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Abstract

Amniotic fluid-derived stem (AFS) cells are an attractive cell source for applications in regenerative medicine due to their proliferation capacity, multipotency, immunomodulatory activity, and lack of significant immunogenicity. In addition, they have the ability to modulate inflammatory responses and secrete therapeutic cytokines. Because of these characteristics, AFS cells have been explored for treatments in wound healing and skin regeneration. Studies show that AFS cells are effective in accelerating wound healing in skin in fetal environments, and more recently in adult wounds. Evidence indicates that delivered cells are often transient, not permanently integrating into the final skin tissue. Instead, they secrete a portfolio of potent growth factors that are integral to skin regeneration and angiogenesis, suggesting a trophic mechanism of augmenting wound healing. These initial works of research suggest that delivery of AFS cells has potential to be an effective cell therapy for facilitating wound healing and should be further considered for clinical use in excessive skin wounds in human patients.

1 Introduction

Extensive burns and full thickness skin wounds can be devastating to patients, even when treated. There are an estimated 500,000 burns treated in the United States each year [1, 2]. The overall mortality rate for burn injury was 4.9 % between 1998 and 2007 and medical costs for burn treatments approach \$2 billion per year [3]. Globally, this statistic increases to 11 million injuries per year [4]. In addition to burns, full-thickness chronic wounds constitute a large patient base, and despite technological advancement of treatments, healing rates remain below 50 % successful [5]. These non-healing chronic wounds are estimated to effect 7 million people per year in the United States, with yearly costs approaching \$25 billion [6]. Patient survival is inversely

proportional to the amount of time required to cover and stabilize a wound. Patients with burns greater than 15–20 % of total body surface area are likely to go into shock without rapid treatment. Furthermore, without sufficient and rapid fluid resuscitation, patient conditions deteriorate and mortality rates increase rapidly [7]. Inadequate treatment regimes result in long-term complications for patients, including open wounds, pain, problems with temperature sensation, loss of feeling, prominent scars, and itching [8].

Patients who suffer from either of these types of injuries benefit from rapid treatments that result in complete closure and protection of the wounds. In particular, burn patients who receive delayed treatments often are subject to extensive scarring that can result in negative long-term physiological effects.

Recent advances have been made in treatments for wound healing; however, the gold standard still employed in the clinic, is an autologous split-thickness skin graft. This involves removing a piece of skin from a secondary surgical site for the patient, stretching the skin, and re-applying the graft on the wound or burn. While this treatment yields a reasonable clinical outcome, if the wound is extensive,

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then the number and size of donor sites are limited, making autografts unusable in cases that require prompt and aggressive measures to maintain the lives of wounded patients. Allografts are an additional option, but suffer from the need of immunosuppressive drugs to prevent immune rejection of the graft. These limitations have thus led to the development of noncellular dermal substitutes, which most often comprises a polymeric scaffold. Examples include INTEGRA® Dermal Regeneration Template (Integra LifeSciences) and Biobrane® (UDL Laboratories), and although such materials result in improved wound healing over untreated controls [9, 10], they are costly to produce and result in relatively poor cosmetic outcomes.

Recent advances in tissue engineering have led to more complex biological skin equivalents that may yield more suitable wound treatment options for patients. Examples include cellularized graft-like products, such as Dermagraft® (Shire), Apligraf® (Organogenesis), and TransCyte® (Advanced BioHealing). These products generally comprise a polymer scaffold patch that is seeded with human fibroblasts and cultured in vitro prior to application. Unfortunately, these grafts are also expensive to produce, and as allografts, can suffer from the same immunological drawbacks discussed above.

