

Human urine-derived stem cells rescue cutaneous manifestation and suppress inflammation and fibrosis *in vitro* and in a mouse model of recessive dystrophic epidermolysis bullosa

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Abstract

Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a life-threatening disease characterized by persistent wound inflammation, tissue fibrosis, and even tumorigenesis in later stages. Despite its significant impact on patient health worldwide, treatment of RDEB has limited options. There is a clear need for new interventions. The goal of this study was to explore the potential efficacy of urine-derived stem cells (USCs) in RDEB.

Methods: We isolated human USCs from healthy donors, and assessed their therapeutic effects on RDEB both *in vitro* with tissue-engineered skin in a three-dimensional co-culture system and *in vivo* with *COL7A1*^{-/-} (RDEB) mice.

Results: USCs showed suppressive effects on expression of inflammation- and fibrosis-related genes involved in RDEB *in vitro* and *in vivo*. USCs could also extend the median life span (from 3 to 5 days), improve the expression of C7, and migrate to various organs of RDEB mice after intrahepatic administration.

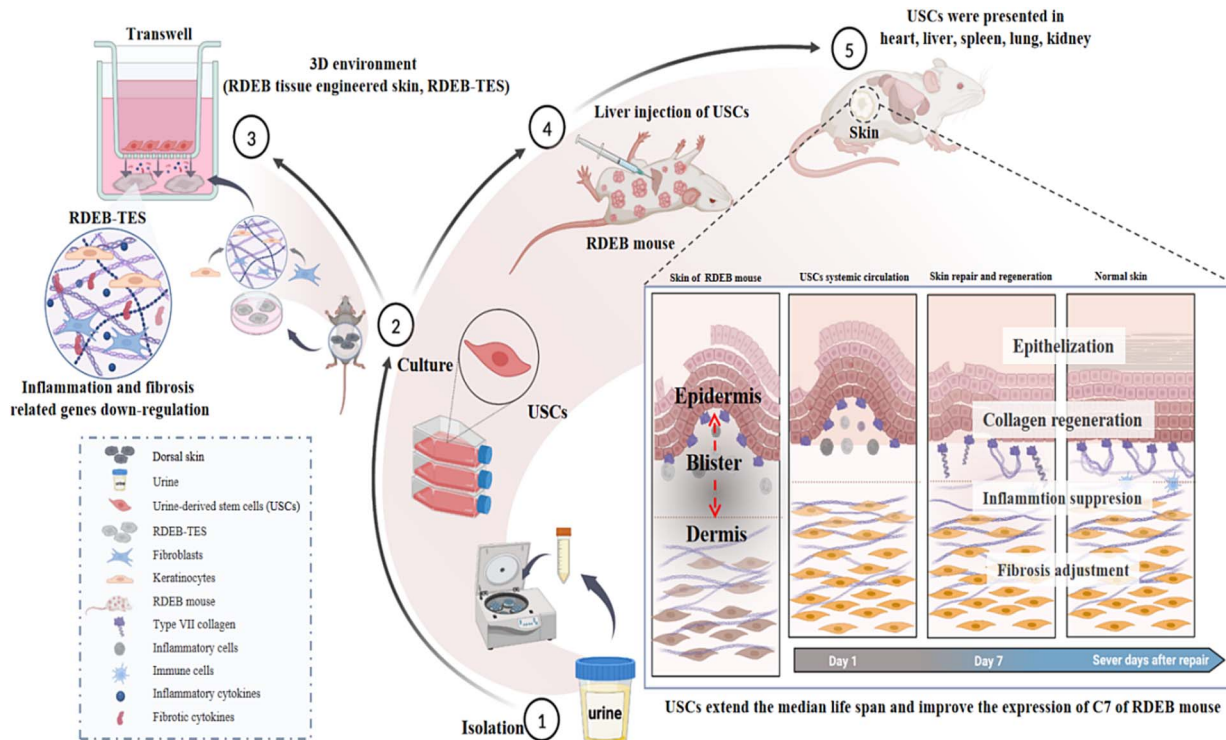
Conclusions: In summary, these results suggest the potential effects of USCs on improving the expression of C7 and would repair of RDEB, which supports the future use of USCs for the treatment of RDEB patients.

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Graphical Abstract



Keywords: Recessive dystrophic epidermolysis bullosa; Urine-derived stem cell; *COL7A1*; Collagen VII; Tissue-engineered skin

Highlights

- This study demonstrates that human USCs have several positive key properties on RDEB both *in vitro* with tissue-engineered skin in a three-dimensional co-culture system and *in vivo* with *COL7A1*^{-/-} (RDEB) mice.
- USCs could improve the expression of C7 at the dermis–epidermis junction of skin from RDEB mice.
- USCs exert a suppressive effect on inflammation and fibrosis progression of keratinocytes and fibroblasts of RDEB mice.

Background

Epidermolysis bullosa (EB), a rare and heterogeneous disease, is distinguished by the mechanical fragility of the skin and mucosa [1]. Recessive dystrophic EB (RDEB) is one of the most life-threatening types of EB with a high mortality rate in young adulthood. Mutations in the *COL7A1* gene, which codes for the type VII collagen (C7)–1 chain and leads to the aberrant expression of C7 produced by keratinocytes and fibroblasts, are involved in the pathogenesis of RDEB [2, 3]. Persistent wound inflammation and tissue fibrosis also lead to a marked increase in the incidence of aggressive cutaneous squamous cell carcinoma (cSCC) in patients with RDEB (90.1% by the age of 55 years) compared to the general population (1% for those aged 50–59 years) [4, 5]. Research has demonstrated that the activation of transforming growth factor-beta (TGF- β) signaling [6–8], abnormal deposition of matrix metalloproteinases (MMPs) [9, 10], epithelial-to-mesenchymal transition, and multiple inflammatory cytokines [11–13] were closely related to the tumorigenesis of patients with RDEB.

The current dominant management strategy for RDEB is amelioration of symptoms, but this provides only a slight benefit in cases of severe RDEB. Researchers have attempted to develop effective treatment strategies for RDEB using various

approaches, such as gene repair and cell therapies. The first attempt to treat seven children with RDEB in a clinic with allogeneic bone marrow transplantation was made in 2007 by Wagner *et al.* Five patients showed improvement in erosions healing and less blistering after the therapy [14]. In the following years, different types of stem cells for the treatment of RDEB were gradually emerged, but to date, almost all stem cell therapies for RDEB patients were in preclinical research or clinical trial stage (Table S1, see online supplementary material) [14–19]. Among various types of stem cells for the treatment of RDEB, mesenchymal stem cells (MSCs), a type of pluripotent stem cell with the capacity for self-renewal and multi-directional differentiation, also showed the potential in changing the disease course of RDEB [17, 20–22]. Currently, the commonly studied MSCs for EB therapy are mainly derived from the bone marrow, umbilical cord, and placenta [6, 17, 18, 23]. However, the acquisition of these MSCs usually involves invasive procedures and limited sources, and some of them also have ethical issues. In 2008, a stem cell population identified in urine (termed urine-derived stem cells [USCs]) exhibiting characteristics of MSCs was first reported by Zhang, *et al.* [24]. USCs can be isolated via a simple, harmless, and low-cost method, and our previous work confirmed that USCs can effectively repair skin defects,

intrauterine adhesions, and bladder injuries [25–27]. All of the aforementioned studies have drawn attention to exploring the therapeutic value of USCs in RDEB.

Additionally, the most commonly used *in vitro* models for RDEB are keratinocytes and fibroblasts derived from the skin of patients/mice with RDEB; however, 2D monolayer cultures of cells have limitations owing to the absence of complicated cell–cell and cell–extracellular matrix interactions. To overcome these limitations, we prepared tissue-engineered skin (TES) [28, 29] by arranging RDEB mice-derived keratinocytes/fibroblasts in acellular dermal matrix (ADM). This RDEB-TES allows the interaction of different cell types with one another and the surrounding environment [30–32], providing us with a 3D physiological research environment.

Based on the aforementioned, this study was conducted as the initial stage in applying USCs in patients with RDEB. We obtained human USCs from healthy donors and demonstrated whether USCs express *COL7A1*. We examined the effects of USCs on the expression of inflammation- and fibrosis-related genes in keratinocytes and fibroblasts from RDEB-TES using a Transwell coculture system. Lastly, the therapeutic effects of USCs on the overall survival, C7 expression, and other functional outcomes in *COL7A1*^{−/−} (RDEB) mice were also studied. We hope that this work can establish a research basis for the application of USCs in RDEB and provide a new alternative to stem cell therapy for the treatment of patients with RDEB.

Methods

Mice

C57BL/6J *COL7A1*^{+/-} mice were crossed with the genotype identified by polymerase chain reaction (PCR) to produce *COL7A1*^{−/−} RDEB mice (Figure S1, see online supplementary material). *COL7A1*^{+/-} mice were generously donated by Dr Chen Ting of the National Institute of Biological Sciences, Beijing. *COL7A1*^{+/-} mice were developed by specifically targeting the c.6485G > A point mutation in exon 80 of mouse *COL7A1* gene [33]. All animal studies were conducted following the procedures approved by the Animal Experiments Committee of Sichuan University (No. 20211261A).

Preparation of urine-derived stem cells

USCs were obtained from five healthy adult (aged 20–30 years) male donors using previously described methodologies [24, 34]. The Ethics Committee on Biomedical Research at West China Hospital of Sichuan University approved this research (2021-1066). All study participants provided written informed consent. The detailed steps and the flow chart of the USCs culture are presented in the Supplement (Figure S2, see online supplementary material). Briefly, 200 ml of urine was collected from each donor, subjected to a treatment regimen involving 1% penicillin and streptomycin (Gibco, USA), and centrifuged at 1500 rpm for 10 min. The resultant supernatant was decanted, and the sediment was subjected to dual rinsing with phosphate-buffered saline (PBS; ZSGB-BIO, China). From the pellet, the cells were seeded in a 25 cm² culture flask containing a nurturing medium composed of the previously introduced constituents [26]. The cells were cultured at 37°C in a humidified incubator with 5% CO₂. When cell clones had

formed, the culture medium was replaced with fresh medium. After that, the medium was replaced every two days. Upon attaining 80%–90% confluency, the cells were passaged at a ratio of 1:3. USCs at the third passage were used for the subsequent experiments.

Characterization of human urine-derived stem cells

Flow cytometry analysis

The nonspecific antigen of USCs was blocked by incubating them with 3% bovine serum albumin (Sigma, USA) for 30 min. Monoclonal antibodies (BD Biosciences) against cluster of differentiation (CD)29-APC, CD34-APC, CD44-FITC, CD45-APC, CD73-PE, CD105-APC, human leukocyte antigen (HLA)-DR-BV510, and HLA-ABC-FITC were incubated for an additional 30 min. At the end of the incubation, the cells were washed with PBS to remove unbound antibody and analyzed by a flow cytometry analyzer (Beckman Cytomics FC500, Beckman Coulter, USA).

Multipotent differentiation potential of urine-derived stem cells

Osteogenic differentiation. USCs cultured at Passage 3 were cultivated in a six-well plate. The medium was changed to osteogenic induction medium (Gibco) when the cells reached 80% confluency. The medium was replaced every 3 days. Alizarin Red S (Sigma) was used to stain the cells after 28 days of induction to identify the calcified extracellular matrix (Supplement file 1 for more details about methods).

