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TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Concise Review: Human Dermis as an Autologous Source of Stem Cells for Tissue Engineering and Regenerative Medicine

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ABSTRACT

The exciting potential for regenerating organs from autologous stem cells is on the near horizon, and adult dermis stem cells (DSCs) are particularly appealing because of the ease and relative minimal invasiveness of skin collection. A substantial number of reports have described DSCs and their potential for regenerating tissues from mesenchymal, ectodermal, and endodermal lineages; however, the exact niches of these stem cells in various skin types and their antigenic surface makeup are not yet clearly defined. The multilineage potential of DSCs appears to be similar, despite great variability in isolation and in vitro propagation methods. Despite this great potential, only limited amounts of tissues and clinical applications for organ regeneration have been developed from DSCs. This review summarizes the literature on DSCs regarding their niches and the specific markers they express. The concept of the niches and the differentiation capacity of cells residing in them along particular lineages is discussed. Furthermore, the advantages and disadvantages of widely used methods to demonstrate lineage differentiation are considered. In addition, safety considerations and the most recent advancements in the field of tissue engineering and regeneration using DSCs are discussed. This review concludes with thoughts on how to prospectively approach engineering of tissues and organ regeneration using DSCs. Our expectation is that implementation of the major points highlighted in this review will lead to major advancements in the fields of regenerative medicine and tissue engineering. STEM CELLS TRANSLATIONAL MEDICINE *2015;4:1–12*

SIGNIFICANCE

Autologous dermis-derived stem cells are generating great excitement and efforts in the field of regenerative medicine and tissue engineering. The substantial impact of this review lies in its critical coverage of the available literature and in providing insight regarding niches, characteristics, and isolation methods of stem cells derived from the human dermis. Furthermore, it provides analysis of the current state-of-the-art regenerative approaches using human-derived dermal stem cells, with consideration of current guidelines, to assist translation toward therapeutic use.

INTRODUCTION

With biomedical research poised to achieve human tissue and organ regeneration, there is an increasing demand for autologous adult stem cell-based therapies. In recognition of this demand, the U.S. Food and Drug Administration (FDA) has published guidelines for somatic cell therapies, detailing the importance of evaluating cell identity, potency, viability, sterility, purity, and general safety [1]. In addition to the well-studied use of dermal fibroblasts as starting material for the production of induced pluripotent stem cells, mounting evidence in the last decade shows that the dermis can provide an accessible and abundant source of adult stem cells [2–6]. Moving toward implementing FDA guidelines into engineering tissues from dermal stem cells (DSCs), it is important to fully characterize and specifically target a particular population, or possibly populations, of DSCs to consistently achieve efficacy, potency, purity, safety, and viability of these cells. After complying with these steps, tissue engineering approaches can be applied toward creating functional organs and tissues from DSCs.

Various groups have performed extensive work focusing on isolation, characterization, and in vitro propagation of DSCs [2–6]. Excitingly, it has been demonstrated that DSCs have potential to differentiate not only along mesenchymal lineages [2–6] but also along the ectodermal

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[4, 7] and endodermal lineages [8]. Differentiation toward these lineages, however, has been shown mostly at the individual cell level or, at most, on small clusters of cells. Moving toward clinically applicable tissue implants, it is prudent to explore how the goal of engineering large quantities of functional tissues may occur using DSCs.

This review discusses recent advances in our understanding of adult stem cells of the dermis, including niche identification and isolation, known characteristic markers, and differentiation capacity. A critical analysis of the methods commonly used for demonstrating multilineage potential discusses their advantages and limitations. Furthermore, DSCs' potential in regenerative medicine, use in tissue engineering, and other possible applications are addressed. This review concludes with suggestions and a vision of how the regeneration of tissues and organs might occur using DSCs.

Anatomy, Embryonic Origin, and Regenerative Potential of Dermis

Prior to discussing the niches in which DSCs reside, it is important to understand the anatomy and embryonic origin of dermis as a whole. DSCs derived from dermis from separate anatomical regions can then be classified by their embryonic origin, yielding hints to their behavior and potency for differentiation.

Integument or skin, the largest organ of the human body, is composed of the epidermis, dermis, and hypodermis [9]. Dermis is distinguished histologically as early as week 6 of human development [10]. During embryonic development, dermis is derived from mesenchyme of three sources: (a) the lateral plate mesoderm, which supplies cells for dermis in the limbs and body wall; (b) the paraxial mesoderm, which supplies cells that form dermis of the dorsum or back; and (c) the neural crest cells, which form the dermis of the face and neck [11] (Fig. 1). Despite differences in origin, the histologic appearance of adult dermis is similar across the body, with the principal cell of dermis being a fibroblast [9]. Despite its large size (the dermis is approximately 10 times thicker than the overlying epidermis), the dermis receives relatively scant attention in the literature. Excitingly, more light was shed recently on various lineages of dermal fibroblasts [12] and their contribution to wound healing [13]. From these works, it is clear that the dermis and its resident fibroblasts are more heterogeneous than was previously thought. Thorough understanding of different fibroblast phenotypes can aid significantly in our understanding of fibrotic processes not only in the skin but also in other organs. Furthermore, it is now recognized that cross-talk between fibroblasts and epidermal structures is crucial in directing the epithelial cell toward specific phenotypes [14, 15].

Although used uniformly among scientists, the term "fibroblast" is somewhat misleading because the cells residing in the adult dermis are terminally differentiated and minimally active metabolically [9]. By definition, a "blast cell" is an immature cell or a precursor cell that is in the earliest stage of development in which it is recognizably committed to developing along a particular cell lineage [16]. Perhaps a more appropriate term for the adult dermal fibroblast would be the "fibrocyte." In this review, however, the term "fibroblast" is applied to the mature cells residing in noninjured dermis to be consistent with the published literature.