Commercially available hydrogel dressings have advantages in that they are often immunologically inert, regulate fluid exchange from the wound surface, and are available as gels, sheets, and impregnated in ordinary cotton gauze pads [10, 11]. For shallow, flat wounds, hydrogel sheets are applied as a primary dressing and remain in place for approximately 4–7 days. For irregular and/or deep wounds, amorphous hydrogels can be used to fill the wound and are held in place with a secondary dressing that is generally changed at least once daily. While these applications have advantages over traditional wound care regimes, the potential for hydrogels to promote wound healing is not limited to inert wound dressings. Other hydrogel materials have been developed that (1) possess crosslinking methods that facilitate deposition and immobilization in situ, and (2) comprise bioactive materials that can improve integration in the wound, can release potent biological signals, and even deliver therapeutic cells to the wound. In order to deliver cells, cell spraying and bioprinting technologies have recently been developed for wound treatment. In these approaches, cells are deposited over the wound, generally mixed in a hydrogel carrier vehicle, via a spray nozzle or print-head. Indeed, in our laboratory, we used a bioprinting device [12] to deposit amniotic fluid-derived stem cells in a full thickness wound healing model with great success [13]. New approaches such as these that permit immediate burn wound stabilization and support functional recovery are necessary. Figure 2.1 summarizes the various wound healing products and technologies discussed above.

| Category | Product | Source/Material | Manufacturer |
|---|--|---|----------------------|
| Donor-Provided | Autograft | Human skin tissue | N/A |
| | Allograft | Human skin tissue | N/A |
| Non-Cellular Dermal Substitutes | Integra™ Meshed Bilayer Wound Matrix | Collagen – Bovine Tendon | Integra LifeSciences |
| | Biobrane | Silicone film, nylon fabric and porcine collagen | UDL Laboratories |
| | Restylane | Hyaluronic acid – Bacterial (<i>Streptococci</i>) | Q-Med |
| Cellular Skin Equivalents | Dermagraft | Polyglactin mesh, human fibroblasts and fibroblast-secreted ECM | Shire |
| | Apligraf | Type I collagen, human dermal fibroblasts and keratinocytes | Organogenesis |
| | TransCyte | Nylon mesh and human fibroblasts | Advanced BioHealing |
| Hydrogels Approved for Use in Humans | Tegagel™ Hydrogel Wound Filler | Calcium alginate Seaweed/bacterial | 3M |
| | Phytacare Alginate Hydrogel wound dressing | Calcium alginate Seaweed/bacterial | CuraPharm |
| | Intrasite gel | Carboxymethyl cellulose and polyethylene glycol | A Smith & Nephew |
| Examples of Materials Currently used for Research | Extracel Hydrogel | Crosslinked hyaluronic acid and gelatin | Glycosan Biosystems |
| | Fibrin-collagen gel blends | Bovine-derived fibrinogen and thrombin, and rat-tail collagen | Various |
| | Silk-based films | Silk fibroin from silkworm cocoons | N/A |

Fig. 2.1 Examples of materials used clinically to treat wounds and experimental materials currently in research stages

Rather than a passive gel dressing that will have a minimal role in the healing process, technologies are needed that will actively promote wound healing by acting as a substrate for endogenous cell migration and proliferation. Cell-based therapies, such as the application of stem cells, may meet these needs.

2 A Brief Introduction to Stem Cells Derived from the Amniotic Fluid

The cell source used in cellular therapies for wound healing is an important consideration that has implications in the cost, speed, and outcome of the treatments. Human keratinocytes are perhaps the optimal cell type to employ as they make up the epidermal zone of skin tissue. However, autologous and

allogeneic keratinocytes suffer from the same drawbacks as their autologous and allogeneic skin graft counterparts, i.e., secondary surgical sites and potential for rejection, respectively. This begs the question: Can we employ cells that are beneficial to wound healing, but may be immunoprivileged despite originating from an allogeneic source?

