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Review

Review

# Urine-derived stem cells: applications in skin, bone and articular cartilage repair

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## Abstract

As an emerging type of adult stem cell featuring non-invasive acquisition, urine-derived stem cells (USCs) have shown great potential for applications in tissue engineering and regenerative medicine. With a growing amount of research on the topic, the effectiveness of USCs in various disease models has been shown and the underlying mechanisms have also been explored, though many aspects still remain unclear. In this review, we aim to provide an up-to-date overview of the biological characteristics of USCs and their applications in skin, bone and articular cartilage repair. In addition to the identification procedure of USCs, we also summarize current knowledge of the underlying repair mechanisms and application modes of USCs. Potential concerns and perspectives have also been summarized.

**Key words:** Urine-derived stem cells, Skin, Bone, Articular cartilage, Tissue engineering, Cell therapy

## Highlights

- Provide an up-to-date overview of the biological characteristics of USCs.
- Summarize current research and propose application modes of USCs in skin, bone and articular cartilage repair.
- Summarize the understanding of the underlying repair mechanisms of USCs.
- Propose further research directions to clarify the safety, efficacy, mechanisms and cost-effectiveness of applying USCs in the context of translational medicine.

## Background

One of the main contributors to the global burden of disease is traumatic injury [1], while surgical wounds and burn injuries affect millions of people worldwide annually [2, 3]. In addition, chronic skin wounds are increasing, such as diabetic foot ulcers [3]. There is a huge demand for wound healing, defect repair and tissue regeneration. During the last decades, mesenchymal stem cells (MSCs) have shown great potential for tissue engineering and regenerative medicine. To date, such cells have been isolated from various tissues [4], e.g. bone marrow, adipose, tendon, placenta, etc. Though with different origins and behaviors, MSCs are believed to have similar characteristics such as cell surface antigen profiles. Adult MSCs are thought to be applicable for various diseases including skin, bone and articular cartilage injuries. Some

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MSCs have already been commercialized and approved by supervisory authorities [5, 6].

As for the roles that exogenous MSCs play in tissue repair, an early hypothesis was that MSCs could differentiate into functional cells to replace damaged cells. However, later studies showed that though in some cases MSCs did function as hypothesized, other mechanisms including MSC/-cell fusion, paracrine effect, organelle transfer, extracellular vesicle-mediated active factors transfer and immune response were often more relevant [7, 8]. The overall outcome of repair is now believed to be a combined result involving multiple mechanisms.

Acquisition of MSCs from various tissues such as bone marrow and adipose tissue usually involves invasive procedures and injuries. As a result, researchers have set their sights on clinically discarded tissues such as the placenta and umbilical cord to isolate MSCs. Recently, urine-derived stem cells (USCs) have drawn much attention for their potential in regenerative medicine. Because of their similarity to MSCs, USCs have been applied in various disease models with promising results. Here, we discuss the biological characteristics of USCs, and then focus on their applications in skin, bone and articular cartilage repair. We also discuss the issues with regard to future studies and applications of USCs in regenerative medicine.

## Review

### Biological characteristics and applications of USCs

Urine has been considered as a new source of adult stem cells. Human kidneys produce ∼180 L of primary filtrate daily, of which only ∼1% is eventually excreted as urine [9]. This process ensures regular removal of metabolic wastes from the blood and maintenance of adequate blood pressure and pH value. Owing to the epithelial lining of the luminal surface in the urinary tract and a conservative estimation that 2000–7000 renal tubular cells are exfoliated daily [10], the sediment of urine is a major source of epithelial cells [11]. In 2008, Zhang *et al*. identified a stem cell population in urine, with an expansion potential for up to ten passages *in vitro* [12]. This stem cell population was later termed urine-derived stem cells or USCs. As the urine produced comes into contact with multiple tissues through the excretion process, the origin of USCs has remained controversial. USCs are positive for CD44, cytokeratin 13 and uroplakin Ia. These markers are also present in basal bladder cells [12]. Because basal cells can self-renew, proliferate and differentiate into intermediate and superficial cells, they are referred to as urothelial progenitor cells or stem cells [13, 14]. Accordingly, USCs are thought to be derived from basal cells [12]. However, subsequent research showed that the USCs derived from female donors who received male kidney transplantation showed X/Y chromosome characteristics, indicating that they are from the upper urinary system [15]. Immunofluorescence assay and real-time PCR assay suggested that USCs may originate from parietal cells or podocytes in the renal glomerulus [15]. In our previous work, according to morphology, we identified and characterized two subpopulations of USCs, and research showed that they have different origins: one of them may be from the renal mesenchyme near the loop of Henle and the distal convoluted tubule, while the other may originate from nephron tubules including Bowman's capsule to the distal convoluted tubule, except the collecting duct [16]. Despite their multiple possible sites of the origin, USCs share similar marker profiles with MSCs, e.g. positive for CD73, CD90 and CD105, but negative for CD11b, CD14, CD19, CD34, CD45 and CD31 [12, 16–18]. Table 1 shows the typical cell surface markers of USCs.

However, it is much simpler to obtain USCs than MSCs. The major steps for the primary culture of USCs include centrifuging the collected urine samples, washing with PBS, resuspending the sediment in a culture medium, and then transferring to culture flasks. After a few days of culture, clones can be observed. Colonies usually appear ∼3–9 days after plating, and the time of the colonies' appearance may be not associated with the age or gender of the donor [19–21]. USCs are usually rice-shaped [17, 21, 22], although spindle-shaped USCs have also been observed [16, 19]. One report showed that urine samples collected continuously for 24 h from a healthy individual could generate up to 140 USC clones, and the average population doubling time from passage 0 forward to passage 8 of fresh USCs is $49.5 \pm 7.2$ h [21]. More than $1 \times 10^8$ USCs may be obtained over three passages, which will suffice for clinical applications [23].

