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Injectable Supramolecular Ureidopyrimidinone Hydrogels Provide Sustained Release of Extracellular Vesicle Therapeutics

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Extracellular vesicles (EVs) are small vesicles secreted by cells and have gained increasing interest as both drug delivery vehicles or as cell-free therapeutics for regenerative medicine. To achieve optimal therapeutic effects, strategies are being developed to prolong EV exposure to target organs. One promising approach to achieve this is through EV-loaded injectable hydrogels. In this study, the use of a hydrogel based on ureido-pyrimidinone (UPy) units coupled to poly(ethylene glycol) chains (UPy-hydrogel) is examined as potential delivery platform for EVs. The UPy-hydrogel undergoes a solution-to-gel transition upon switching from a high to neutral pH, allowing immediate gelation upon administration into physiological systems. Here, sustained EV release from the UPy-hydrogel measured over a period of 4 d is shown. Importantly, EVs retain their functional capacity after release. Upon local administration of fluorescently labeled EVs incorporated in a UPy-hydrogel in vivo, EVs are still detected in the UPy-hydrogel after 3 d, whereas in the absence of a hydrogel, EVs are internalized by fat and skin tissue near the injection site. Together, these data demonstrate that UPy-hydrogels provide sustained EV release over time and enhance local EV retention in vivo, which could contribute to improved therapeutic efficacy upon local delivery and translation toward new applications.

Extracellular vesicles (EVs) are nanosized lipid bilayer-enclosed particles that play major roles in cell-to-cell communication and tissue homeostasis.[1–3] EVs contain specific cargo including genetic material (mRNA, miRNA, lncRNA), proteins, and lipids.[4] They are released by every cell type of the human body studied to date. The ability of EVs to naturally target and cross membrane barriers and deliver their biological cargo intracellularly makes them potentially useful as drug delivery vehicles.[5,6] Moreover, EVs have also gained interest as potential off-the-shelf therapeutics for regenerative medicine applications, since the beneficial effect of progenitor cell therapy has been ascribed to paracrine factors including EVs, mainly due to their anti-inflammatory, antifibrotic, pro-proliferative, and pro-angiogenic characteristics.[7–9] For regenerative medicine applications, EVs may be derived from various cell sources, including mesenchymal stromal cells

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DOI: 10.1002/adhm.201900847

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(MSC), tissue-specific progenitor cells, or induced pluripotent stem cells (iPSC).[10–14] Furthermore, EVs have been considered for application in different patient categories, including peripheral artery disease, cardiomyopathies, chronic kidney disease, and osteoporosis/cartilage degradation.[15–21]

Often, injection of cellular therapeutics demonstrated only modest beneficial outcomes in different patients groups, as a result of retention problems.[22–25] One example is the randomized, controlled BOne marrOw transfer to enhance ST-elevation infarct regeneration (BOOST) trial, where an intracoronary infusion of a single dose of bone marrow cells (BMCs) was performed in patients with acute myocardial infarction.[24] They found an improved cardiac function after six months, however, this beneficial effect was lost after 18 months. Further studies showed that only 5% of the BMCs were retained in the heart after intracoronary infusion, indicating potential retention problems with cellular therapeutics.[26] Similarly, strategies to enhance EV delivery in chronically diseased patients and prolong exposure of EV therapeutics have yet to be optimized to achieve their full potential for true therapeutic efficacy.

Several EV administration routes have been investigated to date, of which intravenous injection is the most widely studied. Unfortunately, intravenously injected EVs are rapidly taken up by the liver and spleen, thereby hampering delivery to other tissues.[27–29] On the other hand, local administration into the diseased organs could be a valuable approach to enhance tissue-specific EV delivery, thereby improving their efficiency and safety profile. However, when investigating cell retention after local injection into porcine hearts,[30] we previously observed an immediate washout of almost all cells via the venous drainage system, resulting in a substantial cell loss. Furthermore, Beegle et al. investigated the retention of VEGF-overexpressing MSC after local intramuscular injection into the hindlimb using bioluminescent imaging,[31] and found that the cell numbers after injection rapidly declined over time, and only less than 0.1% of the total number of injected MSC could be detected after a period of 28 d.

Given this immediate washout of cells, which may also be expected for EVs and will lead to decreased therapeutic exposure, the usage of patches, injectable microcarriers or hydrogels is intensively studied, aiming for increased retention of therapeutics.[32–35] A recent example is the study from Nikravesh et al., showing controlled release of osteoblast-derived EVs using two alginate-based microgels.[34] In addition, the EV release profile was tunable based on physical structuring of the alginate polymers. Incorporation of EVs in a hydrogel could allow for controlled EV release over longer periods, which could even further enhance therapeutic exposure, maximizing their efficacy.