Mesenchymal stem cells (MSCs) have shown therapeutic potential for repair and regeneration of tissues damaged by injury or disease. In particular, MSC treatment of acute and chronic wounds result in accelerated wound closure, increased epithelialization, formation of granulation tissue and angiogenesis [14]. Amniotic fluid-derived stem (AFS) cells are a cell type that shares some characteristics with MSCs, but are perhaps even more potent as they are sourced from an earlier point along the developmental timeline, namely the gestational period. As such, they are an attractive cell source for applications in regenerative medicine due to their high proliferation capacity, multipotency, immunomodulatory activity, and the lack of significant immunogenicity [15, 16]. Unlike embryonic stem cells (ESCs), AFS cells do not form teratomas when injected into immune-deficient mice. Furthermore, AFS cells remain stable and show no signs of transformation in culture. The isolation of AFS cells is a simpler process than that for isolation of MSCs and large numbers of AFS cells can be isolated and expanded from as little as 2 mL of amniotic fluid. These cells proliferate rapidly with doubling times of 30–36 h, and do not require supportive feeder layers [15]. The immunomodulatory and high proliferation properties of AFS cells suggest that they may be appropriate as an effective “off the shelf” cell therapy product for wound healing.

3 Flexibility of Amniotic Fluid-Derived Stem Cells in Regenerative Medicine

In recent years AFS cell-based research for applications in regenerative medicine has become more prevalent in the literature. As with other stem cell types that were established earlier such as MSCs, adipose-derived stem cells (ADSCs), and ESCs, AFS cells have been explored for force induced differentiation into cells of various tissue types, for repairing and replacing damaged or diseased tissues, and antitumor treatments. Additionally, the secretory profiles of AFS cells suggest they may be effective as deliverable “drug factories,” efficiently secreting cytokines and growth factors at target sites such as MSCs have been shown to do [17].

For differentiation purposes, AFS cells have been force induced into a number of lineages, including those of the liver [18], pancreas [19], cartilage [20], fat, muscle, bone, neuronal, and endothelial lineages [15, 21]. In addition to a level of multipotency, AFS cells share several other characteristics with MSCs, including the ability secrete cytokines,

many of which are pro-regenerative or immunomodulatory. The ability to secrete biological molecules such as these makes AFS cells, like MSCs, useful for various repair therapies as well as antitumor treatments. The immunomodulatory activity of AFS cells has been implemented to treat chronic allograft vasculopathy, by decreasing inflammation, thus preventing intimal wall thickening and loss of the graft [22]. This immunomodulatory activity has been further shown in controlled in vitro immune reaction studies, in which AFS cells as well as cell-free supernatants from AFS cell cultures inhibit lymphocyte activation, suggesting the responsible mechanism is through secreted soluble immunomodulatory factors [16]. These characteristics, secretory profiles and low immunogenicity, have also served as motivation to explore the use of AFS cells in anticancer treatments. Like MSCs, AFS cells show a propensity to home to tumor sites with administered intravenously [23]. In one such example, AFS cells expressing interferon beta were administered systemically to mice in a bladder tumor model, where they homed to the tumors and succeeded in inhibiting tumor growth and prolonging survival [24].

This ability to secrete growth factors, immunomodulatory factors, anti-inflammatory molecules, and to migrate to sites of injury has suggested another use in cell therapy—namely can AFS cells be implemented in wound healing treatments in skin?

4 Perinatal Wound Healing

There is a vast amount of literature that argues towards the potential that AFS cells have in wound healing applications, which stems from the phenomenon of perinatal, or fetal, wound healing, in which injuries to a fetus can heal completely scar free [25]. During development the fetus is surrounded by amniotic fluid (AF) within the amniotic sac, the same environment from which AFS cells are isolated. Components of the AF may be responsible for its regenerative properties, and also be able to imbue the regenerative properties inherent in AFS cells. A number of studies have determined that the amniotic fluid has a unique composition that changes over time, supporting scar-free healing at the early stages of gestation, and then transitions to more traditional healing processes at the end of pregnancy. For example, human amniotic fluid was assessed in vitro for its ability to regulate several major proteases involved in wound healing. It was found that amniotic fluid enhanced collagenase, thereby inhibiting collagen formation, a large component of scarring. Conversely, activities of hyaluronidase were inhibited, thereby allowing hyaluronic acid (HA), an anti-inflammatory glycosaminoglycan common to the extracellular matrix (ECM), to be present in high levels [25]. Furthermore, it has been demonstrated that fibrotic healing that occurs in late

gestational and adult wounds is correlated with increased hyaluronidase activity, which decreases HA levels [26]. HA has often been associated with regeneration and anti-inflammation, and is now commonly used in applications demanding such characteristics [27].