Further analysis on the stemness features showed that the USCs displayed detectable levels of telomerase, and they expressed stemness-related genes, such as *SOX2*, *OCT-3/4*, *C-MYC* and *KLF4* [17, 24], which are often of concern for subsequent applications. The USCs did not lead to teratomas or tumors [25], possibly because the mRNA levels of stemness-related genes in the USCs were significantly lower than those in embryonic stem cells [17]. In another study, the USCs did not express *OCT4*, and fully methylated CpG dinucleotides within the *OCT4* promoter were observed [26]. Indeed, the USCs from various donors showed differential mRNA levels of *OCT4* expression [17]. The differences are possibly due to the donor age. The expression of OCT4 at protein level in USCs is even weaker, down to a very low ratio/level [27] or undetectable [17] by immunofluorescence staining. Furthermore, USCs showed self-renewal in which inactive WNT/$\beta$-catenin signaling and active TGF-$\beta$/SMAD2/3 signaling may play an important role [26]. Previous studies proved that USCs are multipotent and could differentiate into bladder cell lineages such as urothelial, smooth muscle and endothelial cells. Other mesodermal cell lineages are also inducible, e.g. chondrocytes, adipocytes and osteocytes [17, 28]. In addition, they could also undergo neurogenic and skeletal myogenic differentiation [17].

Given the above features, USCs have been considered for application in regenerative medicine. They were first introduced in urinary tissue engineering [29, 30]. As adult stem cells, USCs are believed to have potential in regenerative medicine in addition to urological applications. Other potential application areas may include stress urinary

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**Table 1.** The typical cell surface markers of USCs

| Ref. | CD105 | CD73 | CD90 | CD29 | CD146 | CD44 | CD166 | CD133 | CD24 | SSEA-4 | CD34 | CD45 | CD31 | HLA-DR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fu et al. [47] | \ | + | + | + | + | \ | \ | \ | \ | \ | − | \ | \ | − |
| Qin et al. [98] | \ | + | + | + | \ | \ | \ | \ | \ | \ | − | − | \ | \ |
| Pei et al. [64] | W | + | + | + | + | \ | \ | W | + | + | − | − | − | \ |
| Guan et al. [44] | W | + | + | + | \ | + | \ | \ | \ | \ | − | − | \ | \ |
| Guan et al. [99] | \ | + | + | + | \ | + | \ | − | \ | \ | − | − | \ | − |
| Chen et al. [61] | \ | + | + | + | \ | + | \ | \ | \ | \ | − | − | \ | \ |
| Zhang et al. [80] | W | + | + | + | \ | + | \ | − | \ | \ | − | − | \ | − |
| Chen et al. [46] | + | + | + | + | \ | \ | + | \ | \ | \ | − | − | \ | − |
| Xing et al. [101] | \ | \ | + | + | \ | + | \ | \ | \ | \ | \ | − | − | − |
| Cao et al. [81] | \ | + | + | + | + | \ | \ | − | \ | \ | − | − | \ | − |
| Zhang et al. [82] | \ | + | + | + | \ | + | \ | \ | \ | \ | − | − | \ | − |
| Sun et al. [100] | \ | + | + | + | \ | + | \ | \ | \ | \ | − | − | \ | \ |

+ positive expression, − negative expression, \ not tested, *W* weak-positive expression

incontinence [31, 32], erectile dysfunction [18, 33], acute kidney injury [24, 34], chronic kidney disease [35], vascular diseases [22], diabetes mellitus [36, 37], diabetic nephropathy [38, 39], chronic liver injury [40], inflammatory bowel diseases [41], neuron regeneration [42], osteonecrosis of the femoral head [43], bone [44, 45] and cartilage regeneration [46], and skin wounds healing [47]. Induced pluripotent stem cells generated from urine-derived cells or USCs can further expand the application areas [48], such as in cardiac repair [49], dental reconstruction [50], disease modeling and drug screening [51–57]. Moreover, USCs-derived extracellular vesicles also hold promise for the amelioration of various diseases [31, 58–63], and USCs-derived extracellular matrix promotes the differentiation of other stem cells into chondrogenic cells [64, 65].

The above application attempts are all based on the fact that various adult stem cell types have shown similarities in the context of tissue engineering and regenerative medicine. Therefore, it will be interesting to explore the feasibility of using USCs as substitutes for MSCs derived from other tissues in various situations. Since 'MSC' is a joint name, it is unreasonable to compare USCs with 'MSCs' unless a specific origin is defined. Of course, one may expect to find different features in USCs and 'MSCs', and current studies have indeed shown distinct properties of USCs in terms of proliferation, colony-formation and differentiation [66–69]. It is worth noting that most such differences are seen in *in vitro* results. A possible important mechanism *in vivo* is that USCs contain different secretomes [32, 65, 70] which can activate various downstream pathways. As mentioned above, the latest understanding is that differentiation ability is probably not the decisive factor, while the significant advantage of non-invasive acquirement has made USCs a very attractive cell type. It is possible that there will be tradeoffs between acquirement, efficacy and cost when multiple adult stem cell types are considered for particular situations.

In the following sections, the application of USCs in skin, bone and articular cartilage repair are discussed in detail, and the main studies in these fields are summarized in Table 2.

## Applications of USCs in skin repair

**Skin injuries healing** The skin consists of three main layers (epidermis, dermis and hypodermis) containing various appendages, e.g. hair, sweat glands and sebaceous glands. As the largest organ of the human body, it has multiple functions of great importance including protection against foreign pathogens, regulation of body temperature, prevention of dehydration and sensation, as well as production and activation of hormones, neuropeptides and cytokines [71, 72]. The common skin injuries include surgical incisions, burns and chronic ulcers, while wound healing is divided into four stages: hemostasis, inflammation, proliferation and remodeling [73, 74]. Angiogenesis of endothelial cells and collagen deposition of fibroblasts play important roles in the repair of skin defects. For injury healing, the endogenous stem cells of the skin can self-renew remarkably and produce daughter cells capable of differentiation into the relevant cell lineages that participate in the natural cutaneous wound healing process. However, when it comes to serious situations such as severe burn or diabetes mellitus, the repair process may be insufficient to achieve a satisfactory result. Usually, epidermal appendages are lost and scars are generated which are neither functional nor aesthetical. By contrast, exogenous stem cells under such conditions may result in better therapeutic outcomes. Many types of adult stem cells have already been tested for skin repair and regeneration in various acute and chronic skin injuries, including bone marrow-derived MSCs [75–78], adipose-derived stem cells [75], umbilical cord-derived MSCs [78] and placenta-derived stem cells [79].