Adipogenic differentiation. USCs cultured at Passage 3 were cultivated in a six-well plate. The medium was replaced with adipogenic induction medium (Gibco, USA) when the cells reached 80% confluency. The medium was replaced every 3 days. Oil Red O (Sigma) was used to stain the cells after 21 days of induction to visualize the lipid vacuoles (Supplement file 1 for more details about methods).

Chondrogenic induction. A pellet culture approach was used for chondrogenesis differentiation of USCs. In a 15 ml centrifuge tube, 1 × 10⁶ cells were pelleted by centrifugation, and chondrogenic medium (Gibco) was gently poured into the pellets. The pellets were embedded in optimum cutting temperature (OCT) compound after 28 days of induction and fixed in 4% paraformaldehyde (Sigma). Toluidine blue staining was used to stain the 10 μm cryosections, which were then observed under an optical microscope (Leica, Germany) (Supplement file 1 for more details about methods).

Quantitative reverse transcription polymerase chain reaction analysis for *COL7A1* expression in urine-derived stem cells

The total RNA was extracted from USCs using Trizol reagent. A real-time thermocycler (LC96, Roche, Switzerland) with TB Green[®] Premix Ex TaqTM II Mix (TaKaRa, Japan) was used to perform quantitative reverse transcription PCR (q-PCR) in triplicate. The following conditions were specified for q-PCR: 95°C for 30 s, 40 cycles of 95°C for 10 s, and 60°C for 30 s. A 2^{−ΔΔCt} technique was used to compare the relative expression of the genes to glyceraldehyde-phosphate dehydrogenase (GAPDH). Representative reverse transcription PCR (RT-PCR) analysis of the relative expression of *COL7A1* in USCs was also performed. The primers used to detect human *COL7A1* mRNA are listed in Table S2 (see online supplementary material).

Preparation and evaluation of tissue-engineered skin from wild-type and recessive dystrophic epidermolysis bullosa mice

Preparation of acellular dermal matrix from wild-type and recessive dystrophic epidermolysis bullosa mice

The dorsal skin of newborn WT and RDEB mice was digested by 10 mg/mL dispase II (Sigma) for 2 h at 37°C. The dermis was separated from the epidermis. The dermis was decellularized by freezing and thawing three times, added to a solution containing 0.5% Triton X-100 and 20 mM NH₄OH for 24 h, treated with 100 mg/mL DNase I for 4 h, and rinsed with running water for 18 h. The acellular effects were observed using residual deoxyribonucleic acid (DNA) quantification, hematoxylin–eosin (HE) staining, 4,6-diamidino-2-phenylindole (DAPI) staining, and Masson's trichrome staining. Scanning electron microscopy (SEM) (EVO10, Carl Zeiss AG, Germany) was performed to observe the microstructure of ADM.

Preparation of keratinocytes and fibroblasts from wild-type and recessive dystrophic epidermolysis bullosa mice

The epidermis and dermis of newborn WT and RDEB mice were isolated using the aforementioned methods. Epidermis with the basal layer placed downward on a Petri dish was digested in 0.25% trypsin–EDTA buffer for 15 min at 37°C. The basal layer of the keratinocytes was separated from the epidermis. The cells were cultured in CnT-07 medium (CELLnTEC, Switzerland). The medium was changed after 24 h to remove unattached cells, and then every three days. Finally, rabbit anti-cytokeratin 14 (CK14) (Abcam, UK, 1:200) and rabbit anti-C7 (Abcam, UK, 1:500) antibodies were used to incubate keratinocytes. The keratinocytes were then incubated with goat anti-rabbit secondary antibodies (Abcam, USA). The nuclei were stained with DAPI (Sigma). A confocal laser scanning microscope (Nikon, Japan) was used to observe and photograph all the samples ([Supplement file 1](#) for more details about methods).

The dermis was placed in a Petri dish and cut into 0.5 × 0.5 cm²-sized pieces; tissues were cut and explanted into culture dishes in an equilibrium manner with Dulbecco's modified Eagle medium-high glucose (DMEM-HG) (Gibco) containing 10% fetal bovine serum. The medium was changed every 2–3 days. Lastly, anti- α -smooth muscle actin (α -SMA) (Sigma, USA, 1:100) and anti-C7 antibody (Abcam, UK, 1:500) antibodies were used to incubate fibroblasts. The following process was performed as described for keratinocytes ([Supplement file 1](#) for more details about methods).

Preparation and evaluation of wild-type tissue-engineered skin and recessive dystrophic epidermolysis bullosa tissue-engineered skin

Indirect cytotoxicity tests were conducted in accordance with the GB/T16886.5-2003/ISO 10993-5:199 standard. Complete medium with ADM extraction (medium volume (mL):material's total surface area (cm²)=1:6) prepared at 37°C for 24 h was used as an extract solution. The positive and negative controls were composed of complete medium containing 1% phenol and complete medium, respectively. In a 96-well plate, 3 × 10³ cells/well of keratinocytes and fibroblasts were planted beforehand. The culture medium was changed to the corresponding extract solution after the cells were attached to the wall, and the liquid was

changed every other day. Cell proliferation was measured at 1, 3, 5, and 7 days using the Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Japan) at 450 nm.

Next, 1 × 10⁴ keratinocytes of passage 3 derived from WT mice skin were grown on the epidermal side (smooth side) of the acellular scaffold of WT mice (WT-ADM), keratinocytes derived from RDEB mice skin were grown on the epidermal side of the acellular scaffold of RDEB mice (RDEB-ADM); similarly, fibroblasts of passage 3 derived from WT mice skin were grown on the dermal side (rough side) of the WT-ADM; fibroblasts derived from RDEB mice skin were grown on the dermal side of the RDEB-ADM to establish the respective TES. After culturing for 72 h, a confocal laser scanning microscope (Nikon, Japan) was used to observe the growth status of the cells, which were stained with calcein-AM and propidium iodide (Sigma) for 30 min. SEM was used to observe the morphology of growing cells.

Wild-type tissue-engineered skin and recessive dystrophic epidermolysis bullosa tissue-engineered skin coculture with urine-derived stem cells

WT-TES and RDEB-TES established by keratinocytes and fibroblasts grown on the ADM were placed on the lower layer of a six-well transwell plate (Corning, USA), and 1 × 10⁶ USCs were planted on the upper layer and then cocultured for 48 h. Total RNA was isolated from cocultured TES using Trizol reagent. Q-PCR was performed in triplicate to analyze the relative expression of inflammatory and fibrotic factors using a previously described method. As for WT/RDEB-TES established by fibroblasts grown on the ADM, coculture with USCs was also performed following stimulation with 5 ng/mL TGF- β 1 for 24 h. Total RNA was isolated from the cocultured TES using Trizol reagent and analyzed by q-PCR, as described above.

Urine-derived stem cell administration in recessive dystrophic epidermolysis bullosa mice Therapeutic regimen

The buffer, made up of a 1:1 mixture of 0.9% NaCl and 5% human serum albumin containing 10% dextran 40, was used to resuspend USCs at a concentration of 7 × 10⁴ cells/ μ L. Fifteen microliters of the aforementioned solution (1 million cells) were injected into newborn RDEB mouse livers using a 31G insulin syringe (BD Biosciences, USA). RDEB mice that survived 5–7 days were randomly selected for the second injection. The vehicle control was prepared by administering the same volume of thawing buffer. All mice were euthanized on Days 7, 14, 21, and 28 after treatment.

Histological and immunofluorescence analyses

On Days 7, 14, 21, and 28 following USC administration, tissue samples from the selected mice were collected for histological and immunofluorescence investigations. The heart, liver, spleen, lung, kidney, and part of the dorsal skin were sectioned into specimens measuring 4 μ m in diameter after being preserved with 10% neutral formaldehyde and embedded in paraffin. The sections were then stained with HE. Dermis–epidermis junction (DEJ) separation percentage in each section was computed as the average ratio of the total separation/total DEJ of all paws per

finger digit. Image J software 1.8.0 was used for quantification by tracing the DEJ around the perimeter of the biopsies.

Part of the dorsal skin, hind paws, heart, liver, spleen, lung, and kidney were prepared as frozen OCT-embedded tissue and processed into 5 μ m diameter sections. The frozen sections of the dorsal skin were fixed using 10% neutral formaldehyde for a short time and stained with rabbit anti-C7 (OriGene, USA, 1:100), rabbit anti-collagen I (Affinity, USA, 1:200), and rabbit anti-TGF- β 3 (Affinity, USA, 1:200) antibodies. The frozen sections of the heart, liver, spleen, lung, and kidney were fixed using the same method as described before and stained with rabbit anti-HLA-ABC antibody (Abcam, USA, 1:200). Anti-fluorescent quenching coverslips were used to seal the sections that were counterstained with DAPI. A fluorescence microscope (Nikon, Tokyo, Japan) was used to capture images, and ImageJ software 1.8.0 was used for analysis.

Ultrastructural skin examination

Dorsal skin biopsies were prepared for transmission electron microscopy (TEM). Skin samples were fixed overnight with 3% glutaraldehyde. After washing with 0.1 M cacodylate buffer, the skin was incubated for 2 h with 1% osmium tetroxide solution at room temperature. The samples were dehydrated, penetrated, and embedded in an epoxide resin after washing in distilled water. Ultrathin slices were stained with uranyl acetate and lead citrate and then observed under a TEM (Hitachi, Japan).

In vivo imaging to evaluate the distribution of urine-derived stem cells

USCs were transfected with the First Ubiquitin promoter - Green fluorescent protein - Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (FUGW) plasmid (YiBang, China) to label luciferase (Thermo Fisher Scientific, USA), following standard procedures. USCs expressing luciferase were then thawed in a buffer composed of a 1:1 mixture of 0.9% NaCl and 5% human serum albumin containing 10% dextran 40 and injected into the liver of a newborn RDEB mouse. Subsequently, D-fluorescein potassium salt was injected intraperitoneally at different times. The distribution of USCs was dynamically observed using an *in vivo* imaging system (Caliper Life Sciences, USA).

Statistical analysis

Sample sizes for statistical analysis comprised three (*in vitro*) replicates per group at each time point. In the *in vivo* study, 61 mice were in the USC-treated group ($n=61$) and 55 mice in the untreated group ($n=55$). As for statistical analysis, all data were collected from at least triplicated samples and expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using R software (Version 4.3.1) and GraphPad Prism 9.0 software (GraphPad Software, USA). Differences between groups were analyzed using Student's *t*-test or one/two-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test or Dunn's test for multiple comparisons. Survival differences between groups were evaluated using Kaplan–Meier survival analysis, with statistical significance assessed by the log-rank test. A *P*-value < 0.05 was deemed to indicate statistical significance.

