Innovations in Stem Cell Therapy for Diabetic Wound Healing

Jasmine Ho[1,4], Dominic Yue[2], Umber Cheema[1], Henry Hsia[3], Alan Dardik[4]

[1] UCL Centre for 3D Models of Health and Disease, Division of Surgery & Interventional Science, Faculty of Medical Sciences, University College London, Gower Street, London, WC1E 6BT, UK

[2] Plastic Surgery Unit, Chelsea and Westminster Hospital NHS Foundation Trust, 369 Fulham Road, London, SW10 9NH, UK

[3] Division of Plastic Surgery, Department of Surgery, Yale School of Medicine, New Haven, CT 06510, USA

[4] Vascular Biology and Therapeutics Program and the Department of Surgery, Yale School of Medicine, New Haven, CT 06510, USA

Corresponding author: alan.dardik@yale.edu

Key words: Stem & Progenitor Cells; Chronic wounds; Foot Ulcers; Tissue Engineering; Translational Research

Word count: 6598

Abstract

Significance: The global burden of diabetic wounds, particularly diabetic foot ulcers, continues to have large economic and social impact throughout the world. Current strategies are not sufficient to overcome this burden of disease. Finding newer, more advanced regenerative cell and tissue-based strategies to reduce morbidity remains paramount.

Recent Advances: Recent advances in stem cell therapies are discussed. We also highlight the practical issues of translating these advancing technologies into the clinical setting.

Critical Issues: We discuss the use of somatic and induced pluripotent stem cells and the stromal vascular fraction, as well as innovations including the use of 3D bioprinting of skin. We also explore related issues of using regenerative techniques in clinical practice, including the current regulatory landscape and translatability of *in vivo* research.

Future Directions: Advances in stem cell manipulation showcase the best therapeutic resources available to enhance mechanisms of wound healing such as angiogenesis, cell proliferation, and collagen synthesis; potential methods include changing the scaffold microenvironment including relative oxygen tension, and the use of gene modification and nanotechnology. Secretome engineering, particularly the use of extracellular vesicles may be another potential cell-derived therapeutic that may enable use of cell-free translational therapy.

Table of Contents

Deleted:

Deleted:

Deleted:

Deleted:

Deleted:

Deleted:

1.0 Scope and Significance

Diabetes is a highly prevalent metabolic disorder with profound systemic effects; the combination of neuropathy, micro- and macro-vascular disease and poor immune response renders poor wound healing in these patients. These comorbidities prolong inflammation and delay wound closure, leading to increased risk of infection. Difficulty in healing is frequently compounded by tissue ischemia or continual pressure on the site, especially in the foot due to repetitive trauma and poor blood supply in the extremities. If not treated adequately, the natural history leads to amputation.

1.1 Global Burden of Disease

The global economic burden of diabetes is estimated to be a staggering US$760 billion and expected to increase to US$825 billion by 2030.[1] Treatment of diabetic foot ulcers (DFU) cost ten times more compared to non-DFU and has been estimated to be US$9.1 billion annually in the USA alone.[2] Most notably, the direct cost and five year mortality rates of DFU are now comparable to that of cancer.[3] Thus, more innovative therapies to improve diabetic wound healing is critical to reduce the impact of this disease on the patient and society.

2.0 Translational Relevance

Cell-based therapy has gained popularity in recent years due to increasing evidence showing benefit for tissue regeneration and healing. Stem cells have regenerative potential due to their multipotency and various positive paracrine and chemokine effects, both systemically as well as locally. As science advances, various innovative methods of investigating and translating stem cell therapy to wound healing have been developed.

3.0 Clinical Relevance

3.1 Wound Healing Stages

Normal wound healing proceeds through three stages: inflammation, proliferation and remodelling. However, these stages are dysregulated in diabetic wounds due to high systemic plasma glucose, poor immune function confounded by peripheral neuropathy and vascular disease.[4] During each stage, the underlying pathophysiology of the systemic disease further complicates the healing process in multiple ways including:

i. **Inflammation:** glycated extracellular matrix (ECM), neuropathy, poor vascular supply, poor immune response at the wound site, lack of cellular infiltrates to help fight infection, exacerbation of biofilm effects leading to resistance to topical antimicrobial treatment.
ii. **Proliferation:** reduced or lack of cellular infiltrates, platelet aggregation, and matrix formation, leads to poor tissue granulation.
iii. **Remodelling:** Wound site unable to mature and remodel after initial healing due to the above processes leads to wound recurrence and deterioration with potential further involvement of underlying deep structures such as muscle and bone.

3.2 Clinical Need for Advancement

Current treatment strategies are mainly focused on direct wound management via antimicrobial treatment, topical dressings, and pressure-relieving support to promote healing and prevent wound deterioration (**Figure 1**). More advanced wound care technologies help with delaying disease progression and prevention of disability. However, these have only moderate impact to alleviate the burden of disease from these chronic non-healing wounds, with approximately 50% of wounds not responding to current treatment strategies. [4] A systematic review of randomised controlled trials of various wound healing strategies showed that bilayer tissue engineered skin significantly enhanced the healing of chronic wounds compared to simple dressings.[5] This data confirms the need to invest more in research to advance regenerative cell therapy and tissue engineering for chronic non-healing DFU.

4.0 Discussion: Recent advances in stem cell therapy for diabetic wounds

4.1 Advances in stem cell types

4.1.1 Somatic (Adult) Stem Cells

Mesenchymal stem cells (MSC) are the most popular form of somatic stem cells used for therapeutic purposes. MSC are multipotent with self-renewal capacity, and they are found in various organs such as bone marrow, adipose tissue, umbilical cord, placenta and peripheral blood.[6,7] Under the right conditions, MSC differentiate into osteoblasts, chondroblasts, and adipocytes;[8] MSC also differentiate into other cell types including endothelial cells, keratinocytes, and skin appendage cells in an pre-clinical setting.[9,10] More importantly, MSC are trophic factories, producing a wide variety of cytokines and growth factors, such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), angiopoietin-1 (Ang-1), and stromal derived factor-1 (SDF-1; **Figure 2**).[11] All these factors contribute to the various stages of healing, and some of these factors also have immunomodulatory functions, such as increased anti-inflammatory interleukin-10 (IL-10) and IL-4, and decreased tumor necrosis factor- α (TNF-α) and interferon-γ (IFN-γ).[12] These factors also exert antimicrobial effects via peptide cathelicidin LL-37, which are highly beneficial to diabetic wounds prone to infections.[13] As such, MSC are a viable adjunct to tissue engineered constructs and skin regeneration cell therapies; rather than replacing the host cells, MSC can be used to directly affect healing or facilitate regeneration simply by being transplanted into the vicinity of the wound.[14,15] Hence, the functional benefit of adding MSC or MSC-conditioned media directly to a tissue engineered construct has been an ongoing research topic.[16] (see section 4.3)

MSC exhibit immunoprivileged properties, and accordingly show excellent safety for allogeneic transplantation in multiple human clinical trials. A meta-analysis and systematic review of randomised clinical trials showed that autologous stem cell administration to any ulcer size for all ages of patient groups was significantly favourable to heal diabetic ulcers.[17] Bone marrow derived mesenchymal stem cells (BM-MSC) remain the preferred cell type in both pre-clinical and clinical studies of diabetic wounds.[18] This may be due to historical usage and well-published experimental data on the clinical effects and safety of translational use. However, isolation of primary BM-MSC is very invasive and other alternative, more accessible sources, such as fat and placental tissue/umbilical cord are being sought. Adipose-derived MSC (AD-MSC) show similar enhanced wound healing potential compared to BM-MSC, regardless of whether they are taken from a diabetic or non-diabetic host.[19] MSC are a

promising stem cell choice for translational purposes, allowing easily accessible allogenic and autologous cell therapy for diabetic wounds.

Interestingly, in a recent comprehensive analysis of the literature on stem cell therapy for DFU, there was more reported use of peripheral blood stem cells in clinical studies (n=11; 31%) compared to pre-clinical studies (n=2; 4%).[18] Such discrepancies show how the practical considerations for more easily available autologous adult stem cell sources, such as blood and fat, are more likely to be clinically useful. Thus it is not surprising to see a growing interest in the stromal vascular fraction (SVF), a recent addition to the adult stromal/stem cell family.[20] Derived from lipoaspirates, SVF contains a rich cocktail of heterogenous cellular extracts such as MSC, endothelial progenitor cells (EPC), T regulatory cells, macrophages, smooth muscle cells, pericytes and preadipocytes (**Figure 3**). The high concentrations of EPC and MSC gives the SVF a highly desirable number of diverse cell types conducive to regeneration via neoangiogenesis.[21] Han et al. showed 44% increased proliferation and 28% increased collagen synthesis with SVF compared to controls; the follow-up clinical pilot study also showed significant wound closure in patients treated with SVF seeded on a fibrinogen-thrombin carrier (100%) compared to controls (62%).[22] A more recent one year follow up clinical study showed that SVF can be safely used to accelerate wound healing, including neovascularization of the wound bed, and improved terminal vessel run-off in the majority of DFU.[23] These results were shown in a resource-poor clinical setting, highlighting one of the key benefits of using SVF, its ability to be used as a 'point-of-care' therapy as the cells are easily harvested and processed using devices that can be used within a surgical suite. The SVF is then available to be transplanted back into the patient in the same sitting, allowing for the use of a non-expanded autologous cellular product, avoiding the costly GMP laboratory processing that is a major limiting factor for current autologous-based cellular therapies. However, there are still barriers limiting clinical application of SVF, including lack of standardization of extraction protocols (especially between mechanically and enzymatically processed cells), problems with high cost of FDA-approved collagenase for enzymatic digestion, and possible risk of contamination during processing. Despite these barriers, the future of SVF use is promising given its unique potential as an intraoperative cell-based therapy. More robust and focused research on advancing the technology around processing and administering SVF will help both scientists and clinicians develop more confidence in its personalised therapeutic potential.