**The application of USCs** Several application strategies of USCs have been shown to be effective in full-thickness skin defect repair. Zhang *et al*. used the bioactivity of bioglass to enhance the skin defect repair ability of USCs by promoting the paracrine effect [80], which showed that pretreatment of USCs can improve their therapeutic efficacy. The use of USCs in conjunction with membrane materials has also shown therapeutic effectiveness. Fu *et al*. demonstrated that USCs seeded on polycaprolactone/gelatin nanofibrous membranes could enhance skin defect repair by promoting angiogenesis in the

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**Table 2.** Applications of USCs in skin, bone and articular cartilage repair

| Applied tissue | Animal model | Defect | Dosage | Materials | Pretreatment | Outcome | Possible repair mechanisms | Ref. |
|---|---|---|---|---|---|---|---|---|
| Skin | New Zealand white rabbit | Full-thickness skin defects, 2 cm × 2 cm | $2 \times 10^4$ USCs/24-well plate-sized membranes | Polycaprolactone/gelatin nanofibrous (PCL/GT) membranes | N/A | Faster wound closure, increased re-epithelialization, collagen formation, and angiogenesis | USCs secrete VEGF and TGF-$\beta$1 promoting angiogenesis | [47] |
| Skin | BALB/C nude mouse | Full-thickness skin defect, 10 mm in diameter | $1 \times 10^6$ USCs/100 $\mu$L PBS (subcutaneous injection) | N/A | Treated by diluted bioglass ionic (BG) extracts | Better wound healing ability, improved angiogenesis, more collagen deposition, and the collagen structure is closer to that in the mouse | BG ionic extracts activate the paracrine effects (VEGF-KDR) between USCs and recipient cells (endothelial cells and fibroblasts) in wound healing | [80] |
| Skin | Streptozotocin-induced diabetic C57BL/6 mouse | Full-thickness skin defect, 6 mm in diameter | 200 $\mu$g USCs-Exos/100 $\mu$L PBS (intraperitoneal injection) | N/A | N/A | Accelerated wound healing, higher rates of re-epithelialization, more collagen deposition, improved cell (keratinocytes, fibroblasts and vascular endothelial) proliferation, less scar formation and improved angiogenesis | Exosomes (Exos) from USCs could effectively enhance the proliferation, migration and tube formation of vascular endothelial, promoting angiogenesis via transferring DMBT1 protein | [61] |
| Skin | Sprague–Dawley rats | Full-thickness skin defect, 2 cm in diameter | $5 \times 10^3$ USCs/96-well plate-sized membranes | Surface-structured bacterial cellulose nanofiber (S-BC) membranes | N/A | Accelerated wound healing, faster re-epithelialization, more collagen production and neovascularization | The substance secreted from USCs and the effect of S-BC on the adhesion and proliferation of vascular endothelial cells promote angiogenesis | [81] |
| Skin | BALB/C nude mouse | Full-thickness skin defect, 8 mm in diameter | $1 \times 10^6$ USCs/SIS membrane (10 mm in diameter) | Porcine small intestine submucosa (SIS) | The composites were pretreated with hypoxia (1% $O_2$) for 24 h | Accelerated neovascularization, facilitated re-epithelialization, promoted skin appendage regeneration, improved the quality of collagen deposition and enhanced the wound healing | Hypoxic preconditioning enhanced composites secreting a large amount of growth factors (VEGF, EGF and bFGF) for enhancing wound angiogenesis at the early stage of wound healing | [82] |
| Bone | N/A | N/A | USCs | N/A | Fresh medium containing 4 $\mu$g/mL silver nanoparticles (AgNPs) treated for 24 h | Promoted osteogenic differentiation of USCs | The AgNPs themselves, rather than the released silver ions, lead USCs into osteogenic differentiation via activating RhoA, inducing actin polymerization and increasing cytoskeletal tension | [98] |
| Bone | Nude mouse | Ectopic bone formation (muscle pockets in hindlimbs) | $5 \times 10^5$ USCs/scaffold ($5 \times 5 \times 3$ mm) | Poly (lactic-co-glycolic acid)/calcium silicate composite (PLGA/CS) porous scaffold | One week culture *in vitro* | Induced osteogenic differentiation, ingrowth of blood vessels into scaffolds | CS induces the osteogenic differentiation of USCs through the Wnt/$\beta$-catenin signaling pathway | [44] |
| Bone | Sprague–Dawley rats | 6 mm critically sized femoral defect | $5 \times 10^5$ USCs/scaffold ($5 \times 5 \times 6$ mm) | $\beta$-TCP porous scaffold | Composites cultured in osteogenic differentiation media for 7 days | Increased new osseous formation, 5 out of 11 transplants completely bridged the critical-size bone defect | USCs can adhere, proliferate and differentiate into osteoblasts on a $\beta$-TCP scaffold | [45] |
| Bone | Nude mouse | Ectopic bone formation (muscle pockets in hindlimbs) | USCs (concentration not mentioned) | Porous ceramic scaffold made of $\beta$-tricalcium phosphate ($\beta$ -TCP) | Lentiviral vectors-bone morphogenetic protein 2 (BMP2) gene transduction | Increased osteogenic activity of USCs, these transfected cells can undergo osteogenic differentiation without osteogenic medium *in vitro*, observed ectopic bone formation, USCs differentiate into osteoblasts | BMP2 gene transduction | [99] |

*(Continued)*



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**Table 2.** Continued

| Applied tissue | Animal model | Defect | Dosage | Materials | Pretreatment | Outcome | Possible repair mechanisms | Ref. |
|---|---|---|---|---|---|---|---|---|
| Bone | New Zealand white rabbit | Critical-sized segmental bone defects model (the ulna bone together with the periosteum) | $6 \times 10^5$ USCs/scaffold ($\Phi$ 5 × 5 mm) | Surface mineralized biphasic calcium phosphate (BCPs) ceramics scaffold | The composites were cultured in osteogenic differentiation media for 7 days | Promoted the formation of new bone and accelerated the maturation of new bone in ulna defects | Scaffold provided a favorable microenvironment that enabled USCs to adhere and proliferate, early (ALP, BMP2, and RUNX2) and late (OCN) osteogenic gene marker were continuously and significantly upregulated | [101] |
| Bone | Sprague–Dawley rats | Skull defects | $1 \times 10^5$ USCs/hydrogel (5 mm in diameter) | Methacrylated solubilized decellularized cartilage (MeSDCC) hydrogel | USCs were infected with $10^{-6}$ mol/L BMP2 for 21 days | Increased bone formation, larger bone area | FAK plays a key role in regulating BMP2 enhanced osteogenic differentiation of USCs, the underlying mechanism might be the activation of AMPK and Wnt signaling pathways | [100] |
| Bone | Sprague–Dawley rat | Glucocorticoid-induced osteonecrosis of the femoral head | 500 $\mu$g USCs-EVs/200 $\mu$L PBS (tail intervenous injection) | N/A | N/A | Prevention of early stage osteonecrosis, rescued angiogenesis impairment, reduced apoptosis of cells, prevented trabecular bone destruction and improved bone microarchitecture | TIMP1 and DMBT1, respectively, partly mediate the anti-apoptotic and pro-angiogenic effects of extracellular vesicles from USCs (USCs-EVs) | [43] |
| Articular cartilage | N/A | N/A | BMSCs | N/A | Seeded on USCs-ECM for one passage | ECM deposited by USCs (USCs-ECM) could recharge senescent BMSCs toward chondrogenic differentiation | The Wnt11-mediated noncanonical signaling pathway might be responsible for USCs-ECM mediated BMSCs rejuvenation in terms of chondrogenic potential | [64] |
| Articular cartilage | New Zealand white rabbit | Knee-joint cartilage defect model, 5 mm in diameter | $1 \times 10^7$ USCs/1 mL HA (injection into cartilage-defect knee joints) | 1% Hyaluronic acid solution (HA) | N/A | More neocartilage formation that matures over time, showed the expression of collagen type II and synthesized proteoglycans | USCs are able to differentiate into chondrocytes with characteristic deposition of aggrecan and collagen II | [46] |
| Articular cartilage | N/A | N/A | $1 \times 10^3$ SDSCs/cm$^2$ | UECM | N/A | Promoted proliferation and chondrogenic potential of SDSCs | Biophysical and biochemical cues (UECM is softer than others and contains different growth factors and collagen) | [65] |