Results

Urine-derived stem cells met the criteria for defining multipotent mesenchymal stem cells

In USC culture plates, cell colonies were observed 7–14 days after the initial plating. A spindle- or rice-like morphology was observed in the majority of adhering cells (Figure S3a, see online supplementary material). USCs were positive for the antigens CD29, CD44, CD73, CD105, and HLA-ABC and negative for the antigens CD34 and HLA-DR according to flow cytometry analysis (Figure S3b, see online supplementary material). USCs could undergo osteogenic, adipogenic, and chondrogenic differentiation, as shown by the positive staining for Alizarin Red, Oil Red O, and Toluidine blue in osteogenic, adipogenic, and chondrogenic media, respectively (Figure S3c, see online supplementary material). Hence, USCs met the criteria for defining multipotent MSCs. Q-PCR and RT-PCR analyses showed that USCs expressed *COL7A1* (Figure S4, see online supplementary material).

Construction of wild-type tissue-engineered skin and recessive dystrophic epidermolysis bullosa tissue-engineered skin

Characterization of wild-type acellular dermal matrix and recessive dystrophic epidermolysis bullosa acellular dermal matrix

Figure 1a shows a clear view of WT-ADM and RDEB-ADM, which are white and semitransparent. DNA quantification verified that the average DNA content of WT-dermis, RDEB-dermis, WT-ADM, and RDEB-ADM was 1362 ± 32.15 ng/mg, 1445 ± 29.05 ng/mg, 37.43 ± 2.56 ng/mg, and 40.93 ± 2.06 ng/mg, respectively (Figure 1b). The average DNA content of WT-ADM and RDEB-ADM satisfied the criteria for decellularization (<50 ng/mg). HE and DAPI staining revealed that no recognizable residual nuclear debris was left in WT-ADM and RDEB-ADM, and Masson staining of ADM confirmed that the arrangement of collagen fibers in ADM was not only similar to that of the dermis but also intact without obvious fracture (Figure 1c). SEM observations revealed that the collagen fibers on the smooth sides of WT-ADM and RDEB-ADM were neatly aligned in a certain direction. The rough side surface of WT/RDEB-ADM was multi-porous, and collagen fiber bundles were loosely interlaced (Figure 1d).

Characterization of keratinocytes and fibroblasts

Keratinocytes isolated from the epidermis of WT and RDEB mice had cobblestone-like morphology. Immunofluorescence staining for anti-CK14 antibody was positive in WT/RDEB keratinocytes but, for anti-C7 antibody, was positive only in WT keratinocytes (Figure 2a). The fibroblasts of WT and RDEB mice presented spindle-like or triangular-like morphology, and immunofluorescence staining of anti- α -SMA antibody was positive. Only WT-fibroblasts were immunofluorescence-positive for anti-C7 antibody (Figure 2b).

Characterization of wild-type tissue-engineered skin and recessive dystrophic epidermolysis bullosa tissue-engineered skin

Figure 2d shows a graphical model of the TES preparation process. Over time, the proliferation of keratinocytes and fibroblasts increased in each group containing different

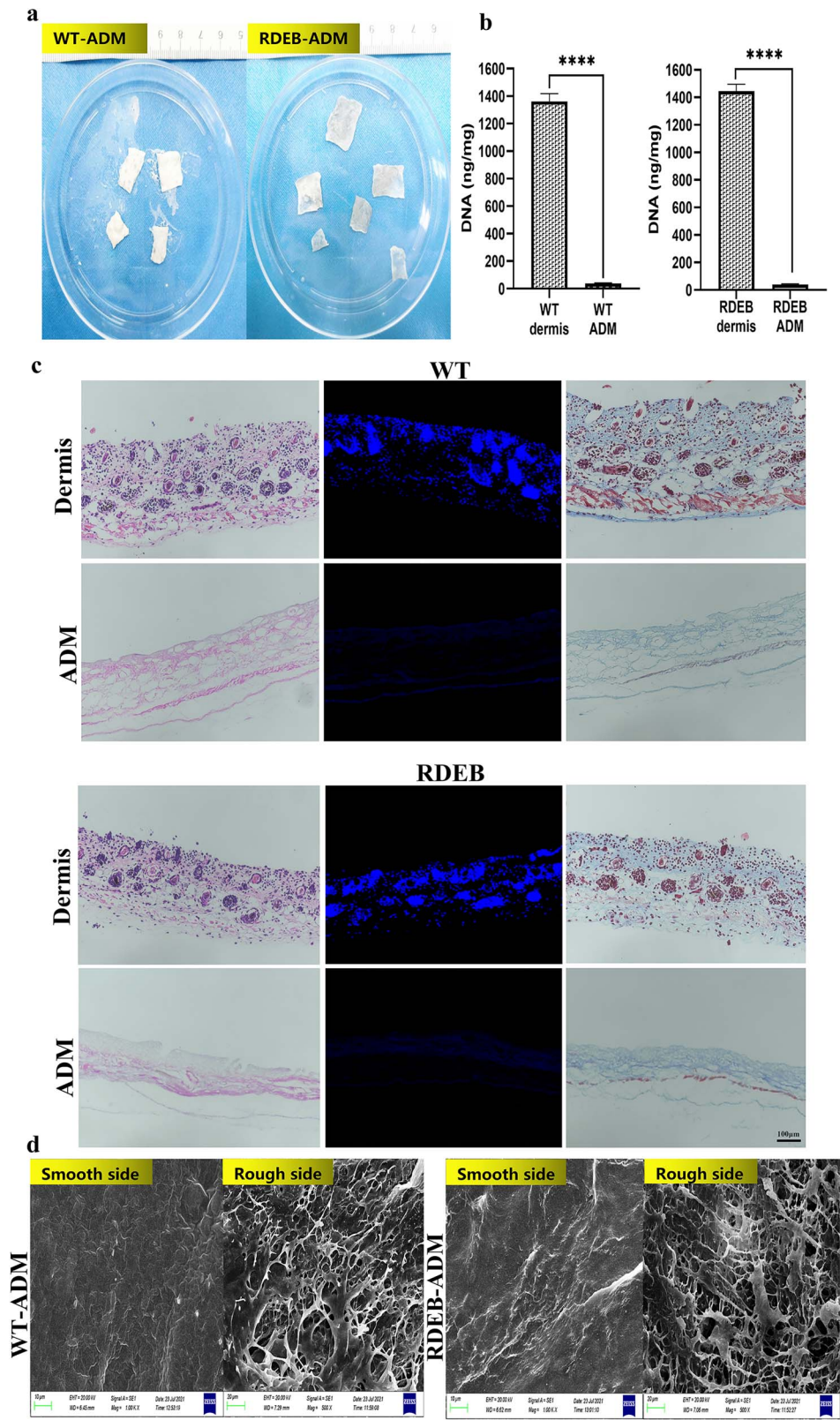


Figure 1. Evaluation of WT-ADM and RDEB-ADM. **(a)** Gross view of the WT-ADM and RDEB-ADM. **(b)** DNA quantitative determination of WT/RDEB dermis and ADM. **(c)** HE, DAPI, and Masson staining of WT/RDEB dermis and ADM. **(d)** SEM observation of the WT-ADM and RDEB-ADM (scale bar: 10 μ m). **** $P < 0.0001$. *WT* wild-type, *RDEB* recessive dystrophic epidermolysis bullosa, *ADM* acellular dermal matrix, *DNA* deoxyribonucleic acid, *HE* hematoxylin-eosin, *DAPI* 4, 6-diamidino-2-phenylindole

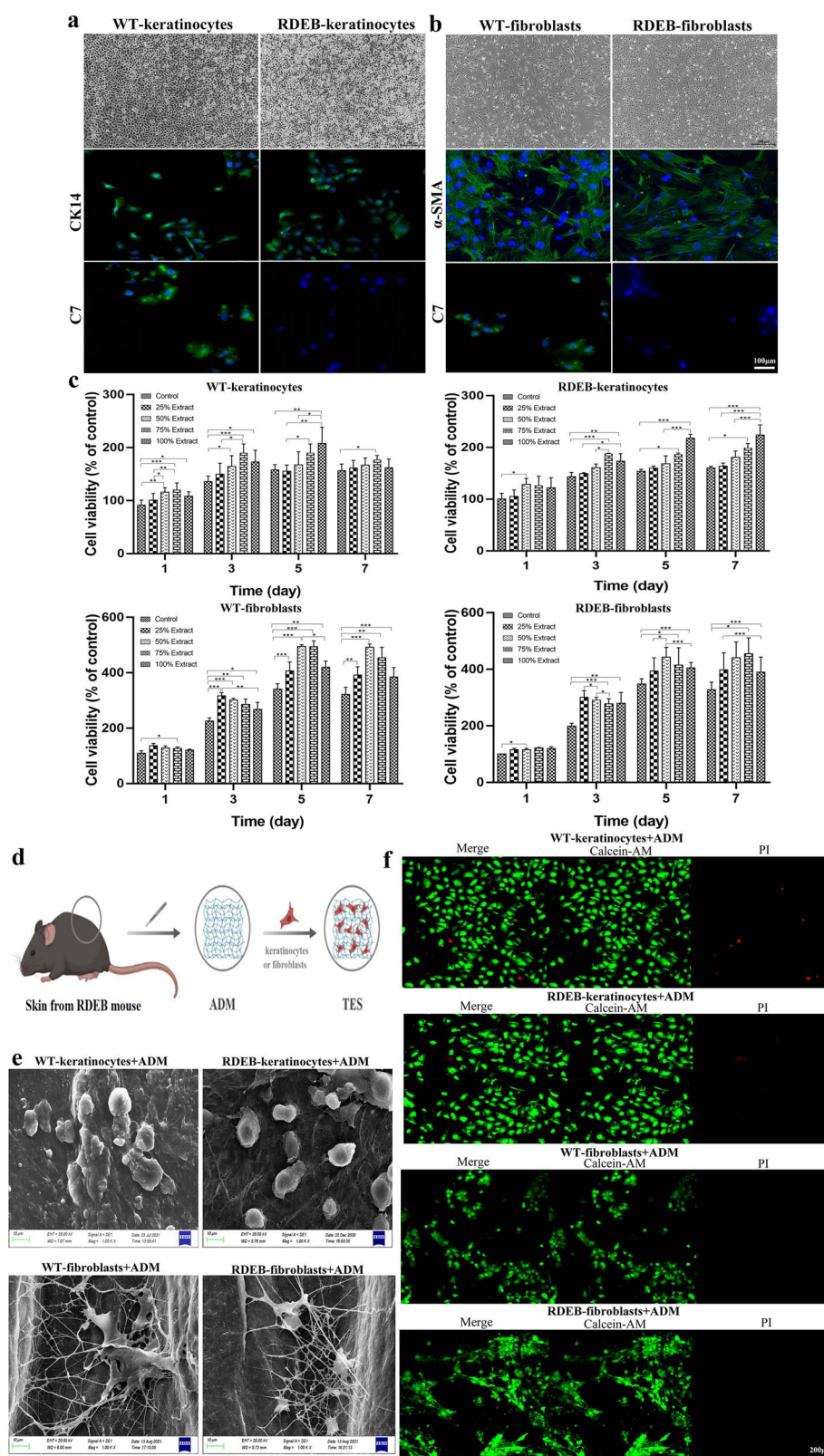


Figure 2. Evaluation of WT-TES and RDEB-TES. **(a, b)** Characterization (scale bar: 200 μ m) and immunofluorescence staining (scale bar: 100 μ m) of WT/RDEB keratinocytes and fibroblasts. **(c)** CCK-8 results about proliferation of the keratinocytes and fibroblasts at Days 1, 3, 5, and 7 in different concentrations of ADM extracts. **(d)** A graph model of TES preparation process. **(e)** SEM observation (scale bar: 10 μ m), and **(f)** live/dead staining (scale bar: 200 μ m) of keratinocytes and fibroblasts seeded on ADM for 3 days. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. WT wild type, RDEB recessive dystrophic epidermolysis bullosa, TES tissue-engineered skin, ADM acellular dermal matrix, SEM scanning electron microscopy, CK 14 cytokeratin 1, C7 collagen VII, α -SMA α -smooth muscle actin, CCK-8 cell Counting Kit-8, AM acetoxymethyl ester, PI propidium iodide