Skin is capable of completely restoring itself histologically within 4 weeks following a small injury [17]. This important regenerative capacity is made possible by stem cells residing in various niches of the skin and by stem cells recruited to the injury site by growth factors and cytokines [18]. Epidermal regeneration and detailed description of epidermal stem cell niches is beyond the scope of this review because these topics have been reviewed elsewhere [19–21]. Traditionally, it was thought that dermal regeneration following a wound is achieved by homeostatic expansion of fibroblast/myofibroblast populations derived from resident tissue cells [18]; however, over the past 10–15 years, mounting evidence has suggested that fibroblasts/myofibroblasts also may be derived from a variety of other sources. These sources include dedifferentiation of epithelial cells by a process known as "epithelial-mesenchymal transition" as well as bone marrow- and tissue-derived mesenchymal stem cells [18, 22, 23]. Furthermore, plasticity and contribution of hair follicle cells to dermal wound healing was also demonstrated [24]. Consequently, the precise source of the cells responsible for dermal repair remains elusive, especially in relation to the known dermal or nondermal stem cell niches. It is likely that stem cells from multiple niches contribute to dermal regeneration.

The Niches of Dermal Stem Cells

Dermal stem cells identified thus far reside in a number of distinct niches. Historically, among the first niches described were the hair follicle dermal papilla (HFP) and the connective tissue dermal sheath (DS) [25–28] (Fig. 2). HFP- and DS-associated cells have potential to differentiate along multiple mesenchymal [28, 29] and neuronal and glial lineages [3].

A traditionally considered epidermal stem cell niche, the hair follicular bulge region, has recently been demonstrated to contain stem cells of neural crest origin (e.g., human epidermal neural crest stem cells [EPI-NCSCs]) [30–32]. Although precise localization of these cells has yet to be determined, they were reported to be located "by the epidermal outer root sheath" and were strongly associated with the dermal sheath of the hair follicle bulge area [32]. These cells were capable of differentiating into all major neural crest derivatives, including bone, cartilage, neurons, Schwann cells, myofibroblasts, and melanocytes [32, 33] (Fig. 2).

The isolation of cells capable of multilineage differentiation from glabrous skin (hairless), such as foreskin [5, 34], raises the hypothesis that hair follicular niches are probably not the only source of dermal stem cells. CD146-positive (CD146+) dermal vascular pericytes from different anatomic locations of human skin, including foreskin, (Fig. 2) have been shown to differentiate along adipogenic, chondrogenic, and osteogenic lineages [35]. The perivascular niche was also recognized as harboring stem cells in adipose tissue, placenta, skeletal muscle, and pancreas, among others [2, 35, 36].

Cells residing in stroma of sweat glands (Fig. 2) recently joined the list of multipotent stem cells of the dermis [37]. These cells were capable of differentiating along adipogenic, osteogenic, and chondrogenic lineages [37].

It is evident that multiple stem cell niches exist in human dermis, and cells residing in them possess capacity of differentiation into various lineages, potentiating their use in tissue and organ regeneration besides the skin. Importantly, determination of characteristics uniform to all of these cells would aid our understanding of their biology and provide a useful tool for isolation and purification of these cells from dermis.

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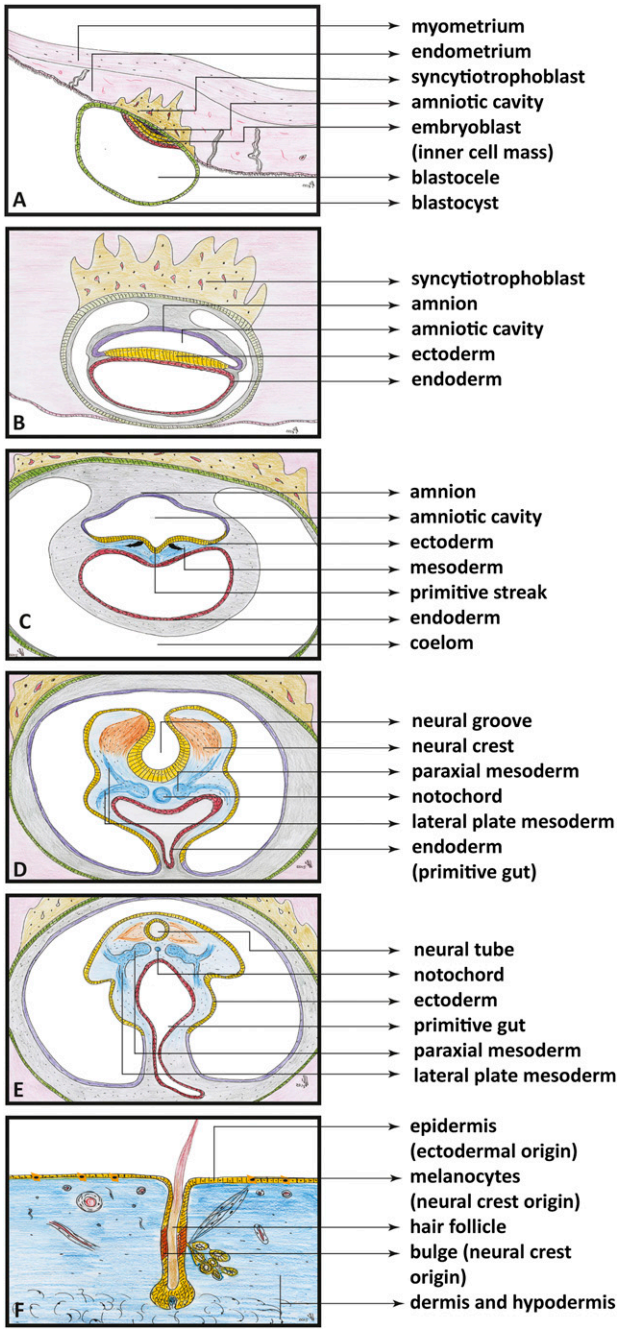

