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Design and characterization of matrix metalloproteinase-responsive hydrogels for the treatment of inflammatory skin diseases

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Enzyme-responsive hydrogels, formed by step growth photopolymerization of dicysteine peptide linkers with alkene functionalized polyethylene glycol, provide interesting opportunities as biomaterials and drug delivery systems. In this study, we developed stimuli-responsive, specific, and cytocompatible hydrogels for delivery of anti-inflammatory drugs for the treatment of inflammatory skin diseases. We designed peptide linkers with optimized sensitivity towards matrix metalloproteinases, a family of proteinolytic enzymes overexpressed in the extracellular matrix of the skin during inflammation. The peptide linkers were crosslinked with branched 4-arm and 8-arm polyethylene glycols by thiol-norbornene photopolymerization, leading to the formation of a hydrogel network, in which the anti-inflammatory Janus kinase inhibitor tofacitinib citrate was incorporated. The hydrogels were extensively characterized by physical properties, in vitro release studies, cytocompatibility with fibroblasts, and anti-inflammatory efficacy testing in both an atopic dermatitis-like keratinocyte assay and an activated T-cell assay. The drug release was studied after single and multiple-time exposure to matrix metalloproteinase 9 to mimic inflammatory flare-ups. Drug release was found to be triggered by matrix metalloproteinase 9 and to depend on type of crosslinker and of the polyethylene glycol polymer, due to differences in architecture and swelling behavior. Moreover, swollen hydrogels showed elastic properties similar to those of extracellular matrix proteins in the dermis. Cell studies revealed limited cytotoxicity when fibroblasts and keratinocytes were exposed to the hydrogels or their enzymatic cleavage products. Taken together, our results suggest multi-arm polyethylene glycol hydrogels as promising matrix metalloproteinase-responsive drug delivery systems, with potential in the treatment of inflammatory skin disease.

Statement of significance

Smart responsive drug delivery systems such as matrix metalloproteinase-responsive hydrogels are excellent candidates for the treatment of inflammatory skin diseases including psoriasis. Their release profile can be optimized to correspond to the patient’s individual disease state by tuning formulation parameters and disease-related stimuli, providing personalized treatment solutions. However, insufficient cross-linking efficiency, low matrix metalloproteinase sensitivity, and undesirable drug release kinetics remain major challenges in the development of such drug delivery systems. In this study, we address shortcomings of previous work by designing peptide linkers with optimized sensitivity towards matrix metallopro-

Abbreviations: AD, atopic dermatitis; APMA, 4-aminophenylmercuric acetate; CCL-2, chemokine ligand 2; CMP, comprehensive multiphas; DMEM, Dulbecco’s modified eagle medium; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; HIRF, homogenous time-resolved fluorescence; HR-MAS NMR, high-resolution magic angle spinning nuclear magnetic resonance; JAK, Janus kinase; LAF, lithium phenyl-2,4,6-trimethylbenzonorphosphinate; MMP, matrix metalloproteinase; NB, norbornene; PBS, Dulbecco’s phosphate buffer; PEG, polyethylene glycol; PEG-2-NB, polyethylene glycol amid conjugated norbornene; PEG-DA, polyethylene diacrylate; RT, room temperature; TIMP, tissue inhibitors of matrix metalloproteinases; TSP-d4, trimethylsilylpropanoic acid.

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teinases and high cross-linking efficiencies. We further provide a proof-of-concept for the usability of the hydrogels in inflammatory skin conditions by employing a drug release set-up simulating inflammatory flare-ups.

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1. Introduction

The skin is the largest organ and comprises three main layers, epidermis, dermis, and hypodermis. Beyond its key role to serve as a protective physical barrier, the skin possesses versatile immune and sensory properties [1]. To maintain homeostasis across the skin, a complex cellular cross-talk is established between the skin barrier, innate immunity, and adapted immunity [2]. Dysfunction of any of these crucial components may lead to inflammatory disorders [3]. Collectively, skin, and subcutaneous disorders are ranked as the fourth leading cause of non-fatal disease burden worldwide [4]. Dermatological manifestations have gained growing significance for global health due to their increasing prevalence and substantial healthcare costs related to treatment and quality-of-life of the patients [5,6]. Fortunately, fundamental advances have been made in revealing the underlying mechanisms of the pathogenesis of inflammatory skin diseases, laying the ground for a range of new therapies to help patients gain disease control [7]. For instance, biological drugs for specific targeting of inflammatory hallmarks (e.g., cytokines), and innovative formulations to enhance the administration and efficacy of small molecules have revolutionized disease management for many patients [8,9]. Despite this progress, the complexity of the inflammatory skin diseases is not fully understood, and there is still no cure available. The current prevailing socio-economic impact and the unpredictable course of these diseases, as well as the poor treatment adherence [6] call for improved and personalized therapies to advance the treatment outcome [3].

Stimuli-responsive drug delivery systems have been intensively investigated in attempts to improve patient health by enhancing drug delivery to the target site, achieving disease-controlled release kinetics, optimizing therapeutic efficacy, and facilitating patient-compliant solutions [10–13]. Among such delivery systems, stimuli-responsive step-growth hydrogels have attracted considerable interest as versatile biomaterials because of their cytocompatibility, tunable properties with modular components, and their potential to entrap a variety of therapeutic compounds (ranging from small molecules to macromolecules) under mild conditions [14–17]. Drug release from such systems may be induced by exogenous (e.g., heat, light, magnetic fields) or endogenous (e.g., redox potential, pH, enzymes) stimuli. In particular, endogenous stimuli are promising as these can be linked to pathological states, enabling disease-controlled drug release. The remarkable discrepancy observed in expression and activity of numerous key enzymes in health and disease makes these robust stimuli for on-demand drug delivery [18,19].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases with a key homeostatic role in remodeling and extracellular signaling [20]. Under physiological conditions, the activity and expression of MMPs are tightly controlled and regulated by tissue inhibitors of metalloproteinases (TIMPs), cytokines, hormones, and cell-cell interactions [21,22]. Thus, most MMPs are present in a low quantity and show limited activity in healthy tissues. However, during pathological conditions such as cancer, arthritis, and inflammatory skin diseases (e.g., psoriasis, atopic dermatitis (AD), and hidradenitis suppurativa) the regulation of MMPs becomes dysfunctional, resulting in a distinctive expression pattern and highly upregulated activity [23]. For example, MMP-9 and MMP-7 have both been found to be overexpressed in psoriasis patients, while levels of MMP-8 display a 22-fold increase in AD lesions, and MMP-2 and MMP-9 were highly up-regulated in skin biopsies from patients with hidradenitis suppurativa [22,24–26].