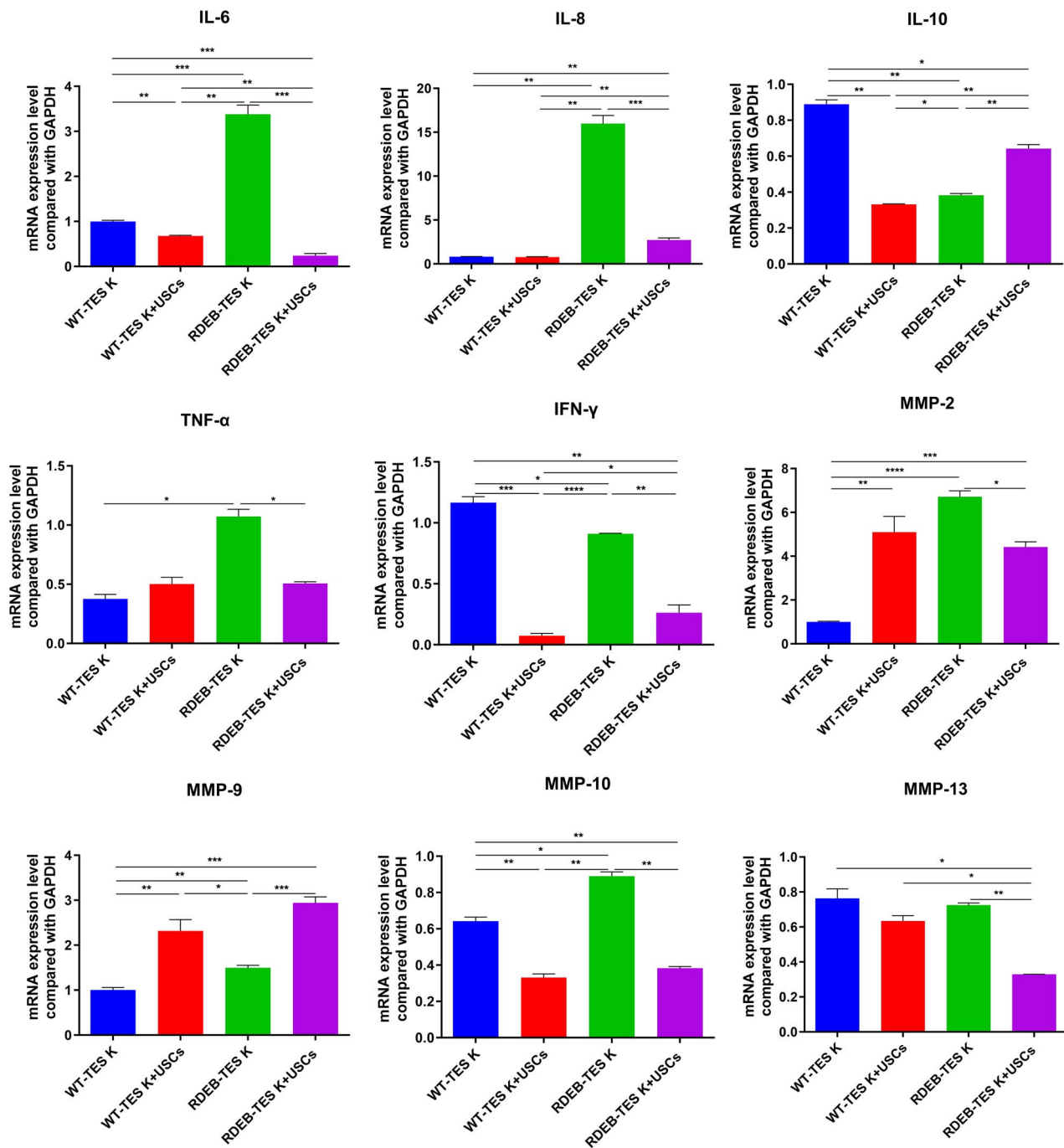


Figure 3. Effect of USCs on the expression of inflammation- and MMP-related genes of keratinocytes from TES. Q-PCR analysis for the relative expression of inflammation- and MMP-related genes of keratinocytes in WT-TES and RDEB-TES (WT-TES K represents keratinocytes in WT-TES; WT-TES + USCs K represents keratinocytes in WT-TES coculturing with USCs; RDEB-TES K represents keratinocytes in RDEB-TES; RDEB-TES + USCs K represents keratinocytes in RDEB-TES coculturing with USCs). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. USCs urine-derived stem cells, MMPs matrix metalloproteinases, TES tissue-engineered skin, q-PCR quantitative reverse-transcription polymerase chain reaction, WT wild type, RDEB recessive dystrophic epidermolysis bullosa, K keratinocytes, m-RNA messenger-ribonucleic acid, GAPDH glyceraldehyde-phosphate dehydrogenase, IL interleukin, TNF α tumor necrosis factor- α , IFN- γ interferon- γ .

concentrations of ADM, as shown by the CCK-8 assay (Figure 2c). This indicates that the ADM scaffolds did not inhibit the proliferation of keratinocytes and fibroblasts. We also performed live/dead staining and SEM assays to assess cell attachment and growth on the ADM scaffolds. After seeding keratinocytes and fibroblasts on the ADM for 3 days, SEM and live/dead staining results showed that a large number of keratinocytes and fibroblasts adhered to smooth and rough surfaces of the ADM showing good cell growth

status, respectively, and some keratinocytes and fibroblasts even exhibited pseudopodia extension (Figure 2e and f).

Urine-derived stem cells suppress inflammation and delay fibrosis progression of keratinocytes and fibroblasts from recessive dystrophic epidermolysis bullosa tissue-engineered skin

After coculturing USCs and TES, q-PCR results showed that the expression of inflammatory cytokine-related genes such

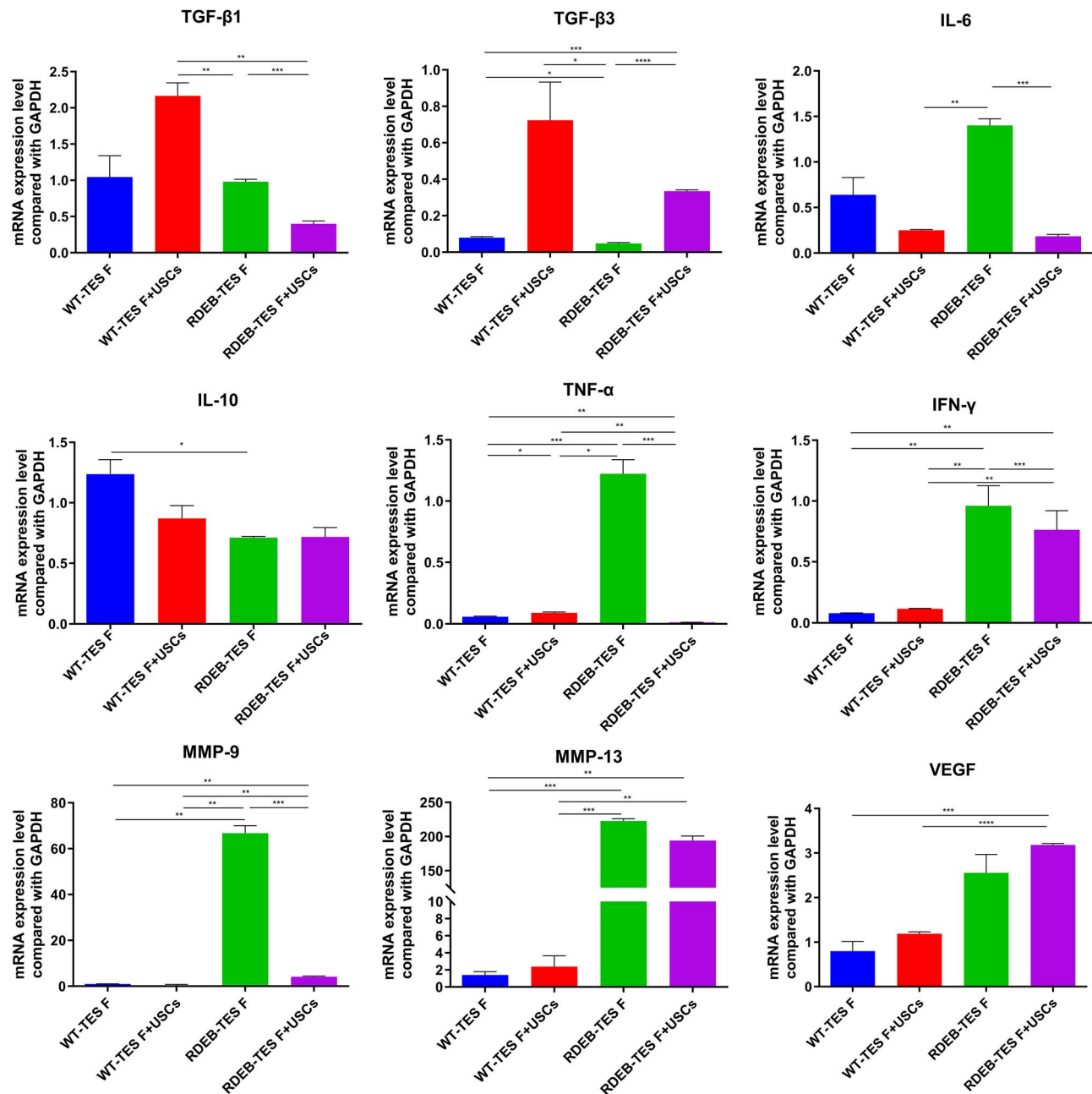


Figure 4. Effect of USCs on the expression of inflammation- and fibrosis-related genes of fibroblasts from TES. Q-PCR analysis for the relative expression of inflammation- and fibrosis-related genes of fibroblasts in WT-TES and RDEB-TES under basal conditions (WT-TES F represents fibroblasts in WT-TES; WT-TES + USCs F represents fibroblasts in WT-TES coculturing with USCs, RDEB-TES F represents fibroblasts in RDEB-TES, RDEB-TES + USCs F represents fibroblasts in RDEB-TES coculturing with USCs). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. USCs urine-derived stem cells, TES tissue-engineered skin, q-PCR quantitative reverse-transcription polymerase chain reaction, WT wild type, RDEB recessive dystrophic epidermolysis bullosa, F fibroblasts, m-RNA messenger-ribonucleic acid, GAPDH glyceraldehyde-phosphate dehydrogenase, TGF transforming growth factor, IL interleukin, TNF- α tumor necrosis factor- α , IFN- γ interferon- γ , MMP matrix metalloproteinase, VEGF vascular endothelial growth factor

as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ , and MMP-related genes such as MMP-2, MMP-10, and MMP-13 in keratinocytes of the RDEB-TES + USCs group was downregulated compared to keratinocytes in the RDEB-TES group ($P < 0.05$). In contrast, IL-10 was upregulated in keratinocytes of the RDEB-TES + USCs group ($P < 0.05$) (Figure 3). Q-PCR analysis of fibroblasts showed that USCs significantly decreased the expression of TGF- β 1, IL-6, TNF- α , IFN- γ , MMP-9, and MMP-13 but upregulated the expression of IL-10 and vascular endothelial growth factor (VEGF) in the RDEB-TES + USCs group under both the basal condition

(Figure 4) and the TGF- β 1-activated condition (Figure 5) ($P < 0.05$).

