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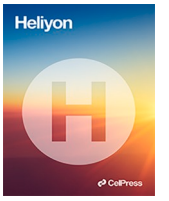

Review article

Urine-derived stem cells: Promising advancements and applications in regenerative medicine and beyond

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ABSTRACT

Currently, stem cells are a prominent focus of regenerative engineering research. However, due to the limitations of commonly used stem cell sources, their application in therapy is often restricted to the experimental stage and constrained by ethical considerations. In contrast, urine-derived stem cells (USCs) offer promising advantages for clinical trials and applications. The noninvasive nature of the collection process allows for repeated retrieval within a short period, making it a more feasible option. Moreover, studies have shown that USCs have a protective effect on organs, promoting vascular regeneration, inhibiting oxidative stress, and reducing inflammation in various acute and chronic organ dysfunctions. The application of USCs has also been enhanced by advancements in biomaterials technology, enabling better targeting and controlled release capabilities. This review aims to summarize the current state of research on USCs, providing insights for future applications in basic and clinical settings.

1. Introduction

1.1. The stem cells and the urine-derived stem cells

Stem cells, which are undifferentiated cells with no specific function, hold immense potential in various scientific and medical fields. Their unique ability to differentiate into any cell type within an organism, coupled with their capacity for self-renewal, has sparked great interest in the scientific community [1,2]. Stem cells can be classified into different categories based on their developmental stage and differentiation potential. Embryonic stem cells, derived from early-stage embryos, possess the highest level of differentiation potential and are capable of giving rise to any cell type in the body. In contrast, adult stem cells are found in mature tissues and have a more limited range of potential cell types they can differentiate into [2,3]. Moreover, stem cells can be further categorized based on their differentiation potential. Totipotent stem cells, such as fertilized eggs, have the incredible ability to develop into complete organisms. Pluripotent stem cells, like embryonic stem cells, have the potential to differentiate into multiple cell types. Monopotent stem cells, such as neural stem cells and hematopoietic stem cells, can only differentiate into one or two closely related cell types [1]. This unique characteristic empowers researchers to delve into prospective avenues within the realms of regenerative medicine, disease modeling, and drug development. The profound implications of stem cell research extend the promise of

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revolutionizing healthcare, offering unprecedented opportunities to enhance global quality of life [4].

The genesis of human life traces back to a solitary cell known as the fertilized egg, heralded as the most totipotent stem cell type—termed totipotent stem cells [5]. Human embryonic stem cells (hESCs) made their debut in 1998, unveiled by James Thomson's research group, 17 years after the advent of mouse embryonic stem cells [6]. Embryonic stem cells stand poised as a potential wellspring for cultured organs and tissues, presenting avenues for the replacement or repair of damaged structures. The fertilized egg and embryonic stem cells, owing to their exceptional capacity for complete and multidirectional differentiation, emerge as invaluable subjects of research. Their specificity, however, renders them subject to ethical, moral, religious, and legal constraints, impeding their unrestricted advancement [7]. Conversely, another class, adult stem cells, quietly resides in various tissues and organs of the human body [8]. These cells, prevalent in fully developed individuals, retain their undifferentiated state, endowed with the ability to self-renew and differentiate along diverse paths. They play a pivotal role in cellular and tissue repair. Adult stem cells can either perpetuate their undifferentiated status through continued division or undergo transformation into progenitor cells, dividing a finite number of times before culminating in specialized cells. Notably, adult stem cells carry no risk of malformation, bypass the intricacies associated with fetal stem cell manipulation, exhibit reduced ethical concerns, and demonstrate low immunogenicity, thereby establishing adult stem cells as the preeminent cell type in cell therapy applications [9].

The nomenclature "mesenchymal stem cell (MSC)" should be distinguished and not used interchangeably with "mesenchymal stromal cells (MSC)" [10]. Due to their remarkable multidirectional differentiation potential, urine-derived stem cells (USCs) garnered attention as early as 2011. Zhang et al. achieved a pioneering extraction and characterization of progenitor cells expressing urothelial, smooth muscle, endothelial, and mesenchymal markers from urine [11]. These isolated USCs demonstrated successful differentiation into osteogenic, lipogenic, and chondrogenic lineages [12]. Fresh urine samples must be collected promptly, ideally within a short timeframe of less than 5 h. Prolonged storage can lead to alterations in pH levels and other urinary constituents, potentially impacting the activity of USCs [13]. The isolated USCs fulfill the essential criteria outlined by the International Society for Cellular Therapy (ISCT) for designated mesenchymal stem cells. They exhibit positive expression for CD44, CD73, CD90, CD105, CD146, and CD271, while notably lacking expression of hematopoietic and endothelial markers including CD11b, CD14, CD19, CD20, CD34, CD45, CD79a, and HLA-DR. Additionally, these cells demonstrate the capability to undergo in vitro differentiation into adipocyte, chondrocyte, and osteoblast lineages [10,14]. Given that industrial-scale production has not been realized, research involving USCs necessitates a sequential process. Initially, urine specimens must be collected, followed by morphological analysis through isolation and culture. Subsequently, surface marker identification, encompassing both negative and positive markers, becomes imperative. Finally, confirmation of chondrogenic, osteogenic and adipogenic differentiation further constitutes an integral aspect of the research endeavor. While initial investigations predominantly centered on USC extraction from healthy populations, ongoing research expanded the scope to encompass diverse pathological conditions, including acute kidney injury, hemophilia A (HA), phenylketonuria (PKU), Down syndrome (DS), and X-linked Alport syndrome (X-LAS) [15–19]. Notably, individual USCs exhibit impressive expansion capabilities, with the capacity to multiply up to 60–70 times while maintaining long telomeres. These cells express markers associated with pericytes and mesenchymal stem cells. Furthermore, USCs display differentiation potential across various lineages, including endothelial, osteogenic, chondrogenic, adipogenic, skeletal myogenic, and neurogenic differentiation pathways [20,21]. Importantly, USCs, obtained non-invasively, are on par with well-known adipose-derived mesenchymal stem cells (MSCs) in terms of their capacity for proliferation and differentiation [22]. In the realm of urinary tract engineering, the differentiation of urothelial-derived stem cells (UDSCs) predominantly focuses on three distinct cell types: urothelial cells, smooth muscle cells, and endothelial cells. Similarly,