Figure 1. The developmental steps from the blastocyst stage to the development of somites, lateral line mesenchyme, and neural crest. **(A):** Day 8 of development. Blastocyst implantation into uterine mucosa is shown. **(B):** Days 8–14 of development. Amniotic cavity enlarges, ectoderm becomes apparent, and endoderm completely covers the cavity of primitive gut (yolk sac cavity). **(C):** Days 15–19 of development. Primitive streak appears, indicating the beginning of gastrulation, in which the third layer of the embryo forms (the mesoderm). Formation of mesoderm occurs through a process of cellular migration of ectodermal cells downward (curved arrows) at the level of the primitive streak. **(D):** Days 19–21 of development. Ectoderm folds in to form a neural groove. Before complete closure of the neural groove, a group of cells detach to form a neural crest. Mesodermal plate divides into notochord, paraxial mesoderm (future somites), intermediate mesoderm (future nephros), and lateral plate mesoderm. **(E):** Day 48 of development. The neural groove is now closed (neural tube); the mesoderm further expands and develops to give rise to muscles, bones, fat, and dermis. **(F):** Mature skin. The adult skin components are represented in colors matching their embryonic origin as shown in previous panels. Despite uniform histological appearance, the dermis of the head is of neural crest origin, the dermis of the dorsal skin is of paraxial mesoderm origin, and the dermis of the body wall and extremities is of lateral plate origin. The epidermis throughout the body is of ectodermal origin. The melanocytes and the stem cells of the bulge portion of the hair follicle are of neural crest origin.

Methods of Isolation and Purification of Stem Cells From Dermis and Their Unique Characteristics

Unlike the bone marrow's mesenchymal stem cells, for which consensus criteria have emerged [38], isolation methods and phenotypic characteristics of DSCs are more heterogeneous (Table 1). In this section, the most commonly used methods of DSC isolation and their characteristics are discussed. They include selective culturing, microdissection, and immunosorting using surface markers.

Selection of progenitors by specific culture conditions is one of the techniques used for isolation of stem cells from the dermis. This strategy, used for the isolation of skin progenitors (SKPs) [5], involves enzymatic digestion of dermis as a whole to obtain individual cells. Selection of progenitor cells follows by placing the whole dermal population in serum-free "neurosphere" growth medium that facilitates SKP growth in spherical aggregates [5]. Characterization of SKPs revealed consistent expression of nestin, fibronectin, and vimentin proteins [39] (Table 1). For facial-haired skin, in vivo location of SKPs was traced to follicular papilla and, further, to the neural crest origin [39]. Nonetheless, cells expressing the same set of surface markers and exhibiting identical multipotency have also been isolated from non haired glabrous skin, such as foreskin [40], and the exact niches for these cells remain to be determined [40].

A microdissection technique is used for the isolation of human bulge cells, in which the bulge portion of the hair follicle is selectively excised from the bulk skin. Explanted bulge tissues are then cultured in conditions that facilitate stem cells to "migrate out" and adhere to the substrate to yield human EPI-NCSCs [32, 41]. Because these cells that migrate out were demonstrated to be CK15 negative, it is possible that their migratory ability separates them from CK15-positive stem cells that are also known to reside in the bulge of hair follicles [19]. Stem cells from the stromal component of the sebaceous gland are also isolated using microdissection [37]. Markers characteristic of these two cell types are summarized in Table 1. Interestingly, nestin is expressed by both bulge stem cells and sebaceous stromal stem cells. The main advantage of this technique is that it provides a more precise definition of the niche from which the stem cells originate than bulk enzymatic digestion of the whole dermis; however, microdissection is tedious and yields fewer primary cells [32, 37]. Consequently, the feasibility of this isolation technique for tissue engineering, for which large quantities of cells are required, may be limited.

Another popular isolation technique relies on selection of cells based on expression of specific surface markers via immunosorting. This technique is particularly intriguing because if the markers chosen are truly DSC specific and are not found on other cell types of the skin, this approach can potentially simplify the isolation and purification of DSCs. It was shown, for example, that CD271+ cells isolated and purified by magnetic immunosorting had significantly higher potential to differentiate along all three mesenchymal lineages [6].

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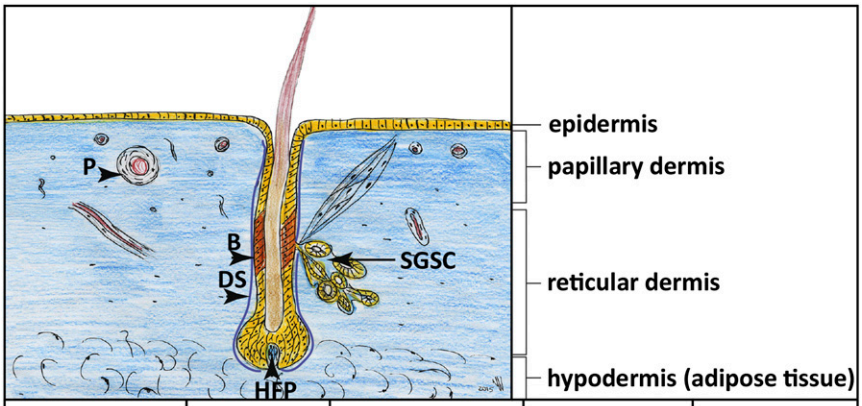

HFP	DS	B	P	SGSC
Osteogenic, Adipogenic, Chondrogenic, Neuronal, Glial	Osteogenic, Adipogenic	Osteogenic, Chondrogenic, Myofibroblastic, Melanocytic, Neuronal, Schwann cell	Osteogenic, Chondrogenic, Adipogenic, Myogenic	Osteogenic, Chondrogenic, Adipogenic

Figure 2. Dermal stem cell niches and the potential of stem cells residing in them to differentiate along different lineages. Schematic of skin section containing a hair follicle. The chart lists major tissue lineages that were derived from dermis stem cells according to niche (HFP [3, 5, 28], DS [28], B [32], P [2, 35], and SGSC [37]). Abbreviations: B, hair follicle bulge; DS, dermal sheath; HFP, hair follicle papilla; P, pericytes; SGSC, sebaceous gland stromal stem cells.