Urine-derived stem cells rescue cutaneous manifestation and suppress inflammation and fibrosis of recessive dystrophic epidermolysis bullosa mice

Urine-derived stem cells rescue cutaneous manifestation and extend survival time of recessive dystrophic epidermolysis bullosa mice

Figure 6a shows obvious blood blisters on the paws and chest of newborn RDEB mice. After 7 days of USC treatment, the

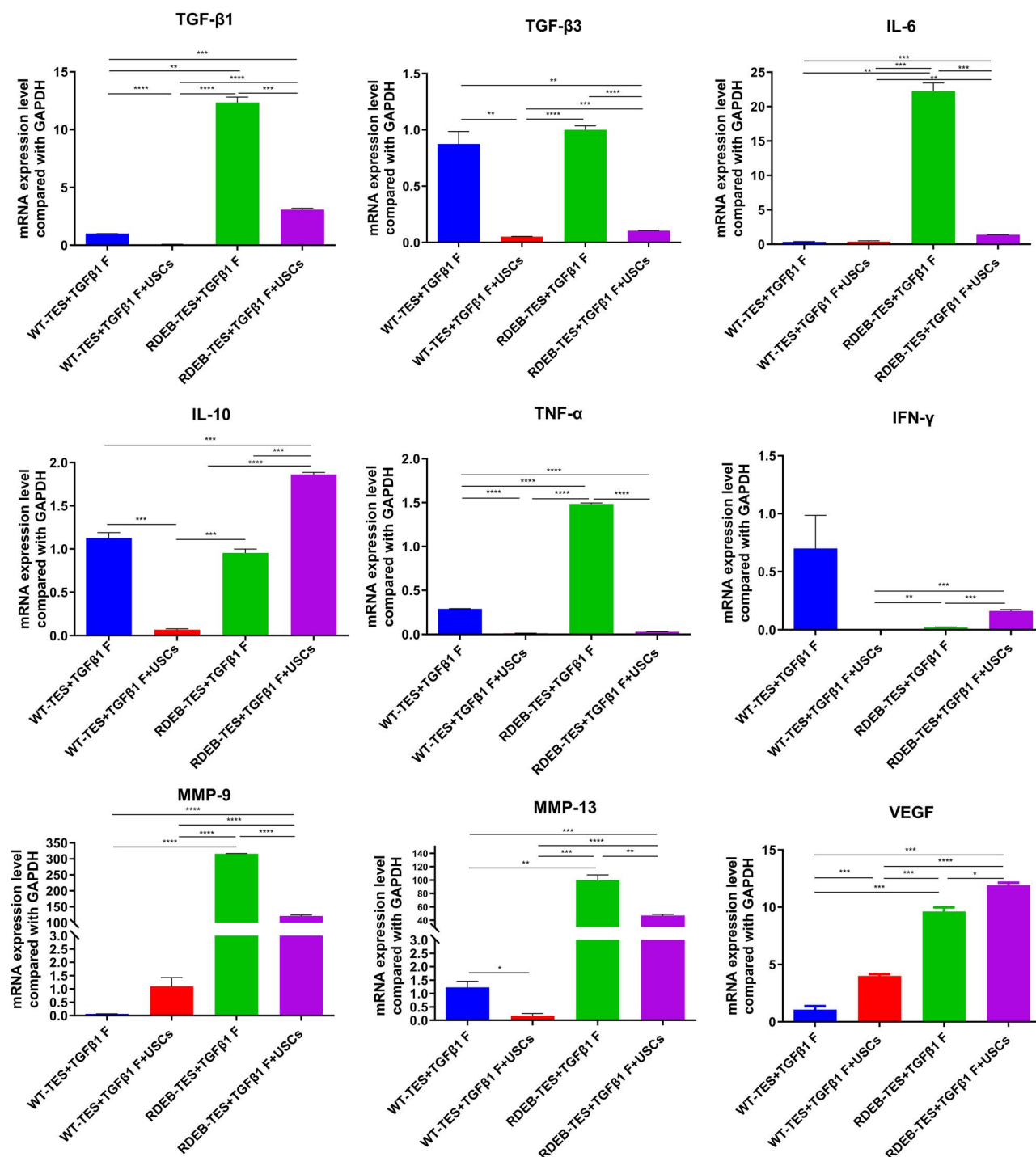


Figure 5. Effect of USCs on the expression of inflammation- and fibrosis-related genes of TGF-β1 activated fibroblasts from TES. Q-PCR analysis for the relative expression of inflammation- and fibrosis-related genes of fibroblasts in WT-TES and RDEB-TES under TGF-β1 activated conditions (WT-TES F represents fibroblasts in WT-TES; WT-TES + USCs F represents fibroblasts in WT-TES coculturing with USCs, RDEB-TES F represents fibroblasts in RDEB-TES, RDEB-TES + USCs F represents fibroblasts in RDEB-TES coculturing with USCs). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. USCs urine-derived stem cells, TES tissue-engineered skin, q-PCR quantitative reverse-transcription polymerase chain reaction, WT wild type, RDEB recessive dystrophic epidermolysis bullosa, F fibroblasts, m-RNA messenger-ribonucleic acid, GAPDH glyceraldehyde-phosphate dehydrogenase, TGF transforming growth factor, IL interleukin, TNF-α tumor necrosis factor-α, IFN-γ interferon-γ, MMP matrix metalloproteinase, VEGF vascular endothelial growth factor

skin lesions of newborn RDEB mice significantly improved. No new blisters, blood blisters, or finger deformities were found on Days 14, 21, and 28 in USC-treated RDEB mice. Kaplan–Meier survival curve analysis (Figure 6b) showed that USC administration extended the median

life span of RDEB mice from 3 to 5 days ($P < 0.0001$), and the survival probability of USC-treated RDEB mice was higher than that of the RDEB group at each time point. Almost 11% of recipient mice survived to adulthood (>3 weeks).

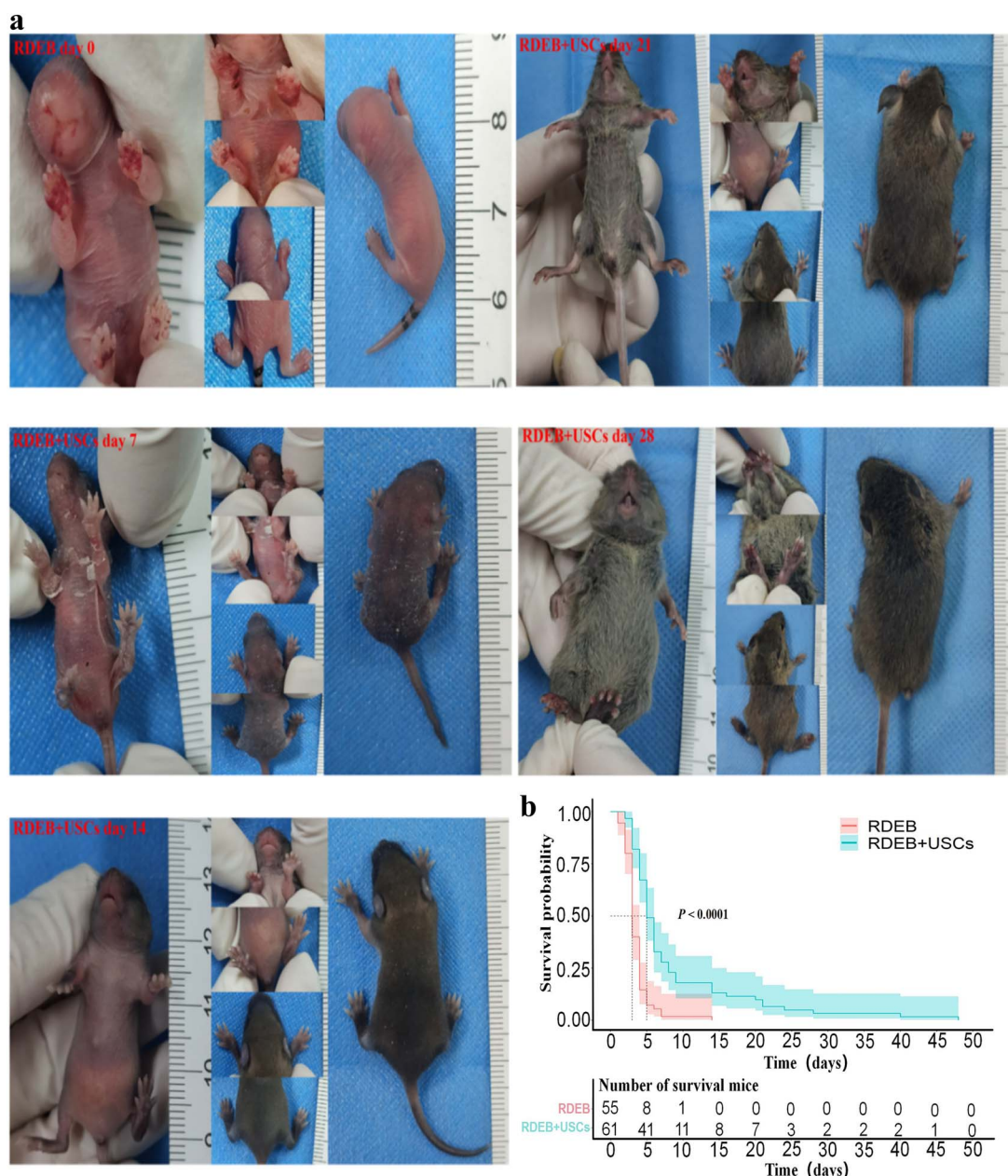


Figure 6. Changes in skin lesions and survival analysis of RDEB mice before and after USC treatment. **(a)** Skin phenotypes of RDEB newborn mice and RDEB mice treated with USCs on Days 7, 14, 21, and 28; **(b)** Kaplan–Meier survival curve analysis of the USC-treated RDEB mice ($n = 61$) and untreated RDEB mice ($n = 55$). *WT* wild type, *RDEB* recessive dystrophic epidermolysis bullosa, *USCs* urine-derived stem cells

Urine-derived stem cells improve dermis–epidermis junction of recessive dystrophic epidermolysis bullosa mice

HE staining showed that the DEJ separation rates of the front and hind paw skin of USC-treated RDEB mice were improved and were significantly lower than those of untreated RDEB mice on Days 7 and 14. Because RDEB mice did not survive for >14 days, the DEJ separation rates of the paw skin could not be compared on Days 21 and 28 (Figure 7a–c).