Injectable hydrogels are among the most promising candidate systems to increase EV local retention. These systems are either based on natural (e.g., extracellular matrix-derived or collagen/fibrin-based) materials that closely mimic the host tissue, or synthetic materials (e.g., poly(ethylene glycol) (PEG) or poly(N-isopropylacrylamide)-based) that are easily tunable, have controllable biochemical properties, and might be less vulnerable to batch-to-batch variation.[36,37] Moreover, various injectable hydrogels are available that differ in composition and mechanical and gelation properties induced by changes in physiological conditions such as temperature, ionic strength, and pH.[37]

One highly potential injectable material is the ureido-pyrimidinone (UPy) supramolecular hydrogelator (UPy-hydrogel).[38] This gel consists of polymers comprising a poly(ethylene glycol) (PEG) backbone telechelically coupled to two UPy units that can form transient supramolecular networks by dimerization of the UPy moieties by four fold hydrogen-bonding and concomitant stacking into nanofibrous structures. This nanofiber formation is facilitated by additional urea groups introducing lateral hydrogen bonding in a hydrophobic pocket provided by additional alkyl spacers. The nanofibrous structures form the transient network by entanglements and supramolecular crosslinking between the nanofibers.[38] The UPy-hydrogel undergoes a solution-to-gel transition when the pH is switched from high to neutral, that is, from basic to neutral environment, with a threshold at pH ≈ 8.5, dependent on the concentration of UPy-polymers. This unique property allows gelation upon injection into physiological systems. The molecular weight of the PEG polymer can also be tuned, resulting in UPy-hydrogels with different functional properties,[39] of which the characteristics have been reported extensively before.[38–42]

Over the past years, UPy-hydrogels have also been investigated as controlled release system for different applications, including for growth factor and miRNA delivery.[40,42] Here, we investigated the use of UPy-hydrogel as a platform for EV delivery, aiming to prolong local delivery of EVs to targeted organs.

To investigate if EV delivery can be prolonged when EVs are incorporated into UPy-hydrogels, we used EVs released from cardiac-derived progenitor cells as they have well-known functionalities.[21,43–45] EV preparations were characterized based on size distribution profile, measured by nanoparticle tracking analysis (NTA) before encapsulation as well as after release from UPy-hydrogel, and by expression of EV markers (**Figure 1**A+B). According to NTA, EVs displayed a classical size distribution profile peaking at 100 nm, as we have seen before.[43] Moreover, encapsulation and release of EVs from UPy-hydrogels did not affect their size distribution profile. Western blot analysis showed an enrichment of typical EV markers such as Alix, CD81, and CD63 as compared to cell lysates. In contrast, calnexin, an endoplasmatic reticulum marker, was undetectable in EVs, confirming a lack of contamination with other membrane compartments.

To study the use of UPy-hydrogels for sustained EV delivery, EVs were loaded into UPy-hydrogels and transferred to an insert of a transwell system containing medium in the bottom compartment. In order to determine the kinetics of EV release from UPy-hydrogels, conditioned medium was sampled at several time points. Subsequently, a bead capture assay was performed to measure EV release in the conditioned medium. When using magnetic beads coated with antibodies directed against the EV markers CD9, CD81, or CD63 and their corresponding fluorescent detection antibodies, we were able to detect different EV marker proteins being released from cardiac progenitor cells (CPCs) (Figure S1, Supporting Information). To assess EV release from UPy-hydrogels, EVs were captured using CD63 antibody-coated magnetic beads and fluorescently labeled with antibodies directed against CD63 (Figure 1C).