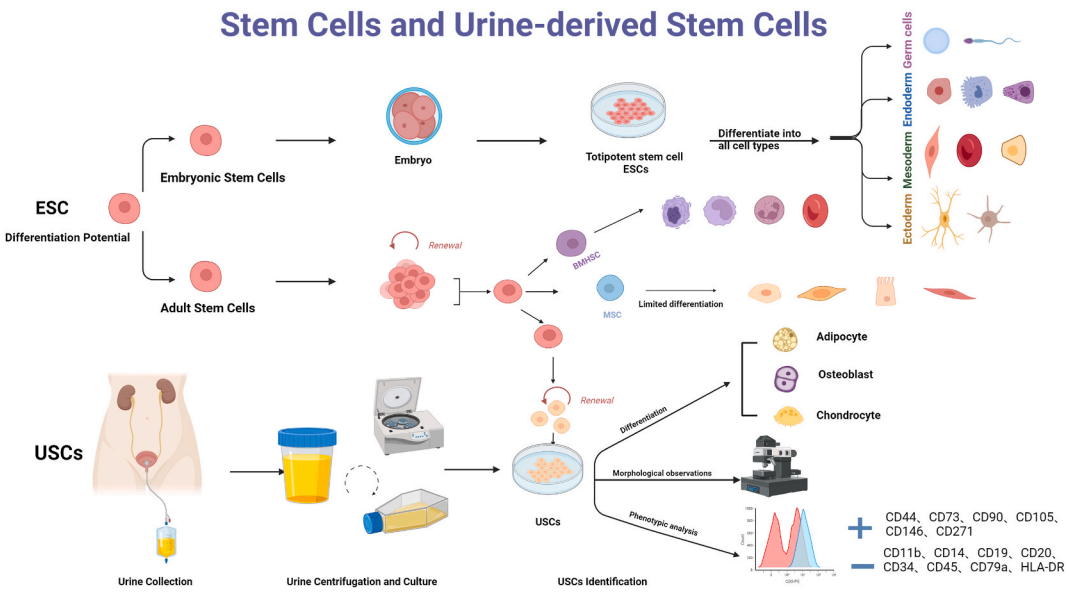

Fig. 1. Stem cell differentiation capacity and identification of USCs.

Bharadwaj and colleagues recovered intraoperative urine specimens from the upper urinary tract, revealing the expression of urothelial epithelial-specific genes and proteins, such as uroplakin-Ia, uroplakin-III, cytokeratin-7 (CK-7), and cytokeratin-13 (CK-13) [23]. These cells exhibit significant potential to differentiate into urethral myocytes and smooth muscle cells, making them valuable candidates for applications in urinary tract regeneration and tissue engineering [24]. See Fig. 1.

1.2. Stem cells associated extracellular vesicles and induced pluripotent stem cell

Extracellular vesicles (EVs) are membranous vesicles released by cells into the extracellular space. They play a crucial role in intercellular communication. They are involved in the regulation of various physiological and pathological processes. EVs facilitate intercellular communication by transferring vesicular proteins, lipids, nucleic acids and other substances, primarily to regulate processes such as cytokine production, cell proliferation, apoptosis and metabolism [25]. These extracellular vesicles are widely distributed in various body fluids such as blood, saliva, cerebrospinal fluid, urine, lymphatic fluid, breast milk and semen. Among these, blood and urine contain a significant number of EVs. Several mechanisms and substances are involved in the release of EVs, including the ESCRT complex, tetraspanin, neutral sphingomyelinase 2, phospholipid relocalisation and actin cytoskeleton depolymerisation [26–30]. Extracellular vesicles can be broadly classified into three groups: exosomes, microvesicles and apoptotic vesicles. In addition, oncosomes has also been identified [31]. Extensive research has demonstrated the benefits of using extracellular vesicles in disease diagnosis and treatment [17–19]. The clinical utility of mesenchymal stem cells (MSCs) is largely attributed to their paracrine and immunomodulatory properties. There is ongoing debate regarding stem cell-associated extracellular vesicles, with most studies suggesting their beneficial effects [25,32–37].

We have made amazing progress in our understanding of cell proliferation and differentiation. Induced pluripotent stem cells (iPSCs) hold immense significance in basic medical research. These cells are generated through the reprogramming of mature somatic cells and share a key characteristic with embryonic stem cells (ESCs) – the ability to differentiate into multiple cell types. Kazutoshi Takahashi successfully induced the transformation of mature mouse cells into pluripotent stem cells that possess characteristics similar to embryonic stem cells by introducing four transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) through lentiviral replication [38]. This cell source is more accessible compared to fertilized eggs and embryonic stem cells, as it retains stem cell properties and has been extensively utilized in areas such as disease mechanisms and therapies [39,40]. In contrast to in vitro two-dimensional cell culture or animal models, the directed differentiation of human induced pluripotent stem cells offers the capability to generate patient-specific three-dimensional renal organs in vitro. This approach proves invaluable for modeling various forms of genetic, developmental, and metabolic kidney diseases, both acute and chronic in nature [41]. USCs are a good cell source for generating iPSCs, and they can also be directly transformed into specific cell lines [42]. The differentiation of human urine-derived induced pluripotent stem cells (UhiPSCs) into hepatocyte-like cells provides a valuable model for simulating PCSK9-mediated hypercholesterolemia [43]. Carmen et al. Use of