Table 1. Summary of the most common human dermal stem cell niches with a brief description of stem cell isolation method and relevant markers

Niche	Method of stem cell isolation	Surface markers	Intracellular markers	Citation
Hair follicle papilla	Enzymatic digestion of dermis as a whole → selective spherical culture	CD90+, CD29+, CD44+, CD49e+, CD54+, CD160+, CD34−	Nestin, vimentin, fibronectin	[5]
Hair follicle bulge	Microdissection → explant culture → 2D expansion	LGR6+, CD34+, CK15−	ETS1, MSX2, THOP1, CRMP1, UBE4B, MYO10, CRYAB, nestin, ADAM 12, SOX10	[32, 119]
Sweat gland stromal stem cells	Microdissection → explant culture → 2D expansion	Iα6	Nestin, GFAP, A-smooth muscle actin, vigilin	[37]
Perivascular stem cells	Enzymatic digestion → immunologic sorting	HD-1+ ($\alpha1\beta1$ integrin), CD146+, CD73+, CD90+, CD105+, CD31−, CD45−	Nestin, vimentin	[2, 35, 36, 42]

Abbreviations: 2D, two-dimensional; GFAP, glial fibrillary acidic protein.

Similarly, CD146+ cells isolated by immunosorting could be differentiated along all three mesenchymal lineages and were traced to reside in the perivascular niche [2, 35]. These CD146+ pericytes also expressed other mesenchymal stem cell markers, such as CD90, CD73, and CD105 [2]. Along similar lines, multilineage potential was demonstrated by pericytes selected with $\alpha1\beta1$ integrin-specific antibody HD1 [42]. Considering the various markers that have been investigated, sorting with double and triple labeling of surface markers may be an even more precise way of isolating DSCs, especially because coexpression of markers has been observed (e.g., CD73/CD90, CD271/CD73, CD73/CD105) [6]. In addition, it is possible for stem cells residing in different niches to express similar markers, allowing stem cells from multiple niches to be targeted at the same time, leading to greater cell yields.

Demonstration of the Multilineage Potential of DSCs

A complete analysis of the DSC literature should offer a critical look at whether it has been sufficiently shown that DSCs are, indeed, stem cells. One of the prevailing definitions of stemness is the capability for self-renewal and multilineage differentiation [43]. Many methods are used to demonstrate multilineage potential of stem cells, and each has its limitations and advantages. In this section, the most common methods used for examining the stemness of DSCs are discussed.

The ability to clonally expand is one of the methods commonly used to demonstrate stemness [44]. Terminally differentiated cells will senesce after several divisions, whereas stem cells will continue to replicate and maintain their multilineage differentiation ability [45]. The ability to form colonies has been

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demonstrated by stem cells derived from the hair follicle bulge and papilla [5, 32] and by pericytes from multiple tissue types [2]. Although dermal pericytes are likely capable of clonal expansion, this trait has yet to be demonstrated [36]. Because clonal behavior is also a feature of unsorted dermal fibroblasts [46] and neoplastic cells [47, 48], additional methods are necessary for demonstrating stemness.

Another common method to demonstrate stemness relies on expression of transcription factors commonly identified in embryonic stem cells. Transcription factors expressed by embryonic stem cells such as NANOG, OCT4, KLF4, and SOX2 are usually assayed [43, 45]. Reverse transcription polymerase chain reaction and immunofluorescence are the most commonly used techniques. Regardless of the technique used, signal intensity is the key, and it is important to compare the signal intensity of embryonic transcription factor expression in a novel population relative to a population of actual embryonic stem cells. This is critical because dermal fibroblasts without any particular stem cell selection have been shown to express these transcription factors when cultured in vitro [46, 49]. Unfortunately, only a few publications have compared signal intensity between populations of isolated DSCs with embryonic stem cells [32].

Another method frequently used to demonstrate stemness is to provide evidence of multilineage differentiation. This is achieved by seeding cells in culture conditions containing lineage-inductive factors. Based on the definition of mesenchymal stem cells (MSCs), differentiation into chondrocytes, adipocytes, and osteocytes needs to be demonstrated for multilineage potential [38]. This is often accomplished using histochemistry assays. Although all studies describing DSCs routinely include various combinations of these assays, they often omit a comparison of morphological traits to those of the target tissues. Likewise, immunohistochemistry (IHC) results, if provided, are often incomplete, at times lacking appropriate positive and negative controls. These considerations should be made for all stem cell studies, not just those using DSCs.

Most commonly, adipogenic differentiation is demonstrated by Oil Red O staining of intracellular fat globules. Although Oil Red O was validated for staining of triglycerides and cholesteryl oleate in tissue culture, this method is not specific for adipose cells. This is because the presence of intracytoplasmic fat droplets is not specific for adipocytes (Fig. 3). Many cells are known to accumulate lipid droplets, especially under compromised metabolic conditions [50]; therefore, methods more specific for demonstrating adipocytic differentiation are desired. Immunohistochemistry for adiponectin [51] or leptin [52] (proteins produced exclusively by adipocytes), for example, can be used in the differentiation assays instead of or in addition to Oil Red O. Alternatively, adiponectin expression can be demonstrated by flow cytometry [53].

Osteogenic differentiation is traditionally demonstrated by von Kossa [54] or Alizarin red staining. As with Oil Red O, von Kossa is a nonspecific tissue stain used to demonstrate the presence of a variety of calcium, chloride, phosphate, sulfate, and carbonate salts [54]. By itself, von Kossa is not sufficient to demonstrate formation of bone in vitro [55] (Fig. 3). Likewise, Alizarin red, used for identification of calcium deposits, is not sufficient to claim bone formation [56, 57]. Osteogenic differentiation should be supported by demonstration of bone-specific matrix proteins or proteins exclusively produced by osteoblasts, such as osteocalcin [58], along with demonstration of characteristic tissue morphology, such as osteocytes encased by osteoid (unmineralized organic portion of the bone matrix). Osteocalcin expression by IHC, for example, was successfully demonstrated on osteoinduced dermal fibroblasts seeded onto synthetic scaffolds [59]. Presentation of the osseous morphology (e.g., through hematoxylin and eosin staining) would further support the target tissue phenotype.

Finally, chondrogenic differentiation is classically obtained by Alcian blue staining of micromasses or pellets induced by culturing in chondrogenic media; however, Alcian blue is not specific for cartilage. At pH 2.5, Alcian blue stains sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins). At pH 1, it stains mainly sulfated mucopolysaccharides and sulfated sialomucins. Many tissues beside cartilage contain Alcian blue-positive mucopolysaccharides (Fig. 3). A more reliable method to demonstrate cartilage differentiation would be demonstration of immunoreactivity for cartilage-specific matrix proteins, such as collagen type II, with simultaneous demonstration of a lack of immunoreactivity for collagen type I. The latter was used for demonstration of chondrogenic differentiation of DSCs from goat dermis [60].