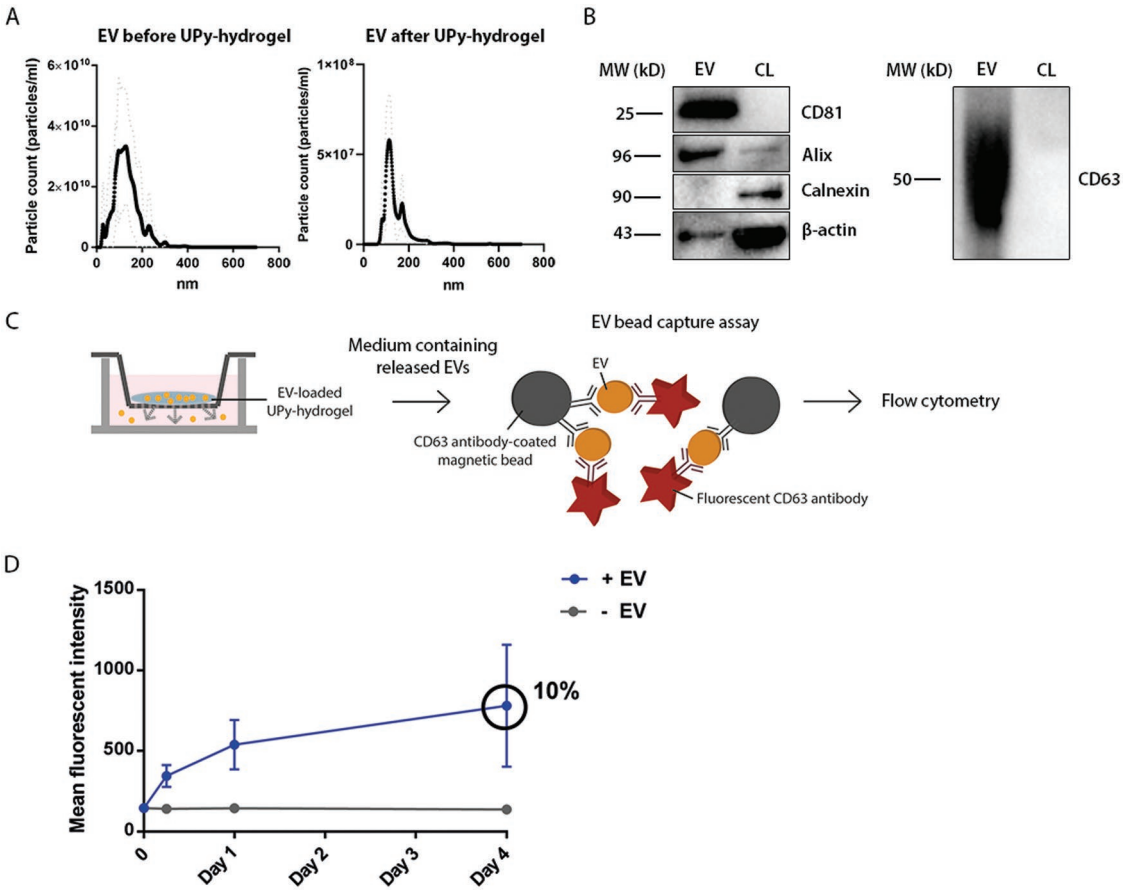

Figure 1. Sustained release of EVs from UPy-hydrogel in vitro. CPC-derived EVs were isolated using ultrafiltration followed by size-exclusion chromatography. A) Nanoparticle tracking analysis (NTA) was performed to show EVs' size distribution before encapsulation and after release from UPy-hydrogel. NTA revealed an average EV size of 100 nm, which was not affected by incorporation into and release from UPy-hydrogels. Results are presented as mean ± SD (black line and dotted line, respectively). B) The presence of typical EV markers CD81, Alix, and CD63 in our EV preparation was confirmed using Western blot analysis. The endoplasmic reticulum membrane protein calnexin was not present in our EVs. C) To evaluate EV release from UPy-hydrogel, conditioned medium containing the released EVs were collected at several time points up to 4 d. An EV bead capture assay was performed to assess EV release based on CD63+fluorescent intensity. D) ≈10% of the initial EVs were released from UPy-hydrogel after 4 d, calculated by using a standard curve of multiple known EV concentrations. Data are displayed as mean ± SD of three replicate experiments. Abbreviations: CL = cell lysate, CPC = cardiac progenitor cell, MW = molecular weight.