In this study, we explored MMP-responsive multi-arm polyethylene glycol (PEG) hydrogels for on-demand delivery of the anti-inflammatory Janus kinase (JAK) inhibitor tofacitinib citrate to improve the treatment of chronic inflammatory skin diseases. We addressed challenges seen in prior studies, such as insufficient cross-linking efficiency in hydrated environments, low MMP sensitivity and specificity, as well as undesirable release kinetics, by designing biscysteine peptide linkers optimized for MMP cleavage, and incorporating these into multi-arm PEG amide conjugated norborne (PEG-a-AB) hydrogels by step-growth photopolymerization [14,21,27–33]. The MMP-sensitive peptide linkers were designed to have an optimized susceptibility for MMPs based on site specificity profiling of MMPs by placing leucine in P1, glycine in P3and proline in P3 [21,33]. Moreover, amide-functionalized NB was used in this study due to its higher hydrolytic stability compared to conventional ester-functionalized NB [34]. We compared the resulting MMP-responsive hydrogels to non-responsive control hydrogels, characterizing them in terms of degree of cross-linking, swelling behavior, elastic modulus, and release kinetics. To provide a proof-of-concept specifically for inflammatory skin indications, we investigated release kinetics in a set-up simulating inflammatory flare-ups. We further conducted cytocompatibility studies with fibroblasts and verified the anti-inflammatory activity of tofacitinib citrate released from the hydrogels in an atopic dermatitis (AD)-like keratinocyte assay and in an activated T-cell assay. Collectively, the results demonstrate that the MMP-responsive hydrogels have a potential as drug delivery systems for inflammatory skin diseases, which indeed represents a proactive and personalized approach.

2. Materials and methods

2.1. Materials

4-arm PEG-a-NB (10 kDa) and 8-arm PEG-a-NB (10 kDa) were purchased from Creative PEGworks (NC, USA). Custom-made peptide linkers (peptide linker A: CVPGG↓LAGAC and B: CGPGG↓LAGGC) with a purity of >95% were obtained from Bachem AG (Budendorf, Switzerland). Murine MMP-9 (909-MM-010, pro-form: 92kDa) and fluorogenic peptide substrate 1 (Mca-PLGL-Dpa-AR-NH₂, ES001) were both from R&D Systems (MN, USA), while Kel-F® inserts for NMR analysis were from Bruker (MA, USA). Dulbecco’s modified eagle medium (DMEM), Dulbecco’s phosphate-buffered saline (PBS, no calcium, no magnesium), trypsin-EDTA (ethylenediaminetetraacetic acid) (0.25% trypsin), GlutaMax medium (Gibco) and nitrocellulose dialysis membranes (25 mm, 9 kDa cut-off) were all from Thermo Fisher Scientific Inc. (Darmstadt, Germany). Cell-counting cassettes (Via1-CassetteTM) were from ChemoMetec (Denmark). The Cisbio human chemokine ligand 2 (CCL-2) kit, and the Human IL-2 AlphaLisa detection kit were purchased from PerkinElmer (Codolet, France). A T-cell activation/expansion kit was purchased from Milenyl Biotec.
(Bergisch Gladbach, Germany) and the 384 multi-spot detection tissue culture IL-4 kit was purchased from Meso Scale Diagnostics (Maryland, USA). All other materials and reagents were obtained from Sigma-Aldrich, Darmstadt, Germany.

2.2. Preparation of hydrogels

Empty hydrogels were prepared by reacting multi-arm PEG-α-NB polymer (4-arm or 8-arm) (Fig. 1A) with either MMP-sensitive peptide linker A (CVPGG↓LAGAC), MMP-sensitive peptide linker B (Fig. 1B) (CGPGG↓LAGGC), or non-responsive PEG dithiol linker (Fig. 1C) in PBS at room temperature (RT). The polymer and the peptide were dissolved separately in PBS (pH = 7.4) containing 0.05% (w/w) lithium phenyl-2,4,6-trimethylbenzylphosphinate (LAP) and vortexed (IKA® VIBRAX VXR Basic) for 5 min at 800 rpm. The polymer solution was then mixed with the peptide solution and vortexed to obtain the final precursor formulation containing 2.5%, 5%, 10% or 15% (w/w) polymer concentration with the peptide linker added in a 1:1, thiol:ene stoichiometric ratio. All formulations were made in triplicate in cylinder-shaped plastic molds (100 µl) with a hemispherical bottom and cross-linked by irradiating the samples with UV light (Spectrolite® E Series UV lamp, 230 V) at 365 nm for 30 s at a distance of ~1 cm. The same cross-linking procedure was applied for tofacitinib citrate-loaded hydrogels; however, tofacitinib citrate (Fig. 1D) was dissolved at a concentration of 0.25 mg/ml in PBS (pH = 7.4) containing 0.05% (w/w) LAP before addition of solid form polymer and peptide. The thiol: norbornene photopolymerization process [15] is depicted in Fig. 1E.

2.3. Investigation of cross-linking degree of hydrated hydrogel formulations

$^3$H high resolution magic-angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy was performed using a Bruker Avance 600 HD III (14.1 T) spectrometer equipped with a 4 mm double-tuned comprehensive multiphase (CMP) MAS probe to investigate the cross-linking degree of hydrated multi-arm PEG hydrogels. The precursor formulations were formulated as described in section 2.2.; however, PBS was exchanged with D$_2$O, and 1 mg/ml trimethylsilylpropanoic acid (TSP-d4) was added as an internal standard. Two different photo-initiators (0.05% w/w Irgacure 2959 or 0.05% w/w LAP) at different UV exposure times (30 s, 1 min, 5 min), four different multi-arm PEG concentrations (2.5%, 5%, 10% and 15% (w/w)) and three different crosslinkers (MMP-sensitive peptide linker A, MMP-sensitive peptide linker B or non-responsive PEG dithiol linker) were investigated. Prior to analysis, 30 µl of the precursor formulation were slowly transferred
into a Kel-F® insert. After exposure to UV light, the Kel-F® insert was placed in a 4 mm (o.d.) ZrO2 rotor. The 1H NMR data were recorded using single pulse experiment, a recycle delay of 5 s, an acquisition time of 2.73 s, a spin rate of 6000 Hz, 64 scans and a temperature of 298 K. The software Bruker Topspin 3.6.2 was used for data acquisition and MestreNova 14.2 for spectrum analysis and quantification. The cross-linking degree in UV-exposed hydrogels was quantified by normalizing the peak corresponding to the double C=C bond in NB (5.96 to 6.30 ppm) to the internal standard (-0.04 to 0.05 ppm) and comparing it to the normalized NB peak in the precursor formulations. For hydrogels with ~100% cross-linking, a complete disappearance of the NB peak (~ noise signal 8.85 to 9.40 ppm) was observed. Samples were analyzed in triplicate.