To summarize, histological assays, although often used, can potentially lead to erroneous conclusions and thus cannot be used as the singular basis for determination of lineage differentiation. Instead or in addition to histochemical techniques, more specific IHC, immunofluorescence, or enzyme-linked immunosorbent assay methods need to be used. In this context, it is important to emphasize that IHC need to be performed on thinly sectioned specimens with appropriate positive and negative tissue controls. A common mistake, for example, is to use a sample with no primary antibody labeling as a single negative control; although a good control for unspecific background labeling by secondary antibodies, it is not sufficient just by itself. An additional negative control should be a tissue known to have no expression of examined marker [61]. The studies describing DSCs include various assays, but a cautious approach should be followed in interpreting results based on positive Alcian blue, von Kossa, and Oil Red O stains without the morphologies characteristic of cartilage, bone, and fat. Because collagen type II is rarely found in tissues aside from cartilage, it should be used more often in showing a chondrogenic phenotype.

Safety and Biocompatibility

In establishing regulatory processes for stem cell-derived products, the FDA has compiled guidance documents [1]. In these documents, stringent requirements need to be applied to ensure the safety of stem cell-derived products. The cell type, purity, potential introduction of adventitious infectious agents, site-specific integration, tumorigenicity, potency, and duration of effect are the main safety concerns when dealing with autologous stem cell-based therapies.

Neoplastic or malignant transformation is a legitimate concern when dealing with cells capable of self-renewal and multilineage differentiation. Because classic embryonic stem cell markers such as SOX2, KLF4, and OCT4 are often overexpressed in malignant tumors [62], caution must be used when using cells expressing these markers. Although not reported for DSCs, it has been demonstrated that human multipotent embryonic cells are prone to acquire karyotypic aberrations when cultured in vitro and have the potential to form teratomas on in vivo implantation [63–67]. Furthermore, several reports indicated malignant transformation of bone marrow-derived MSCs following long-term

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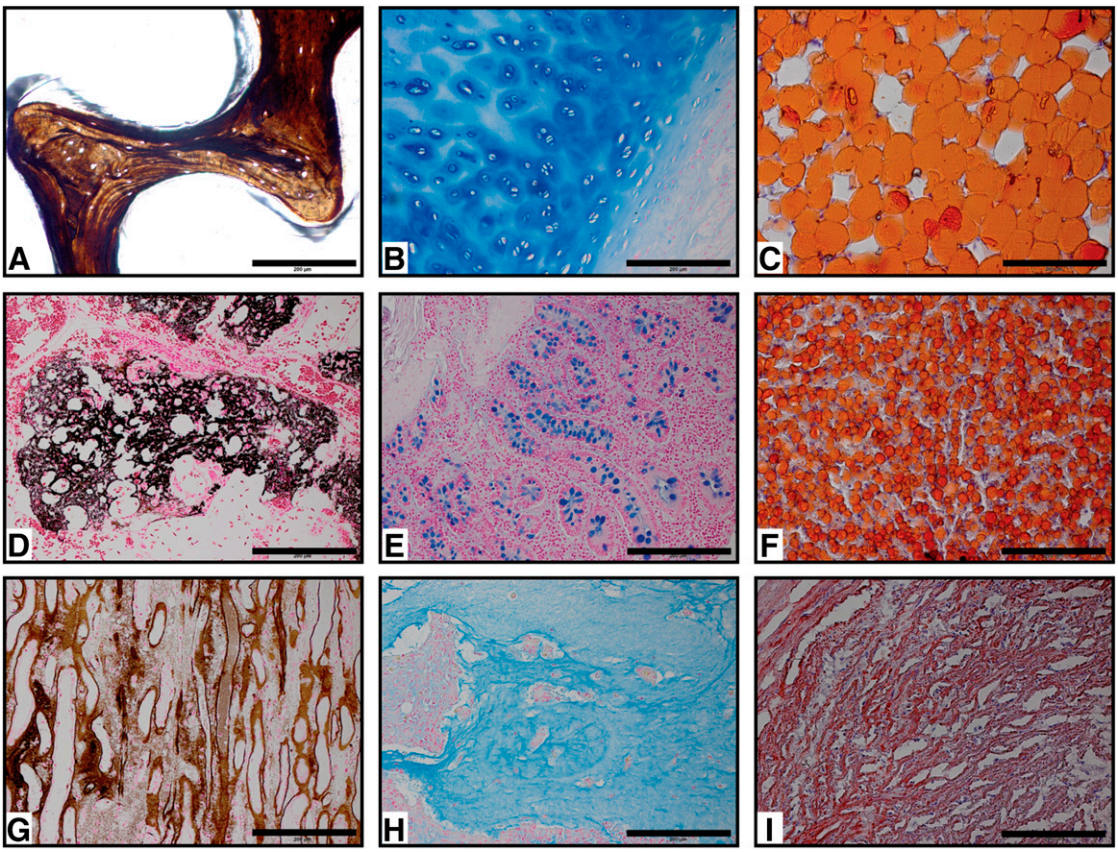

Figure 3. Histochemical stains are not specific enough for demonstration of lineage differentiation. **(A–C):** Examples of native bone, cartilage, and adipose tissues stained with von Kossa, Alcian blue, and Oil Red O, respectively. **(A):** A nondecalcified equine trabecular bone embedded in Technovit resin, stained with von Kossa. **(B):** Tracheal bovine cartilage stained with Alcian blue (pH 1) and counterstained with nuclear fast red. **(C):** A cryosection of human adipose tissue stained with Oil Red O. **(D):** Avian lung affected by metastatic mineralization, stained with von Kossa and counterstained with nuclear fast red. **(E):** Normal canine colon stained with Alcian blue (pH 1) and counterstained with nuclear fast red, highlighting the goblet cells filled with mucopolysaccharides. **(F):** A cryosection of bovine liver affected by severe lipidosis, stained with Oil Red O. **(G):** Canine kidney affected by severe tubular mineralization, stained with von Kossa. **(H):** Canine oral neoplasm with myxomatous metaplasia, stained with Alcian blue (pH 1) and counterstained with nuclear fast red. **(I):** A cryosection of atherosclerotic plaque from a blood vessel of a primate, stained with Oil Red O. Scale bars = 200 μm.

culture ex vivo [68, 69]. The changes most frequently reported are gain of chromosomes 12, 17, and X (aneuploidy), but other karyotypic changes also have been reported [65, 67, 70, 71]. To address these concerns, a nude mouse model for assessment of the biosafety profile of stem cell-derived cell transplants has been developed [72].