The molecular weight of the PEG block within UPy-hydrogels can be varied which may result in different kinetics of release. Therefore, first, EV release patterns from UPy-hydrogels with a PEG block of 10 kg mol^{-1} (UPy10k) or 20 kg mol^{-1} (UPy20k) were compared, which revealed a delayed EV release from the UPy10k-hydrogels as compared to UPy20k-hydrogels (Figure S2, Supporting Information).[38] Moreover, the release pattern of the UPy10k-hydrogels showed less variation. Therefore, we decided to continue with the UPy10k-hydrogels for future experiments. Next, EV signals in conditioned medium were assessed for UPy-hydrogels with and without EVs at different time points (Figure 1D). The percentage of EVs that was released from the gel after 4 d was estimated to be ≈10%, as assessed using a standard curve of known EV concentrations. A gradual and sustained release of EVs from UPy-hydrogel was observed over a period of several days rather than an immediate burst of EVs, indicating its potential to prolong EV delivery. When measuring long-term release of EVs from UPy-hydrogels, we found that EVs are continuously released from the hydrogel for up to 2.5 weeks (Figure S3, Supporting Information). In contrast, Hernandez et al. investigated EV release from extracellular matrix-derived (ECM) hydrogels and found that the majority of released EVs were already detected 1 d after encapsulation, thus the release profile of EVs from our UPy-hydrogel seems favorable as compared to EV release from ECM hydrogels.[35] EV release from ECM hydrogels varied from 25 to 45% after 3 d, depending on hydrogel tissue source. Furthermore, when incorporating miRNA or antimiR molecules in UPy-hydrogels, Bakker et al. observed near complete release from UPy-hydrogels within 2 d.[42] This release could be delayed by using cholesterol-conjugated molecules, suggesting an affinity of cholesterol to the UPy-hydrogel network. EVs are much larger in size and also contain high levels of cholesterol, which could possibly explain the more gradual release of EVs compared to miRNA or antimiR molecules. Since our method of EV detection is based on the expression of CD63, we cannot exclude a bias for a specific EV population. However, since we were able to detect multiple EV markers using this method

(Figure S1, Supporting Information), and as we are mainly interested in release of EVs from UPy-hydrogels and its application in vivo in general, we assume the release profile is similar for other EV populations of the same size and composition.

Importantly, to achieve EVs' full therapeutic potential, they should remain biologically active after release from hydrogels. EVs have the ability to transfer their biological cargo, including proteins and RNA, between cells after EV uptake by the recipient cell.[46] Therefore, to ensure that EVs maintain their integrity after release from UPy-hydrogels, we assessed EV uptake by human microvascular endothelial cells (HMEC-1). Moreover, since CPC-EVs that were used in this study have been shown to induce phosphorylation of ERK1/2, we used ERK1/2 activation in HMEC-1 as a second outcome parameter to determine EV functionality.[43] PKH26-labeled EVs were loaded into UPy-hydrogels, placed in a transwell insert and cocultured with HMEC-1 in the bottom well (**Figure 2**A). After 4 d, PKH26-labeled EVs were visible in the HMEC-1 cocultured with EV-loaded UPy-hydrogels, whereas UPy-hydrogels without EVs did not show any positive PKH26-staining (Figure 2B). In addition, we evaluated if EVs maintained their functional ability to activate signaling pathways in targeted HMEC-1 after being released from UPy-hydrogel. Medium containing EVs released from UPy-hydrogels, collected after one week but also after two weeks, retained the ability to activate ERK signaling as evidenced by an increased pERK/ERK ratio in HMEC-1 upon exposure (Figure 2C). When compared to fresh EVs, EVs released after one week can activate ERK1/2 to the same extent as fresh EVs (Figure S4, Supporting Information). When released after two weeks, EVs still retain the ability to activate ERK signaling, although to a lower extent than fresh EVs, which should be further investigated in future studies. Together, these data indicate that EV function is preserved after release from UPy-hydrogels. We showed that after release from UPy-hydrogels EVs retain the ability to be taken up by recipient cells and activate internal signaling pathways, indicating a preserved biological function of EVs.

The abovementioned results confirmed a sustained EV release from UPy-hydrogels in vitro. To achieve increased therapeutic exposure in vivo however, improving local EV retention