2.4. Swelling behavior and mechanical properties

Weight gain studies were performed using an analytical balance (ME54T/00 Mettler Toledo®) to investigate the degree of swelling after cross-linking. Empty PEG hydrogels (100 μl) were formulated as described in Section 2.2. After cross-linking, the hydrogels were gently pushed out of the hydrogel mold with a wetted spatula, weighed and placed in an Eppendorf tube with 1.4 ml PBS (pH = 7.4). Between each weighing, the dispersed hydrogels were stored in a shaking incubator at 350 rpm and 37°C. Prior to weighing, the hydrogels were transferred from the Eppendorf tube to a petri dish and gently blotted with a tissue. Hydrogels were weighed at 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h and 96 h of swelling. Samples were analyzed in triplicate at RT.

The mechanical properties of the PEG hydrogels were determined using an Instron universal testing machine (Instron 5564, Massachusetts, US) equipped with 100 N load cell and compression anvils (Instron, 50 mm, 10 kN). The analysis was carried out at RT at a compression speed of 10 mm/min. The test was programmed to stop compressing at 80% of the maximum load cell force [35]. The elastic modulus (Young’s modulus) was calculated from the tangent slope of the stress/strain curves, and the stress/strain values at the fracture point were extracted. Newly crosslinked (initial), swollen (48 h in PBS) and MPP-9 exposed (20 nM, 48 h in PBS) hydrogels were tested in triplicate. Before each compression test, the dimensions of the hydrogels were measured and used in the calculation of the elastic modulus in the BlueHill Universal software (Version 4.08, Instron, Massachusetts, US).

2.5. In vitro release studies

In order to analyze the release of tofacitinib citrate from the hydrogel, Franz diffusion cells with a screw cap donor chamber were used with a nitrocellulose membrane (molecular cut-off: 9 kDa) to separate the donor (2 ml) and receptor (5 ml) chambers [12]. The hydrogels were loaded with 0.25 mg/ml tofacitinib citrate (solubility in PBS at pH = 7.4; 0.75 mg/ml) in a final hydrogel volume of 100 μl (n=3). Each experiment was carried out at 37°C using PBS (pH = 7.4) in the receptor chamber at a stirring rate of 350 rpm. Prior to the experiments, tofacitinib citrate-loaded formulations were prepared and cross-linked as described in Section 2.2. The receptor chamber was filled with 5 ml PBS and the nitrocellulose membrane fixed between the receptor and the donor compartments. The screwcap on the donor chambers was removed and hydrogels added on top of the membrane together with 2 ml PBS, either in the absence (control) or presence of activated MPP-9. Samples were collected directly into sealed HPLC vials by automatic controlled sampling needles at 5 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, 48 h, 72 h, 96 h and 120 h. The kinetic release data obtained were fitted to the following kinetic release models: zero order [36], first order [37], Higuchi [38], Korsmeyer-Peppas [39] and Nixon-Crowell [40]. A quenched fluorescence assay (R&D Systems) was conducted to confirm the function of the MMP-9 batch before MMP-responsive release testing. The assay buffer was prepared with 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, and 0.05% (w/v) Brij-35 with a pH adjusted to 7.4.

For experiments mimicking flare-ups, activated MMP-9 was added multiple times at a concentration of 20 nM in the beginning of the experiment (0 h) and an additional 20 nM at 23 h, 47 h, 71 h and 95 h, respectively. Automatic sampling was performed after 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 28 h, 32 h, 48 h, 52 h, 54 h, 72 h, 76 h, 80 h, 96 h, 100 h, 104 h and 120 h. In order to ensure complete accounting of tofacitinib citrate, a mass balance study was performed after termination of the flare-up release study (Table S1). In brief, the hydrogels were transferred from the donor chamber to a glass vial containing 20 ml PBS with 100mM L-cysteine methyl ester hydrochloride (pH = 3.0) for 14 days, with a magnetic stir bar (250 rpm) at RT. After degradation of the hydrogels, the concentration of tofacitinib citrate was measured with HPLC as described below.

Tofacitinib citrate in both release samples and mass balance study samples was quantified using HPLC (Waters ACQUITY UPLC H-class system, Massachusetts, US). The separation was carried out on a reverse-phase C18 column (Waters Acquity BEH, 1.7 μm, 2.1 mm × 50 mm). The mobile phase consisted of 10 mM ammonium acetate with a gradient (water:acetate) going from a 90:10 (% ratio) to a 70:30 (% ratio) within 3 min, and then to 15:85 (% ratio) within 3 min, maintaining the 15:85 (% ratio) for 5 min and going back to 90:10 (% ratio) within 5 min. Samples of 5 μl were injected into the column. All HPLC measurements were performed at a column temperature of 40°C and a flow rate of 0.5 ml/min. With this method, tofacitinib citrate eluted after 2 min and was detected at 287 nm. Data collection and processing were conducted in the software Empower 3 Pro (Waters Corporation, version 7.21.00).