An additional safety concern is related to the fibroblast contamination of dermal stem cell cultures because fibroblasts are the principal and most abundant cells in the dermis. Although fibroblasts may have the potential to be clonally expanded [46], to be differentiated along all three mesenchymal lineages [46, 73–75], and to express embryonic stem cell markers [49], these cells have also been reported to malignantly transform in culture conditions by acquiring chromosomal aberrations and aneuploidy [76]. It would be ideal to identify and remove fibroblasts in the early stages of DSC isolation. The main challenge in negative selection of these cells is in cross-reactivity of their surface markers with other, potentially desirable cell types; it remains to be determined whether DSCs also express the same markers. Multiple groups have recognized the need for identification of fibroblasts and markers specific to fibroblasts (Table 2).

An appeal of using DSCs in tissue engineering is the generation of autologous implants. Although adventitious agents are less of a concern when autologous cells are used, they can nonetheless be introduced during the manufacturing process. When nonautologous feeder cell lines are used, for example, there is an increased risk of introducing adventitious agents; therefore, feeder cell lines must be screened for potential pathogens. In addition, animal-derived products routinely used for cell culture, such as fetal bovine serum (FBS), may contain prion pathogens [77] if not properly certified or can potentially provoke an immune response [78]. These concerns are addressed by constant improvement and development of good manufacturing practice (GMP) techniques. Although not yet optimal for large-scale expansion, serum-free and xeno-free GMP techniques have been developed and tested on bone marrow-derived and adipose-derived stem cells [79]. FBS can be used in clinical expansion of MSCs in GMP facilities if it is properly certified. It would be important to apply similar GMP techniques to DSCs; however, because of the wide variety of niches from which DSCs may be derived and the number of isolation and culture techniques that currently exist for isolating DSCs, scale-up of DSCs faces different challenges than other, more well-characterized stem cells. For use

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Table 2. Examples of markers reported to be fibroblast specific and their cross-reactivity with other cell types

Marker	Intracellular/surface expression	Cross-reactivity with other cell types present in the dermis
FSP-1 (S100A4) [120]	Surface expression	Tissue macrophages [121]
AminopeptidaseN (CD13) [122]	Surface expression	Endothelial cells [123]
HSP47 [124]	Intracellular	Not reported
TE-7 [125]	Surface	Basal epidermal cells
1B10 [126]	Surface	Tissue macrophages and circulating monocytes [126]
Prolyl-4-hydroxylase (5B5) [125]	Intracellular (ER)	Myoepithelial cells, acinar cells, plasma cells, dendritic cells [127]
CD26/Dpp4 [12, 13]	Surface	T cells [128]
DLK1 [12]	Surface, neonatal dermis only	Not reported
PDGFRα, CD140A [12]	Surface	Adipose-derived stem cells, pericytes [129, 130]

In the Marker column, each fibroblast marker is listed in relation to the group that described it. References to other groups that described the same marker in cell types other than fibroblasts are provided in the column entitled, "Cross-reactivity with other cell types present in the dermis." Because surface expression is generally preferred for live cell selection, the column labeled "Intracellular/surface expression" specifies whether the marker is expressed on the surface or intracellularly. None of the markers listed in this table has yet been reported for dermal skin cells.
Abbreviations: DLK1, delta-like homolog 1; Dpp4, dipeptidyl peptidase 4; ER, endoplasmic reticulum; FSP-1, Fibroblast specific protein 1; PDGFRα, platelet-derived growth factor receptor α.

in therapy, sufficient MSCs and adipose-derived stem cells can be isolated by their ability to adhere to plastic combined with verification of their phenotype by flow cytometry [80]. Scale-up and improvements of the process can proceed focusing on this isolation methodology. As this review shows, there are many ways to isolate ad grow DSCs from their various niches. Until uniform isolation and culture methods can be identified for DSCs, efforts for scale-up may lack focus and efficiency. Better characterization of these cells and development of more uniform culturing methodologies that, ideally, target more than one niche would be of great benefit to the field.

DSCs in Tissue Engineering and Regenerative Medicine

As discussed previously, DSCs can be differentiated into multiple lineages. Although researchers have yet to take advantage of the full potential of these cells, exciting work is ongoing in vitro and in vivo to translate DSC-derived products to clinical use. This section presents and discusses examples of recent advancements of DSC use in regenerative medicine research.

Both terminally differentiated dermal cells and stem cells have been used extensively in skin and hair follicle regeneration. The FDA-approved product Apligraf (Organogenesis, Canton, MA, http://www.organogenesis.com) consists of dermal fibroblasts seeded onto collagen type I and overlaid by keratinocytes and showed promising results in clinical trials for treatment of chronic skin wounds associated with diabetes [81]. Many current efforts also use HFP and bulge cells (Fig. 2) to generate hair. Generation of hair follicles, for example, was achieved in a rat model by autologous whisker-derived HFP cells that were expanded in vitro into multilayered sheets and then implanted subepidermally [82]. Likewise, human hair follicle neogenesis has been shown by subepidermal implantation of HFP cells that were expanded in three dimensions. Interestingly, HFP cells cultured in two dimensions were not capable of follicle neogenesis [83]. Hair follicles were also successfully generated by implantation of rat vibrissae bulge stem cells [84]. Bulge stem cells from one hair follicle were sufficient to generate multiple hair follicles, sebaceous glands, and interfollicular epidermis. Using both HFP- and bulge-derived stem cells, full-thickness human skin grafts have been generated by seeding onto decellularized porcine dermal scaffolds [85]. Grafted onto nude mice, successful integration and formation of hair buds were observed [85]. Microdissection to harvest HFP and bulge cells can be laborious, however, and regeneration has also been attempted with other DSC types. Formation of hair follicles, sebaceous glands, epidermis, and dermis was achieved in a nude mouse model by implanting a cell mixture of epidermal cells, CD34+ cells selected by a microfluidic device, and dermal fibroblasts [86]. Although unsorted skin cells have yielded successful products and treatments for the skin, hair follicle regeneration likely requires stem cells or the selection and enrichment of subpopulations of skin-derived cells. As previously discussed, epidermal-dermal cross-talk appears to be extremely important in directing the differentiation of the adnexal structures [14]. Consequently, further identification of the mediators of this cross-talk would significantly aid future attempts to regenerate tissues or both ectodermal and mesodermal lineages.