*N/A* not applicable, *BMSC* bone marrow stromal cell, *SDSC* synovium-derived stem cells, *β-TCP* β-tricalcium phosphate, *UECM* ECM deposited by USCs, *USCs* urine-derived stem cells
$\Phi$ The diameter and height of 5 mm



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wound [47]. Cao *et al*. also reported that surface-structured bacterial cellulose loaded with the USCs could accelerate skin wound healing by promoting angiogenesis [81]. One of our recent studies showed that USCs-seeded porcine small intestine submucosa (USCs-SIS) biomaterial accelerated full-thickness skin defect repair in a streptozotocin-diabetic rat model. Moreover, such biomaterial promoted the regeneration of skin appendages at the center of the wound. Notably, further decellularization of the USCs-SIS did not significantly compromise the effectiveness of repair in the same *in vivo* model, which highlighted a greater application potential of such re-decellularized biomaterial as an off-the-shelf product for acute skin injuries (Wang Z. L. *et al*., in preparation). In another of our recent studies, a tissue-engineered skin patch that consisted of porcine SIS and hypoxic pretreated USCs could accelerate full-thickness skin defect repair and promote skin appendage regeneration in a nude mouse model [82]. Chen *et al*. also showed that the exosomes released by USCs could accelerate full-thickness skin defect healing in a streptozotocin-induced diabetic mouse model, and exosomal DMBT1 from USCs may play a crucial role through promoting angiogenic responses [61]. Taken together, it seems that USCs exert bioactivity in wound healing by paracrine effects and exosomes. Of note, membrane materials seem to be beneficial for the adhesion and proliferation of USCs, and pretreatment can enhance the effect of USCs. Moreover, combining the proper material forms (e.g. membrane-shaped materials) with certain pretreatments may create a synergistic effect. An effective application mode of USCs for skin defect repair is shown in Figure 1. However, other mechanisms may also be involved, and more experimental evidence is needed.

## Applications of USCs in bone repair

**Osteanagenesis** Bone has an irregular and anisotropic hierarchical structure. Repeating osteon units of collagen fibers and calcium phosphate crystals make up the outer cortical bone, and an interconnecting framework of trabeculae surrounding a marrow space forms the inner cancellous bone [83]. Bone serves many key functions, such as load-bearing, movement, hematopoiesis, calcium homeostasis, acid/base buffering and cytokine storage [83, 84]. It also has high inherent regeneration capacity. However, nonunion and scar tissue formation can happen due to insufficient spontaneous healing under the situation of large bone defects that usually result from complex trauma, tumor resection and other diseases, i.e. critical-size defects (CSDs) which are defined as a deficiency of a length exceeding 2–2.5 times the diameter [85]. In such cases, surgical reconstruction with allogeneic or synthetic bone grafts is required. Although the gold standard for reconstructing large skeletal defects remains the transplantation of autogenous bone, the drawbacks are apparent, such as limited supply and secondary injuries. Instead, a promising alternative is tissue-engineered bone grafts.

For tissue-engineered bone, the essential scaffold material should be biocompatible, osteoconductive, osteoinductive, osteogenic, resorbable or degradable, and it should have proper mechanical properties [86]. Furthermore, different material properties can affect the behavior of the cells seeded onto the scaffold [87–89]. Meanwhile, much attention should be paid to the seeded cells themselves. Autologous osteoblasts are a choice, but their availability is hampered by prolonged timespan, limited source and sometimes bone-related disease [90]. In general, stem cells can be expanded remarkably, although adult stem cells have more advantages than embryonic stem cells with regard to ethics and safety concerns. As an adult stem cell type and derived from bone marrow, bone marrow-derived MSCs (BMSCs) are considered for bone tissue engineering. Bruder *et al*. [91, 92] provided the first proof for the possible application of BMSCs for the reconstruction of long segmental defects in larger animals. One of our previous studies showed that the BMSCs accelerated the repair of a tissue-engineered bone constructed in a rhesus monkey model [93]. And one our previous clinical case with 12-year follow-up of tissue-engineered ribs for chest wall reconstruction demonstrated the feasibility of BMSCs-seeded tissue-engineered bone construct for promoting functional bone regeneration in humans [94]. In addition to BMSCs, other adult stem cells such as adipose-derived stem cells [95], umbilical cord MSCs [96] and dental-derived MSCs have also been applied in bone tissue engineering [97]. The use of USCs has also been reported in bone tissue engineering.