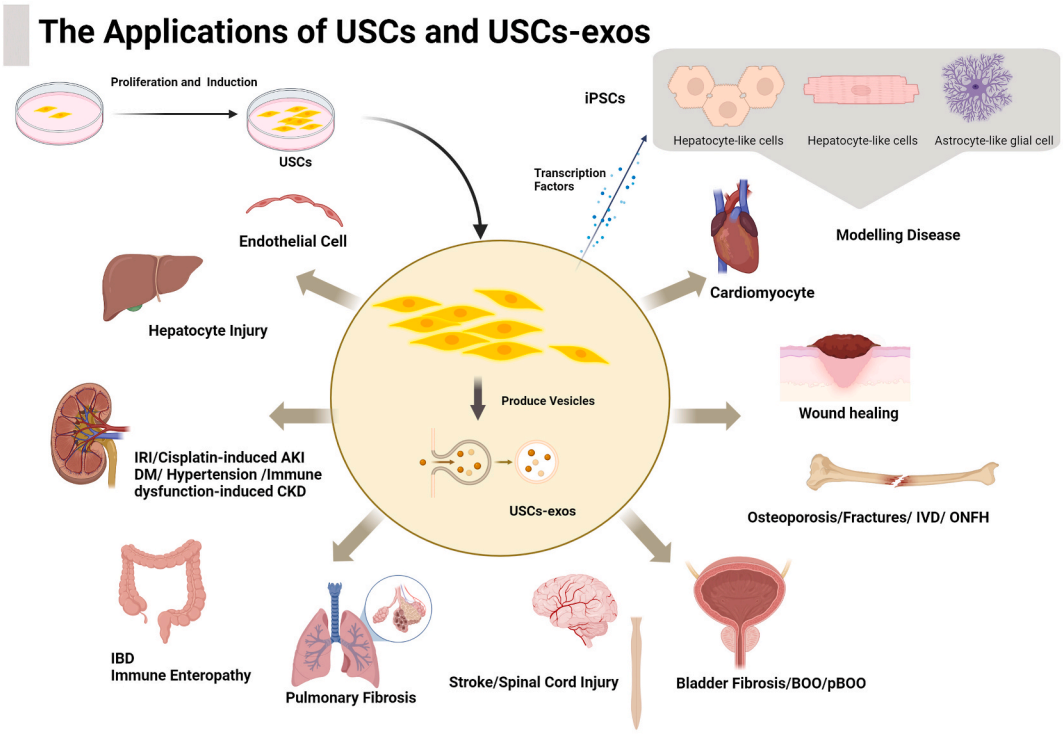

Fig. 2. USCs and USCs-EVs act in multiple organ diseases.

urine samples as a source of somatic cells to generate iPSC lines from paediatric patients with brain tumors (BT-iPSC) compared with iPSCs obtained from urine samples from non-tumor paediatric patients (nonT-iPSC). These BT-iPSCs exhibited comparable morphology, pluripotency, and the ability to differentiate into the three blastomeres when compared to iPSCs obtained from paediatric patients without tumors (non-T-iPSCs). This result and technique offer a nearly limitless source of starting material for cellular therapeutics and introduce a new perspective for regenerative medicine [44]. Furthermore, genetically engineered induced cells offer models for understanding the basis of rare diseases, including those associated with abnormal nerve cell development such as Hemophilia A (HA), phenylketonuria (PKU), and Down syndrome (DS). Moreover, there are ongoing studies exploring the therapeutic potential of exosomes derived from these cells for various disease-related conditions, Fig. 1 [45,46]. See Fig. 2.

2. Protection of kidney injury

2.1. Acute kidney injury (AKI)

Acute kidney injury (AKI) can stem from diverse causes, including reduced renal blood flow due to factors such as hypoperfusion, exposure to nephrotoxic substances, kidney inflammation, or urinary tract obstructions.

Ischemia/reperfusion injury (IRI) stands as a predominant trigger for AKI, characterized by a lack of effective treatments. The experiments by Li et al. revealed that miR-146a-5p within USC-exos targeted and degraded the 3′ UTR of interleukin-1 receptor-associated kinase 1 (IRAK1) mRNA. This action inhibited the activation of the nuclear factor (NF)-κB signaling pathway, curbing the infiltration of inflammatory cells, reducing the inflammatory response to injury from ischemia-reperfusion [47]. In a separate study, Zhang et al. demonstrated that miR-216-5p in USC-exos targeted phosphatase and tensin homologs (PTEH), leading to reduced Akt phosphorylation. This protective mechanism proved effective against AKI induced by hypoxia/reoxygenation [48]. Additionally, Sun validated that TUG1, an lncRNA abundant in USC-Exos, regulated ACSL4 mRNA stability through interaction with SRSF1, influencing ferroptosis and offering renoprotection in an IRI-induced acute kidney injury mouse model [49].

In conditions where kidney damage is attributed to drugs or toxins, USC exhibits remarkable protective effects. A foundational study conducted demonstrated the capacity of USC to mitigate cisplatin-induced acute kidney injury. This protection primarily manifests through the downregulation of the expression levels of pro-inflammatory cytokines, specifically TNF-α and IL-6, as well as apoptosis-associated proteins like BAX and cleaved caspase-3 [50].

2.2. Chronic kidney injury (CKD)

Chronic kidney injury (CKD) often results from conditions such as diabetes mellitus (DM), hypertension, and immune dysfunction, causing irreversible harm to kidney cells. In the animal experiments, Zhang et al. demonstrated that USCs possess anti-inflammatory, antioxidative stress, and antifibrotic properties. These attributes make them effective against nephrotoxic drugs and renal ischemic dual-toxicity-induced chronic renal injury, thereby playing a protective and therapeutic role in renal injury [51]. Moreover, Gao et al. explored the treatment of mice with diabetic nephropathy by inducing USC differentiation into nephron progenitor cells. This approach enhances kidney therapy by reducing glomerular hypertrophy, tubulointerstitial fibrosis, and markers like blood urea nitrogen, serum creatinine, and albuminuria. It also lowers inflammation/fibrosis ratios while bolstering renal regenerative capacity [52]. In a study, Kim et al. investigated the mechanisms underlying USCs' ability to inhibit fibrosis in HK-2 cells, which is associated with CKD progression. Their experiments revealed that USCs possess a high capacity to express Klotho protein, while in an in vitro model, they suppress fibrosis by inhibiting transforming growth factor (TGF)-β in human renal proximal tubule cells [53–56].

2.3. Bladder dysfunction and urological tissue engineering

Urine-derived stem cells, sourced from the urinary system, possess unique attributes as a novel stem cell source. These cells exhibit high expandability, self-renewal capacity, and paracrine properties [20]. In a pioneering study, Wu et al. demonstrated the potential of USCs when combined with a modified three-dimensional porous small intestinal submucosal scaffold and layered cocultures, as an alternative cell source for applications in cell-based tissue engineering, including urethral reconstruction and other urological tissue repairs [57]. USCs stand as an ideal candidate for cell therapy in bladder tissue repair [58]. While scaffolds designed to capture USCs have been investigated to facilitate endothelial healing and smooth muscle sarcomere regeneration, they have yet to be fully explored as advantageous alternative materials for bladder repair [59,60]. Notably, Wan et al. demonstrated that induced USC cells exhibit superior fine structure compared to noninduced cells, making them a promising choice for in vivo urethral tissue repair and in vitro urethral or bladder modeling [61]. Genome sequencing analysis revealed the potential involvement of miR-142 and miR-9a in regulating multiple pathways and cytokine interactions in partial bladder outlet obstruction (pBOO) [62]. Additionally, Wu et al. elucidated that exosomes derived from human USCs containing NRF1 could mitigate bladder fibrosis by modulating the miR-301b-3p/TGFβR1 pathway. Thoes discovery represents a novel therapeutic direction for the treatment of bladder outlet obstruction (BOO) [63].