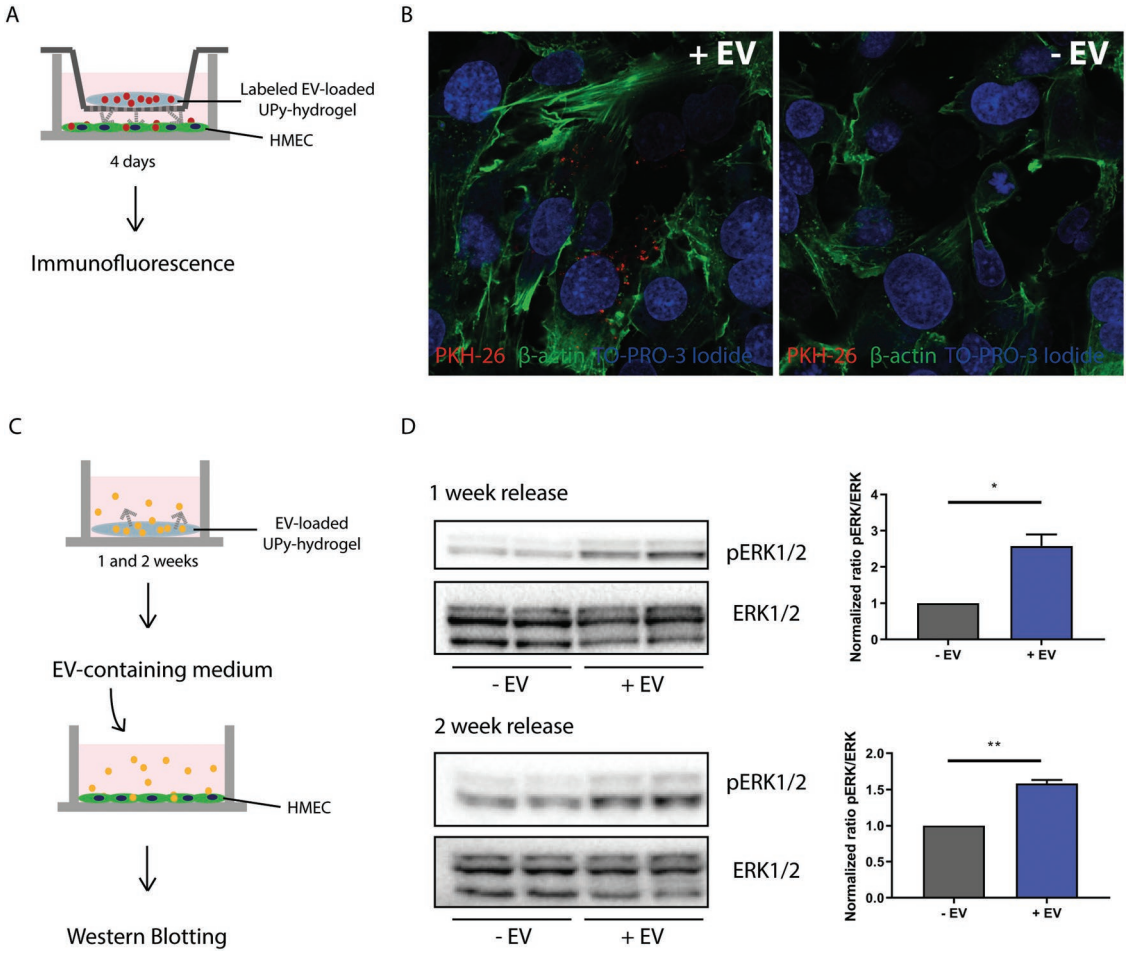

Figure 2. EVs retain their functionality after release from UPy-hydrogel. A) In vitro setup to determine uptake of PKH26-labeled EVs by HMEC-1 after release from UPy-hydrogels. B) Fluorescent image of HMEC-1 cocultured with either empty or EV-loaded UPy-hydrogels showing that EVs can be taken up by HMEC-1 after release from UPy-hydrogel. C) In vitro setup to determine if EVs maintain their ability to activate ERK signaling. D) EVs collected after one or two weeks after release retain the ability to induce phosphorylation of ERK1/2. Data are displayed as mean ± SD. * represents $p < 0.05$ and ** $p < 0.01$ using an unpaired Student's *t*-test. Abbreviations: ERK1/2 = extracellular signal-regulated kinase 1/2, HMEC-1 = human microvascular endothelial cells.