Urine-derived stem cells increase the expression of C7 and improve ultrastructure of the basement membrane zone of recessive dystrophic epidermolysis bullosa mice. Immunofluorescence staining with anti-C7 antibody showed that C7 could not be detected in the BMZ of the dorsal skin

of RDEB mice. After treatment with USCs for 7 days, C7 was detected in the BMZ of the dorsal skin of RDEB mice. Discontinuous C7 continued to be detected in the BMZ of the treatment group until Days 14, 21, and 28 (Figure 7d). Additionally, TEM observations revealed that the ultrastructure of the BMZ was incomplete and that the anchor fibrils disappeared in RDEB mice. In the skin of RDEB mice treated with USCs for 7, 14, 21, and 28 days, the ultrastructure of the lamina densa was significantly improved, and immature anchor fibrils emanating from the lamina densa were observed. However, compared to untreated RDEB mice, although anchoring filaments showing wisp-like structures were observed after USC treatment, no anchoring fibrils with normal morphology were observed (Figure 8).

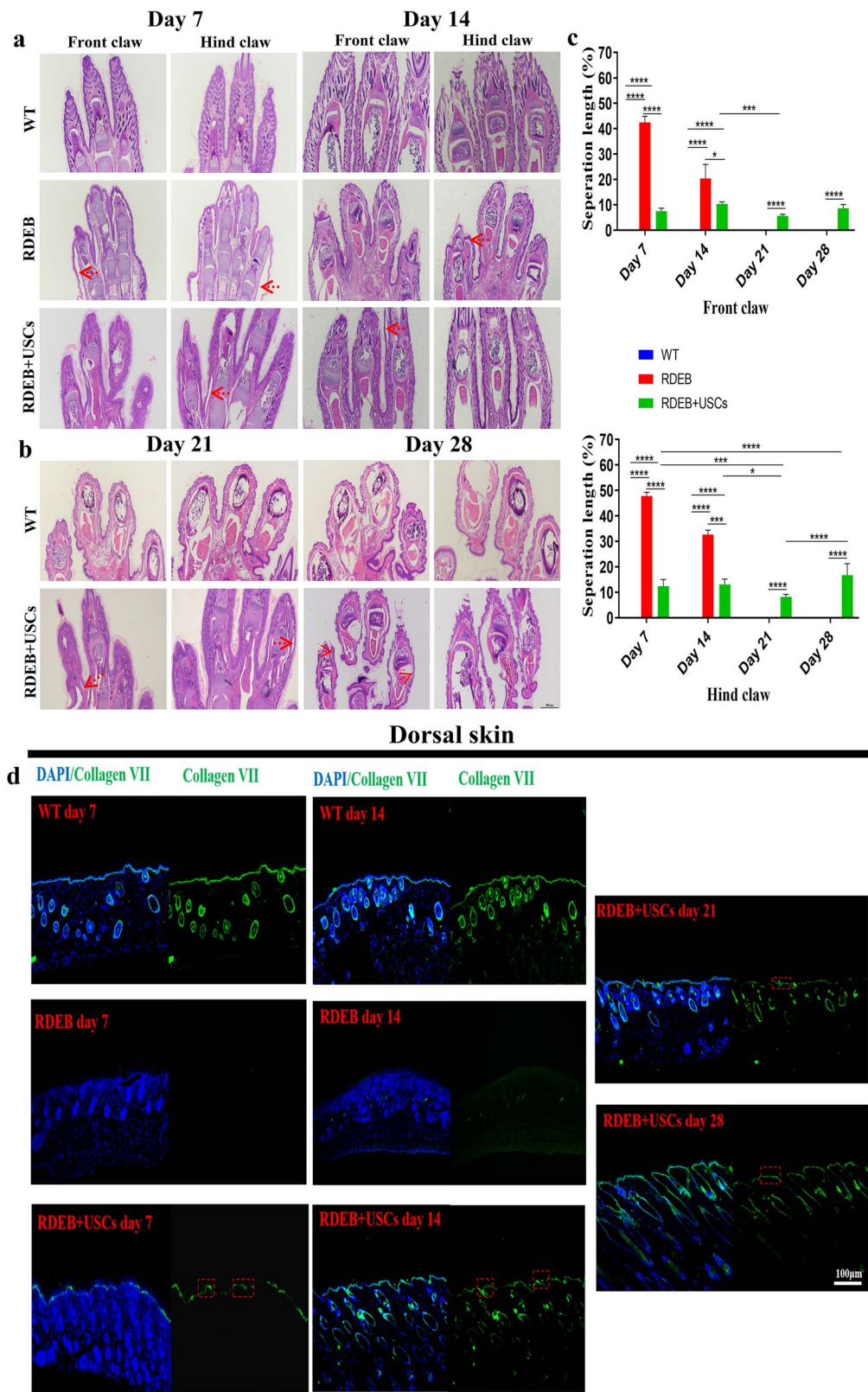


Figure 7. Changes in histopathology of RDEB mice before and after USC treatment. **(a, b)** HE analysis and **(c)** quantitation of the percentage of DEJ separation in the skin of the WT, RDEB, and RDEB + USCs mice on Days 7, 14, 21, and 28 and **(d)** immunofluorescence staining for anti-C7 antibody in the dorsal skin of WT, RDEB, and RDEB + USCs mice on Days 7, 14, 21, and 28 (scale bar: 500 µm & 100 µm). * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. WT wild type, RDEB recessive dystrophic epidermolysis bullosa, USCs urine-derived stem cells, HE hematoxylin–eosin, DEJ dermis–epidermis junction, C7 collagen VII, DAPI 4, 6-diamidino-2-phenylindole

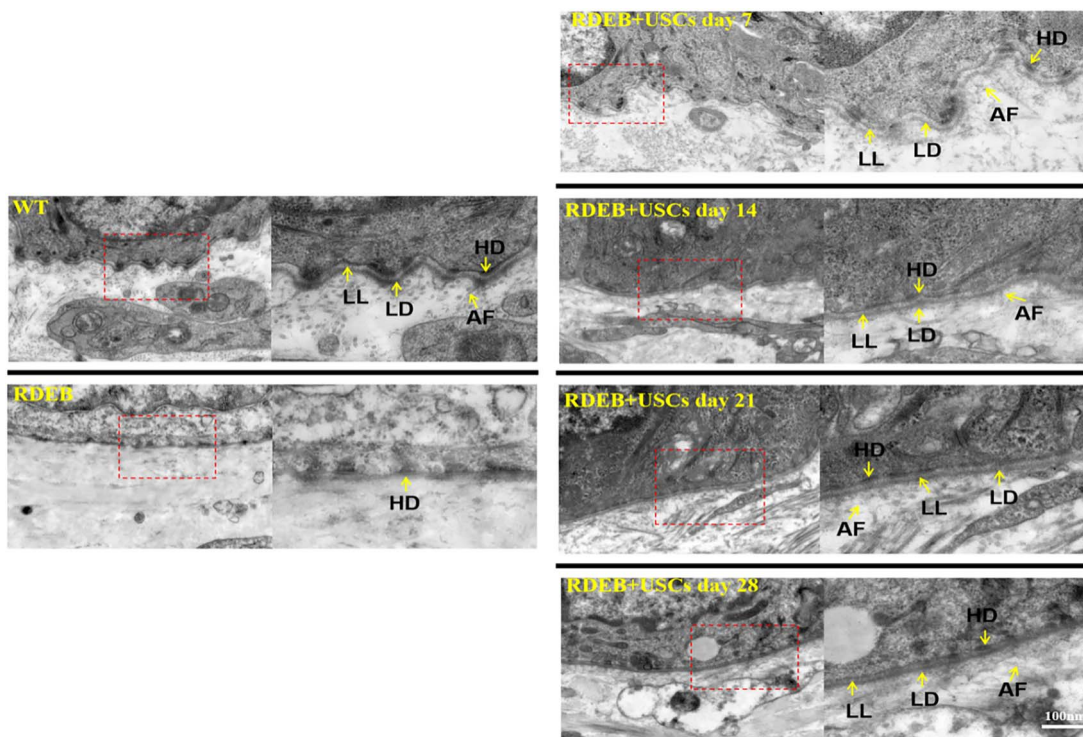


Figure 8. Changes in ultrastructure of DEJ of RDEB mice before and after USC treatment. TEM analysis of skins of WT, RDEB, and RDEB + USC mice on Days 7, 14, 21, and 28 (scale bar: 100nm). WT wild type, RDEB recessive dystrophic epidermolysis bullosa, USC urine-derived stem cells, DEJ dermis–epidermis junction, TEM transmission electron microscopy, AF anchoring fibril, HD hemidesmosome, LD lamina densa, LL lamina lucida

Urine-derived stem cells suppress inflammation and delay fibrosis progression in recessive dystrophic epidermolysis bullosa mice

The results of collagen I immunofluorescence staining showed that the expression of collagen I in USC-treated RDEB mice was significantly reduced compared to that in untreated RDEB mice on Days 7 and 14 ($P < 0.05$), and the expression of collagen I in the paws continued to decrease on Days 21 and 28 (Figure 9a). The expression of TGF- $\beta 3$ in the paws of RDEB mice was significantly increased on Days 7 and 14 after USC treatment ($P < 0.05$), then increased on Day 21 but decreased on Day 28 (Figure 9b). Q-PCR results showed decreased expression of TGF- $\beta 1$, IL-1 β , IL-6, IL-8, IFN- γ , MMP-2, and MMP-13 and increased gene expression of TGF- $\beta 3$, anti-inflammatory cytokines like IL-10, and VEGF in the skin of RDEB mice treated with USCs for 7 days compared to RDEB mice ($P < 0.05$) (Figure 9c). In addition to the similar changes in the genes mentioned above, the expression of TNF- α was also decreased 14 days after USC treatment ($P < 0.05$) (Figure 9d).

Urine-derived stem cells migrate to different organs of recessive dystrophic epidermolysis bullosa mice after intrahepatic injection

In vivo imaging showed that the fluorescence signals were mainly located in the chest and abdomen at 15 and 40 min and 2 h after the intrahepatic administration of USCs, and the fluorescence signals gradually decreased with time. Fluorescence signals were detected in the paws, neck, and tail after 6 h and 1 day and finally disappeared after 11 days (Figure 10a). Further immunofluorescence staining showed that HLA-ABC-positive USCs were present in the heart, liver,

spleen, lung, and kidney of RDEB mice after 7 days of USC treatment and decreased gradually on Days 14, 21, and 28 in the heart and liver (Figure 10b and c). Notably, USC distribution was also observed in the gastrointestinal tract of RDEB mice on Days 7, 14, 21, and 28 after USC injection (Figure S5). These results indicate that USCs migrate to different organs of the RDEB through the circulation after intrahepatic injection.

Discussion

The study is the first to report the potential efficacy of human-derived USCs, a novel MSC source for the treatment of RDEB mice. USCs can not only be harvested via a noninvasive, painless procedure and possess a high proliferative capacity [35] but also express COL7A1. These features make USCs an ideal option for treating RDEB. Schosserer *et al.* successfully extracted USCs from urine derived from patients with EB in 2015 [36], providing a basis for obtaining autologous USCs from patients with RDEB or their families to avoid immune responses in the future. In addition, this study confirmed a significant improvement in the life span, blistering phenotype, and C7 expression in RDEB mice following USC administration.