In cases of interstitial cystitis (IC), a condition with an unknown etiology, USCs demonstrated the ability to inhibit oxidative stress, dampen inflammatory responses, and mitigate apoptotic processes in a protein/lipopolysaccharide (PS/LPS)-induced interstitial cystitis animal model, resulting in a partial restoration of bladder function and histological structure [64]. Moreover, Sun et al. successfully induced the differentiation of USC cells into cells resembling Cajal-like cells (ICC-LCs). Lentiviral-HCN4-transfected cells exhibited morphological changes characteristic of ICC-LCs, along with visible hyperpolarization-activated cyclic nucleotide-gated

potassium channel (HCN) current amplitude and density. These USC-induced cells play a pivotal role in the regulation of spontaneous bladder contractions by contributing to their pacing function [65].

3. Protection and regeneration of bone and related diseases

Urine-derived stem cells exhibit remarkable tissue differentiation capabilities akin to traditional stem cells, including lipogenic, osteogenic, and chondrogenic properties [66–68]. Moreover, in vitro, USCs display a robust proliferative capacity, comparable to that of bone marrow-derived mesenchymal stem cells (MSCs), while both have demonstrated similar effectiveness in cartilage repair in vivo [66]. USCs and their exosomes have demonstrated therapeutic potential in mitigating various bone-related ailments, such as osteoporosis, bone fractures, intervertebral disc degeneration, osteonecrosis of the femoral head (ONFH), and expediting rotator cuff healing [69–77].

By leveraging macrophage membranes (MM) to encapsulate USC-derived stem cells, Xie and his team developed exosomes with targeted delivery to osteolytic zones. This innovative approach enhances the therapeutic efficacy of exosomes in mitigating wear particle-induced calcific osteolysis [69,72]. Furthermore, USC-derived extracellular vesicles (USC-EVs) have been shown to deliver miR-26a-5p to osteoprogenitor precursor cells, promoting their differentiation, enhancing osteoblast activity, and inhibiting osteoclast activity. This mechanism helps prevent diabetic osteoporosis (DOP) by inhibiting HDAC4 activation of the hypoxia-inducible factor 1 subunit alpha (HIF-1α)/vascular endothelial growth factor A (VEGFA) pathway [70]. The focal adhesion kinase (FAK) mediates BMP2-enhanced osteogenic differentiation of human USCs by activating adenosine 5′-monophosphate-activated protein kinase and Wnt signaling pathways [78]. Additionally, a combination of USCs and BMP2-CSM/Col I hydrogel has proven effective in enhancing bone regeneration [79]. Chen et al. identified collagen triple helical repeat sequence 1 (CTHRC1) and osteoprotegerin (OPG) contained in USC-EVs as essential proteins that promote osteoclast and osteoblast resistance [76]. Furthermore, the osteogenic differentiation of USC in the presence of calcium silicate (CS) is associated with the Wnt/β-catenin signaling pathway [80].

Human urine-derived stem cell exosomes have demonstrated anti-senescence effects, promotion of cell proliferation, and modulation of the extracellular matrix (ECM) in degenerating intervertebral discs. This beneficial impact is attributed to the abundance of MATN3 protein in USC-exos, which stimulates the proliferation of nucleus pulposus cells (NPCs) and ECM synthesis by activating TGF-β [73]. USC-exos have also shown significant potential in ameliorating endoplasmic reticulum (ER) stress, inhibiting excessive activation of the unfolded protein response (UPR), and reducing apoptosis and discoidal degradation [81]. Additionally, USC-EVs exhibit protective effects in preventing glucocorticoid-induced ONFH by providing pro-angiogenic proteins, including Deleted in malignant brain tumors 1 (DMBT1) and tissue inhibitor of metalloproteinases 1 (TIMP1) [75]. Furthermore, USC-EVs demonstrate a synergistic effect with phosphate (β-TCP) biomaterials. USCs on β-TCP scaffolds promote bone regeneration in segmental femoral defects in rats [82].

4. Protection and regeneration of other vital organs

Liu's research team targeted the stem cell properties of USCs by focusing on endothelial cells, a key component in vascular regeneration. Their experimental findings demonstrated that USCs induced the expression of specific endothelial cell markers (CD31, vWF, eNOS) at significantly higher levels in vitro and in vivo. USCs also formed complex tubular networks with tight junctions, exhibited migration and invasion abilities, and produced nitric oxide (NO) [83].

The heart, a vital organ in the human body, has seen successful induction of USCs into cardiomyocytes. Urine-derived cardiomyocytes retain characteristic features and have been utilized for mechanistic studies and drug discovery since 2014 [84]. Furthermore, Gao et al. employed clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9) technology to integrate a triple-fusion (TF) reporter gene into the AAVS1 locus in human urine-derived induced pluripotent stem cells (hiPSCs), generating TF-hUiPSCs. These cells enhanced cardiomyocyte glucose metabolism and promoted angiogenic activity [85]. USCs have demonstrated an inhibitory effect on both the histological destruction and functional decline of the heart in cases complicated by diabetes mellitus (DM) [86,87]. A study by Mariam et al. is particularly exciting as it reprogrammed cells obtained from urine samples, resulting in derived cardiomyocytes (CMs) that exhibited proper expression of atrial and ventricular myofilament proteins and ion channels, making them a new cellular model for studying patient-specific arrhythmia mechanisms [88]. Urine-derived induced pluripotent stem (iPS) cell lines have emerged as a valuable cellular platform for studying the pathogenesis, drug therapy, and gene therapy in patients with ventricular septal defects and heart failure [89].