4.1.2 Induced Pluripotent Stem Cells

Although MSC are a promising source of cells for therapeutic purposes, they do not have unlimited proliferative ability, limiting their application in the field of regenerative medicine. Pluripotent stem cells are a valuable source of stem cells for regenerative purposes due to their proliferative capability and potential to differentiate into multiple cell lineages. Embryonic stem cells (ESC) have relatively poor translational potential due to ethical concerns around the source as well as risk of tumorigenicity. Induced pluripotent stem cells (iPSC) are engineered via the reprogramming of somatic cells into a pluripotent state. iPSC were first developed by Takahashi and Yamanaka in 2006 as the solution to the limited available pool of adult stem cells.[24] Adult somatic cells are reprogrammed into iPSC by inducing the expression of four transcription factors, typically either Oct4/Sox2/KLF4/c-Myc or Oct4/Sox2/NANOG/LIN28, via viral or non-viral delivery systems.[27-29] This negates any of the ethical issues faced by ESC. Perhaps the greatest benefit for iPSC is the unlimited source of differentiated cells that can be used for therapeutic applications to promote wound healing (**Figure 4**). Human iPSC (iPSC) can overcome many barriers associated with

autologous cell therapy. iPSC derived from the skin of healthy patients and those with recessive dystrophic epidermolysis bullosa can differentiate into functional dermal fibroblasts and keratinocytes that can be transplanted into 3D skin models for therapeutic uses. [28,29] Similarly, iPSC-derived fibroblasts from patients with DFU were functionally similar to iPSC derived from healthy patients and distinct from the original primary cells. When seeded onto 3D tissues, the iPSC-derived fibroblasts showed engraftment onto the wound and facilitated diabetic wound closure compared with primary DFU fibroblasts.[30] This data shows the potential of autologous cell sources for stem cell therapies to treat DFU.

Most recently, Gorecka et al. showed that human iPSC, derived from neonatal fibroblasts and then differentiated into smooth muscle cells (SMC), and delivered via a collagen matrix, accelerated wound healing and neoangiogenesis of the wound bed in a splinted diabetic murine model.[31] These cells were still present in the wound bed 7 days post-implantation, accelerated wound closure and secreted higher levels of pro-angiogenic cytokines compared to adipose-derived MSC (ADSC) controls. There was also associated immunomodulatory changes with the macrophage population in the wound area compared to ADSC and acellular scaffolds; there were decreased numbers of M1-type macrophages, associated with pro-inflammation, and increased numbers of M2-type macrophages, which are anti-inflammatory in nature and thought to be more pro-regenerative and hence more desirable for wound healing. This data was extended to show similar improved neovascularization with the use of human iPSC-SMC therapy in the murine ischemic hind limb model, adding to the increasingly promising therapeutic potential of iPSC.[32]

Since the dermal layer is a highly vascularised section of skin, it is no surprise that chronic diabetic wounds with poor blood supply do not heal properly with conventional treatment strategies. Hence, optimising neoangiogenesis to the wound site is another critically important therapeutic objective. Co-culture of mesenchymal-derived stem cells with endothelial or endothelial progenitor cells offers hope of an enhanced vascular connection by providing the necessary raw materials for a quick microvascular assembly. However, endothelial cells are highly immunogenic,[33] and thus it may not be possible to rely solely on allogenic sources for these cells. Using autologous iPSC-derived endothelial cells could help solve this problem by enabling a cell source originating from the patients themselves. Abaci et al. showed the successful creation of vascular networks using iPSC-derived endothelial cells combined with a novel 3D bioprinting technique for skin tissue engineering; this human skin equivalent formed an epithelial layer and vascularised successfully with fully formed endothelial-lined vessels in a murine model.[34] Shen et al. used iPSC-endothelial cells from both type 1 diabetic and healthy patients to generate a vascularised skin construct that were perfused by the host vasculature within 3 days of implantation.[35] This combination of tissue engineering technologies may enable personalised off-the-shelf vascularised skin substitutes in wound healing.

While promising, the general efficacy of stem cell therapy, including MSC and iPSC, is often difficult to determine due to the phenotypic variation of cells that occurs between donors and even within the same individual.[36,37] In addition, there are safety concerns around potential mutagenicity in culture and potential tumorigenicity of undifferentiated cells once implanted.[27] Nevertheless, it is anticipated that advancing technology and further research will improve somatic cell induction technology to enable stricter, more reliable phenotype profiling and characterisation of iPSC. In addition, more specific isolation of therapeutically active populations of cells may help develop more clinically efficacious applications.

4.2 Advances in stem cell delivery

There are several methods to deliver stem cells to the site of interest but the lack of standardization makes it challenging to compare their efficacy.[18] However, due to the systemic nature of diabetes, administration of stem cells for diabetic wounds may be beneficial by either systemic or local routes.

Systemic administration can be performed by injection of stem cells directly into the circulation or intramuscularly. Systemic administration of stem cells can also potentially help with alleviating some of the systemic complications of diabetes, such as re-vascularization of the ischemic limb using adipose derived MSC or iPSC-smooth muscle cells,[32,38] thus potentially promoting the overall healing environment. It is worth noting that in studies using intravenous infusion, a large proportion of injected MSC are trapped in the lungs.[39] This contributes to the short-lived presence of MSC after administration; avoidance cells being trapped in the lungs may improve the survival of circulating MSC. Arterial injections, such as through the femoral artery, carotid artery, left ventricle or renal artery, may avoid this issue.[40–43] The arterial route improves delivery of MSC to organs and sites of interest; however, this route increases risk of complications due to its increased invasiveness compared to intravenous infusion.

After administration of cells, some studies have also shown poor cell engraftment at the injury site despite evidence of positive therapeutic benefits.[16,38] One study showed poor engraftment of cells with local injection of BM-MSC to wound sites, with only 2.5% of the original cells detected at 28 days.[44] To improve engraftment, a cell-matrix carrier can be used for local administration at the wound site. Scaffold products can contain biological materials such as collagen hydrogels or bioengineered synthetic material to also help support and heal wounds; they can also aid local/topical delivery of stem cells to the area of regenerative need. Tissue engineered skin substitutes can be created using different types of biomaterials with spatially distributed stem cell types to replicate the lost skin and underlying tissue. Several hydrogel and polymer scaffolds have been developed to induce activity and maintain MSC viability to promote the production of angiogenic, immunomodulatory, matrix remodelling, or other regenerative cytokines.[45] Another recent innovation is the laboratory processing of autologously harvested full thickness skin, producing a product that is then topically applied back onto the patient's wound.[46] This method may stimulate endogenous stem cell populations from the harvested skin; early studies suggest its utility in a variety of chronic wounds, including diabetic wounds.[47]

Recent advancements in single-cell transcriptomic analysis has made it possible to detect and select more regenerative subpopulation of MSC with enhanced healing capacity from different donor populations. Khong et al. showed that young BM-MSC, which contained a higher proportion of cells with higher expression of genes involved in tissue regeneration, healed wounds faster compared to aged BM-MSC.[48] Huang et al. presented a method of standardization of umbilical cord-derived MSC using transcriptome profiling to show lack of heterogeneity on a genetic level within the donor cell populations.[49] Data produced from this exciting field of transcriptional profiling and analysis with single-cell RNA sequencing may help identify regenerative potential of donor stem cells and potentially help screen and reduce donor variability of stem cells delivered clinically, via the use of subpopulation markers. Additionally, the use of single-cell transcriptomics can identify genes differentially expressed among different spatial regions of a wound, potentially guiding researchers and clinicians to deliver patient- and gene-specific therapy to promote wound healing in individual patients.

4.3 Advances in stem cell manipulation for skin regeneration

Stem cell manipulation can help host cells home to the wound site via increasing circulating stem cells, enhancing integration of cells into the wound site, differentiating stem cells into the cells of choice and increasing angiogenesis. Neovascularization of the wound is by far the most important objective to wound healing as it is the limiting factor for all the downstream healing processes that enhance wound regeneration. The following sections discuss various advances in stem cell manipulation to achieve this goal.