2.6. Fibroblast cytotoxicity assay

Murine fibroblasts (3T3 cells) were grown in T-175 flasks with DMEM growth medium (10% fetal bovine serum (FBS) + 1% Penstrep) at 37°C in a 5% CO2 humidified incubator. Fibroblasts were harvested at passage 2 - 4 with a confluence of 70% - 80%. They were then seeded in a 96-well plate with density of 4.0 × 10⁴ cells/well and allowed to attach to the bottom 24 h prior to experimental start. Empty hydrogels formulated as described in Section 2.2., and subsequently placed in an Eppendorf tube with 1.4 ml DMEM growth medium without and with 20 nM activated MMP-9, respectively, for 24 h at 37°C. After incubation, the growth medium without (control) and with hydrogel breakdown products was transferred into Eppendorf tubes for storage (Fig. S1). The samples were stored at 4°C for a day to neutralize the MMP activity. Controls (multi-arm PEG polymer, photolinker A and B, non-responsive linker, photoinitiator LAP, MMP-9, PBS) were dissolved in DMEM medium and stored in the same way.

On the day of the experiment, DMEM samples (cleavage products and controls) were heated to 37°C in a bead bath incubator (Lab Armor LLC, Texas, USA). Thereafter, 96-well plates with fibroblasts were prepared by removing the growth medium by aspiration, and 150 μl sample was added to each well in triplicate. The plates were stored at 37°C in a 5% CO2 humidified incubator (one plate for 2 h and the other one for 24 h). Controls with cells in DMEM growth medium (negative control) and DMEM medium without any cells (positive control) were included in both 96-well plates. After the desired exposure time, the samples/DMEM media were removed and repopulated with 150 μl fresh DMEM medium. To investigate the cell viability, 15 μl PrestoBlue® (resazurin based assay) was added to each well and incubated for 4 h at RT (n = 3).
Fluorescence was measured at 560 nm/590 nm using a microplate reader (Perkin Elmer EnVision® 2104).

2.7. Atopic dermatitis-like keratinocyte assay and activated T-cell assay

For anti-inflammatory testing, samples with tofacitinib citrate released from 5% (w/w) 4-arm and 8-arm hydrogels were prepared in the diffusion setup described in Section 2.5., where PBS was exchanged with EpiLife™ medium (AD-like keratinocyte assay) or GlutaMAX medium (activated T-cell assay) in both donor and receptor chambers. Briefly, tofacitinib citrate-loaded hydrogels were transferred to the donor chamber and samples collected after 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h for the AD-like keratinocyte assay and after 1 h, 4 h, 8 h, 24 h, and 72 h for the activated T-cell assay. The experiments were conducted either without or with 20 nM MMP-9 added to the donor chamber at time = 0. Control samples, containing free tofacitinib citrate (1 nM to 1 μM) in the desired medium, were prepared for evaluation in the keratinocyte assay (Fig. S2A) and the T-cell assay (Fig. S3A-B). All samples were stored at 4°C protected from light and heated to 37°C prior to incubation with the cells.

LEO Pharma Open Innovation has developed an AD-like keratinocyte model, in which keratinocytes are stimulated with T-cell cytokines, characteristic of the inflammatory skin disease AD [41]. The assay allows investigation of the ability of compounds to inhibit levels of CCL-2 released by stimulated keratinocytes, providing an indication of its efficacy in AD [41]. Employing this assay, human epidermal keratinocytes isolated from adult skin (HEKa) were grown in 775 flask in EpiLife™ medium and kept in a 5% CO₂ humidified incubator. Keratinocytes were harvested at passage 3-6 and seeded into 384-well plates using a multi-drop cassette. The cells were seeded in a density of 3500 cells/well in a volume of 40 μl medium. The plates were incubated for 24 h at 37°C. On the following day, 20 μl of each sample (tocracinib citrate released from the hydrogels or free tofacitinib citrate), as well as the positive control (terfenadine (NN6394A), ~100% inhibitory effect) and negative control (0.1% DMSO (w/w), ~0% inhibitory effect), were added to the keratinocytes, respectively, and incubated at 37°C for 2 h. Thereafter, the keratinocytes were stimulated with a cytokine mixture (final well concentration: 10 ng/ml IL-4, IL-13, IL-22 and 1 ng/ml INF-γ) for 48 h at 37°C. After incubation with the cytokine mixture, 3 μl of the supernatant from each well was transferred to a white ProxiPlate-384. 7 μl of CCL-2 homogeneous time-resolved fluorescence (HTRF) detection reagent was added to each well. The CCL-2 standard curve can be seen in Fig. S2B. The plates were sealed and incubated overnight at RT protected from light. The plates were read at 620 nm/665 nm the following day using a microplate reader (Perkin Elmer EnVision® 2104).

In the activated T-cell assay, the activation of CD4+ T-cells by antigen presenting cells was mimicked by adding anti-biotin MACSiBead beads with immobilized antibodies binding to CD3, CD28 and CD2. Consequently, the stimulation leads to activation of various intracellular transduction pathways, resulting in production of cytokines (e.g., IL-4 and IL-2) and proliferation of the T-cells [48]. The CD4+ cells were seeded in a concentration of ~ 4 x 10⁴ cells/ml in a 384 well plate and stimulated with beads at a final concentration of 1 bead/cell for 2 days kept in a 5% CO₂ humidified incubator at 37°C. On day 3, the T-cells were incubated with the release samples containing tofacitinib citrate for 1 h, as well as the positive (no stimulation + 0.1% DMSO) and negative (bead stimulation + 0.1% DMSO) controls. IL-4 was detected by a multiplex detection assay, whereas IL-2 was measured by HTRF, and cell viability by PrestoBlue (1:10). Cell viability measurements can be found in Fig. S4 in the Supplementary Material.

2.8. Statistical analysis

Statistical analysis and graphing were conducted in GraphPad Prism (8.1.1, GraphPad, San Diego, California). The student’s t-test was performed to compare two experimental groups, while one-way ANOVA was used for comparing more than two groups. Data are presented as mean ± standard deviation (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001 and ****p-value <0.0001) with n = 3.