The USCs have been successfully extracted from patients with brain disorders. In neurologically relevant studies, USCs have proven valuable in neuroprotection and therapy. Exosome-derived miR-21-5p from USCs has been shown to attenuate the symptoms of Rett syndrome by modulating the EPha4/TEK axis [44,90]. USC-exos carrying ANGPTL3 can enhance functional recovery of the spinal cord after injury by promoting angiogenesis [91,92]. In an animal model of cardioversion ischemic-hypoxic encephalopathy, USCs facilitated neurological function recovery by promoting the expression levels of brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) while inhibiting cerebral edema [85]. Urinary epithelial cell (UEC)-derived induced pluripotent stem cell (iPSC)-developed cerebral organoids (COs) demonstrated a robust capacity for neurogenesis and astrogliogenesis [93]. Urine-derived iPSCs may hold promise for cell transplantation therapy in stroke by enhancing neurogenesis and alleviating neurological deficits via the miR-26a/HDAC6 axis [94,95].

Although less is known about the relationship between USCs and the lung. The miRNAs found in urine-derived microvesicles correlate with fibrotic phenotypes in idiopathic pulmonary fibrosis (IPF) [96]. Many of the functions of USCs can be harnessed through various reprogramming methods. It has been observed that urine-derived induced pluripotent stem cells (UiPSCs) can differentiate into

alveolar type II (AT II) cells, which could hold value for cellular therapy in lung diseases [97]. For instance, the induced pluripotent stem cell (iPSC) line WMUi017-A was generated by reprogramming urine cells from a 5-year-old male X-linked retinoschisis (X-RSY) patient with the hemizygous PQBP1 gene mutation p. P609A (c.1825C > G) using the commercial Sendai virus reprogramming system [98].

In a liver characterization study aimed at assessing the biosignature of end-stage liver disease patient-derived USCs, independent of the underlying liver disease, the transplantation of these cells demonstrated partial repair of liver injury [99]. While associations of USCs with the gastrointestinal tract require further exploration, one study has suggested that USCs may attenuate inflammation in a rat model of inflammatory bowel disease (IBD) by suppressing the Th1/Th17 immune response [100]. Small intestine submucosa (SIS) is commonly employed as a soft tissue scaffold, and under hypoxic conditions, USC + SIS composites have demonstrated enhanced wound healing potential [101,102]. See Table 1.

5. Association with tumor, immune and inflammatory responses

Extracellular vesicles (EVs) play a pivotal role in immune regulation, and given the close association between stem cells and EVs, USCs may also hold significant potential in modulating the body's immune system in this manner [34,103,104]. Remarkably, several studies have shed light on the immunoregulatory capabilities of USCs. USCs-derived exosomes, for instance, are enriched in cellular molecules critical for B-cell stimulation, differentiation, and humoral immunity, including BAFF, APRIL, IL-6, and CD40L [105]. Additionally, USCs have demonstrated the ability to suppress Th1/Th17 immune responses in a PGE2-dependent manner [100]. It is anticipated that future research will delve deeper into the mechanistic aspects of USCs' immunoinflammatory-related functions. The USCs have not been extensively studied in common rheumatological and immunological diseases such as systemic lupus erythematosus, rheumatoid arthritis, and ankylosing spondylitis. However, research on various stem cells, including MSCs and adipose stem cells, in the context of immune-related diseases is abundant. The exploration of USCs in this area may just be at its initial stages.

Similarly, in the field of oncology, there is limited research on urinary-derived stem cells (USCs) compared to other types of stem cells such as mesenchymal stem cells (MSCs). The investigation of USCs in oncology may still be in its early stages. Although fewer studies have explored the relationship between USCs and tumor diseases, research conducted by Chen et al. has revealed that renal clear cell carcinoma cells can influence the differentiation of USCs into carcinoma-associated fibroblasts through the RUNX3/TGF-β1 signaling axis [106]. Luo et al.'s study utilized sequencing and biological analyses to reveal that urinary-derived stem cells (USCs) derived from clear cell renal cell carcinoma (ccRCC) patients exhibited heightened proliferative and invasive capabilities. Additionally, these cancer patient-derived USCs expressed several genes associated with ccRCC. However, minimal differences in pathways

Table 1
The role of USCs in some diseases.

Disease		Mechanism	Substances
Kidney disease	AKI	NF-κB signaling pathway ACSL4 mRNA stability through interaction with SRSF1	miR-146a-5p in USC-exos miR-216-5p in USC-exos TUG1 in USC-exos PTEH
	Cisplatin-induced acute kidney injury	Downregulation of the expression levels of pro-inflammatory cytokines	TNF-α, IL-6, BAX and cleaved caspase-3
	CKD	Anti-inflammatory, antioxidative stress, and antifibrotic properties;	Klotho protein
Bladder disease	Interstitial cystitis (IC)	Urethral reconstruction and other urological tissue repairs; facilitate endothelial healing and smooth muscle sarcomere regeneration (scaffolds designed to capture USCs)	
	BOO/pBOO	Multiple pathways and cytokine interactions	miR-301b-3p/TGFβR1 in USC-exos miR-142 and miR-9a
	Bladder contractile dysfunction disease	Into cells resembling Cajal-like cells (ICC-LCs)	regulation of spontaneous bladder contractions by contributing to their pacing function
Bone and Related Diseases	Diabetic osteoporosis (DOP)	inhibiting HDAC4 activation of the HIF-1α/VEGFA pathway	miR-26a-5p in USC-exos
	Intervertebral discs degeneration	stimulates the proliferation of NPCs and ECM synthesis by activating TGF-β	MATN3 protein in USC-exos
	Glucocorticoid-induced ONFH	providing pro-angiogenic proteins	DMBT1 and TIMP1 in USC-exos
Other Vital Organs	Rett syndrome	modulating the EPha4/TEK axis	miR-21-5p in USC-exos
	Spinal cord injury	promoting angiogenesis	ANGPTL3 in USC-exos
	Cardioversion ischemic-hypoxic encephalopathy	inhibiting cerebral edema	promoting the expression levels of BDNF and VEGF
	Idiopathic pulmonary fibrosis (IPF)	correlate with fibrotic phenotypes	miRNAs in USC-exos
	Inflammatory bowel disease (IBD)	suppressing the Th1/Th17 immune response	

[a]AKI: Acute kidney injury; CKD: Chronic kidney injury; PTEH: Phosphatase and tensin homologs; BOO: Bladder outlet obstruction; pBOO: Partial bladder outlet obstruction; HIF-1α: Hypoxia-inducible factor 1 subunit alpha; VEGFA: Vascular endothelial growth factor A; NPCs: Nucleus pulposus cells; BDNF: Brain-derived neurotrophic factor.

were observed between cancer patient-derived USCs and those derived from normal patients, as analyzed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment [107].