4.3.1 Scaffold microenvironment and engineering

Scaffold matrix can be used to manipulate the stem cell microenvironment to enhance its pro-regenerative capability. Dash et al. showed that the scaffold collagen fibrillar density can modulate the secretory function of seeded human iPSC-vascular smooth muscle cells to promote regenerative qualities of the cells.[50] The dense fibrillar collagen increased pro-healing cytokines, such as VEGF, IL-8, IL-10, TGFβ, and keratinocyte growth factor (KGF), as well as increased endothelial cell proliferation and migration. Surface modification of scaffolds using bioactive polymers can also affect stem cell behaviour. For example, the addition of laminin and hyaluronic acid increase cell survival and upregulation of VEGF cytokines from seeded cells.[51,52] Further progress in this field is likely to involve a cross-disciplinary approach to produce personalised tissue engineered constructs by using patient specific cells, biomimetic matrices (e.g. collagen, gelatin, elastin, fibrin etc.) and bioactive polymers (e.g. hyaluronic acid, glycosaminoglycans, laminin, silk protein etc.) to promote regenerative healing (**Figure 5**).[53] Additionally, the intermolecular bonds within the scaffold microenvironment that contributes to its physical properties affect cell proliferation and differentiation.[54] MSC cultured in polyacrylamide hydrogels with an increasing stiffness gradient from 0.1 to 25 kPa (within parameters of normal tissue stiffness) commit to different cell lineages of neurons, myoblasts or osteoblasts; cells in a 'softer' matrix (~0.1–1 kPa) committed to a neurogenic lineage, compared to cells on a 'stiffer' matrix (~8–17 kPa) that committed to a myogenic lineage.[55] Matrix stiffness affects collagen deposition by resident cells, with low density 'softer' matrix simulating greater collagen production by fibroblasts *in situ*.[56] Recent advances in nanotechnology also highlight the importance of physical properties of the scaffold, such as porosity, to manipulate cell fate to accelerate wound healing.[57] Hence, altering the mechanical properties of matrix may help implanted stem cells sense mechanical environmental cues, which can in turn facilitate cell migration and differentiation for enhancement of the different phases of wound healing.

Tissue engineering of skin with cells is complex. The skin's ECM environment, from the specific basket-weave structure of the epidermis to the changes in architecture in specific layers of skin, allowing in each layer for the distinct cell populations to form structures including the vascular and neural networks as well as sweat glands, make engineering of this multi-layered tissue incredibly challenging. Bioprinting of skin is an advanced tissue engineering strategy that aims to deliver stem cells directly to the wound site to help overcome some of the above challenges; bioprinting allows quick and effective replacement of skin in the surgical suite.[58] Several pre-clinical studies have already established technology that allows for the *in situ* assembly of skin cells mixed within bio-ink carrier/gel into a complex 3D skin graft.[59] In addition, engineering pigmented skin by incorporation of melanocytes or melanin can match the graft to the recipient site.[60] Skardal et al. showed that

bioprinting of cells directly onto skin defects enhances wound healing in nu/nu mice compared to control hydrogel.[61] These data suggest that improved wound healing may be due to the trophic factors, rather than cell-to-cell interactions, consistent with previous studies. With newer and more innovative technologies in scaffold engineering, scientists are now able to alter the microenvironment in which these stem cells reside. Combined with 3D-bioprinting, Kim et al. used skin-derived ECM with intrinsic cytokine and growth factor properties to enhance epidermal organization, dermal ECM secretion, barrier function as well as pre-vascularization of the engineered skin patches.[62] This encourages differentiation in the cell lineage of choice and enhances the regenerative potential of the stem cells delivered to increase wound healing potential. Abaci et al. also showed viable vascular networks using iPSC-derived endothelial cells and novel 3D bioprinting.[34] This may pave the way to future tissue engineering of ready-to-vascularise skin.

4.3.2 Hypoxic pre-conditioning

Hypoxic preconditioning of cells improves wound healing via enhancement of pro-angiogenic properties in MSC.[45] Hence, priming of the cells via hypoxic induction may be useful to optimize angiogenesis on demand when cells are transplanted. Pre-conditioning of cells in hypoxia was proposed by Rosova et al. to integrate into steps of a pre-transplantation protocol, enabling cells to resist apoptotic stimuli when transplanted into a hostile *in vivo* environment with severe hypoxia, such as ischemic tissues from chronic wounds or other sites of injury.[63] Another advantage of hypoxia preconditioning for MSC is the maintenance of stemness and multipotency, which could have therapeutic benefits when used in areas where regeneration of tissue is desired.[64]

4.3.3 Gene modification

Stem cells can also be genetically modified to increase their regenerative and therapeutic potential. The use of iPSC creates an opportunity for further genetic modifications to correct the gene defects. Teo at al. proposed that technologies such as zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) or clustered regularly interspaced short palindromic repeat (CRISPR), can be used to repair mutated gene sequences associated with diabetes *in vitro* before transplanting back into diabetic patients.[65] The ability to genetically correct iPSC from the elderly diabetic donor and remove defects associated with diabetes and aging may help yield better autologous cell-base therapeutic products for clinical translation. Song et al. showed that introduction of v-Myc via lentiviral gene transfer into human ADSC increased secretion of VEGF and *in vitro* vasculogenesis.[66] Preclinical studies by Fierro et al. also confirmed that MSC can be safely genetically engineered using a lentiviral vector to produce high levels of VEGF to increase angiogenic potency in a mouse hind limb ischemic model.[67],[68] The same group extended this model to show good medical practice, demonstrating the feasibility of a clinically viable product to bring to the patient's bedside.[69] It is anticipated that this form of cellular therapy can also be extended to include healing of chronic wounds clinically.

4.3.4 Nanotechnology

Nanotechnology can enhance stem cell potential and differentiation. Photosensitive and biomimetic core-shell nanofibrous scaffolds when seeded with adipose-derived stem cells showed that differentiation into epidermal keratinocytes after stimulation with light.[70]

Nanoparticles can also be used to deliver genetic material to cells. Yang et al. showed that a nonviral, biodegradable polymeric nanoparticle delivered the VEGF gene to MSC and embryonic stem cell-derived cells; treated stem cells had markedly enhanced VEGF production, cell viability, and engraftment into target tissues. When implanted on a scaffold and transplanted into mice models, there were two to four-fold-higher vessel densities after 2 weeks compared to control; four weeks after intramuscular injection into murine ischemic hindlimbs, the genetically modified MSC substantially enhanced angiogenesis and limb salvage while reducing muscle degeneration and tissue fibrosis. These results show that engineered stem cells with biodegradable polymer nanoparticles may be therapeutic tools treating ischemic disease as well as showing potential for wound healing.[71]

4.3.5 Secretome engineering

As eluded to above, the trophic effects of MSC are thought to contribute towards the therapeutic effects of transplanted cells in translational studies. Collectively these substances are known as secretomes. Secretomes are composed of soluble factors such as cytokines, chemokines and growth factors, as well as insoluble nano/microscopic extracellular vesicles (EV). Similar to MSC, data from iPSC studies suggests the paracrine effect of cellular secretomes promotes wound healing rather than direct cellular interactions.[27]

There is now a growing field to understand how to harness the effect of these substances from cell supernatant, particularly separating the soluble factors from insoluble EV.[72] EV are released from cells as microvesicles or exosomes depending on the extrusion modality (**Figure 6A**). EV contain nucleic acids, proteins and lipids from the cells of origin and act as important mediators for intracellular crosstalk, which is seen as a key advantage over soluble secretomes (**Figure 6B**).[73] The culture microenvironment can heavily influence EV production; parameters such as cell density, cell age, culture system geometry, hypoxia and mechanical stress all have an impact on the cargo transference and downstream therapeutic effects of EV.[74] Hence, EV can be exploited from cells pre-conditioned in *in vitro* microenvironments known to increase its therapeutic potential for wound healing.[75] The highly modifiable nature of EV explains why research into engineering EV derived from MSC and iPSC is gaining more traction, particularly regarding the upgraded performance seen in iPSC-derived EV compared to adult MSC.[76]

Early data from Zhang et al. showed that exosomes derived from human iPSC-MSC facilitated cutaneous wound healing in rats by promoting collagen synthesis and angiogenesis.[77] Exosomal miRNA obtained from umbilical cord MSC, human amniotic epithelial cells, and human umbilical cord blood plasma improve wound healing by accelerating re-epithelialization, reduced scar widths, and enhanced angiogenesis in murine models.[78] Exosomes derived from PRP enhance angiogenesis and improve cutaneous healing compared with control groups in diabetic rats.[79] EV have also been loaded as bioactive molecules onto tissue engineered scaffolds for wound healing. Tao et al. showed accelerated wound healing via enhanced re-epithelialization, angiogenesis, and collagen production in a diabetic rat model after using controlled-release exosomes derived from miR-126-3p-overexpressing synovium MSC combined with chitosan matrix.[80] Particularly relevant to chronic diabetic wounds, Wang et al, engineered an antibacterial multifunctional FHE hydrogel (F127/OHA-EPL) with controlled release of impregnated exosomes derived from ADSC.[81] These engineered exosomes facilitated diabetic wound healing compared to hydrogel or exosomes alone, and have the potential for complete skin regeneration via restoration of skin appendages and reduction of scar tissue. These positive effects of EV were

reviewed in a recent meta-analysis and systematic review of pre-clinical literature of the efficacy of MSC-EV to promote diabetic wound healing. The analysis showed a significant effect on the healing of diabetic wounds, particularly when enriched in non-coding RNAs or microRNAs compared to controls. There was also a positive improvement on other outcomes such as blood vessel density and number, scar width, and re-epithelialisation. These data form a strong rationale for translational use of MSC-EV in clinical studies.[82]