3. Results and discussion

3.1. Cross-linking degree in multi-arm PEG hydrogels

1H HR-MAS NMR spectroscopic measurements enabled quantitative investigation of the degree of cross-linking in hydrated multi-arm PEG hydrogels after photopolymerization. Firstly, we found that the photo-initiator LAP was more efficient than Irganix 2959, resulting in a ~100% disappearance of the C=C double bond in the multi-arm PEG functionalized NB groups within 30 s (Fig. S5). This finding is in agreement with existing literature, where LAP has demonstrated to exhibit enhanced cross-linking efficiency over Irganix 2959 for a range of linear norbornene functionalized macromers (e.g., PEG, hyaluronic acid, gelatin) [14]. Therefore, LAP was chosen as the preferred photo-initiator for further investigation of the MMP-responsive and non-responsive multi-arm PEG hydrogels. Secondly, we noted that the degree of cross-linking changed remarkably with the PEG polymer concentration, but only slightly with the type of cross-linker (Table 1). Irrespective of the cross-linker, almost complete cross-linking was seen for hydrogels with polymer concentrations of 5% and 10% (w/w) of both 4-arm and 8-arm PEG hydrogels, respectively. In comparison, the hydrogels with a 15% (w/w) polymer concentration exhibited a significantly lower cross-linking degree, namely ~81% for 4-arm and ~87% for 8-arm PEG hydrogels crosslinked with the non-responsive linker (Table 1). Finally, the 2.5% (w/w) PEG hydrogels showed cross-linking degrees of ~95% for both 4-arm and 8-arm PEG hydrogels, respectively, across all three crosslinkers. Due to the high viscosity of the 15% (w/w) precursor formulation, the mobility of the polymer decreases. This has been described to influence the propagation of thiol radicals across the alkene groups, resulting in a slower formation of a nearly complete crosslinked network [44,50]. At the other end of the polymer concentration range, it has been found that a higher degree of unreacted end-groups could result from low polymer concentrations for statistical reasons. This in turn leads to a higher degree of diffusivity and an increased drug release of both proteins and small molecules from step-growth photopolymerized hydrogels [29,51,52]. Corresponding characteristics have been found for Michael-addition reacted multi-arm PEG hydrogels, where either too low or too high PEG polymer concentration may result in incompletely cross-linked hydrogel networks [50]. To achieve an efficient cross-linking degree and avoid a high degree of drug diffusivity throughout the network, a range of norbornene-thiol reacted hydrogels have been found to be most attractive in concentrations ranging from 4% to 10% (w/w) due to the low UV exposure time needed to reach optimal crosslinked networks [29,31,34,44,53]. Our findings are in agreement with these previous studies, and, therefore, the hydrogels with polymer concentrations of 5% and 10% (w/w) were selected, while the PEG hydrogels with 2.5% and 15% (w/w) polymer concentrations were excluded from swelling- and mechanical properties studies, release testing and in vitro cell studies.

After UV exposure for 30 s, the degree of cross-linking was found to be higher than 94% and similar for peptide linkers A and B (Table 1). The size of the terminal residues and their hydrophilicity have been shown to hamper the rate of cross-linking.
in click-chemistry reacted hydrogels [14]. Taking this into account, the amino acid sequence in peptide linker B was optimized to avoid steric hindrance affecting cross-linking. At the peptide linker termini, the residues alanine and valine in peptide linker A were changed to glycine residues to obtain peptide linker B. By optimizing the sequence of peptide linkers for MMP cleavage, a high susceptibility for enzymatic cleavage can be realized [49]. However, this requires that the peptide linkers get incorporated in a fashion, in which a nearly complete network can be established. This can be achieved by minimizing steric hindrance of functional end-groups [14]. Through this, unspecified drug release can be suppressed and then triggered by MMP cleavage for hydrogels containing both peptide linkers A and B.

### 3.2. Swelling and mechanical properties of multi-arm PEG hydrogels

Next, the swelling behavior was investigated for 5% (w/w) and 10% (w/w) 4-arm and 8-arm PEG hydrogels after immersing these in PBS for 48 h at 37°C, directly after cross-linking (Fig. 2) and for 96 h (Fig. S6A-C). In contrast to conventional procedures that include freeze-drying before evaluating swelling properties [54], our procedure allowed direct monitoring of the relationship between network composition and hydration under biologically relevant conditions. The increase in weight gain for hydrogels containing peptide linker A, peptide linker B and the non-responsive linker followed similar kinetics across the different hydrogel formulations. However, hydrogels with a multi-arm PEG concentration of 10% (w/w) were found to display a higher swelling capacity compared to the 5% (w/w) hydrogels. After 8 h, the 10% (w/w) 4-arm hydrogels showed more than a 6-fold higher water uptake compared to 8-arm hydrogels (Fig. 2A-C). Hence, it can be concluded that an increase in multi-arm PEG concentration leads to an increased capacity to hold imbibed water. In contrast, an increase in PEG multi-arm connectivity, from 4-arm to 8-arm, results in a lower weight gain, indicating that the increased network connectivity constrains swelling and water uptake, in agreement with previous findings [14,55]. In another study, it was found that the size of multi-arm PEG microparticles dispersed in an aqueous solution increased in size to a higher extent when lowering the complexity of the PEG polymer arms. For instance, the increase in particle size for 3-arm PEG microparticles was ~10 fold higher compared to 8-arm PEG microparticles [55].

All hydrogels exhibited a visibly decreased swelling rate after about 8 h incubation and after 48 h no further increase in weight gain was observed (Fig. 2A-C). When the hydrogels were fully swollen, there was no significant difference in the weight gains between hydrogels containing MMP-sensitive peptide linkers A or B. Due to the insignificant difference in weight gain and the slightly higher degree of cross-linking, peptide linker B was chosen over peptide linker A for further investigation. The 4-arm formulations with the non-responsive linker showed a significantly higher weight gain compared to the hydrogels with the MMP-sensitive linkers (Fig. 2D). For the MMP-sensitive linker B, a significant difference was observed only between 5% and 10% (w/w) 4-arm PEG hydrogels (Fig. 2E). For the non-responsive linker, however, there was a significant difference between the 5% and 10% (w/w) for both 4-arm and 8-arm PEG formulations (Fig. 2F). The non-responsive linker has a slightly higher molecular weight compared to peptide linkers A and B, and due to its PEG polymer backbone it is more hydrophilic, which may explain the increased weight gain under hydration [56].