Significant work has been done on DSCs in the field of neuroregeneration. These strategies often consist of direct injection of undifferentiated DSCs or injections of DSCs differentiated into specific neural-related lineages (e.g., Schwann cells). Examples in the murine model include SKP injection to promote axonal regeneration of cutaneous nerves [87] and injection of SKPs differentiated into Schwann cells (SKP-SCs) to promote myelin restoration of the tibial nerve in a rat model [88]. For rats with transected and an immediately repaired sciatic nerve, allogeneic SKP-SC treatment recovered thermal sensitivity [89]. SKP-SCs also improved nerve regeneration across a 12-mm gap created in the sciatic nerve of Lewis rats when applied in combination with freeze-thawed nerve graft [90]. A similar study demonstrated that SKP-SC therapy improved behavioral recovery of rats after acute, chronic, and nerve graft repair beyond the current standard of microsurgical nerve repair [91]. In summary,

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SKP-SCs can support functional restoration by stimulating superior axon regeneration, myelination, and electrophysiological recovery.

Neuroregeneration using EPI-NCSCs has also been explored extensively in animal models. Allogeneic whisker-derived EPI-NCSCs injected perilesionally into murine spinal cord following injury, induced by contusion, revealed that injected cells expressed both neuronal, some glial cell markers [92] and substantially restored touch perception [93]. Another study showed that allogeneic, green fluorescent protein-labeled (GFP-labeled), CD34+, murine vibrissae-derived, colony-forming cells transplanted into severed spinal cord led to significant hind-limb locomotor function recovery compared with untransplanted control mice. The GFP-labeled cells expressed glial fibrillary acidic protein, suggesting glial differentiation [94]. Implantation of human hair follicle-derived stem cells that were nestin and CD34 positive and K15 negative (K15−) likewise led to functional recovery of impinged sciatic nerve of nude mice [95]. Both bulge- and papilla-derived follicular stem cells have also been shown to express nestin, to differentiate into neuronal and glial cells, to contribute to spinal cord repair, and to enhance locomotor recovery [96]. It seems that stem cells associated with hair follicle niches have excellent potential for Schwann cell regeneration. It would be extremely valuable to explore the ability of these cells to regenerate tissues of other lineages.

The ability of DSCs to differentiate into multiple lineages has also been exploited for hematopoietic regeneration and for in vitro pharmacological screening. Allogeneic DS and dermal follicular papilla cells derived from rat vibrissa were reported to generate hematopoietic colonies in vitro and to contribute to all blood lineages in vivo for at least 13 months after transplantation. These transplanted cells were reported to retain their regenerative potential when transferred to secondary recipients, potentially indicating a primitive stem cell nature [97]. An interesting approach has been developed to use DSCs in pharmacological studies by using them to generate monolayers of cells with sufficient hepatic features for hepatotoxicity screening [8]. SKPs isolated from foreskin were induced into hepatic phenotype by exposure to hepatogenic growth factors and cytokines. Human SKP-derived hepatic progenitor cells (hSKP-HPCs) expressed progenitor and adult hepatic cell markers, key biotransformation enzymes, and influx and efflux drug transporters. The response of hSKP-HPCs to acetaminophen was comparable to that of primary human hepatocytes [8], leading to the possibility of using DSCs in liver regeneration efforts. These experiments demonstrated the enormous plasticity of DSCs and highlighted the benefits of further exploration of their potential for generation of parenchymatous organs such as kidney or pancreas or treatment of bone marrow diseases. Further stringent studies with clonal analysis are needed to definitively explore this proposed property of DCSs.

Promising results also exist in in the field of cartilage tissue engineering. DSCs derived from dermis of goat skin and further selected by rapid adherence were shown to be chondroinducible either by aggregate culture on aggrecan-coated plates [98] or by culturing in monolayer in chondroinductive culture medium [60]. Tissues formed using these cells were biochemically and histologically comparable with native cartilage [60, 98] and demonstrated robust mechanical properties [60]. DSCs derived from neonatal human foreskin, mixed with decalcified bone dust, and seeded onto human skin-derived collagen sponges were shown to chondrodifferentiate by chondroitin 4-sulfate proteoglycan analysis and toluidine blue staining [99]. Culturing chondrocytes encapsulated in alginate under hypoxia yielded increases in collagen II and aggrecan gene expression and matrix glycosaminoglycan concentration [100]. Likewise, hypoxia promoted greater extracellular matrix synthesis by DSC-derived constructs compared with constructs grown in normoxia [101]. In addition, increased metachromatic staining and chondroitin 4-sulfate concentration were observed in constructs produced from human foreskin-derived fibroblasts seeded onto human skin-derived collagen sponges mixed with decalcified bone powder and cultured under hypoxic conditions [102]. Many biochemical and biomechanical stimuli have been discovered so far for use in cartilage tissue engineering using chondrocytes, and the studies using hypoxia have promise for translating this wealth of knowledge to the use of DSCs in cartilage tissue engineering.