The advantage of EV over cell-based therapy lies in the ability to properly characterise and modify the therapeutic materials within EV to have a more measurable and predictable dose-response effect *in vivo*. (**Figure 7**) EV can also be stored long-term without the loss of function, which is a key point to consider for the manufacturing, storage, and product shelf life of a commercial product.[73] Hence, the use of EV is a highly promising cell-free alternative to the current wound healing paradigm, avoiding potential complications and risks of using stem cells in patients when used alone, or combined with stem cell therapy to further enhance therapeutic output from both approaches.[83] Nonetheless, translating the secretome as therapy also presents with its own challenges. It requires a scalable, reproducible process capable of being compatible with good manufacturing practice (GMP) procedures.[84] This is a highly exciting and progressive field, with researchers describing promising manufacturing technique such as ultrafiltration and freeze-drying to produce stable 'off-the-shelf' powder from cellular secretomes with low batch variability.[85],[86] With time, this field will likely see more combination of advancing therapies from the above described technologies, such as the use of tissue engineered scaffolds with engineered exosomes tailored specifically to aid chronic wound healing.[87]

5.0 Wider clinical applications for stem cell derived therapies

The therapies described above are focused on the treatment of diabetic disease processes, but there are also a multitude of other clinical situations where they can be useful.[88],[89] Stem cell derived therapies affect many bio-molecular pathways, such as angiogenesis and the production of pro-healing cytokines and growth factors, positively impacting on the repair mechanisms of the skin and its adnexal structures. Due to common physiological mechanisms in the skin, there are wider off-target effects beyond wound healing. Most relevant to this article's scope would be the wider applications in skin wound healing and conditions of the skin adnexal structures. For the latter, stimulating hair regrowth in androgenic alopecia (AGA) with hair tissue engineering and stem cell therapies is a relatively new approach. Autologous cell-based treatments can be used to regenerate hair follicles by reversing biological mechanisms that cause AGA, regenerating mature hair follicles and neogenesis of whole hair follicles.[90],[91]

Complex skin defects, not necessarily diabetic in origin, that are full thickness have been treated with a variety of reconstructive options enhanced with stem cells.[92] Although this is a relatively new area and many studies are experimental in nature, it shows great promise to a future that combines stem cell derived therapies with one or more traditional reconstructive methods, innovatively expanding the 'reconstructive ladder'.[93] There is evidence for positive effects of ADSC on radiation-induced skin injury.[94] Some complex defects may require fat grafting and there is some evidence that MSC can enhance fat graft take.[95] The use of such therapies have also been investigated in conjunction with other techniques, such as platelet rich plasma (PRP)[96], and combination with an acellular dermal matrix.[97]

5.1 Regulatory environment

Clinical use of stem cells is a relatively new area of medicine and is highly dynamic with a wide range of research and therapeutic applications. This has led to regulatory issues surrounding the clinical adoption of some stem cell therapies. Some physicians use stem cell therapies for a variety of indications, and some may be ambiguous regarding claims, processes and clinical efficacy. The U.S. Food and Drug Administration (FDA) recently published regulatory guidelines that cover stem cell treatments and includes safety requirements and manufacturing standards. Some of the key factors regulated are product quality and efficacy, clinical safety, processing integrity and public health safety (including prevention of communicable disease).[98,99]

Products should ideally be subject to regulations and require premarket approval unless certain exceptions are satisfied; product manufacturers would need to seek approval from the FDA for a 'drug' or 'medical device'. This process has high costs of both money and time, and involves significant investment, clinical trials and regulatory processes before a final approval. In brief, the exceptions for this process include a minimal level of manipulation of human cells, tissues, and cellular and tissue-based products (HCT/P), intention for homologous use, absence of combining with another compound, certain intended usages (e.g. non-systemic effect or autologous use).

The FDA considers minimal manipulation of HCT/P as processing that does not alter the relevant biological characteristics of the cell. The criteria for homologous use of HCT/P is that it performs same basic function in the recipient site as in the donor. Homologous use provides more basis for predictable product behaviour. For the manufacturing process, the FDA exemption states that the HCT/P should not be combined with other cells, tissues or compounds (except for water, crystalloids, inert sterilising, preserving, or storage agents). Generally, this is a significant issue for enzymatic digestion that is part of SVF isolation, but less of a concern with mechanical processing. There are different isolation methods described to satisfy quality and exemption criteria to help bring such technologies into clinical practice.[100] However, no universal standardization of these methods exist, which will undoubted cause variability in the final product. Thus, more studies are needed to determine clinical effectiveness of products such as SVF isolated products to standardize quality control and regulatory adherence.

Many of the applications for stem cells may have a systemic effect or are dependent upon the metabolic activity of living cells for their primary function, but would be for autologous use, which fits within the exemption. Some legal implications involve the above criteria. For example, SVF derived from ADSC may not be considered under the homologous use category as the product to be implanted is not structurally the same as the product harvested. Also, the implanted stem cells are intended to perform functions beyond the basic functions of the stem cells in their pre-harvested state.

The safety of stem cells is another major regulatory consideration, particularly with potential tumorigenesis of transplanted cells *in vivo*. The main risk of this lies with presence of residual undifferentiated cells in the final cell therapy product and potential cell transformation of cells and the activation of tumorigenic networks, such as during cell expansion, cell transduction with integrating vectors, or differentiation/activation within the manufacturing process.[101] Tumorigenesis is mainly seen in human pluripotent iPSC and ESC therapy, of which ESC is usually considered the regulatory standard for both iPSC and ESC. A 2008

FDA briefing document discussed the risks associated with human ESC and in a further published guidance in 2013, recommended vigorous pre-clinical testing to reduce the risk of tumorigenicity in the final cell therapy product.[102,103] These include sensitive *in vitro* assays, such as flow cytometry and quantitative RT-PCR, to detect undifferentiated cell contaminant in therapeutic end product, as well as *in vivo* testing on animals to detect tumour formation.[101] On the clinical level, the screening of patients for risk factors of tumorigenicity, such as the use of immunosuppressants and underlying cancer, can also help with minimising tumorigenicity in carefully selected patient groups.[101] Other strategies that have been described to reduce tumorigenicity include 1. use of immunologic targeting for elimination of undifferentiated cell population using cytotoxic agents;[104] 2. chemical or pharmacological ablation of tumorigenic cells;[105] 3.genetic modification of iPSC to interfere with tumour-inducing genes, to introduce 'suicide' genes or to enable cell labelling and separation.[106,107] However, detailed discussions of these strategies are beyond the scope of this review.

5.2 Future directions for use of stem cell derived clinical research

Despite continued basic research, there are many remaining questions. Understanding gaps and discrepancies in the field and refining future research is important to obtain translatable research data with greater clinical impact.

A discrepancy and source of heterogeneity in *in vivo* diabetic wound stem cell research involves the heavy reliance on murine models compared to other animal models. It is widely understood that the murine wound healing is quite different from the human process. Mouse and rat skin heal mainly by contraction due to the presence a layer of the skin called the panniculus carnosus, which is absent in humans. Yet in a comprehensive review of wound healing, 85% of published literature used a murine model (52% mouse; 33% rat) compared to only 5% that used a pig model.[108] Many researchers attempt to minimise the effect of murine contractile wound healing via the use of the splinted wound model; however, only 8.4% of these diabetic murine model studies are splinted compared to 32% unsplinted.[108] Such discrepancies will likely hinder the clinical relevance of such published data. Hence, within cost and ethical restrictions, other more well-designed animal models with similar human-like wound healing physiology, such as the porcine skin, ought to be considered more frequently.[109] Understandably, large animal models have their own challenges as diabetic models in other animals are more poorly described compared to the mouse and rat models.

Advances in iPSC technology might provide a solution to discrepant animal and human data by using a patient's own somatic cells. iPSC-derived cells can differentiate into multiple cell types to allow microengineering of functional yet complex 3D tissue structures or organoids within its own microfluidic devices, otherwise known as skin-on-a-chip.[110,111] There are only a handful of studies have shown vascularised human skin equivalent structures[111], such as the use of nylon thread to create fully endothelialized vascular channels.[112] However, Abaci et al. utilized iPSC-derived endothelial cells to form vascularised human skin equivalent as a functional organoid.[34] Examples of skin-on-a-chip technology stimulating a disease process was first shown with a device resembling the true architectural layers of the skin using the channels for each skin layer, to investigate effects of skin inflammation and edema via the application of TNF-α to the dermal layer.[113] Organoids that closely resemble human skin may recapitulate physiological dysregulation such as seen in diabetes, similar to other organ-on-a-chip diabetic models.[114] This technology may enable pre-clinical *in vitro* testing of diabetic wound healing that would negate the use of animals.

Published clinical studies on stem cell therapies in DFU mainly focus on the use of adult MSC therapies.[18] Significant heterogeneity still exists within these studies and variability in delivery methods as well as discordance between preclinical and clinical studies have confounded their interpretation and conclusive results. Nevertheless, results from meta-analysis of RCT of stem cell therapy in diabetic wounds have shown autologous stem cell therapy to be safe, effective and promising treatment for DFU.[17],[115] However, to use other emerging stem cell types such as iPSC-based therapy, there will need to be additional larger, multi-centre, randomised, double-blinded controlled trials. Ideally these studies would also help optimise cell processing, protocols, reproducible outcome measures and better biomarker-based diagnostics. Understanding how to utilise stem cells in the most effective way, within regulatory structures, is still a challenge. The FDA has created guidelines and updates, but due to the dynamic and fast-paced innovation that accompanies this field of medicine, there will be continued grey areas that will be discussed and investigated.