Amniotic fluid-based fetal wound healing studies have yielded a knowledgebase consisting of growth factors, cytokines, and extracellular matrix components that have been further explored to minimize scarring in adult wounds. Indeed, it has been shown that amniotic fluid, containing high levels of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF), was a potent stimulant of both fetal and adult skin fibroblast growth [28]. A number of clinical trials have been instigated, but none have reached the level of FDA approval [29]. However, the concept of using the fetal developmental environment to “fool” the body into scar-free healing holds merit.

5 Amniotic Fluid-Derived Stem Cells in Wound Healing

Secretory profiles of AFS cell-conditioned media contain a number of cytokines known to be important in wound healing, such as vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), and transforming growth factor beta (TGF- β) [30]. This suggests that AFS cells delivered to wound sites may be able to secrete these factors in situ, contributing to wound healing in cell therapy administrations. MSCs have been shown to have a paracrine role in wound healing, secreting similar factors [31]. Interestingly, MSCs have also been documented as being able to integrate permanently by differentiating into cells of myofibroblast phenotype in vivo [32] and acting as vasculature-supporting

pericytes in vitro [33], another potential mechanism by which AFS cells could potentially impact wound healing.

As discussed above, the environment within the amniotic sac itself may be the main component in effective wound healing, but until recently it was unknown whether a cellular component of the environment was important. In a fetal lamb wound model, fetuses receive an infusion of autologous AFS cells into the amniotic sac. Access to wounds was either not impeded, or made impermeable to cells using exterior titanium chambers. AFS-impermeable wounds showed significantly slower healing rates. Furthermore elastin content in those wounds were significantly decreased compared to wounds that were accessible to the AFS cells, further indicating the effectiveness of the cells themselves in wound healing, albeit in a fetal environment [34].

Our laboratory has a track record in pioneering isolation, characterization, and applications of AFS cells [15, 19, 21, 35, 36]. However, until recently no one had explored the use of human AFS cells in healing of severe wounds in adults. We recently demonstrated the use of AFS cells using a full thickness wound model in mice [13]. We employed a bioprinting device previously developed in house [12] to deliver AFS cells to wounds within a fibrin-collagen gel vehicle, and compared this treatment to MSCs or no cells delivered in the same gel delivery vehicle. AFS cells and MSCs showed similar potency in topographical wound closure (Fig. 2.2), significantly accelerating wound closure compared to the cell-free control. However, upon histological inspection, we observed that AFS cells induced significantly greater vascularization than both MSC and cell-free treatments (Fig. 2.3a–h) during the short 2-week time course. Additionally, vasculature in AFS-treated wounds also appeared more mature, displaying robust smooth muscle actin surrounding larger vessels and a lack of extravasated red blood cells (Fig. 2.3i–n). Extravasated