Instead of DSCs, efforts have been made in using dermis cell isolates that did not undergo characterization as stem cells and that were primarily isolated just by their ability to grow as plastic-adhered monolayers. These include bone, tendon, and striated muscle tissue engineering and repair. Autologous dermal fibroblasts, isolated by a crawl-out technique and induced into osteoblast differentiation, were seeded onto a porous intramedullary prosthetic component. The investigators observed enhanced osseointegrative properties of the prosthesis seeded with osteo-induced fibroblasts compared with the prosthesis alone [103]. Autologous dermal fibroblasts isolated by enzymatic digestion from abdominal dermis and expanded as monolayer have also been used to form tendon implants by seeding onto polyglycolic acid. Used in a porcine flexor digital superficial tendon defect, these engineered tendons possessed tensile strength at 75% of native tissue values. Immunohistological evaluation revealed that the dermal fibroblasts no longer expressed collagen type III, characteristic of the dermis, suggesting a possible switch to tenocyte phenotype [104]. Injection of cultured autologous dermal fibroblasts, isolated from hip dermis by enzymatic digestion, have also been explored for human treatment in a prospective clinical pilot study for lateral epicondylitis [105] and a randomized controlled clinical trial for treatment of patellar tendinopathy [106]. In all cases, the injections yielded improvements in clinical scoring and/or response to pain and function [105, 106]. Injections of cells isolated from the dermis via explant culture have also been found to contribute to muscle regeneration following crush/freeze injury [107]. Consequently, dermis-derived cells have been used for bone, tendon, and muscle tissue engineering and regeneration efforts, but these studies lack characterization of the cells used. The roles of DSCs (i.e., stem cells) in these repair or tissue-generation efforts are currently unclear. It is plausible to assume that the cells used in all of the cited studies contain a high concentration of pericytes because the contribution to myogenic and osseous regeneration was demonstrated by pericytes isolated from human muscle tissue and by pericytes isolated from other human tissues, such as bone marrow, placenta, pancreas, and fat [2, 108]. Essentially, it is likely that stem cells have been included in the above-mentioned studies, and the potential exists for muscle, bone, and tendon tissue engineering and regeneration to be more effective if purified stem cells were used.

Finally, a new frontier of using DSCs in tissue engineering and regenerative medicine lies in taking advantage of their immunomodulatory effects. The immunomodulatory effects of bone marrow-derived MSCs have been studied extensively and are thoroughly summarized elsewhere [109]. MSCs have been proposed to have low immunogenicity due to lack of surface expression of major

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histocompatibility complex class II, B7-1, B7-2, CD40, or CD40L molecules [110]. In addition, on stimulation by inflammatory mediators, MSCs have been reported to produce anti-inflammatory cytokines such as interleukin 10 (IL-10), transforming growth factor β1, and hepatocyte growth factor (HGF) [111–113]. Through paracrine action of these mediators, MSCs were shown to reduce effector activity of innate antigen-presenting cells [114] and of T and B lymphocytes and to reduce proliferation of the latter two [111, 115]. Although not as extensively studied, DSCs have strikingly similar capabilities of immunomodulation and immunogenicity. It was shown that hSKPs express HLA-ABC molecules but not HLA-DR, rendering them poorly immunogenic, similar to the MSC literature. In addition to the poor immunogenic properties, hSKPs also have immunosuppressive properties. Specifically, hSKPs reduced the intensity of allogeneic mixed lymphocyte response in vitro, and suppression of T and B cells in vivo was evident by reduced interferon-γ (INF-γ) levels and reduction of human IgG secretion, respectively. These effects were proposed to be exerted by decreasing INF-γ secretion, stimulating IL-10 production, and downregulating costimulatory (CD27, CD13, CD154) surface molecules by T cells, and by highly expressing heme oxygenase-1 (CD274), secreting HGF, leukemia inhibitory factor, and PGE2 by hSKPs upon exposure to inflammation and allogeneic activated T lymphocytes. These effects of suppression are similar to those described for MSCs. In addition, cotransplantation of hSKPs and human peripheral blood lymphocytes into SCID mice showed a significant impairment of the graft-versus-host response at 1 week after transplantation, with a 60% increase in survival time [116]. The graft-versus-host response was also suppressed when using MSCs in a murine model [117].

In addition to the many similarities observed between MSCs and DSCs in terms of immunomodulation, CD90+ DSCs were shown to induce FoxP3 expression in T-cell receptor complex-stimulated CD25+2CD4+CD45RA+ T cells in the absence of CD28 coligation in a cell contact-dependent manner. These T cells possessed an effective suppressive capacity in vitro [118]. In short, the potential for immunomodulatory effects of DSCs is only beginning to be unraveled, and many of the findings using MSCs are now being shown for DSCs. Further studies exploring the capacity of these cells in the treatment of immune-mediated diseases and inflammatory conditions would be of benefit to the field of regenerative medicine.

Conclusion

Multiple niches of stem cells exist in the dermis, and the potential of DSC use in tissue engineering and regenerative medicine is almost limitless; however, efforts should be directed toward better characterization of cells residing in each niche, with a goal of identifying common markers. At the same time, it would be prudent to examine whether cells from a particular niche have better potential to generate or regenerate a particular tissue type and, if so, what features of these cells provide them with this unique ability. In addition, given a divergent embryologic origin of dermis in different anatomical locations of human skin, it remains to be determined if the anatomical location from which the dermis stem cells are isolated plays a role.

It is apparent from the examples provided that generation of specific structures like hair follicles or myelinating glial cells, for example, may require more purified populations of stem cells compared with uncharacterized dermal fibroblasts, whereas generation of other tissues like tendon or muscle may be achieved with the latter. It would be of tremendous value to explore whether better and more robust tissues can be engineered or whether better or faster regeneration of tissues and organs can be achieved with purified DSCs. Understanding and addressing these issues would greatly benefit and tremendously improve the fields of tissue engineering and regenerative medicine and hopefully bring us closer to the era in which tissues could be generated or regenerated from autologous cell sources on demand.

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Author Contributions

N.V.: study concept and design, acquisition of data, drafting of manuscript; B.A. and J.A.N.: critical revision of manuscript for important intellectual content; J.C.H.: study concept and design, critical revision of manuscript, study supervision; K.A.A.: study concept and design, study supervision, critical revision of manuscript for intellectual content

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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