In numerous studies on the tumor immune microenvironment, alongside cancer stem cells, another class of multipotent stem cells originating from tissues like bone marrow, known as mesenchymal stem cells, have been demonstrated to influence tumor growth, invasion, and metastasis. These cells also play a role in the regulation of the tumor microenvironment [108,109]. The intricate interactions between stem cells, including USCs, and immune cells within the tumor microenvironment play a crucial role. Stem cells, in this context, regulate the activity and function of immune cells, such as T cells and macrophages. This holds significant implications for understanding tumor immune evasion and the effectiveness of anti-tumor immunotherapy. Different types of stem cells exert influence on tumor cell proliferation, angiogenesis, and inflammatory responses, impacting the biological characteristics of tumors, signaling pathways within the microenvironment, and the production of cytokines. In a study by Ting et al., the capacity of human adipose-derived stem cells (ADSCs) to mitigate inflammation and enhance the immunomodulatory potential of ADSCs in tumorigenesis was demonstrated [110]. The immune-related regulatory mechanisms of USCs in the tumor microenvironment remain largely unknown.

6. Urine-derived stem cells and their extracellular vesicles

With the recent studies on the mechanisms of action of USCs in various disease models, it becomes evident that extracellular vesicles, particularly exosomes derived from USCs, play a pervasive role in the regulatory mechanisms [111,112]. The majority of studies still center around the urinary system, including conditions like acute/chronic kidney disease and bladder-related diseases. However, there is an increasing number of applications in the realms of bone and joint diseases, as well as neurological injury repair [77,113–115]. As we discussed in the previous article, USC-derived exosomes have demonstrated a protective effect against ischemia-reperfusion injury (IRI)-induced renal injury. In Xirui Li's study, it was found that MiR-146a-5p within these exosomes targets the 3′ UTR of IRAK1, consequently inhibiting NF-κB signaling activation and reducing inflammatory cell infiltration [47]. In a study led by Cristina Grange's team, animal experiments revealed that extracellular vesicles derived from USCs, particularly those enriched with Klotho, exhibited a protective effect against acute kidney injury induced by glycerol injection [116]. Furthermore, studies related to the protective role of USCs-derived extracellular vesicles or exosomes in various chronic kidney diseases are particularly promising. Current research indicates that glomerular mesangial cells and multinucleated cells play crucial roles in the pathogenesis of diabetic nephropathy (DN). In a study led by Nie's team, USCs exosomes were injected into male diabetic model rats, leading to a relative reduction in the activation of the mTOR signaling pathway in the exosome-intervened group. Additionally, there was an increase in the protein expression of Bcl-2 in renal tissues, resulting in a certain degree of kidney protection [117]. Another significant finding is that USCs alleviate high glucose-induced podocyte injury through the delivery of miR-16-5p via exosomes [118]. The circRNA ATG7 present in USCs-derived exosomes was transferred to modulate the SOCS1/STAT3 signaling pathway through miR-4500. This process promoted the polarization of macrophages towards the M2 phenotype and inhibited the progression of diabetic nephropathy (DN) [119]. Given the wide range of substances, including nucleic acids, proteins, and metabolites, covered by exosomes, current research indicates that they play a role in regulating renal diseases across various pathways and aspects. Much remains unknown about the regulatory role of the substances encompassed in vesicles in disease, and it is possible that this type of intercellular signaling could also be a more reliable therapeutic tool.

Furthermore, in diseases related to bone and joints, USCs exosomes have demonstrated more protective effects. Exosomes containing microRNA-26a-5p inhibit osteoclastogenesis by regulating the HIF-1α/VEGFA axis. Additionally, these exosomes contain collagen triple-helix repeat-containing 1 (CTHRC1) and osteoprotegerin (OPG) proteins, thereby preventing osteoporosis [76,120]. hUSC-Exos demonstrated the ability to overexpress miR-140-5p and mediate increased secretion of vascular endothelial growth factor A (VEGFA) and extracellular matrix (collagen II and aggrecan). This plays a protective and regulatory role in knee osteoarthritis (KOA), enhancing cartilage regeneration and subchondral bone remodeling [77]. Recent studies conducted by Xie's team have advanced the targeting of exosomes derived from USCs. They have innovatively employed macrophages to provide immunological camouflage for targeted delivery of macrophage membrane-encapsulated urine-derived stem cell-derived exosomes (MM-Exos) to the cleft zone. This

Table 2
Some functions of USC-derived exosomes.

Substances	Function
miR-146a-5p	Inhibiting NF-κB signaling activation and reducing inflammatory cell infiltration Increased secretion of VEGFA and extracellular matrix (collagen II and aggrecan)
miR-16-5p	Suppress VEGFA expression and podocytic apoptosis
smiRNA-26a-5p	Inhibit osteoclastogenesis by regulating the HIF-1α/VEGFA axis
circRNA ATG7	Modulate the SOCS1/STAT3 signaling pathway, promoting the polarization of macrophages towards the M2 phenotype
Klotho protein	Accelerated renal recovery, stimulating tubular cell proliferation, reducing the expression of inflammatory and injury markers, and restoring endogenous Klotho protein loss
Unknown	Reduced the activation of the mTOR signaling pathway, reduced the autophagy of their kidney cells, increased the protein expression of Bcl-2
CTHRC1 and OPG proteins	Preventing osteoporosis
MATN3 protein	Slowed down disc degeneration, while simultaneously promoting nasopharyngeal carcinoma cell proliferation and extracellular matrix (ECM) synthesis

approach enhances the therapeutic efficacy of exosomes for peripheral-prosthetic clefts [69]. Osteonecrosis of the femoral head (ONFH) induced by glucocorticoid (GC) treatment has also demonstrated benefits from USC-derived extracellular vesicles (EVs) [75]. Endoplasmic reticulum stress in intervertebral disc degeneration (IDD) was also found to be modulated by USCs-derived exosomes (USCs-Exos). These exosomes inhibited unfolded protein response (UPR) hyperactivation, apoptosis, and disc degeneration by regulating the AKT and ERK signaling pathways [81]. However, the following year, Zhu Guo's study again demonstrated that USCs-Exos transferring MATN3 protein slowed down disc degeneration, while simultaneously promoting nasopharyngeal carcinoma cell proliferation and extracellular matrix (ECM) synthesis [73].