**The application of USCs** Although Wu *et al*. have shown that USCs have inferior osteogenic differentiation capability [66], the abundant source and non-invasive acquisition of the USCs have endowed them with great potential for bone injury repair. Many studies have been conducted to enhance the osteogenic properties of USCs. Qin *et al*. showed that silver nanoparticles could enhance the osteogenic differentiation of USCs by activating RhoA, inducing actin polymerization and increasing cytoskeletal tension [98]. Guan *et al*. showed that *BMP2* gene transduction could enhance the osteogenic potential of USCs [99]. Sun *et al*. showed that FAK could regulate BMP2-induced osteogenic differentiation of USCs *in vitro* and *in vivo*, and the activation of AMPK and Wnt signaling pathways might be responsible [100]. Furthermore, scaffolds with proper mechanical properties may be beneficial for osteogenesis. Guan *et al*. have successfully repaired a segmental femoral defect in a rat model by combining USCs with $\beta$-tricalcium phosphate scaffold [45]. They also used calcium silicate (CS) to induce the osteogenic differentiation of USCs, and the Wnt/$\beta$-catenin pathway is involved in the process. In *in vivo* implantation, the USCs-seeded poly(lactic-co-glycolic acid)/CS scaffold had high expression of osteocalcin [44]. Xing *et al*. used surface mineralized biphasic calcium phosphate ceramics seeded with USCs to repair segmental bone defects in a rabbit model, and showed that USCs/scaffold composites could promote the formation and maturation of new bone in ulna defects by providing a favorable microenvironment [101]. Moreover, the extracellular vesicles secreted by USCs have a certain

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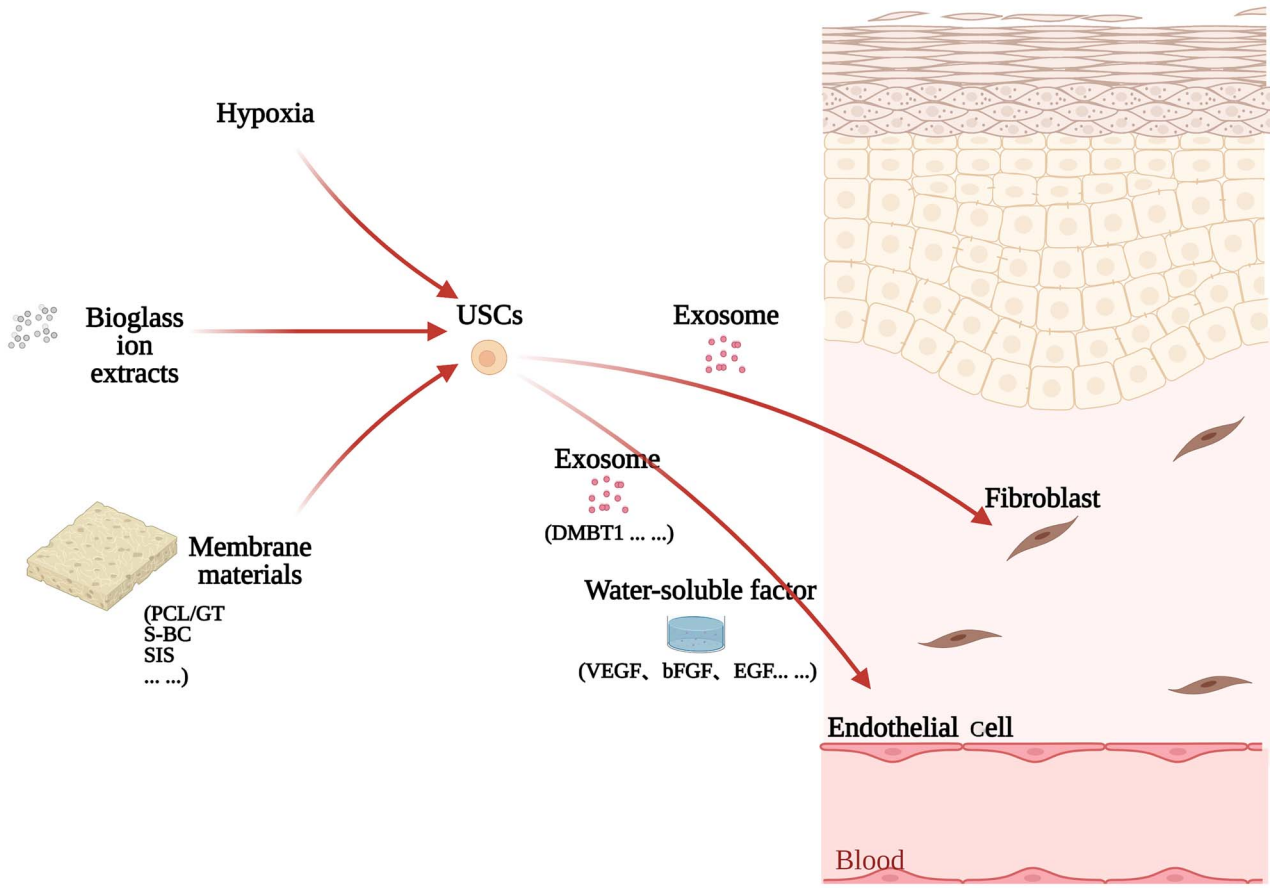



**Figure 1.** An effective application mode of USCs in skin defect repair. Pretreatment can improve the performance of USCs. The secretions of USCs have therapeutic effects. USCs cultured with the membrane materials may show a synergistic effect. A variety of cellular strategies may impact on bioactivities through different pathways, and ultimately act on the effector cells to repair the skin defect. *PCL/GT* polycaprolactone/gelatin, *S-BC* surface-structured bacterial cellulose, *SIS* porcine small intestine submucosa

effect of preventing osteonecrosis. Chen *et al*. showed that the extracellular vesicles secreted by USCs prevented early-stage glucocorticoid-induced osteonecrosis in a rat model, and the underlying mechanism could be the delivery of pro-angiogenic DMBT1 and anti-apoptotic TIMP1 [43]. Altogether, researchers have paid extensive attention to the effective osteogenic differentiation of USCs that already have great advantages as seed cells. Various strategies including treatment with nanoparticles, ion extracts of CS powder or BMP2 protein, as well as gene transduction (such as *BMP2*) can improve the osteogenic differentiation of USCs. Besides, scaffolds with proper mechanical properties are crucial for tissue-engineered bones. Furthermore, the extracellular vesicles of USCs may also be applied in bone injuries. The current effective application mode of USCs in bone defect repair is shown in Figure 2.