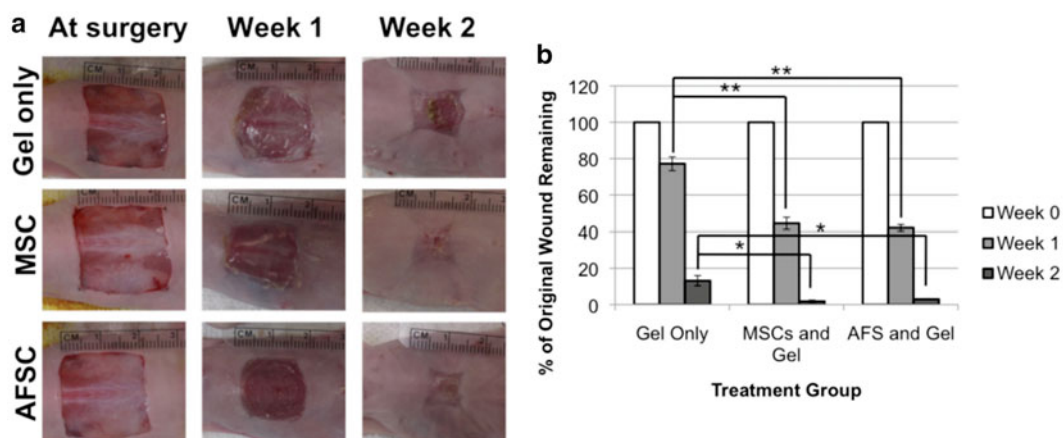


Fig. 2.2 Wound closure rates are increased in AFS- and MSC-treated mice. (a) Gross histology images illustrating wound closure in Gel-only, MSC, and AFS treatments. (b) Percentage of unclosed wound remaining at surgery, 1 week, and 2 weeks. Significance: * $p < 0.05$; ** $p < 0.01$