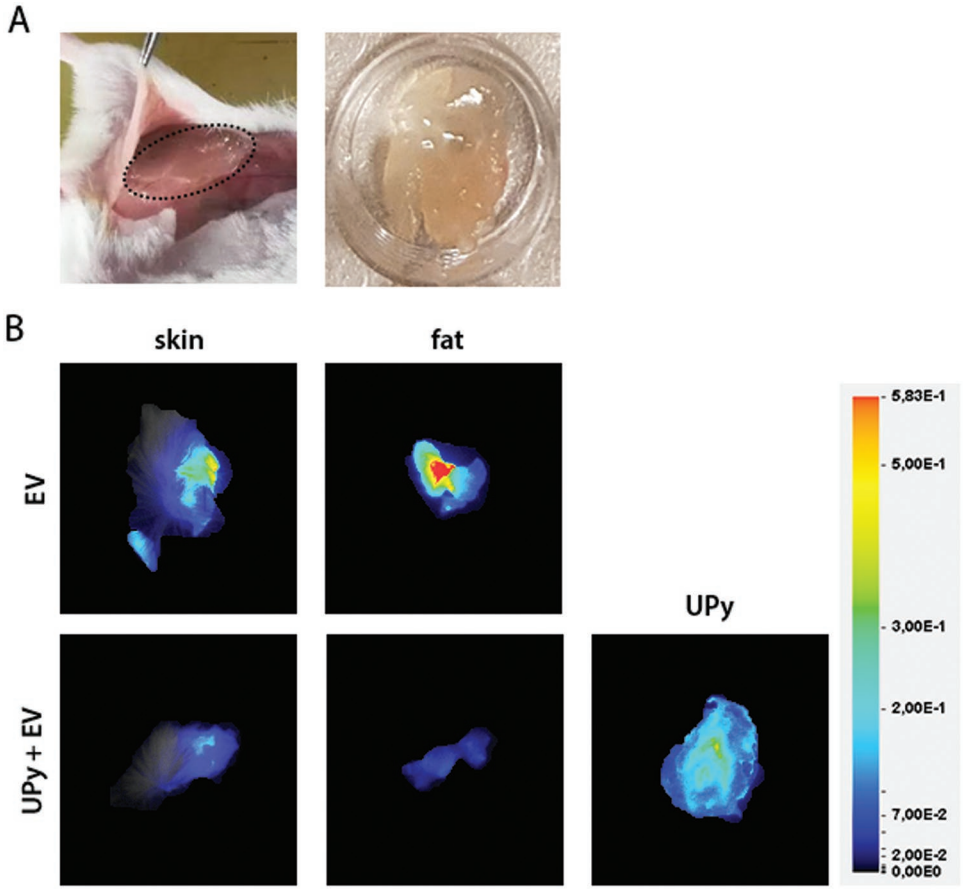

Figure 3. In vivo retention of EVs in UPy-hydrogels. A) Subcutaneously injected UPy-hydrogel can be detected as a solid-like gel 3 d after administration. B) Fluorescent images of skin, fat, or UPy-hydrogel from mice treated with either EV alone or UPy + EV. In the EV group, most of the fluorescent signal was present in surrounding skin and fat, whereas in the UPy + EV group EVs were retained in the UPy-hydrogel.

would also be of great importance. Therefore, we evaluated the feasibility of UPy-hydrogel to improve local retention of EVs in an in vivo model. UPy-hydrogel loaded with fluorescently labeled EVs (UPy + EV) or fluorescently labeled EVs alone (EV) were injected subcutaneously in mice. Solid-like UPy-hydrogels could be detected in all mice 3 d after subcutaneous injection (**Figure 3**A and Figure S5, Supporting Information). UPy-hydrogels, skin, and fat tissue near the injection site were excised and fluorescent signals (800 nm) were visualized using a Pearl Imager (Li-cor). In the UPy + EV group, most of the fluorescent signal could be detected within the UPy-hydrogel, whereas in the EV group highest fluorescent signals were present in surrounding tissues including skin and subcutaneous fat tissue (Figure 3B). These data clearly show that EV retention in vivo can be improved by encapsulating EVs in UPy-hydrogels compared to EV treatment alone.

Our study may also potentially be extended to other biomedical applications, such as therapeutic drug delivery, where EVs are regularly being used as drug carriers.[47,48] The injectable nature of UPy-hydrogels makes them an attractive candidate for local clinical applications. For example, Bastings et al. showed that growth factor-loaded UPy-hydrogels could be delivered into the infarcted myocardium by catheter-guided injections.[40] Furthermore, the ability to visualize a gadolinium(III)-DOTA labeled UPy-hydrogel with contrast enhanced MRI could enhance injection accuracy when applying this to patients, emphasizing feasibility of UPy-hydrogels for translational purposes.[41]

Near-infrared fluorescently labeled EVs were used in this study, which allows for studying their biodistribution in different tissues in vivo, but does not allow true quantification. A study by Royo et al. used radioactive labeling of EVs with Na[124I]I and detection with positron emission tomography (PET) to quantify EV biodistribution in vivo.[49] The use of radioactive-labeled EVs to investigate in vivo biodistribution will make quantification possible, however, this method requires specific technologies to be available. EV release kinetics from UPy-hydrogel in vivo therefore remains to be investigated.

Interestingly, Gangadaran et al. observed an additional beneficial effect on blood perfusion and formation of new blood vessels in a hind-limb ischemia model when treated with mesenchymal stromal cell derived EVs (MSC-EV) in matrigel compared to MSC-EV alone.[17] In addition, when combining endothelial progenitor-derived EVs with a shear-thinning hydrogel, Chen et al. showed increased therapeutic efficacy after myocardial infarction compared to EV treatment alone.[10] These studies indicate the potential additional value of sustained EV release on tissue repair. Whether sustained EV

release from UPy-hydrogels increases therapeutic efficacy in vivo compared to a single EV dose remains to be investigated.