## Applications of USCs in articular cartilage repair

**Articular cartilage defect repair** Cartilage consists of chondrocytes and the surrounding extracellular matrix. Chondrocytes embedded in articular cartilage receive nutrition diffused through the matrix. According to matrix composition, cartilages can be classified into three types (elastic cartilage, fibrocartilage and hyaline cartilage), and each of them makes up different cartilage tissues. Elastic cartilage is commonly seen in auricula and fibrocartilage in the intervertebral disk. Articular cartilage is hyaline cartilage which is aneural and avascular. The articular cartilage serves several critical functions for body movement, including providing a low-friction gliding surface, acting as a shock absorber and minimizing peak pressures on the subchondral bone. Articular cartilage has a very limited healing capacity, while damage from trauma or degeneration often results in gradual tissue deterioration, leading to debilitating joint pain, functional impairment and degenerative arthritis. The current treatment of articular cartilage defects includes total joint replacement for end-stage degenerative joint pathology, bone-marrow stimulating techniques and mosaicplasty for early lesions [102, 103]. To attain long-term clinical outcomes, cell-based strategies have been proposed, which hold promise for stimulating the regeneration of cartilage. Autologous chondrocytes are naturally considered for cell-based cartilage therapies. Indeed, there are commercially available products such as ChondroCelect and Carticel [104]. However, the challenge of obtaining high cell density and maintaining the differentiation state

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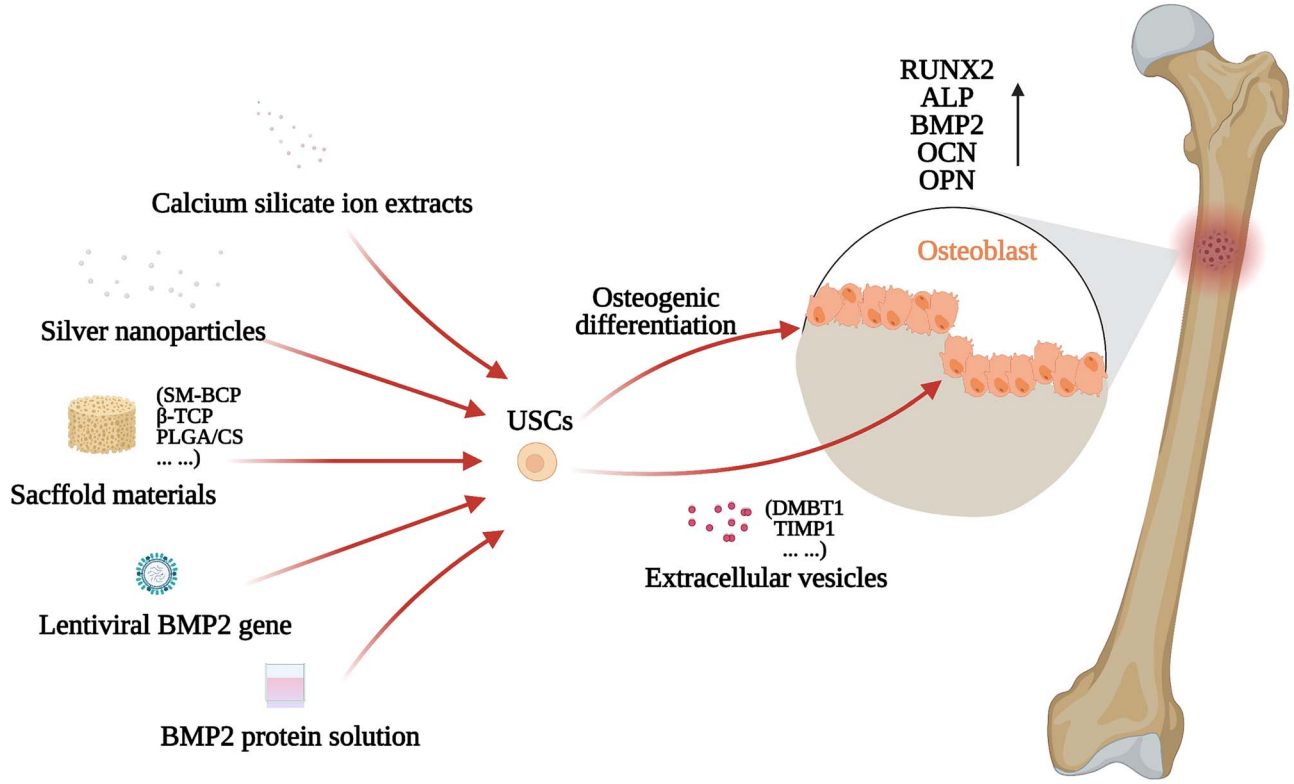



**Figure 2.** The current application mode of USCs in bone injuries healing. Pretreatment and transduction could improve the performance of USCs. The extracellular vesicles secreted by USCs have a therapeutic effect. USCs cultured with the scaffold materials may show a synergistic effect. A variety of cellular strategies may exert bioactivities through different pathways, and ultimately act on effector cells to repair the bone injury. *SM-BCP* surface mineralized biphasic calcium phosphate, *β-TCP* β-Tricalcium phosphate, *PLGA/CS* poly(lactic-co-glycolic acid)/calcium silicate composite

of the cells have prompted the quest for other cell sources. Likewise, MSCs are considered as alternatives for cell-based therapies in cartilage damage [105]. An important characteristic of MSCs is their chondrogenesis potential. The use of MSC-derived chondrocytes has been widely reported [106]. As discussed above, exogenous stem cells may participate in tissue repair in different ways. Usage of the MSCs *per se* has also been widely reported [107]. USCs have also been used for the repair of the cartilage defects.

**The application of USCs** Chen *et al*. made a compound by combining hyaluronic acid (HA) with USCs, and they injected the compound into the knee joint with a cartilage defect in a rabbit model. The result showed that the compound stimulated more neocartilage formation compared with single USCs, HA or normal saline [46]. Currently, USCs are mainly reported to be chondrogenic [17, 46, 47], but some are also reported to be non-chondrogenic [64]. This may be due to the difference in origin, status and culturing process of the cells. Our previous study also showed that different populations of USCs have different chondrogenic abilities [16]. Although some research showed that the USCs are non-chondrogenic, the extracellular matrix (ECM) deposited by the USCs could promote the chondrogenic capacity of the bone marrow stromal cells [64] and synovium-derived stem cells [65], indicating that the non-chondrogenic USCs can still participate in cartilage repair indirectly. Besides, Pei *et al*. showed that the supernatant of USCs contained an abundance of 31 human cytokines [64]. Li *et al*. showed that the USCs-derived ECM (USCs-ECM) is softer than the ECM deposited by adipose-derived stem cells, synovium-derived stem cells or dermal fibroblasts. Also, USCs-ECM contains different collagen (COL4A1, COL4A2) and growth factors (LOXL2, TGM2, PXDN) [65]. This may explain why USCs can promote chondrogenic differentiation of other stem cells. Taken together, USCs may be directly or indirectly involved in cartilage repair, and it may be beneficial to use a mixture of injectable materials with USCs. Furthermore, biophysical and biochemical cues may also contribute to the signals for the proliferation and chondrogenic differentiation of stem cells. As for the effectiveness of extracellular vesicles derived from USCs for cartilage regeneration, more research is still required. The current effective application mode of USCs is shown in Figure 3.