RDEB mice have blood blisters on the skin at birth, followed by secondary erosion and infection; thus, mice usually cannot survive for >2 days without treatment [23, 37]. Because of premature death in RDEB mice, increasing the median life span and long-term survival are important for evaluating the therapeutic efficacy of USCs in animal models. In our study, the median life span of RDEB mice was extended to 5 days after intrahepatic administration

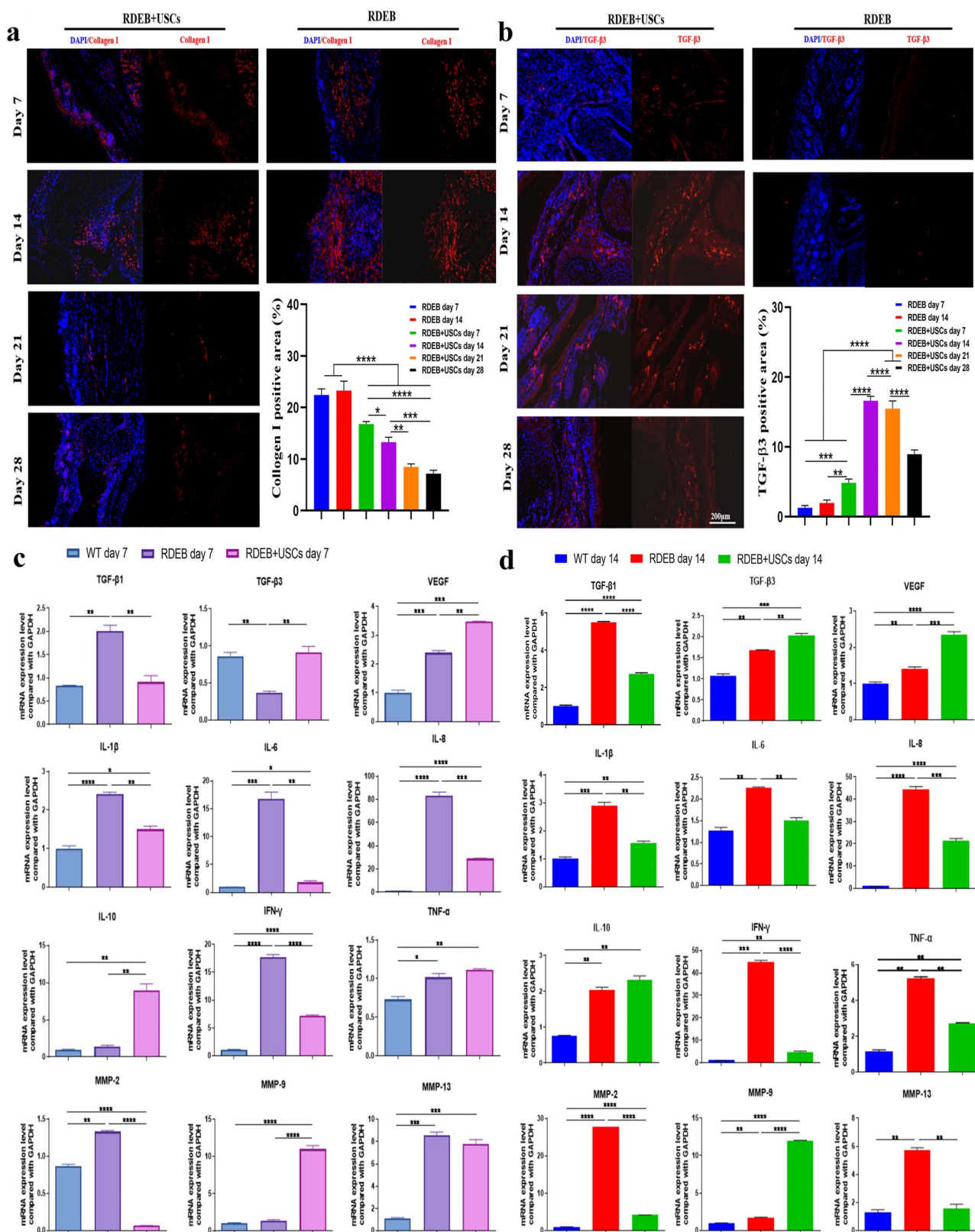


Figure 9. Changes in inflammation and fibrosis in RDEB mice before and after USC treatment. Immunofluorescence staining and quantitation of (a) anti-collagen I, (b) anti-TGF-β3 antibodies of hind paw skin of WT, RDEB, and RDEB + USCs mice on Days 7, 14, 21, and 28 (scale bar: 200 μm). (c, d) Q-PCR analysis for the relative expression of inflammatory, fibrotic cytokine-related genes of skin from RDEB mice treated with USCs at Days 7 and 14. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. WT wild type, RDEB recessive dystrophic epidermolysis bullosa USCs, urine-derived stem cells, DAPI 4, 6-diamidino-2-phenylindole, q-PCR quantitative reverse transcription polymerase chain reaction, m-RNA messenger-ribonucleic acid, GAPDH glyceraldehyde-phosphate dehydrogenase, TGF transforming growth factor, VEGF vascular endothelial growth factor, IL interleukin, IFN-γ interferon-γ, TNF-α tumor necrosis factor-α, MMP matrix metalloproteinase

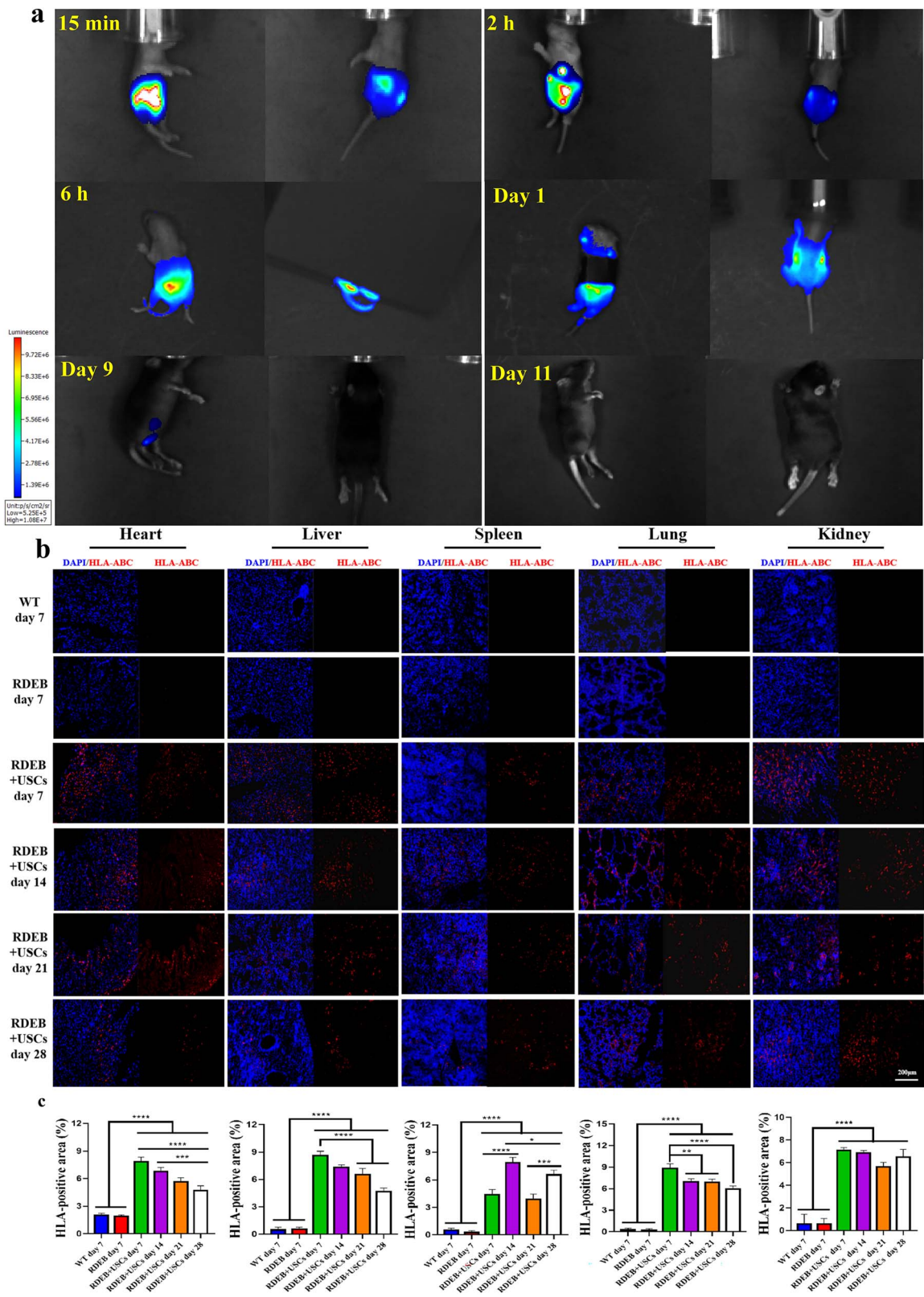


Figure 10. USC distribution *in vivo* in RDEB mice. **(a)** *In vivo* bioluminescent imaging of the RDEB mice treated with USCs at different time points; **(b)** immunofluorescence staining and **(c)** quantitation of anti-HLA-ABC antibody in heart, liver, spleen, lung, and kidney of WT, RDEB, and RDEB + USCs mice on Days 7, 14, 21, and 28. *WT* wild type, *RDEB* recessive dystrophic epidermolysis bullosa, *USCs* urine-derived stem cells, *min* minute, *h* hour, *HLA-ABC* human leukocyte antigen-ABC, *DAPI* 4, 6-diamidino-2-phenylindole

of USCs. This phenomenon suggests that USCs themselves have regenerative activity in RDEB mice to a certain extent. We speculated that this may be related to the improvement of blister formation in the skin, oral cavity, and digestive tract of some recipients, resulting in better feeding after UCS infusion. Besides, USCs could release anti-inflammatory and immunomodulatory cytokines (such as regulated on activation in normal T cell expressed and secreted [RANTES], growth-regulated oncogene- α [GRO- α], and granulocyte macrophage colony-stimulating factor [GM-CSF]) [38, 39], which may also improve the survival of RDEB mice.