Most of the literature reviewed on the effects of human urine-derived stem cell-derived exosomes (hUSCs-Exos) on a variety of diseases is mostly protective. This includes the neurological system, which has poor regenerative capacity but can also benefit from the therapeutic effects of hUSCs-Exos [90,114]. Despite the numerous beneficial effects demonstrated by hUSCs-Exos in various diseases, it is important to consider the possibility of adverse effects that may arise. Further research is needed to explore potential risks and limitations associated with the use of hUSCs-Exos in different therapeutic contexts. See Table 2.

7. Some functions associated with hydrogels

Hydrogel possesses characteristics such as rapid dissolution in water and the ability to retain a substantial amount of water without decomposing. This gel material closely resembles the structure of biological tissue, consisting of a highly hydrophilic three-dimensional network with cross-linked structures that enable it to swell and retain considerable water content [121]. Hydrogels are broadly divided into natural and synthetic types, and can also be classified based on factors such as raw material source, cross-linking method, polymerization method, charging characteristics, environmental response and degradability. Additionally, hydrogels can be classified as physical or chemical gels, with chemical gels characterized by irreversible bonding within the hydrogel network. Since their inception in 1894, hydrogels have evolved and gained advanced features, thanks to technological advancements and expanding applications. Hydrogels find predominant applications in various fields, including tissue engineering scaffolds, cell encapsulation, controlled drug release systems, wound dressings, and drug treatments [122–131].

Indeed, hydrogels serve as effective carriers for targeted delivery and enrichment of stem cells and their exosomes, including those derived from urinary stem cells (USCs). The use of hydrogels in this context provides a controlled and supportive environment for stem cells and their secreted factors, facilitating their therapeutic applications in regenerative medicine and targeted therapies. Liu et al. conducted experiments using an animal model. They elevated the expression of vascular endothelial growth factor (VEGF) in USCs, which were then implanted into nude mice via a collagen-I gel. Their findings demonstrated that this approach improved the survival of the transplanted cells, recruited resident cells, and promoted myogenic phenotype differentiation of USCs, alongside enhancing vascularization and innervation in the graft site [132]. The utilization of hydrogels, such as collagen gel and hyaluronic acid heparin gel, as scaffolds for stem cells, including urinary stem cells (USCs), is promising for addressing issues related to urinary incontinence. These hydrogels provide a supportive matrix for stem cells, aiding in their localization to specific anatomical sites and promoting their therapeutic effects. The incorporation of growth factors into these hydrogels further enhances their regenerative potential. The targeted implantation of such hydrogel-supported stem cells can contribute to the repair and regeneration of tissues associated with urinary incontinence, such as muscles, blood vessels, and peripheral nerves around the urethra. This approach holds potential for innovative treatments in the field of urology. Their team also discovered that the release of myogenic growth factors from a heparin-hyaluronic acid gel (hp-HA gel) could augment in vivo cell survival, in-growth, and myogenic differentiation of USCs. This was associated with improved graft vascularization, innervation, and regenerative properties [133].

Hydrogel-bound USCs can play a role in cell communication at designated sites. Biocompatibility is of paramount concern in tissue regeneration and stem cell transplantation engineering.

Zeng et al. developed an injectable porcine cartilage-derived decellularized extracellular matrix (ECM) hydrogel designed for repairing cartilage defects. This hydrogel exhibited superior biocompatibility and immunomodulatory abilities [134]. Hydrogels as carriers for transporting USCs have yielded innovative approaches to addressing traditionally challenging medical conditions. CD133+USC-Exos encapsulated in hydrogel complexes have shown promise as a therapeutic strategy for rectifying rectal cancer (RC) healing, based on the potential of stem cell exosomes [135]. Optimized hydrogel-loaded USCs have shown promise in enhancing epithelialization of the vagina after vaginoplasty through the sustained release of extracellular vesicles. The key molecule, miR-126-3p, found in these vesicles, inhibits the expression of Spred1 and PIK3R2, promoting the migration and differentiation of VK2 cells. This activation subsequently triggers the ERK1/2 and AKT signaling pathways [136].

A novel injectable light-triggered hydrogel, characterized by excellent tissue adhesion and adaptive properties, was developed. The piGEL-sEVs exhibited robust binding to tissues, facilitated by covalent bonds and physical adhesion. In animal experimental models, this composite system demonstrated sustained release of piGEL-hUSC-sEVs enriched with miR-126-3p. Notably, these vesicles were effectively internalized by vaginal cells, fostering accelerated morphological and functional recovery of vaginal mucosal defects. The targeted and controlled release capabilities of hydrogels have also shone in basic bone regeneration experiments. Lu et al. designed a novel composite hydrogel, GelMA-HAMA/nHAP, incorporating gelatin acrylate (GelMA) and hyaluronic acid methacrylate (HAMA) with the addition of nano-hydroxyapatite (nHAP) to enhance mechanical properties. This hydrogel efficiently encapsulated USC-EXOs, displaying excellent biocompatibility and effectively promoting bone regeneration by facilitating osteogenesis and angiogenesis simultaneously [115]. Additionally, a combination of USCs and BMP2-CSM/Col I hydrogel has proven effective in enhancing bone regeneration [79]. The exploration of USCs and hydrogels represents a burgeoning area of research, primarily centered on the synergistic interplay between stem cells and hydrogels. Leveraging the diverse chemical and physical attributes of hydrogels not only augments the inherent functions of USCs within the body but also imparts additional immunomodulatory and sustained-release

capabilities. In comparison to alternative biomaterials, hydrogels exhibit promising potential to outperform in niche applications within the realm of medical research and practice. See Fig. 3.

8. Conclusion and perspective

In conclusion, USCs offer a promising and non-invasive resource with excellent stem cell properties, making them a valuable asset in the field of regenerative engineering. While research involving stem cells often faces ethical considerations, USCs present more possibilities in this regard, and numerous researchers are dedicated to advancing innovative research and applications for these cells.