6.0 Summary

Stem cells improve wound healing by their paracrine as well as cellular effects. Stem cells secrete cytokines in response to microenvironmental cues, which can promote healing via chemotaxis of other cell types, angiogenesis and immunomodulatory functions as well as extracellular matrix remodelling. iPSC are a promising source of stem cells to be used for regeneration of diabetic wounds. Their innate ability to differentiate into other cell types of interest with fewer issues concerning the ethics, supply or source make them incredibly favourable for use in difficult to heal chronic wounds with complex tissue loss. The SVF is also a potential translational solution for cellular therapy in wound healing given its point-of-care applications with a patient's own autologous cells. There are also new advances in technology to help with stem cell manipulation to enhance wound healing via improving neoangiogenicity of transplanted cells. In addition, secretomes such as extracellular vesicles from stem cells are a promising cell-free strategy or adjunctive therapy to achieve wound healing in difficult to heal chronic diabetic wounds. There are increasing numbers of difficult-to-treat conditions that may benefit from such therapeutic approaches. Chronic wounds are likely to be an area of early benefit, with a relatively larger body of existing research, problem prevalence, dire clinical need, financial burden and detrimental psychological and self-care implications. As this field expands, further research needs to be done to understand the exact mechanism of the therapeutic effects of stem cell therapy, to evaluate clinical safety of such therapies and to focus on innovating and validating clinical GMP manufacturing technologies for translational purposes.

Take Home Messages

1. There are two main types of stem cells for translational use: somatic (adult) stem cells and pluripotential stem cells.
2. Stem cell therapy accelerates diabetic wound healing mainly via paracrine action, increasing angiogenesis and regulating immune function as well as remodelling the extracellular matrix of the wound site.
3. iPSC are a promising source of somatic stem cells available from a patient's own cells to enable an essentially unlimited cell source for therapeutic purposes, without the ethical concerns of their embryonic counterparts. iPSC can also contribute to emerging skin-on-a-chip technologies to replace *in vivo* experimental models.
4. The stromal vascular fraction is another promising and highly translatable adult autologous cell source.

5. Combination of stem cells, tissue engineered scaffold and gene and nanotechnologies for delivery of stem cells *in vivo* can further potentiate diabetic wound healing.
6. Secretomes such as extracellular vesicles from stem cells may be an important cell-free or cell-complimenting strategy to promote diabetic wound healing.

Author Confirmation

I confirm that I have the written consent of all contributing authors and that all authors accept complete responsibility for the contents of the manuscript.

Author Disclosure and Ghostwriting

We have no conflict of interest or ghostwriters to declare.

Funding Statement

Dr Jasmine Ho is funded by the Medical Research Council (MRC) UK grant MR/N002113/1 and Rosetrees Trust. She has also received additional funding from the UCL Doctoral School for participating in the Yale-UCL Collaborative Scholar Exchange Programme. Dr. Alan Dardik is funded by the US National Institute of Health (NIH) grant R01-HL144476.

About the Authors

Jasmine Ho, MBBS, BSc, MSc, MRCS, is an academic surgeon and clinical research fellow at the Centre for 3D Models of Health and Disease at University College London. She is also engaged in research with Yale University through Dardik's Lab via the Yale-UCL Collaborative Scholar Exchange Programme. Her research interests include vascularization of tissue-engineered constructs for translational applications.

Dominic Yue, MBBS, BSc, FEBHS, FRCS(Plast), is a Consultant Plastic Surgeon at the Chelsea and Westminster Hospital in London, UK. He has a clinical and academic interest in complex wound healing with innovative strategies. His specialty is in hand and burn surgery.

Umber Cheema, BSc (Hon), PhD, is a Professor of Bioengineering at the UCL Centre for 3D Models of Health and Disease, Division of Surgery and Interventional Sciences in the UK. Her research interests include engineering vascular networks in 3D-engineered tissues to further understand the role that native matrix density and composition have in this biological process.

Henry Hsia, MD FACS, is an Associate Professor of Surgery (Plastic) and Founding Director of Yale Regenerative Wound Healing Center. He is a Board Certified Plastic Surgeon at Yale School of Medicine who has dedicated his professional life to finding innovative wound treatments for patients through basic and clinical research.

Alan Dardik, MD PhD, is a Professor of Surgery and of Cellular and Molecular Physiology, as well as Vice Chair of the Department of Surgery, at the Yale School of Medicine. He leads a translational research program that examines the role of stem cells in wound healing.

Abbreviations and Acronyms

ADSC = adipose-derived stem cell
Ang-1 = angiopoietin-1
bFGF = basic fibroblast growth factor
BM-MSC = bone marrow derived mesenchymal stem cell
DFU = diabetic foot ulcer
ECM = extracellular matrix
EPC = endothelial progenitor cells
ESC = Embryonic stem cell
EV = extracellular vesicle
hiPSC = human induced pluripotent stem cell
IFN-γ = interferon-γ
IL-4 = interleukin-4
IL-8 = interleukin-8
IL-10 = interleukin-10
iPSC = induced pluripotent stem cell
KGF = keratinocyte growth factor
miRNA = micro ribonucleic acid
MSC = mesenchymal stem cell
PDGF = platelet derived growth factor
RNA = ribonucleic acid
SDF-1 = stromal derived factor-1
SVF = stromal vascular fractions
TGF-β = transforming growth factor-β
TNF-α = tumour necrosis factor- α
VEGF = vascular endothelial growth factor

References

1. IDF. International Diabetes Federation. IDF Diabetes Atlas 9th Edition. 9th ed. 2019. Accessible from: https://www.diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf.
2. Hicks CW, Selvarajah S, Mathioudakis N, Sherman RE, Hines KF, Black JH, et al. Burden of Infected Diabetic Foot Ulcers on Hospital Admissions and Costs. Ann Vasc Surg. 2016 May 1;33:149–58. doi: 10.1016/j.avsg.2015.11.025.
3. Armstrong DG, Swerdlow MA, Armstrong AA, Conte MS, Padula W V., Bus SA. Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer. Vol. 13, Journal of Foot and Ankle Research. BioMed Central Ltd.; 2020. p. 1–4. doi: 10.1186/s13047-020-00383-2.
4. Mustoe TA, O'Shaughnessy K, Kloeters O. Chronic wound pathogenesis and current treatment strategies: A unifying hypothesis. Vol. 117, Plastic and Reconstructive Surgery. Plast Reconstr Surg; 2006. doi: 10.1097/01.prs.0000225431.63010.1b.
5. Jones JE, Nelson EA, Al-Hity A. Skin grafting for venous leg ulcers. Vol. 2013, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2013. doi: 10.1002/14651858.CD001737.pub4.
6. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in

virtually all post-natal organs and tissues. J Cell Sci. 2006 May 9;119(11):2204–13. doi: 10.1242/jcs.02932.
7. Nombela-Arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. Nat Rev Mol Cell Biol. 2011;12(2):126–31. doi: 10.1038/nrm3049.
8. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci. 2000 Apr 1;113(7):1161–6. doi: 10.1242/jcs.113.7.1161.
9. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal Stem Cells Are Recruited into Wounded Skin and Contribute to Wound Repair by Transdifferentiation into Multiple Skin Cell Type. J Immunol. 2008 Feb 15;180(4):2581–7. doi: 10.4049/jimmunol.180.4.2581.
10. Li H, Fu X, Ouyang Y, Cai C, Wang J, Sun T. Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. Cell Tissue Res. 2006;326(3):725–36. doi: 10.1007/s00441-006-0270-9.
11. Chen L, Tredget EE, Wu PYG, Wu Y, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008 Apr 2;3(4). doi: 10.1371/journal.pone.0001886.
12. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005 Feb 15;105(4):1815–22. doi: 10.1182/blood-2004-04-1559.
13. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, et al. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells. 2010 Dec;28(12):2229–38. doi: 10.1002/stem.544.
14. Yu JR, Navarro J, Coburn JC, Mahadik B, Molnar J, Holmes JH, et al. Current and Future Perspectives on Skin Tissue Engineering: Key Features of Biomedical Research, Translational Assessment, and Clinical Application. Vol. 8, Advanced Healthcare Materials. Wiley-VCH Verlag; 2019. doi: 10.1002/adhm.201801471.
15. Motegi S ichiro, Ishikawa O. Mesenchymal stem cells: The roles and functions in cutaneous wound healing and tumor growth. Vol. 86, Journal of Dermatological Science. Elsevier Ireland Ltd; 2017. p. 83–9. doi: 10.1016/j.jdermsci.2016.11.005.
16. Gnecchi M, Danieli P, Malpasso G, Ciuffreda MC. Paracrine mechanisms of mesenchymal stem cells in tissue repair. In: Methods in Molecular Biology. Humana Press Inc.; 2016. p. 123–46. doi: 10.1007/978-1-4939-3584-0_7.
17. Guo J, Dardik A, Fang K, Huang R, Gu Y. Meta-analysis on the treatment of diabetic foot ulcers with autologous stem cells. Vol. 8, Stem Cell Research and Therapy. BioMed Central Ltd.; 2017. doi: 10.1186/s13287-017-0683-2.
18. Lopes L, Setia O, Aurshina A, Liu S, Hu H, Isaji T, et al. Stem cell therapy for diabetic foot ulcers: A review of preclinical and clinical research. Vol. 9, Stem Cell Research and Therapy. BioMed Central Ltd.; 2018. doi: 10.1186/s13287-018-0938-6.
19. Guo J, Hu H, Gorecka J, Bai H, He H, Assi R, et al. Adipose-derived mesenchymal stem cells accelerate diabetic wound healing in a similar fashion as bone marrow-derived cells. Am J Physiol - Cell Physiol. 2018 Dec 1;315(6):C885–96. doi: 10.1152/ajpcell.00120.2018.
20. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded

adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013 Jun 1;15(6):641–8. doi: 10.1016/j.jcyt.2013.02.006.
21. Guo J, Nguyen A, Banyard DA, Fadavi D, Toranto JD, Wirth GA, et al. Stromal vascular fraction: A regenerative reality? Part 2: Mechanisms of regenerative action. Vol. 69, Journal of Plastic, Reconstructive and Aesthetic Surgery. Churchill Livingstone; 2016. p. 180–8. doi: 10.1016/j.bjps.2015.10.014.
22. Han SK, Kim HR, Kim WK. The treatment of diabetic foot ulcers with uncultured, processed lipoaspirate cells: A pilot study. Wound Repair Regen. 2010 Jul;18(4):342–8. doi: 10.1111/j.1524-475X.2010.00593.x.
23. Carstens MH, Quintana FJ, Calderwood ST, Sevilla JP, Ríos AB, Rivera CM, et al. Treatment of chronic diabetic foot ulcers with adipose-derived stromal vascular fraction cell injections: Safety and evidence of efficacy at 1 year. Stem Cells Transl Med. 2021; doi: 10.1002/sctm.20-0497.
24. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006 Aug 25;126(4):663–76. doi: 10.1016/j.cell.2006.07.024.
25. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell. 2007 Nov 30;131(5):861–72. doi: 10.1016/j.cell.2007.11.019.
26. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science (80-). 2007 Dec 21;318(5858):1917–20. doi: 10.1126/science.1151526.
27. Gao X, Gorecka J, Cheema U, Gu Y, Wu Y, Dardik A. Induced pluripotent stem cells in wound healing. In: Recent Advances in iPSCs for Therapy, Volume 3. Elsevier; 2021. p. 269–90. doi: 10.1016/b978-0-12-822229-4.00003-6.
28. Itoh M, Kiuru M, Cairo MS, Christiano AM. Generation of keratinocytes from normal and recessive dystrophic epidermolysis bullosa-induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2011 May 24;108(21):8797–802. doi: 10.1073/pnas.1100332108.
29. Itoh M, Umegaki-Arao N, Guo Z, Liu L, Higgins CA, Christiano AM. Generation of 3D Skin Equivalents Fully Reconstituted from Human Induced Pluripotent Stem Cells (iPSCs). PLoS One. 2013 Oct 11;8(10). doi: 10.1371/journal.pone.0077673.
30. Kashpur O, Smith A, Gerami-Naini B, Maione AG, Calabrese R, Tellechea A, et al. Differentiation of diabetic foot ulcer-derived induced pluripotent stem cells reveals distinct cellular and tissue phenotypes. FASEB J. 2019 Jan 1;33(1):1262–77. doi: 10.1096/fj.201801059.
31. Gorecka J, Gao X, Fereydooni A, Dash BC, Luo J, Lee SR, et al. Induced pluripotent stem cell-derived smooth muscle cells increase angiogenesis and accelerate diabetic wound healing. Regen Med. 2020 Feb 1;15(2):1277–93. doi: 10.2217/rme-2019-0086.
32. Gao X, Gao M, Gorecka J, Langford J, Liu J, Luo J, et al. Human-Induced Pluripotent Stem-Cell-Derived Smooth Muscle Cells Increase Angiogenesis to Treat Hindlimb Ischemia. Cells. 2021 Apr 2;10(4):792. doi: 10.3390/cells10040792.
33. Cerilli J, Brasile L, Galouzis T, Lempert N, Clarke J. The vascular endothelial cell antigen system. Transplantation. 1985 Mar 1;39(3):286–9. doi: 10.1097/00007890-198503000-00016.
34. Abaci HE, Guo Z, Coffman A, Gillette B, Lee WH, Sia SK, et al. Human Skin Constructs

with Spatially Controlled Vasculature Using Primary and iPSC-Derived Endothelial Cells. Adv Healthc Mater. 2016 Jul 20;5(14):1800–7. doi: 10.1002/adhm.201500936.
35. Shen YI, Cho H, Papa AE, Burke JA, Chan XY, Duh EJ, et al. Engineered human vascularized constructs accelerate diabetic wound healing. Biomaterials. 2016 Sep 1;102:107–19. doi: 10.1016/j.biomaterials.2016.06.009.
36. Koga K, Wang B, Kaneko S. Current status and future perspectives of HLA-edited induced pluripotent stem cells. Vol. 40, Inflammation and Regeneration. BioMed Central Ltd; 2020. doi: 10.1186/s41232-020-00132-9.
37. Rady D, Abbass MMS, El-Rashidy AA, El Moshy S, Radwan IA, Dorfer CE, et al. Mesenchymal Stem/Progenitor Cells: The Prospect of Human Clinical Translation. Vol. 2020, Stem Cells International. Hindawi Limited; 2020. doi: 10.1155/2020/8837654.
38. Mi HM, Sun YK, Yeon JK, Su JK, Jae BL, Yong CB, et al. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem. 2006;17(5–6):279–90. doi: 10.1159/000094140.
39. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012 Sep 26;3(SEP):297. doi: 10.3389/fimmu.2012.00297.
40. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: Feasibility, cell migration, and body distribution. Circulation. 2003 Aug 19;108(7):863–8. doi: 10.1161/01.CIR.0000084828.50310.6A.
41. Feng CJ, Perng CK, Lin CH, Tsai CH, Huang PH, Ma H. Intra-arterial injection of human adipose-derived stem cells improves viability of the random component of axial skin flaps in nude mice. J Plast Reconstr Aesthetic Surg. 2020 Mar 1;73(3):598–607. doi: 10.1016/j.bjps.2019.10.001.
42. Namestnikova DD, Gubskiy IL, Revkova VA, Sukhinich KK, Melnikov PA, Gabashvili AN, et al. Intra-Arterial Stem Cell Transplantation in Experimental Stroke in Rats: Real-Time MR Visualization of Transplanted Cells Starting With Their First Pass Through the Brain With Regard to the Therapeutic Action. Front Neurosci. 2021 Mar 2;15:179. doi: 10.3389/fnins.2021.641970.
43. Thomson AL, Berent AC, Weisse C, Langston CE. Intra-arterial renal infusion of autologous mesenchymal stem cells for treatment of chronic kidney disease in cats: Phase I clinical trial. J Vet Intern Med. 2019 May 1;33(3):1353–61. doi: 10.1111/jvim.15486.
44. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal Stem Cells Enhance Wound Healing Through Differentiation and Angiogenesis. Stem Cells. 2007 Oct 1;25(10):2648–59. doi: 10.1634/stemcells.2007-0226.
45. Lee DE, Ayoub N, Agrawal DK. Mesenchymal stem cells and cutaneous wound healing: Novel methods to increase cell delivery and therapeutic efficacy. Vol. 7, Stem Cell Research and Therapy. BioMed Central; 2016. doi: 10.1186/s13287-016-0303-6.
46. Mundinger GS, Mundinger GS, Armstrong DG, Smith DJ, Sailon AM, Chatterjee A, et al. Autologous Homologous Skin Constructs Allow Safe Closure of Wounds: A Retrospective, Noncontrolled, Multicentered Case Series. Plast Reconstr Surg Glob Open. 2020;8(5). doi: 10.1097/GOX.0000000000002840.
47. Armstrong DG, Orgill DP, Galiano R, Glat PM, Carter M, Zelen CM, et al. Complete