Next, the mechanical properties of the MMP-sensitive hydrogels cross-linked with peptide linker B were investigated under three different conditions: (i) newly cross-linked (initial), (ii) fully swollen in PBS for 48 h without MMP-9 (swollen), and (iii) after exposure to 20 nM activated MMP-9 (MMP-9 exposed). Prior to testing of the mechanical properties, the dimensions of the hydrogels were measured (Table 2). Both newly cross-linked and swollen hydrogels (48 h) displayed shape memory as they recovered to their original shape after being deformed. Besides solely polymeric materials it has been reported that semi-crystalline polymer networks that contain water swellable moieties, such as PEG hydrogels, exhibit shape-memory capabilities especially in aqueous environments [57]. Upon mechanical testing, it was observed that the 4-arm hydrogels undergo more pronounced dimensional changes compared to 8-arm ones, which was in line with the weight gain results from the swelling experiments. After mechanical testing, the Young’s moduli were calculated (Fig. 3) and stress and strain values determined (Fig. S7A-B). Expectedly, the Young’s moduli were found to decrease on hydration (Fig. 3), which is consistent with previous findings for other thiol-norbornene hydrogels [14,16,55]. Notably, there was a significant difference in the Young’s moduli of swollen and MMP-9 exposed formulations for 5% (w/w) multi-arm PEG hydrogels, but not for 10% (w/w) multi-arm PEG hydrogels. Likely, this is due to the intrinsically lower number of peptide linkers to cleave compared to 10% (w/w) multi-arm PEG hydrogels, and potentially also due to the larger mesh sizes for the 5% (w/w) hydrogels, allowing more efficient MMP diffusion through the hydrogel network [58]. Furthermore, the initial state of the hydrogels showed a higher Young’s modulus for 10% (w/w) compared to 5% (w/w) for both 4-arm and 8-arm cross-linked hydrogels. Previously, it has been demonstrated that thiol-norbornene hydrogels with lower swelling ratios have higher Young’s moduli, which supports our findings [59]. Moreover, 8-arm hydrogels were stiffer and more brittle than 4-arm hydrogels. It has been shown that varying the PEG multi-arm complexity from low to high generates more dense and stiff structures with increasing Young’s modulus [55]. Overall, it was found that our PEG hydrogels, especially in the swollen state, have stiffness equivalent to skin and protein fibers in the skin [60,61].

### Table 1

<table>
<thead>
<tr>
<th>PEG arm</th>
<th>Polymer concentration</th>
<th>Cross-linking degree</th>
<th>(i) Non-responsive</th>
<th>(ii) Peptide linker A</th>
<th>(iii) Peptide linker B</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-arm</td>
<td>2.5</td>
<td>95.2 ± 1.0</td>
<td>94.1 ± 2.1</td>
<td>95.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>99.8 ± 0.2</td>
<td>~100</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>97.5 ± 1.0</td>
<td>99.3 ± 0.5</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>81.1 ± 2.3</td>
<td>~100</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td>8-arm</td>
<td>2.5</td>
<td>95.3 ± 0.9</td>
<td>95.2 ± 2.6</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>~100</td>
<td>~100</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>~100</td>
<td>99.1 ± 1.3</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>86.9 ± 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Hydrogel swelling shown as weight gain of hydrogels containing (A) peptide linker A, (B) peptide linker B, and (C) non-responsive linker. (D) Weight gain of hydrogels after 48 h for the different crosslinkers. (E - F) Weight gain differences for hydrogels containing peptide linker B or non-responsive linker at different polymer concentrations after 48 h of swelling, respectively. N = 3.

Table 2
Hydrogel dimensions before mechanical characterization. Initial, swollen, and MMP-9-exposed refer to newly crosslinked hydrogels, equilibrium swollen hydrogels (48 h) and equilibrium swollen hydrogels exposed to 20 nM MMP-9 (48 h), respectively. N = 3.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Hydrogel dimensions [mm]</th>
<th>MPP-9 exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Swollen</td>
</tr>
<tr>
<td>5% 4-arm PEG (w/w)</td>
<td>d=5.2, h=4.0</td>
<td>d=8.3±0.2, h=6.5±0.2</td>
</tr>
<tr>
<td>10% 4-arm PEG (w/w)</td>
<td>d=5.2, h=4.0</td>
<td>d=8.9±0.2, h=6.6±0.3</td>
</tr>
<tr>
<td>5% 8-arm PEG (w/w)</td>
<td>d=5.2, h=4.0</td>
<td>d=6.5±0.1, h=4.5±0.1</td>
</tr>
<tr>
<td>10% 8-arm PEG (w/w)</td>
<td>d=5.2, h=4.0</td>
<td>d=6.9±0.2, h=4.8±0.2</td>
</tr>
</tbody>
</table>
3.3. Release kinetics and MMP responsiveness

Having characterized the hydrogel properties, MMP-9-triggered release of tofacitinib citrate from 5% (w/w) 4-arm and 8-arm hydrogels cross-linked with either the MMP-responsive peptide linker B or the non-responsive linker was investigated. The 5% (w/w) PEG hydrogels were selected over 10% (w/w) PEG hydrogels due to the moderate swelling behavior, which provide access to the MMP-responsive peptide linkers, while minimizing the drug diffusivity and non-specific release. In analogy with the findings on MMP-9 effects on the mechanical integrity of the hydrogels, the 5% (w/w) hydrogels displayed a significant change in Young's modulus when exposed to MMP-9, whereas there was no such change observed for 10% (w/w) hydrogels.

In the absence of MMP stimulus, a cumulative release of 32% and 25% of the total amount of tofacitinib citrate in the hydrogels was seen after 48 h incubation for 4-arm and 8-arm MMP-responsive PEG hydrogels, respectively (Fig. 4A). Upon addition of 20 nM MMP-9, in contrast, the cumulative release of tofacitinib citrate reached 67% and 49% for 5% (w/w) 4-arm and 8-arm PEG hydrogels, respectively (Fig. 4A). As a control, the non-responsive 4-arm and 8-arm PEG hydrogels were tested in parallel and no significant difference in cumulative release was found between MMP-9-exposed hydrogels and controls. After 48 h, the cumulative release for the non-responsive PEG hydrogels was ~37% and ~21% for 4-arm and 8-arm PEG hydrogels, respectively (Fig. 4B).