## Perspectives

**Basic biological characteristics of USCs** Some fundamental biological issues, such as immunoregulatory activities and carcinogenesis risks, have not been fully explored for the understanding of the effectiveness and safety of USCs. Our recent study showed that USCs could not stimulate the proliferation of allogeneic peripheral blood mononuclear cells (PBMCs)

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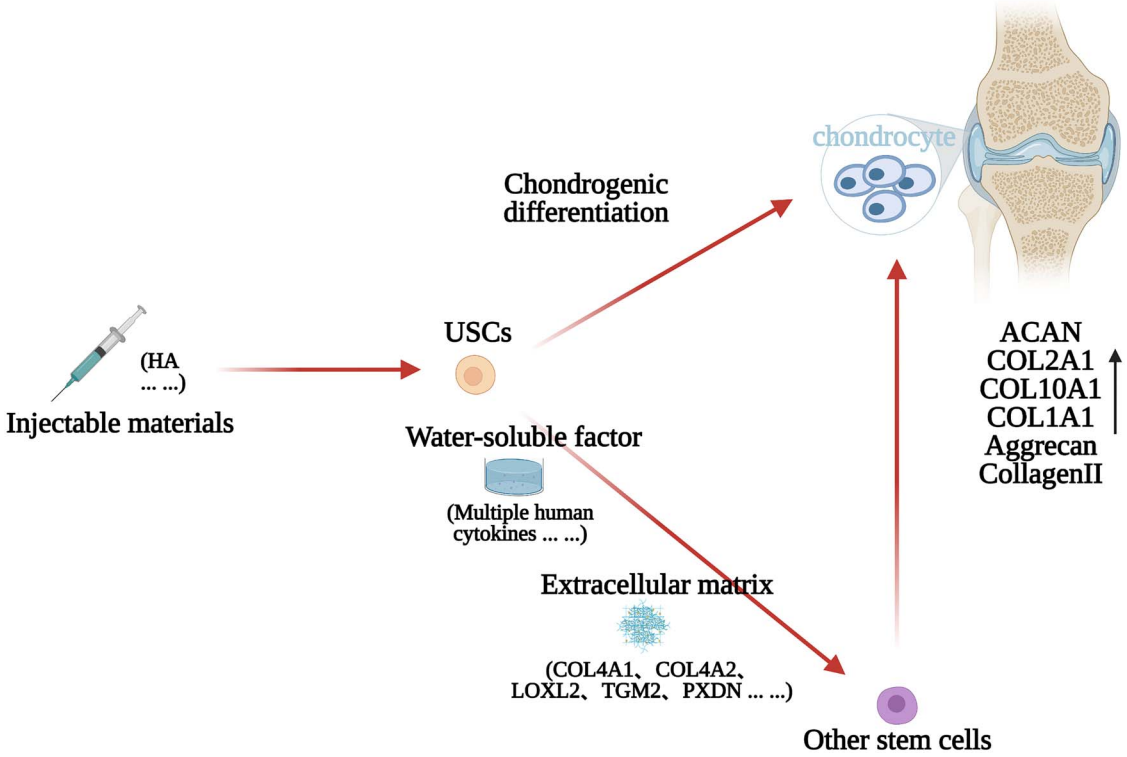



**Figure 3.** The current effective application mode of USCs in articular cartilage injuries healing. The secretions of USCs have therapeutic effects. USCs cultured with injectable materials may show a synergistic effect. A variety of cellular strategies may exert bioactivities through different pathways, and ultimately act on the effector cells to repair the articular cartilage injury. *HA* hyaluronic acid, *USCs* urine-derived stem cells

but suppressed the phytohemagglutinin-induced activation of PBMCs, which demonstrated the low immunogenicity and moderate immunoregulatory activity of USCs (Gao *et al*., unpublished results). As the immunomodulatory effects are probably of great significance, such effects of USCs need to be addressed [41]. In addition, our previous study showed that subpopulations of the USCs have different characteristics [16]. Delineation of the unique features of USCs subpopulations is required to provide evidence for USC-based therapy. Meanwhile, basic research on the source of USCs is of significance, as such research may give some answers as to what kinds of people are suitable for supplying USCs, how to obtain USCs properly and whether there are some specific markers of USCs. We believe that single-cell sequencing combined with lineage tracing may offer some hints.

**Flow-line production of USCs** A flow-line production and application patterns of USCs are summarized in Figure 4. Production of clinical-grade stem cells should follow strict good manufacturing practice (GMP) of medical products guidelines. The safety and effectiveness of the USCs need to be monitored. In general, the production of USCs involves donor selection, cells harvesting method, medium formulation, cells amplification method, quality control criteria and so on. First, donors' conditions such as age, disease and medication may influence the biological characteristics of the USCs obtained. Gao *et al*. found that the USCs from younger donors showed higher proliferation ability, less senescence and stronger osteogenic differentiation capacity, although USCs from all ages have shown potential for bone regeneration [108]. Schosserer *et al*. have noted a higher rate of success for isolating USCs from male donors compared with females (70 vs. 42%) [109]. Considering the difference between male and female genitals, attention should be paid to preventing the risk of contamination when collecting urine samples from females to avoid the period of menstruation and the first micturition of the day [48], and to cleaning the pudendum and the labia with pre-moistened wipes [48] and moist anti-bacterial toilettes [110]. Second, the method of cell harvesting can also influence the final therapeutic outcome [103], and it is necessary to optimize the culture protocol of the USCs [111]. Third, as medium formulation varies with laboratory, the differences mainly being related to the serum concentration and nutrient factor types, for instance, the presence or absence of adenine, epinephrine, or hydrocortisone [47, 61, 82], the necessary ingredients need to be determined.