wound closure following a single topical application of a novel autologous homologous skin construct: first evaluation in an open-label, single-arm feasibility study in diabetic foot ulcers. Int Wound J. 2020 Oct 1;17(5):1366–75. doi: 10.1111/IWJ.13404.
48. Khong SML, Lee M, Kosaric N, Khong DM, Dong Y, Hopfner U, et al. Single-Cell Transcriptomics of Human Mesenchymal Stem Cells Reveal Age-Related Cellular Subpopulation Depletion and Impaired Regenerative Function. Stem Cells. 2019 Feb 1;37(2):240–6. doi: 10.1002/STEM.2934.
49. Huang Y, Li Q, Zhang K, Hu M, Wang Y, Du L, et al. Single cell transcriptomic analysis of human mesenchymal stem cells reveals limited heterogeneity. Cell Death Dis 2019 105. 2019 May 8;10(5):1–12. doi: 10.1038/s41419-019-1583-4.
50. Dash BC, Setia O, Gorecka J, Peyvandi H, Duan K, Lopes L, et al. A Dense Fibrillar Collagen Scaffold Differentially Modulates Secretory Function of iPSC-Derived Vascular Smooth Muscle Cells to Promote Wound Healing. Cells. 2020 Apr 14;9(4). doi: 10.3390/cells9040966.
51. Assi R, Foster TR, He H, Stamati K, Bai H, Huang Y, et al. Delivery of mesenchymal stem cells in biomimetic engineered scaffolds promotes healing of diabetic ulcers. Regen Med. 2016 Apr 1;11(3):245–60. doi: 10.2217/rme-2015-0045.
52. Cerqueira MT, Da Silva LP, Santos TC, Pirraco RP, Correlo VM, Reis RL, et al. Gellan gum-hyaluronic acid spongy-like hydrogels and cells from adipose tissue synergize promoting neoskin vascularization. ACS Appl Mater Interfaces. 2014 Nov 26;6(22):19668–79. doi: 10.1021/am504520j.
53. Dash BC, Xu Z, Lin L, Koo A, Ndon S, Berthiaume F, et al. Stem cells and engineered scaffolds for regenerative wound healing. Vol. 5, Bioengineering. MDPI AG; 2018. doi: 10.3390/bioengineering5010023.
54. Jeon O, Alsberg E. Regulation of Stem Cell Fate in a Three-Dimensional Micropatterned Dual-Crosslinked Hydrogel System. Adv Funct Mater. 2013;23(38):4765–75. doi: 10.1002/adfm.201300529.
55. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell. 2006 Aug 25;126(4):677–89. doi: 10.1016/J.CELL.2006.06.044.
56. Pizzo AM, Kokini K, Vaughn LC, Waisner BZ, Voytik-Harbin SL. Extracellular matrix (ECM) microstructural composition regulates local cell-ECM biomechanics and fundamental fibroblast behavior: A multidimensional perspective. J Appl Physiol. 2005 May;98(5):1909–21. doi: 10.1152/JAPPLPHYSIOL.01137.2004.
57. Jiang S, Li SC, Huang C, Chan BP, Du Y. Physical Properties of Implanted Porous Bioscaffolds Regulate Skin Repair: Focusing on Mechanical and Structural Features. Adv Healthc Mater. 2018 Mar 1;7(6):1700894. doi: 10.1002/ADHM.201700894.
58. Bauermeister AJ, Zuriarrain A, Newman MI. Three-dimensional printing in plastic and reconstructive surgery a systematic review. Vol. 77, Annals of Plastic Surgery. Lippincott Williams and Wilkins; 2016. p. 569–76. doi: 10.1097/SAP.0000000000000671.
59. Manita PG, Garcia-Orue I, Santos-Vizcaino E, Hernandez RM, Igartua M. 3D Bioprinting of Functional Skin Substitutes: From Current Achievements to Future Goals. Pharmaceuticals. 2021 Apr 14;14(4):362. doi: 10.3390/ph14040362.
60. Ng WL, Qi JTZ, Yeong WY, Naing MW. Proof-of-concept: 3D bioprinting of pigmented human skin constructs. Biofabrication. 2018 Jan 24;10(2). doi: 10.1088/1758-5090/aa9e1e.

61. Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted Amniotic Fluid-Derived Stem Cells Accelerate Healing of Large Skin Wounds. Stem Cells Transl Med. 2012 Nov;1(11):792–802. doi: 10.5966/sctm.2012-0088.
62. Kim BS, Kwon YW, Kong JS, Park GT, Gao G, Han W, et al. 3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: A step towards advanced skin tissue engineering. Biomaterials. 2018 Jun 1;168:38–53. doi: 10.1016/j.biomaterials.2018.03.040.
63. Rosova I, Dao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells. 2008;26(8):2173–82. doi: 10.1634/stemcells.2007-1104.
64. Grayson WL, Zhao F, Izadpanah R, Bunnell B, Ma T. Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. J Cell Physiol. 2006 May 1;207(2):331–9. doi: 10.1002/jcp.20571.
65. Teo AKK, Wagers AJ, Kulkarni RN. New opportunities: Harnessing induced pluripotency for discovery in diabetes and metabolism. Vol. 18, Cell Metabolism. Cell Press; 2013. p. 775–91. doi: 10.1016/j.cmet.2013.08.010.
66. Song SH, Lee MO, Lee JS, Jeong HC, Kim HG, Kim WS, et al. Genetic modification of human adipose-derived stem cells for promoting wound healing. J Dermatol Sci. 2012 May;66(2):98–107. doi: 10.1016/j.jdermsci.2012.02.010.
67. Fierro FA, Kalomoiris S, Sondergaard CS, Nolta JA. Effects on proliferation and differentiation of multipotent bone marrow stromal cells engineered to express growth factors for combined cell and gene therapy. Stem Cells. 2011 Nov;29(11):1727–37. doi: 10.1002/stem.720.
68. Beegle JR, Magner NL, Kalomoiris S, Harding A, Zhou P, Nacey C, et al. Preclinical evaluation of mesenchymal stem cells overexpressing VEGF to treat critical limb ischemia. Mol Ther - Methods Clin Dev. 2016 Mar 16;3:16053. doi: 10.1038/mtm.2016.53.
69. Fierro FA, Magner N, Beegle J, Dahlenburg H, Logan White J, Zhou P, et al. Mesenchymal stem/stromal cells genetically engineered to produce vascular endothelial growth factor for revascularization in wound healing and ischemic conditions. Transfusion. 2019 Feb 1;59(S1):893–7. doi: 10.1111/trf.14914.
70. Jin G, Prabhakaran MP, Ramakrishna S. Photosensitive and biomimetic core-shell nanofibrous scaffolds as wound dressing. Photochem Photobiol. 2014;90(3):673–81. doi: 10.1111/php.12238.
71. Yang F, Cho SW, Son SM, Bogatyrev SR, Singh D, Green JJ, et al. Genetic engineering of human stem cells for enhanced angiogenesis using biodegradable polymeric nanoparticles. Proc Natl Acad Sci U S A. 2010 Feb 23;107(8):3317–22. doi: 10.1073/pnas.0905432106.
72. Eleuteri S, Fierabracci A. Insights into the Secretome of Mesenchymal Stem Cells and Its Potential Applications. Int J Mol Sci 2019, Vol 20, Page 4597. 2019 Sep 17;20(18):4597. doi: 10.3390/IJMS20184597.
73. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. Vol. 23, Molecular Therapy. Nature Publishing Group; 2015. p. 812–23. doi: 10.1038/mt.2015.44.
74. Patel DB, Santoro M, Born LJ, Fisher JP, Jay SM. Towards rationally designed biomanufacturing of therapeutic extracellular vesicles: impact of the bioproduction microenvironment. Vol. 36, Biotechnology Advances. Elsevier Inc.; 2018. p. 2051–9.

doi: 10.1016/j.biotechadv.2018.09.001.
75. Hu P, Yang Q, Wang Q, Shi C, Wang D, Armato U, et al. Mesenchymal stromal cells-exosomes: a promising cell-free therapeutic tool for wound healing and cutaneous regeneration. Burn Trauma. 2019 Dec 1;7. doi: 10.1186/s41038-019-0178-8.
76. Jeske R, Bejoy J, Marzano M, Li Y. Human pluripotent stem cell-derived extracellular vesicles: Characteristics and applications. Vol. 26, Tissue Engineering - Part B: Reviews. Mary Ann Liebert Inc.; 2020. p. 129–44. doi: 10.1089/ten.teb.2019.0252.
77. Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. J Transl Med. 2015 Feb 1;13(1). doi: 10.1186/s12967-015-0417-0.
78. Hu Y, Rao SS, Wang ZX, Cao J, Tan YJ, Luo J, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. Theranostics. 2018;8(1):169–84. doi: 10.7150/thno.21234.
79. Guo SC, Tao SC, Yin WJ, Qi X, Yuan T, Zhang CQ. Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. Theranostics. 2017;7(1):81. doi: 10.7150/THNO.16803.
80. Tao S-C, Guo S-C, Li M, Ke Q-F, Guo Y-P, Zhang C-Q. Chitosan Wound Dressings Incorporating Exosomes Derived from MicroRNA-126-Overexpressing Synovium Mesenchymal Stem Cells Provide Sustained Release of Exosomes and Heal Full-Thickness Skin Defects in a Diabetic Rat Model. Stem Cells Transl Med. 2017 Mar;6(3):736–47. doi: 10.5966/sctm.2016-0275.
81. Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, et al. Engineering Bioactive Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Diabetic Wound Healing and Complete Skin Regeneration. Theranostics. 2019;9(1):65. doi: 10.7150/THNO.29766.
82. Bailey AJM, Li H, Kirkham AM, Tieu A, Maganti HB, Shorr R, et al. MSC-Derived Extracellular Vesicles to Heal Diabetic Wounds: a Systematic Review and Meta-Analysis of Preclinical Animal Studies. Stem Cell Rev Reports. 2021; doi: 10.1007/s12015-021-10164-4.
83. Jarrige M, Frank E, Herardot E, Martineau S, Darle A, Benabides M, et al. The Future of Regenerative Medicine: Cell Therapy Using Pluripotent Stem Cells and Acellular Therapies Based on Extracellular Vesicles. Vol. 10, Cells. NLM (Medline); 2021. p. 240. doi: 10.3390/cells10020240.
84. Manandhar S, Kothandan VK, Oh J, Yoo SH, Hwang J, Hwang SR. A pharmaceutical investigation into exosomes. J Pharm Investig 2018 486. 2018 Mar 8;48(6):617–26. doi: 10.1007/S40005-018-0391-7.
85. Bari E, Perteghella S, Di Silvestre D, Sorlini M, Catenacci L, Sorrenti M, et al. Pilot Production of Mesenchymal Stem/Stromal Freeze-Dried Secretome for Cell-Free Regenerative Nanomedicine: A Validated GMP-Compliant Process. Cells. 2018 Nov 1;7(11). doi: 10.3390/CELLS7110190.
86. Mocchi M, Bari E, Marrubini G, Bonda AF, Perteghella S, Tartara F, et al. Freeze-Dried Mesenchymal Stem Cell-Secretome Pharmaceuticalization: Optimization of Formulation and Manufacturing Process Robustness. Pharm 2021, Vol 13, Page 1129. 2021 Jul 23;13(8):1129. doi: 10.3390/PHARMACEUTICS13081129.
87. Safari B, Aghazadeh M, Davaran S, Roshangar L. Exosome-loaded hydrogels: a new