Currently, much of the research involving USCs is limited to animal experiments. However, USCs have shown great potential beyond the urinary system, displaying protective effects in various organ systems. Their interaction with hydrogel-related biomaterials has been particularly noteworthy, highlighting their superior targeting and potent regenerative capabilities.

Ongoing research into the induced differentiation of USCs is in full swing, with the potential to unlock even more robust stem cell properties. In both in vitro and in vivo experiments, these cells hold significant promise for the treatment of patients with organ dysfunction. Currently, much of the research involving USCs is limited to animal experiments. USCs have shown great potential beyond the urinary system, displaying protective effects in various organ systems. Their interaction with hydrogel-related biomaterials has been particularly noteworthy, highlighting their superior targeting and potent regenerative capabilities. Ongoing research into the induced differentiation of USCs is in full swing, with the potential to unlock even more robust stem cell properties. In both in vitro and in vivo experiments, these cells hold significant promise for the treatment of patients with organ dysfunction. The future of USCs in regenerative medicine appears bright, and further exploration and innovation in this field are eagerly anticipated.

Ethics approval and consent to participate

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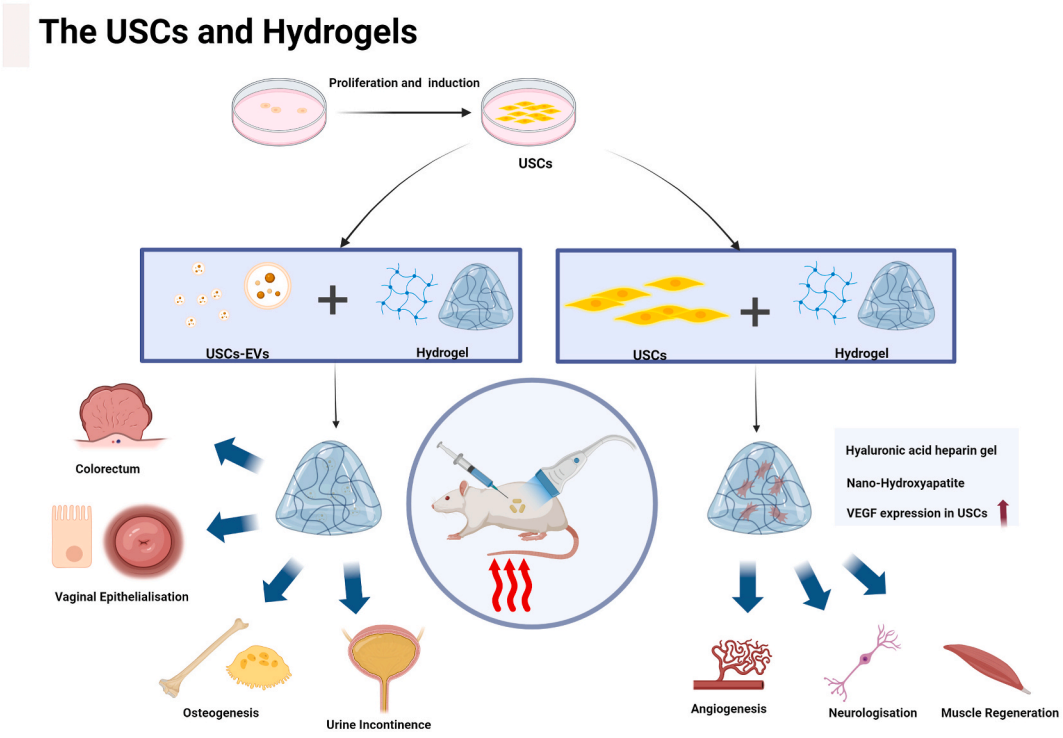

Fig. 3. The USCs and USCs-EVs with hydrogels.

Consent for publication

All the authors have approved the publication.

Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Yao Sun: Writing – review & editing, Writing – original draft, Supervision. **Huiying Zhao:** Supervision. **Shuguang Yang:** Writing – original draft. **Guangjie Wang:** Writing – review & editing. **Leijie Zhu:** Writing – original draft. **Chang Sun:** Writing – original draft. **Youzhong An:** Writing – review & editing, Supervision.

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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Abbreviations

USCs	urine-derived Stem Cell;
hESCs	human embryonic stem cells
TE	
trophoblast	
ICM	inner cell mass
EVs	extracellular vesicles
CK	cytokeratin
HA	hemophilia A
PKU	phenylketonuria
DS	down syndrome
X-LAS	X-linked Alport syndrome
AKI	acute kidney injury
IRI	ischemia/reperfusion injury
USC-exos	USC exosomes
IRAK1	interleukin-1 receptor-associated kinase 1
NF	nuclear factor
PTEH	phosphatase and tensin homologs
CKD	chronic kidney injury
DM	diabetes mellitus
TGF	transforming growth factor
pBOO	partial bladder outlet obstruction
IC	For interstitial cystitis
PS/LPS	protein/lipopolysaccharide
LCs	Cajal-like cells
HCN	hyperpolarization-activated cyclic nucleotide
ONFH	osteonecrosis of the femoral head
MM	macrophage membranes
DOP	diabetic osteoporosis
HIF-1α	hypoxia-inducible factor 1 subunit alpha
VEGFA	vascular endothelial growth factor A
FAK	focal adhesion kinase
CTHRC1	collagen triple-helix repeat containing 1
OPG	osteoprotegerin
CS	calcium silicate
ECM	extracellular matrix
NPCs	nucleus pulposus cells

ER	endoplasmic reticulum
UPR	unfolded protein response
DMBT1	deleted in malignant brain tumors 1
TIMP1	tissue inhibitor of metalloproteinases 1
NO	nitric oxide
TF	triple-fusion
hiPSCs	human induced pluripotent stem cells
CM	cardiomyocyte
BDNF	brain-derived neurotrophic factor
VEGF	vascular endothelial growth factor
UEC	urinary epithelial cell
IPF	pulmonary fibrosis
IBD	inflammatory bowel disease
SIS	small intestine submucosa
hp-HA gel	heparin-hyaluronic acid gel
ECM	extracellular matrix
GelMA-HAMA/nHAP	gelatin acrylate and Hyaluronic acid methacrylate with the addition of nano-hydroxyapatite

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