Moreover, deposition of C7 was detected in the BMZ of skin from USC-treated RDEB mice, and TEM assays further showed that the lamina densa was significantly improved, with wisp-like structures of anchoring fibrils. At present, the mechanism of action of stem cells in EB treatment has not been fully elucidated. Raoufinia *et al.* reported that MSCs were found in the skin, lung, and other organs after bone marrow transplantation [40]. After migrating and homing to damaged skin and other organs, MSCs can mature into fibroblasts (stem cell genes shut off and genes for fibroblast markers including *COL7A1* turn on) and then synthesize functional proteins that are lacking in patients with RDEB [41, 42]. In our study, because RDEB mice completely lacked C7, it was speculated that the detected C7 was only being produced by USCs. However, the identity of C7 and the specific mechanisms underlying its production remain unclear. It is also crucial to note that although some anchoring fibrils were formed, their morphology and quantity were significantly abnormal. This suggests that USCs do not completely reverse the disease phenotype in RDEB mice. Compton *et al.* reported that anchoring fibrils usually require 18 months to reach maturity in human skin regeneration [43]. Therefore, another factor related to this outcome to consider was the possibility that the C7 level obtained from USCs may not have been sufficient or have a considerably short life span for their proper assembly into normal anchoring fibrils. Nevertheless, in our study, discontinuous deposition of C7 was detected at the BMZ within 28 days in USC-treated RDEB mice but was not observed in older mice. The reason for this is probably C7 attenuation and depletion of USCs *in vivo*. Researchers have reported that the half-life of C7 is ~ 1 month [44], which coincides with the period during which we observed C7. The oldest mouse that survived after USC administration in this study was 48 days old. Although feeding behavior was similar to that of a healthy WT mouse, it was smaller and lighter than that of a healthy WT mouse of the same age. This may be related to the gastrointestinal involvement in RDEB mice (Figure S6).

In addition to the absence of C7, inflammation and fibrosis are also characteristic features of animal models and patients with RDEB. Chronic inflammation, fibrosis, and scarring during wound healing place patients with RDEB at a higher risk of developing aggressive cSCC than the general population [45]. Researchers have reported that patients with RDEB have higher TGF- $\beta 1$ levels, abnormal expression of multiple MMPs (MMP-2, 9, and 13) in the serum and skin wounds compared to healthy people [46–49]. In addition, Albregues *et al.* reported that IL-6 family members can trigger the invasive activation of fibroblasts [50, 51], and a higher level of IL-6 expression was found in the plasma of patients with RDEB [52]. In this study, we used a 3D research mode (TES) to evaluate the efficacy of USCs in RDEB

in vitro. Through the Transwell coculturing system, USCs downregulated the levels of IL-6, IL-8, and MMP-2, 10, and 13 in keratinocytes of RDEB-TES. Notably, the IL-6/IL-10 ratio of keratinocytes in RDEB-TES cocultured with USCs decreased nearly 10-fold compared with that in the control. Studies have shown that the higher the ratio of IL-6/IL-10, the worse the prognosis of patients with EB in later stages [53]; therefore, USCs may also be beneficial for the long-term prognosis of RDEB. TGF- $\beta 1$, IL-6, TNF- α , IFN- γ , MMP-9, and MMP-13 of fibroblasts in RDEB-TES also decreased after co-culturing with USCs. As previously mentioned, studies have shown that USCs could regulate inflammation and immune reactions [38, 39]. Additionally, Zhou *et al.* observed that CD4 T-cell proliferation was inhibited and mRNA levels of IFN- γ and IL-17A were significantly reduced after coculturing with USCs [54]. Similarly, Kang *et al.* cocultured USCs with peripheral blood mononuclear cells (PBMCs) and observed a dose-dependent inhibitory effect of USCs on PBMC proliferation [55]. In this study, USCs showed similar suppressive effects on inflammation in RDEB-derived keratinocytes and fibroblasts. In contrast, expression of VEGF in fibroblasts of RDEB-TES was upregulated after coculturing with USCs. Jiang *et al.* demonstrated that USCs could release VEGF via paracrine effect [56], and Ouyang *et al.* found that USCs could provide proangiogenic-related microRNAs, such as miR-10, miR-21, and miR-30, which may result in an increase in VEGF expression [57]. USCs also upregulated the expression of TGF- $\beta 3$ in fibroblasts of RDEB-TES. TGF- $\beta 3$ is an isoform of TGF- $\beta 1$; when TGF- $\beta 3$ is combined with the TGF- β II receptor, different signal transduction pathways promoting scar-free healing are initiated [6, 58, 59]. A previous study demonstrated that the activation of the TGF- β signaling in fibroblasts of RDEB mice occurred as early as 1 week postnatally [6]. Since the fibroblasts in our study were extracted from neonatal mice, we also used TGF- $\beta 1$ to stimulate fibroblasts in TES and then cocultured them with USCs. Except for the expression of TGF- $\beta 3$ in fibroblasts, which was downregulated by USCs under an exogenous TGF- $\beta 1$ -activated condition, other inflammatory and fibrotic-related genes like TGF- $\beta 1$, IL-6, MMP-9, and MMP-13 presented a similar variation trend to the normal condition. The expression of TGF- $\beta 3$ was downregulated by 1-fold, but the TGF- $\beta 1$ level was decreased by 10-fold under TGF- $\beta 1$ -activated conditions. Overall, USCs showed anti-inflammatory and antifibrotic effects against RDEB *in vitro*.

To validate this speculation, we further analyzed the changes in the expression of inflammation- and fibrosis-related genes in the skin of RDEB mice after USC treatment. Q-PCR results of the skin tissue demonstrated that the administration of USCs acted similarly to suppress TGF- $\beta 1$, IL-6, and MMP-2, 13 in RDEB mice on Days 7 and 14. This suggests that USCs play an anti-inflammatory role and delay the progression of fibrosis in a mouse model of RDEB. Actually, USCs have been reported to have anti-inflammatory properties in many diseases. Tian *et al.* reported that USCs can downregulate the expression levels of proinflammatory factors such as INF- γ and IL-1 β in USC-treated rat model of ischemic acute kidney injury [60]. Li *et al.* found that USCs could improve the expression of inflammation-related factors, such as IL-6 and TNF- α , and reduce mast cell infiltration in a rat model of interstitial cystitis [61]. Moreover, USCs can reduce myeloperoxidase (MPO) activity and the number

of MPO+ cells in tissues to reduce neutrophil infiltration [38, 54]. Since the skin of RDEB mice is prone to forming erosions accompanied by the infiltration of a large number of inflammatory cells (including neutrophils), the suppressive effect of USCs on neutrophils may subsequently lead to the downregulation of inflammatory factors. Additionally, Chen *et al.* found that tissue inhibitor of metalloproteinase 1 (TIMP1) (a natural inhibitor of MMPs) was highly enriched in USCs-extracellular vesicles [62], which may explain the suppressive effect of USCs through downregulating the MMP genes. However, it is worth noting that the expression levels of MMP-9 in keratinocytes from RDEB-TES and skin tissues were upregulated compared to those in the control group after USC intervention. MMP-9 is a gelatinase (termed gelatinase A) belonging to the large MMP family. In addition to MMP-9, MMP-2 is also a gelatinase (termed gelatinase B); the two work together to degrade gelatin and various types of collagen. The deletion of MMP-2 may be functionally compensated for by MMP-9 [63]. In our study, because of the inhibition of MMP-2 by USCs, the level of MMP-9 increased as a result of compensation. In fact, MMP-9 increased 1.5-fold in keratinocytes in RDEB-TES cocultured with USCs, but MMP-2 decreased nearly 4-fold compared to the control; an analogous variation trend was also exhibited in the skin tissue from USC-treated mice on Day 14. These results suggest that USCs could still inhibit the degradation of the extracellular matrix in RDEB mice and may play a role in reducing the invasion and metastatic ability of tumors in later periods. However, the causes and specific mechanisms of upregulation of the MMP-9 gene induced by USCs require further study.

We also investigated the distribution of USCs after intrahepatic administration in newborn RDEB mice and detected USCs in the heart, liver, spleen, lung, and kidney after 7 days, and a greater number of USCs were distributed in the heart and lungs. In addition, we did not observe any abnormal changes in the structure of any organ (Figure S7). Lee *et al.* reported that within a few minutes of intravenous injection of MSCs in mice, >80% of them first accumulated in the lungs [64]. *In vivo* imaging examination revealed that the fluorescence signals were mainly located in the chest and abdomen, indicating that USCs injected into the liver first reached the lungs and then circulated to various organs. Importantly, USCs were also detected in the gastrointestinal tract of RDEB mice after treatment. This phenomenon may have vital therapeutic significance for patients with RDEB presenting skin injury as well as esophageal structural involvement. However, we did not perform further analyses of other changes in the gastrointestinal tract before and after treatment, leaving further exploration for future studies.

In summary, in this study, the efficacy of USCs in ameliorating skin lesions and improving the separation rates of DEJ and the expression of C7 in RDEB mice were investigated for the first time. RDEB-TES was successfully prepared *in vitro*. *In vitro* experiments based on RDEB-TES and *in vivo* experiments in RDEB mice showed that USCs manifested the effect of suppressing inflammation and delaying fibrosis. In addition, USCs could migrate to multiple organs of RDEB mice after intrahepatic administration, which may be an ideal “seeded player” for RDEB with multiple system involvement. These study findings reflect the potential of USCs in treating patients with RDEB. There were also some limitations of this study. Due to the poor survival of RDEB mice, a certain number of survivors at specific time points could not be

attained in this study. Therefore, the conclusions drawn at subsequent intervals were based on the data from mice that survived, resulting in a certain degree of selectivity in the outcomes. In future studies, a greater number of RDEB mice will be needed to verify the specific therapeutic effects of USCs in RDEB. The other aspect that needs to be explored is how USCs are engrafted onto the skin and undergo division or proliferation *in vivo*.

Conclusions

This study demonstrates that human USCs have several key properties in treating a mouse model of RDEB. First, USCs suppressed inflammation and delayed fibrosis progression of keratinocytes and fibroblasts in RDEB mice. Second, the life span, DEJ of skin, expression of C7, ultrastructure of the BMZ, and inflammation and fibrosis of RDEB mice were improved after intrahepatic injection of USCs. Third, USCs could migrate to different organs of RDEB without structural damage to vital organs or tumorigenic activity after circulation in RDEB mice. These findings support their future use for the treatment of RDEB. Taken together, this study justifies future clinical research on USCs as a general source of stem cells in patients with RDEB.

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Author contributions

Xingli Zhou (Writing—original draft [lead]), Jie Tan (Writing—original draft [equal]), Yuting Song (Writing—original draft [supporting]), Pengcheng Liu (Project administration [equal]), Xiwen Zhang (Project administration [supporting]), Xun Feng (Data curation, Methodology [supporting]), Yue Xiao (Data curation [supporting]), Yiyi Wang (Formal analysis [equal]), Guoqing Liu (Data curation [equal]), Wen-Qian Zhang (Data curation [equal]), Qingyi Zhang (Data curation [equal]), Yanling Jiang (Project administration [supporting]), Yuan Liu (Data curation [supporting]), Long-Mei Zhao (Data curation [supporting]), Huiqi Xie (Conceptualization [equal]), and Wei Li (Conceptualization [equal])

Supplementary data

Supplementary data are available at *Burns & Trauma Journal* online.

Ethics statement

Study on Biological Characteristics and Tissue Engineering of Urine-Derived Stem Cells was approved in September 2021 by the Ethics Committee on Biomedical Research at West China Hospital of Sichuan University (2021-1066). An animal study entitled Human Urine-derived Stem Cells for the Treatment of Recessive Dystrophic Epidermolysis Bullosa was conducted following procedures approved by the Animal Experiments Committee of Sichuan University in July 2021 (20211261A).

Conflict of interest

None declared.

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Data availability

All data are available in the main text or the supplementary materials.

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