In conclusion, this study shows that EVs are gradually released from UPy-hydrogels and remain associated with hydrogels upon injection in vivo. Furthermore, EVs keep their functionality after release in vitro. Therefore, EV delivery to specific sites may be prolonged using UPy-hydrogel, which could contribute to higher therapeutic effects upon local delivery and translation toward new applications.

Experimental Section

Cell Culture: CPC and HMEC-1 were cultured as described before.[44,50] Cells were incubated at 37 °C (5% CO_2 and 20% O_2) and passaged at 80–90% confluency using 0.25% trypsin digestion. For EV isolation, CPCs were cultured for 3 d, after which medium was replaced with serum-free Medium 199 (Gibco, 31150-022). After 24 h, conditioned medium (CM) was collected.

EV Isolation Protocol: EVs were isolated using ultrafiltration combined with size-exclusion chromatography (SEC).[43] First, cell culture CM was centrifuged at 2000× *g* for 15 min, followed by 0.45 µm filtration to remove residual cell debris. Subsequently, CM was concentrated using 100 kDa molecular weight cutoff Amicon spin filters (Merck Millipore), after which it was loaded onto a S400 highprep column (GE Healthcare, Uppsala, Sweden) and fractionated using an AKTAStart (GE Healthcare) system equipped with an UV 280 nm flow cell. After elution from the column, EV-containing fractions were pooled, 0.45 µm filtered, and concentrated using a 100 kDa Amicon spin filter. EV particle number and size distribution were determined using nanoparticle tracking analysis (Nanosight NS500, Malvern). The camera level was set at 15 and the detection threshold at 5.

EV Labeling: For in vitro experiments, EVs were labeled with PKH-26 using a red fluorescent cell linker kit (Sigma, PKH26GL) according to the manufacturer's protocol. In short, EVs were diluted with Diluent C, followed by incubation with 5×10^{-6} M dye for 15 min at room temperature in the dark. Next, free dye was removed using a Sepharose CL-4B column coupled to an AKTAStart, followed by concentration of EV-containing fractions with a 100 kDa Amicon spin filter. For in vivo studies, EVs were labeled with Alexa Fluor 790 NHS Ester dyes (ThermoFisher, A37569). EVs were incubated with 30×10^{-6} M reactive dye in 0.1 M $NaHCO_3$ in phosphate buffered saline (PBS) and incubated for 45 min at 37 °C while shaking at 450 rpm. After labeling, dye was quenched using a final concentration of 50×10^{-3} M Tris-HCl for 30 min and free dye was removed using a Sepharose CL-4B column coupled to an AKTAStart. EV-containing fractions were concentrated using a 100 kDa Amicon spin filter.

UPy-PEG Hydrogel Preparation and EV Loading: The UPy-PEG polymers with a 10k and 20k PEG-spacer were synthesized as described before (SyMO-Chem BV, Eindhoven, The Netherlands).[38] The hydrogelator powder was dissolved at a high pH (11.7) in PBS to the appropriate concentration. The mixture was vigorously shaken for 1 h at 70 °C until a homogenous mixture was obtained. Subsequently, the mixture was cooled down to RT and pH was confirmed to be ≈9.

EV Release Studies: UPy-hydrogels (10% w/w) of 100 µL were mixed with 10×10^{10} EVs in PBS of pH 9 to prevent presolidification. Liquid UPy-hydrogels were transferred to transwell inserts with 8 µm pore size (Greiner, 662638) and solidified by raising pH to 7.4. Release of EVs from UPy-hydrogels was examined by placing the transwell inserts in wells containing 1 mL of medium. Next, 200 µL of conditioned medium was collected at 6 h, 24 h, and 4 d and replaced with new medium after each time point. Release kinetics were assessed using flow cytometry after capturing EVs with antibody-coated magnetic beads, as described before.[35] In short, EV-containing medium was incubated O/N with either, CD9-, CD81-, or CD63-antibody-coated magnetic beads (ExoCap, JSR Life Sciences) and washed with 2% bovine serum albumin (BSA) in PBS. Subsequently, CD9-, CD81-, or CD63-Alexa647 antibody (CD9, BD Bioscience, 341648, clone M-L13; CD81, BD Biosciences, 551112, clone JS-81; CD63, BD Biosciences, 561983, clone H5C6) in PBS was added and incubated for 2 h at RT while shaking. After washing with 2% BSA in PBS, samples were resuspended in 0.25% BSA in PBS for analysis. Mean fluorescence intensity (MFI) of bead-captured EVs was measured using flow cytometry (BD FACSCanto II).