Clinical applications will usually require lots of cells, therefore, fast and vast proliferation of USCs for extensive usage have posed a great challenge. Microcarrier-based suspension culture may provide a solution [112]. However, whether USCs cultured by this method remain the same or whether the differences between USCs cultured by different methods lead to different therapeutic outcomes is still unknown. Another solution that faces the same dilemma

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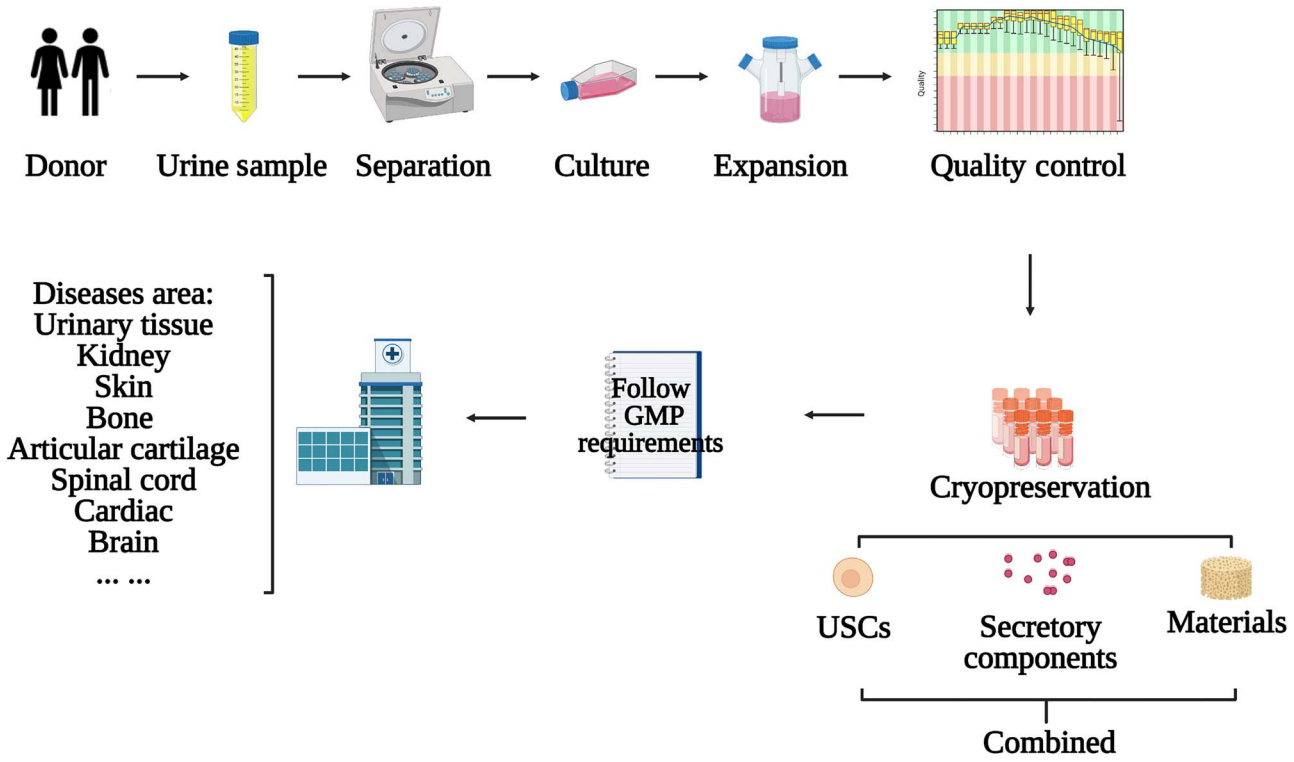



**Figure 4.** Workflow for application of USCs. USCs are obtained by centrifuging urine collected from donors, cultured in the appropriate medium, and then expanded into a large number by using the appropriate method. USCs or their secretory components can be used directly or coupled with the materials according to the target tissue types/diseases. *GMP* good manufacturing practice, USCs urine-derived stem cells

is reconstituting the culture conditions of the USCs by adding certain nutritional supplements, seeding the USCs on other extracellular matrix components, and taking oxygen tension into account to improve the isolation and proliferation of the USCs [67, 113]. Considering batch-to-batch variation, the quality control of the USCs is of great significance. To meet the quality control criteria is a prerequisite for USCs-based cytotherapies and may involve an enormous amount of work. RNA detection of selected gene products, expression analysis of functionally relevant cell surface markers and protein detection of the secretome are suggested as an assay matrix [114].

**Application modes of USCs** When applying USCs in regenerative medicine, the scaffold materials should be considered simultaneously with the cell–scaffold complex depending on the clinical needs and the mechanisms by which stem cells may exert biological functions. For example, the materials used for skin defects should mimic the structure and biological function of the dermis [115]. Tan *et al*. showed that a hydrogel derived from acellular porcine adipose tissue could induce the regeneration of intradermal adipocytes and thereby accelerate wound healing in a nude mice model [116]. In addition to the materials, the strategies of cell manipulation may also vary with application situations. For example, the stem cells used in skin repair may be exempt from further induction, while osteoinduction of stem cells is usually included in bone repair. Moreover, *in vitro* pre-differentiation or *in vivo* differentiation of stem cells based on a controlled release system containing a cocktail of growth factors showed different effects [117]. Indeed, how USCs may participate in the repair process in various tissues remains to be clarified. Accordingly, whether cell induction or other manipulations are necessary remains uncertain. Furthermore, the application of USC secretions such as the extracellular vesicles, exosomes and extracellular matrix, may circumvent the potential risk of using the USCs themselves for treating the disease. It will also take a considerable amount of time to generate enough cells for autologous application. Therefore, it is more practical to apply USCs for treating chronic wound and elective surgical procedures but not acute burns, unless allogeneic applications of USCs prove to be safe, effective and cost-effective. For their non-invasive acquisition, low cost and tremendous application potential, USCs deserve more research and hold great promise for a broader range of applications.

## Conclusions

The demands for wound healing, defect repair and tissue regeneration are ever-growing. Over the last decades, stem cells have shown great potential for regenerative medicine. As an emerging type of adult stem cell featuring non-invasive acquisition, USCs have been successfully applied for cytotherapies and tissue engineering in various disease models. With a

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few reports on skin, bone and articular cartilage repair, USCs have shown their effectiveness already. However, research on USCs is at its infancy stage, and more investigations are still required to answer the basic questions with regard to their origin, immunoregulatory activities and difference between their subpopulations, as well as optimization of their translational issues such as mode of application.

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## Authors' contributions

All authors read and approved the final manuscript. WQZ and JGH contributed equally to the review, and should be viewed as co-first authors.

## Conflicts of interest

None declared.

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