The cumulative release observed in the absence of MMP-9 was lower for the 8-arm than for 4-arm hydrogels cross-linked with peptide linker B or the non-responsive linker, respectively (Fig. 4C-D). As discussed above, higher multi-arm PEG connectivity was found to result in larger capacity to resist dimensional changes due to swelling. The correspondingly slower water diffusion into the hydrogel network is expected to result in a decreased release rate of incorporated drug. For all formulations, the highest release rates were observed within the first 8 h (Fig. 4A-B), which correlated well with the obtained weight gain data. In general, the key mechanistic drivers behind drug release from hydrogels are physical network changes and drug diffusion through the matrix as the hydrogels swell [40,42,43]. The initial increase in release seen in the control conditions at early time points was probably driven mainly by swelling, diffusion and tofacitinib citrate released from the surface of the hydrogels. However, when exposed to MMP-9, an increased release was seen indicating that the hydrogel network has been partly degraded by enzymatic cleavage, resulting in larger mesh size, and allowing higher release rates of any incorporated drug. Drug release induced by hydrogel network cleavage or degradation similar to our study has been investigated for hydrogels against cancer, cardiovascular diseases and rheumatoid arthritis [44,46,47]. In a previous study on intraoral drug delivery, MMP-8 was added to induce a drug release from 10% (w/w) 4-arm PEG-diacrylate hydrogels crosslinked with an MMP-sensitive linker (CGPQGcWGC). It was found that small molecules, such as minocycline hydrochloride, could readily diffuse out of the hydrogel within 5 days. Only when hydrogels were loaded with larger biomacromolecules, such as antimicrobial peptides, burst release could be suppressed to the level that MMP-8-triggered release could be realized [29]. In contrast, the presently investigated system readily allows this also for low-molecular weight drugs such as tofacitinib citrate.

Due to the dimension of the hydrogels, spanning 7mm in diameter, the diffusion coefficient of tofacitinib in intact hydrogels is expected to be moderately suppressed in comparison to that in aqueous solution. The macroscopic diffusion length, in combination with the absence of convection in hydrogels, may substantially slow down release also of small molecules from hydrogels [52].

The release data on the MMP-responsive and non-responsive hydrogels was fitted to several kinetic models in order to elucidate possible drug transport mechanisms. A full overview of the data from the kinetic modeling can be seen in Table 3. In the Korsmeyer-Peppas model the exponent n is geometry dependent and may be indicative of the release mechanism. Depending on the value of n the controlling release mechanism can be described by Fickian diffusion or non-Fickian diffusion [39]. In general, drug release from MMP-exposed hydrogels containing MMP-responsive linkers was best fitted to the Korsmeyer-Peppas model (4-arm; R²=0.968, n=0.54 and 8-arm; R²=0.980, n=0.52), in which the n value indicates that the release was driven by non-Fickian transport as n > 0.45. Likely, however, a combination of mechanisms such as diffusion, swelling, structural cleavage and subsequent erosion collectively governed the drug transport within and from the degrading hydrogels, as observed previously [40,44]. In contrast, the MMP-exposed hydrogels cross-linked with the non-
Fig. 4. Cumulative release of tofacitinib citrate from 5% (w/w) 4-arm and 8-arm PEG hydrogels in PBS at 37°C without and with 20 nM activated MMP-9, respectively. Results are shown for PEG hydrogels cross-linked with (A) MMP-sensitive linker B and (B) non-responsive linker. (C-D) MMP-responsive cumulative drug release after 48 h for hydrogels with MMP-sensitive linker B and non-responsive linker. (E) Cumulative drug release after repeated MMP-9 exposure (↓) of the PEG hydrogels cross-linked with MMP-sensitive linker B. N = 3.

responsive linker displayed a good fit to both the Higuchi model (4-arm; $R^2=0.966$ and 8-arm; $R^2=0.977$), which takes the square root of time and is based on Fick's first law of diffusion, and the Korsmeyer-Peppas model (4-arm; $R^2=0.976$, $n=0.43$ and 8-arm; $R^2=0.955$, $n=0.41$), which is a power law function also used to model swelling-controlled release. Furthermore, calculations revealed that $n < 0.45$ for both 4-arm and 8-arm hydrogels, indicating that the drug transport was driven by Fickian diffusion for the MMP-9 exposed non-responsive hydrogels. Similarly, the release kinetics of tofacitinib citrate from MMP-responsive hydrogels and
Table 3

Results obtained from kinetic modeling of data generated in release studies, in which MMP-9 stimulus was added once. Data from 5% (w/w) 4-arm and 8-arm formulations with the MMP-responsive peptide linker B and the non-responsive linker were modelled both in the absence and presence of 20 nM MMP-9. The fitting to the Korsmeyer-Peppas model includes data up to 60% of the cumulative release, whereas data from the first 24 h were included for fitting to the remaining kinetic models.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Condition</th>
<th>R²</th>
<th>Korsmeyer-Peppas</th>
<th>Hixon-Crowell</th>
<th>Zero order</th>
<th>First order</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-arm PEG</td>
<td>Control</td>
<td>0.856</td>
<td>0.905</td>
<td>0.476</td>
<td>0.627</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td>20 nM MMP-9</td>
<td>0.871</td>
<td>0.968</td>
<td>0.484</td>
<td>0.655</td>
<td>0.380</td>
</tr>
<tr>
<td>8-arm PEG</td>
<td>Control</td>
<td>0.980</td>
<td>0.847</td>
<td>0.710</td>
<td>0.856</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>20 nM MMP-9</td>
<td>0.924</td>
<td>0.980</td>
<td>0.592</td>
<td>0.734</td>
<td>0.497</td>
</tr>
<tr>
<td>4-arm PEG</td>
<td>Control</td>
<td>0.960</td>
<td>0.698</td>
<td>0.595</td>
<td>0.813</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>20 nM MMP-9</td>
<td>0.966</td>
<td>0.976</td>
<td>0.476</td>
<td>0.627</td>
<td>0.371</td>
</tr>
<tr>
<td>Non-responsive</td>
<td>Control</td>
<td>0.971</td>
<td>0.820</td>
<td>0.662</td>
<td>0.829</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td>20 nM MMP-9</td>
<td>0.977</td>
<td>0.955</td>
<td>0.700</td>
<td>0.850</td>
<td>0.604</td>
</tr>
</tbody>
</table>

non-responsive hydrogels under control conditions (i.e., absence of MMP-9) seems to be driven mainly by diffusion. Taken together, these results indicate the mechanism of drug transport and release changed significantly in the presence of MMP-9 for hydrogels containing the MMP-responsive linker.