EV Functionality: For EV uptake experiments, PKH26-labeled EVs (22×10^{10}) were loaded into an UPy-hydrogel and placed into transwell inserts as described above. 1.2×10^5 HMEC-1 cells were plated in the lower compartment. After 4 d, EV uptake by HMEC-1 cells was determined using fluorescent microscopy. Cells were fixed using 4% PFA and incubated with a primary antibody for β-actin (1:1000) (Sigma, A5441) at RT for 1 h, followed by incubation with Alexa Fluor 488 goat anti-mouse secondary antibody (1:2000) (Invitrogen, A11001). Nuclei were stained using TO-PRO-3 Iodide (ThermoFischer, T3605). Fluorescent images were taken using a confocal microscope (Zeiss, LSM 700).

EV functionality was also assessed by determining their effect on ERK1/2 phosphorylation as described before.[21,43–45] In short, 1.2×10^5 HMEC-1 cells were starved for 3 h using basal MCDB131-medium, after which conditioned medium from EV-loaded UPy-hydrogels was added and incubated for 30 min. Cells were lysed using lysis buffer (Roche, 04719964000), followed by centrifugation at 14 000× *g* for 10 min. Protein levels of phosphorylated ERK1/2 and total ERK1/2 were assessed using Western blotting.

Western Blotting: Protein lysates were loaded on precasted Bis-Tris protein gels (ThermoFischer, NW04125BOX) and run for 1 h at 160 V. Next, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, IPVH00010), followed by incubation with antibodies for 42/44 pERK1/2 (1:1000) (Cell Signaling, 43705), 42/44 ERK1/2 (1:1000) (Cell Signaling, 91025), Alix (1:1000) (Abcam, 177840), CD81 (1:1000) (Santa Cruz, sc-166029), CD63 (1:1000) (Abcam, 8219), Calnexin (1:1000) (Tebu-bio, GTX101676), or β-actin (1:7500) (Sigma, A5441). To visualize proteins a chemiluminescent peroxidase substrate (Sigma, CPS1120) was used. Quantification of the images was performed using ImageJ software (1.47 V).

In Vivo Studies: Female Balb/CAnnCrl mice (age 10–12 weeks, weight 20–30 g), originally obtained from the Jackson Laboratory and kept in a breeding facility, were housed under standard conditions with 12 h light/dark cycles and received standard chow and water ad libitum. All experiments were carried out according to the "Guide for the Care and Use of Laboratory Animals," with prior approval by the Animal Ethical Experimentation Committee, Utrecht University, the Netherlands.

Mice were anesthetized by inhalation of 2.0% isoflurane in a mixture of oxygen/air (1:1). Subsequently, 350 µL of 4×10^9 EVs ($N = 2$) or 4×10^9 EV-loaded UPy-hydrogels ($N = 4$) were injected subcutaneously in the right flank using a 25 G needle. The amount of EVs that were injected was 4×10^9. The needle was slowly retracted 30 s after injection to prevent any leakage from the injection site. Mice were euthanized 3 d after subcutaneous injection using sodium pentobarbital (60.0 g kg^{-1}). Skin and fat tissue around the injection site and UPy-hydrogels were excised and fluorescent images were acquired using a Pearl Impulse Imager (Li-cor), as described before.[51]

Statistical Analysis: Data are presented as mean ± SD. Unpaired Student's *t*-test was used for comparison of two groups. Significance levels were set as $p < 0.05$ or $p < 0.01$ as indicated.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors thank Maike Brans (UMC Utrecht), Hemse Al-Khamisi (UMC Utrecht), and Maaike Schotman (TU Eindhoven) for their excellent

technical assistance and useful discussions. This work was supported by the Project EVICARE (No. 725229) of the European Research Council (ERC) to J.P.G.S. and P.Y.W.D., co-funded by the Project SMARTCARE-II of the BioMedicalMaterials institute to J.P.G.S./M.-J.G., the ZonMw-TAS program (No. 116002016) to J.P.G.S./Z.L., the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the Netherlands CardioVascular Research Initiative (CVON): the Dutch Heart Foundation to J.P.G.S./M.-J.G., Dutch Federations of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Sciences. P.V. was funded by a VENI fellowship (No. 13667) from the Netherlands Organisation for Scientific Research (NWO).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

controlled release, drug delivery, exosomes, extracellular vesicles, injectable hydrogels

Received: June 30, 2019
Revised: August 23, 2019
Published online: September 26, 2019

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