cell-free therapeutic approach for skin regeneration. Eur J Pharm Biopharm. 2021 Nov 15; doi: 10.1016/J.EJPB.2021.11.002.
88. Sabol RA, Bowles AC, Côté A, Wise R, Pashos N, Bunnell BA. Therapeutic Potential of Adipose Stem Cells. Adv Exp Med Biol. 2018;1341:15–25. doi: 10.1007/5584_2018_248.
89. Deptuła M, Brzezicka A, Skoniecka A, Zieliński J, Pikuła M. Adipose-derived stromal cells for nonhealing wounds: Emerging opportunities and challenges. Med Res Rev. 2021 Jul 1;41(4):2130–71. doi: 10.1002/MED.21789.
90. Gentile P, Garcovich S. Advances in Regenerative Stem Cell Therapy in Androgenic Alopecia and Hair Loss: Wnt pathway, Growth-Factor, and Mesenchymal Stem Cell Signaling Impact Analysis on Cell Growth and Hair Follicle Development. Cells. 2019 May 16;8(5):466. doi: 10.3390/CELLS8050466.
91. Gentile P, Alves R, Cole JP, Andjelkov K, Van Helmelryck T, Fernandez J, et al. AIRMESS–Academy of International Regenerative Medicine & Surgery Societies: recommendations in the use of platelet-rich plasma (PRP), autologous stem cell-based therapy (ASC-BT) in androgenetic alopecia and wound healing. Expert Opin Biol Ther. 2021;21(11):1443–9. doi: 10.1080/14712598.2021.1908995.
92. Huayllani MT, Sarabia-Estrada R, Restrepo DJ, Boczar D, Sisti A, Nguyen JH, et al. Adipose-derived stem cells in wound healing of full-thickness skin defects: a review of the literature. J Plast Surg Hand Surg. 2020 Oct 1;54(5):263–79. doi: 10.1080/2000656X.2020.1767116.
93. Levin LS. The Reconstructive Ladder: An Orthoplastic Approach. Orthop Clin North Am. 1993 Jul 1;24(3):393–409. doi: 10.1016/S0030-5898(20)31815-0.
94. Huayllani MT, Ruiz-Garcia H, Boczar D, Avila FR, Lu X, Rinker BD, et al. Adipose-Derived Stem Cells Therapy for Radiation-Induced Skin Injury. Ann Plast Surg. 2021 Dec;87(6):639–49. doi: 10.1097/SAP.0000000000003039.
95. Gentile P, Kothari A, Casella D, Calabrese C. Fat Graft Enhanced With Adipose-Derived Stem Cells in Aesthetic Breast Augmentation: Clinical, Histological, and Instrumental Evaluation. Aesthetic Surg J. 2020 Aug 14;40(9):962–77. doi: 10.1093/ASJ/SJZ292.
96. Ni X, Shan X, Xu L, Yu W, Zhang M, Lei C, et al. Adipose-derived stem cells combined with platelet-rich plasma enhance wound healing in a rat model of full-thickness skin defects. Stem Cell Res Ther. 2021 Dec 6;12(1):226. doi: 10.1186/s13287-021-02257-1.
97. Nie C, Zhang G, Yang D, Liu T, Liu D, Xu J, et al. Targeted delivery of adipose-derived stem cells via acellular dermal matrix enhances wound repair in diabetic rats. J Tissue Eng Regen Med. 2015 Mar 1;9(3):224–35. doi: 10.1002/term.1622.
98. U.S. Food and Drug Administration (FDA). FDA Guidance Dcoument : Evaluation of Devices Used with Regenerative Medicine Advanced Therapies Guidance for Industry February 2019. 2019. Accessible from: https://www.fda.gov/media/120266/download.
99. U.S. Food and Drug Administration (FDA). Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use. 2020. Accessible from: https://www.fda.gov/media/109176/download.
100. Gentile P, Calabrese C, De Angelis B, Pizzicannella J, Kothari A, Garcovich S. Impact of the Different Preparation Methods to Obtain Human Adipose-Derived Stromal Vascular Fraction Cells (AD-SVFs) and Human Adipose-Derived Mesenchymal Stem Cells (AD-MSCs): Enzymatic Digestion Versus Mechanical Centrifugation. Int J Mol Sci.

2019 Nov 1;20(21). doi: 10.3390/IJMS20215471.
101. Sato Y, Bando H, Di Piazza M, Gowing G, Herberts C, Jackman S, et al. Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider. Cytotherapy. 2019 Nov 1;21(11):1095–111. doi: 10.1016/J.JCYT.2019.10.001.
102. U.S. Food and Drug Administration (FDA). Guidance for Industry Preclinical Assessment of Investigational Cellular and Gene Therapy Products. 2013. Accessible from: https://www.fda.gov/media/87564/download.
103. Cellular, Tissue and GTAC (CTGTAC) M #45. Cellular Therapies Derived from Human Embryonic Stem Cells - Considerations for Pre-Clinical Safety Testing and Patient Monitoring. 2008. Accessible from: http://www.fda.gov/ohrms/dockets/ac/08/briefing/2008-0471B1_1.pdf.
104. Ben-David U, Nudel N, Benvenisty N. Immunologic and chemical targeting of the tight-junction protein Claudin-6 eliminates tumorigenic human pluripotent stem cells. Nat Commun 2013 41. 2013 Jun 18;4(1):1–8. doi: 10.1038/ncomms2992.
105. Ben-David U, Benvenisty N. Chemical ablation of tumor-initiating human pluripotent stem cells. Nat Protoc 2014 93. 2014 Feb 27;9(3):729–40. doi: 10.1038/nprot.2014.050.
106. Wuputra K, Ku C-C, Wu D-C, Lin Y-C, Saito S, Yokoyama KK. Prevention of tumor risk associated with the reprogramming of human pluripotent stem cells. J Exp Clin Cancer Res. 2020 Dec 3;39(1):100. doi: 10.1186/s13046-020-01584-0.
107. Malecki M. “Above all, do no harm”: Safeguarding pluripotent stem cell therapy against iatrogenic tumorigenesis. Stem Cell Res Ther. 2014 Jun 3;5(3):1–10. doi: 10.1186/SCRT462/TABLES/1.
108. Ho J, Walsh C, Yue D, Dardik A, Cheema U. Current Advancements and Strategies in Tissue Engineering for Wound Healing: A Comprehensive Review. Adv Wound Care. 2017 Jun 1;6(6):191. doi: 10.1089/WOUND.2016.0723.
109. Gordillo GM, Bernatchez SF, Diegelmann R, Di Pietro LA, Eriksson E, Hinz B, et al. Preclinical Models of Wound Healing: Is Man the Model? Proceedings of the Wound Healing Society Symposium. Adv Wound Care. 2013 Feb;2(1):1. doi: 10.1089/WOUND.2012.0367.
110. Zhang Q, Sito L, Mao M, He J, Zhang YS, Zhao X. Current advances in skin-on-a-chip models for drug testing. Microphysiological Syst. 2018;2:1–1. doi: 10.21037/MPS.2018.08.01.
111. Risueño I, Valencia L, Jorcano JL, Velasco D. Skin-on-a-chip models: General overview and future perspectives. APL Bioeng. 2021 Jul 8;5(3):030901. doi: 10.1063/5.0046376.
112. Mori N, Morimoto Y, Takeuchi S. Skin integrated with perfusable vascular channels on a chip. Biomaterials. 2017 Feb 1;116:48–56. doi: 10.1016/J.BIOMATERIALS.2016.11.031.
113. Wufuer M, Lee GH, Hur W, Jeon B, Kim BJ, Choi TH, et al. Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Reports 2016 61. 2016 Nov 21;6(1):1–12. doi: 10.1038/srep37471.
114. Rogal J, Zbinden A, Schenke-Layland K, Loskill P. Stem-cell based organ-on-a-chip models for diabetes research. Adv Drug Deliv Rev. 2019 Feb 1;140:101–28. doi: 10.1016/J.ADDR.2018.10.010.
115. Shu X, Shu S, Tang S, Yang L, Liu D, Li K, et al. Efficiency of stem cell based therapy in the treatment of diabetic foot ulcer: a meta-analysis. Endocr J. 2018;65(4):403–13.

doi: 10.1507/ENDOCRJ.EJ17-0424.