To mimic the flare-up characteristics seen in several inflammatory skin diseases and to investigate the enzymatic release associated with such flare-ups, activated MMP-9 was repeatedly added to the donor chamber of the IVRT set-up (Fig. 4E). Both 4-arm and 8-arm PEG hydrogels were found to respond to the addition of newly activated MMP-9 stimulus, as confirmed by the stepwise increase in cumulative release. Interestingly, for the 4-arm PEG hydrogels the fourth and fifth MMP-9 addition did not contribute to increase in release, whereas for 8-arm PEG hydrogels an increase in cumulative release was also seen after the fourth MMP-9 addition (Fig. 4E). A mass balance study was carried out to measure the remaining tofacitinib citrate in the hydrogels after termination of the flare-up release study (Table S1). For 4-arm PEG hydrogels, a loss of $6.7 \pm 1.9$ % and for 8-arm PEG hydrogels a loss of $13.8 \pm 2.3$ % of tofacitinib citrate were observed. A stagnation in release seen after 60 h for the 4-arm PEG hydrogels in response to MMP-9 may be explained by the loss of tofacitinib citrate in combination with the remaining amount of tofacitinib citrate being entrapped in the core of the hydrogel. Despite these limitations, the results demonstrate that the multi-arm PEG hydrogels indeed were responsive towards MMP-9 also in a flare-up mimicking scenario. To further evaluate the sensitivity of the hydrogels, the drug release could also be investigated using cell strainers or ex vivo skin models, where the hydrogels are in direct contact with the cells producing MMPs [45].

3.4. In vitro cytocompatibility and anti-inflammatory effect

Next, we investigated the cytotoxicity of hydrogel breakdown products after exposure to MMP-9, i.e., polymer and peptide residues (Fig. 5A), as well as to all separate hydrogel components employing 3T3 fibroblasts (Fig. 5B). For this, the metabolic activity of the cells was measured by adding PrestoBlue® reagent, a cell-permeant viability indicator that utilizes the reducing environment in living cells to reduce the non-fluorescent resazurin to the highly fluorescent resorufin. No significant reduction in cell viability was observed for either the MMP-9 generated cleavage products (Fig. 5A) or the separate hydrogel components (Fig. 5B). From this, it can be concluded that both the intact and cleaved hydrogel formulation were essentially non-cytotoxic to fibroblasts under the conditions investigated.

AD is a chronic inflammatory skin disease characterized by the upregulation of T-cell cytokines such as IL-4, IL-13, IL-22 and INF-γ [62]. These cytokines were used in the AD-like keratinocyte assay to stimulate keratinocytes. For this, the anti-inflammatory activity of tofacitinib citrate released from 5% (w/w) PEG hydrogels with MMP-sensitive linker B was tested to measure its inhibitory effect on the levels of CCL-2 released into the supernatant by the keratinocytes (Fig. 6). Tofacitinib citrate is a pan-JAK inhibitor and targets JAK3 and JAK1, leading to modulation of several downstream inflammatory signals (e.g., CCL-2, INF-γ, IL-6, IL-4, IL-2) [63]. Tofacitinib citrate samples were collected at different time points from the release set-up described in Section 2.5, where the 4-arm and 8-arm PEG hydrogels were exposed to growth medium without (Fig. 6A, C) or with 20 nM MMP-9 (Fig. 6B, D). It was found that the release samples collected from experiments without MMP-9 do not fully inhibit the production of CCL-2 in the early sampling times (1 and 4 h) for 4-arm PEG and 8-arm PEG hydrogels (Fig. 6A, C). However, a higher degree of CCL-2 inhibition was seen for the release samples (1 h and 4 h) for the 4-arm hydrogels compared to the 8-arm hydrogels, which correlates with the in vitro release data presented in Fig. 4A. When the hydrogels were exposed to MMP-9, however, the resulting increased tofacitinib citrate concentration present after triggered release was found to result in full inhibition of CCL-2 production at all sampling times for 4-arm PEG hydrogels (Fig. 6B), whereas an increased inhibitory effect was observed for 8-arm PEG hydrogels (Fig. 6D). As a comparison, samples with free tofacitinib citrate (1 nM to 10 μM) were tested, and the EC50 found to be $\sim$45 nM (Fig. 5B). In addition, the anti-inflammatory effect of the hydrogel released tofacitinib citrate was tested in an activated T-cell assay. Measurement of IL-4 and IL-2 levels confirmed the anti-inflammatory effect of the hydrogel released tofacitinib citrate. The degree of inhibition of IL-4 can be seen in Fig. 6E-H and the degree of inhibition of IL-2 can be seen in Fig. 6I-L. The release samples taken at 1 h and 4 h inhibit the IL-4 production more efficiently compared to the IL-2 production. Additional data with release samples up to 72 h can be seen in Fig. S10 for IL-4 and Fig. S11 for IL-2. In conclusion, the entrapment of tofacitinib citrate in multi-arm PEG hydrogels cross-linked by an MMP-sensitive peptide linker and its subsequent release did not compromise its anti-inflammatory activity.
4. Conclusion

In this paper, we have presented a rational design of MMP-responsive hydrogels for treatment of inflammatory skin disease. We have demonstrated that multi-arm PEG-a-NB can be cross-linked by peptide linkers to form hydrogels by thiol-norbornene photopolymerization with a nearly complete cross-linking degree. As a pre-clinical proof-of-concept, we verified that such hydrogels can be designed to deliver tofacitinib citrate in response to MMP-9 exposure. Both concentration and architecture of PEG multi-arms allows the physical and mechanical behavior of the hydrogels to be varied, e.g., for tailoring different indications and application routes. Under the conditions investigated, no cytotoxicity was observed, neither related to the MMP-responsive hydrogels nor to the cleavage products generated during their exposure to MMP-9. In an AD-like keratinocyte assay and an activated T-cell assay, we further confirmed that incorporation of tofacitinib citrate into the hydrogels does not compromise the anti-inflammatory effect of tofacitinib citrate. In conclusion, the MMP-responsive multi-arm PEG hydrogels developed in this study therefore represent a ver-
satellite drug delivery system with easily tailorable components and a simple drug loading mechanism suitable for a range of therapies. Translation into an intradermal depot of such hydrogels seems to be promising with respect to the treatment of flare-up-driven diseases characterized by high MMP activity, including psoriasis, atopic dermatitis, and hidradenitis suppurativa. To drive the development of these systems towards becoming the next generation therapies, however, more research is required in order to optimize administration possibilities, understand the in vivo behavior, stability, and degradation mechanisms.

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**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 paper.

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**Supplementary materials**


**References**


