX, and alginate samples loaded with the drug.

The absorption bands of about 1610 and 1416 cm^{-1} were attributed to the stretching vibrations of asymmetric and symmetric bands of the carboxylate anions of the alginate, respectively. For the NPX, the bands of stretching vibration ν(C=O) of carboxyl groups at 1260 cm^{-1} were attributed to the presence and vibrations of NPX ν(C–H) at about 900 cm^{-1}. A broad peak between 3700 and 3000 cm^{-1} corresponded to the stretching vibration ν(O–H).

For the alginate samples loaded with the drug, the presence of NPX in the samples was indicated by the bands of stretching vibration between 3700 and 3000 cm^{-1} for ν(O–H) and the vibrations of NPX ν(C–H) at about 900 cm^{-1}. This showed that NPX was successfully incorporated into alginate.

Comparison of drug release performance using Franz diffusion cells of different sizes

After confirming that the drug was bound to the fibers, we evaluated the effects of the Franz diffusion cell size on the actual release results. As discussed in the Materials and Methods section, two sizes of Franz diffusion cells were used for this purpose (5 and 15 mL). The release performance was presented in the form of three graphs to provide a better overview, showing drug release as concentration, mass, and percentage of NPX released as a function of time (Fig. 5).

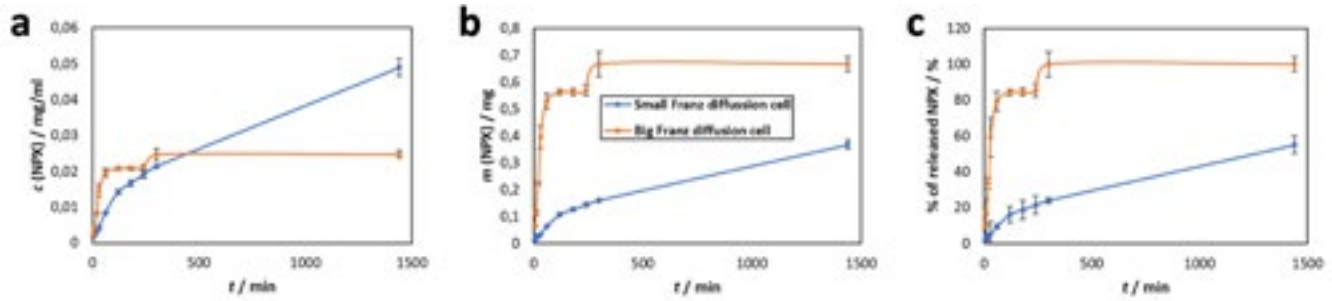

Figure 5. Comparison of release performance between the two differently sized Franz diffusion cells: (a) concentration of NPX released as a function of time, (b) mass of NPX released as a function of time, and (c) percentage of NPX released as a function of time.

DISCUSSION

An optical microscope was used to confirm the actual binding of the drug to the fibers of the alginate wound dressing material (Fig. 3). The figure shows that most of the drug was in the form of crystals. The alginate material was suitable for NPX incorporation because the fibers were almost invisible after attachment. The successful incorporation of NPX was additionally confirmed using FTIR. The latter confirmed that the incorporation did not affect the chemical composition of the drug.

Figure 5 shows that the compartment volume of the used Franz diffusion cells significantly influenced the *in vitro* drug release outcome. The release curves corresponding to different cell volumes differed in all three result representation types. This was true even though they showed the evaluation of samples prepared by the same process (the alginate wound dressings with the incorporated NPX were prepared the same way for all performed release studies). Although the difference in the concentration of released NPX was expected (a larger volume meant a lower concentration; Fig. 5a), the other two graphs were more surprising. Figure 5b shows the released NPX mass as a function of time. The graphs for the two cell volumes not only showed different masses at the respective time points, but also had completely different curve shapes, indicating different release kinetics. The same was true for the third graph, which showed the percentage of NPX released as a function of time (Fig. 5c).

Several possible explanations existed for these drastic changes. First, the size of the Franz diffusion cells might affect the extent of wetting of the samples. The size of the entry point changed for both cell sizes, and hence might have a huge impact, especially in the initial minutes of release. This might explain the difference in the first 30 min of release, where the release curve for the larger cell showed a burst-like release while the release for the smaller cell showed a slower release. The second possible explanation for the changes in the rest of the release curve was most likely related to the wetting, as previously discussed. This could lead to more rapid degradation of the alginate wound dressing material over a longer period. The resulting increased degradation might lead to an increase in the surface area of the portions of the material exposed to the release medium. A larger surface area and a larger number of NPX molecules exposed to the medium could significantly improve the release behavior (as observed in this study).

In this study, we compared the *in vitro* drug release of NPX from a model alginate-based wound dressing from two Franz diffusion cells of different volumes. The different volumes of their receptor compartments allowed the simulation of wounds with higher and lower levels of exudation. We confirmed that the volume of the receptor medium not only affected the amount of drug released in the selected time period but also significantly influenced the release kinetics

and thus the drug release performance. Based on these results, we concluded that the experimental design of *in vitro* drug release tests based on Franz diffusion cells must thoroughly consider the targeted wound type for which the dressings were developed.

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