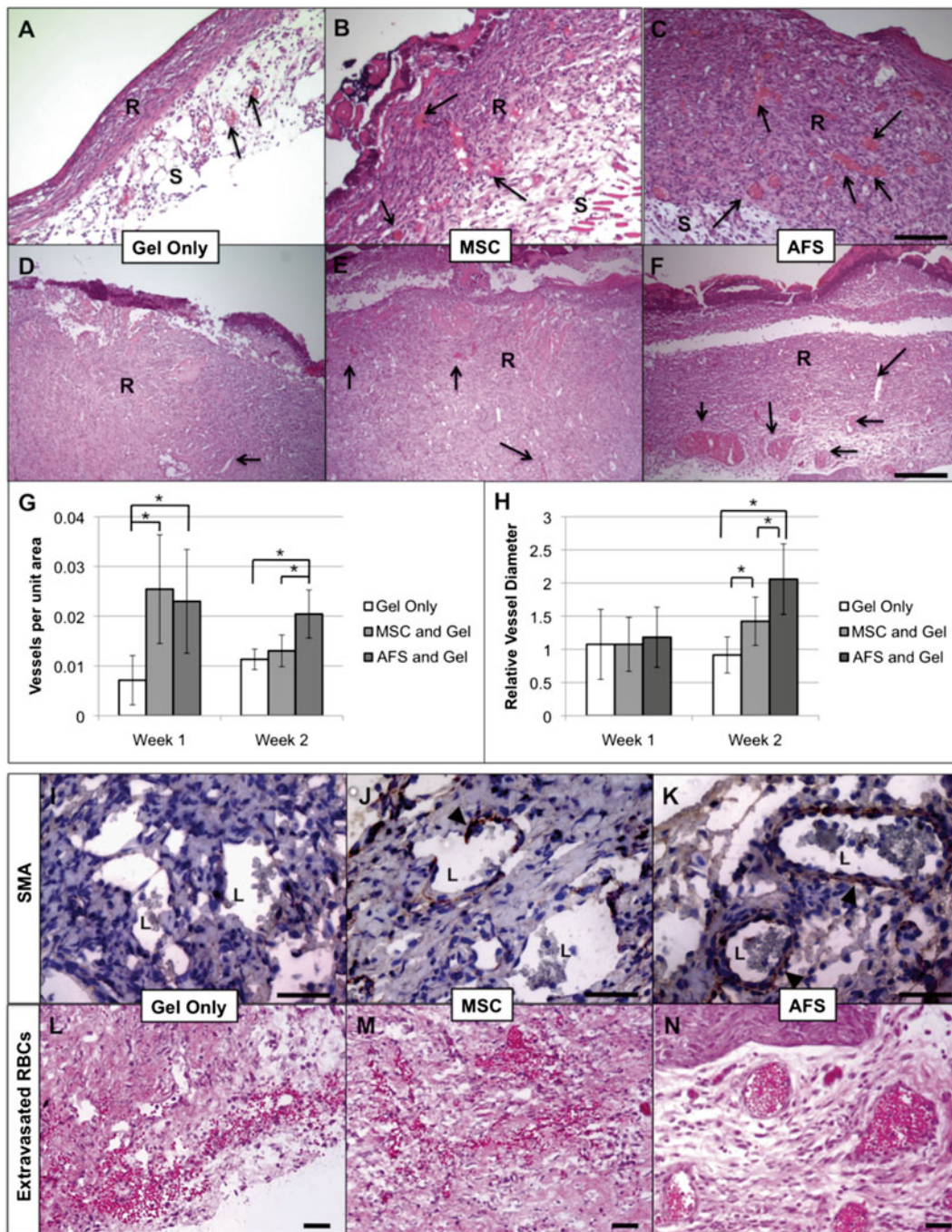


Fig. 2.3 AFS cells induce neovascularization and blood vessel maturation in vivo. H&E staining revealed thicker regenerating tissue w/more blood vessels in AFS and MSC group compared to the gel-only group. (a–c) Week 1; (d–f) Week 2; (a and d) Gel-only; (b and e) MSC; (c and f) AFS. Arrows—vessels. AFS cells increase the number of newly formed vessels and induce formation of larger vessels. (g) Microvessel

density (MVD) and (h) vessel diameter were quantified using histology images and ImageJ software. Significance: $*p < 0.05$. AFS cells induce formation of mature blood vessels. SMA staining around blood vessels in regenerated tissues of gel only (i), MSC (j), and AFS (k) groups. Arrows: Cells expressing SMA. Extravasated RBCs were present in gel only (l) and MSC (m) groups, but not in the AFS (n) group

red blood cells were observed in the other groups, indicating leaky or less mature blood vessel structures. Interestingly, both AFS cells and MSCs were transient in our model as evidenced by tracking of the cells after transfection with green

fluorescent protein (GFP). Cells were present immediately after treatment, 4 days after treatment, and 7 days after treatment—albeit in fewer numbers. However, no evidence of either cell type was found after day 7 (Fig. 2.4).

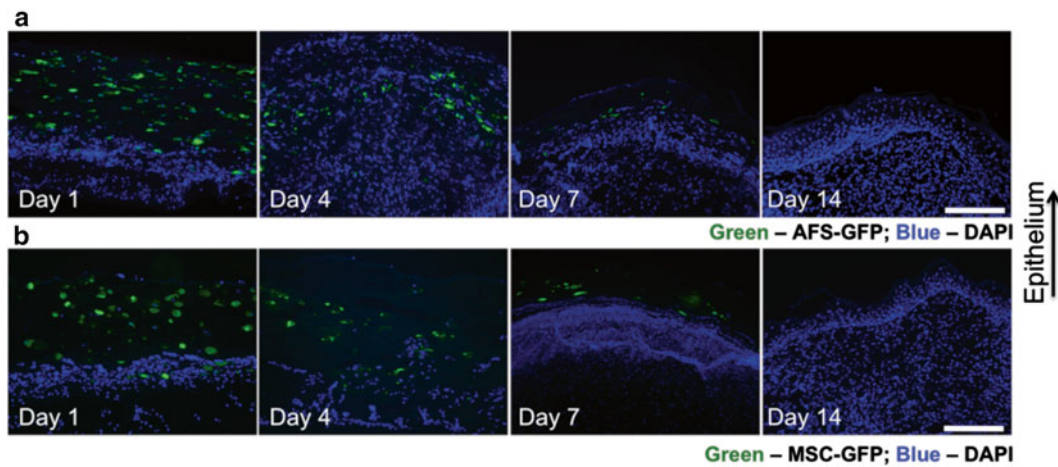


Fig. 2.4 AFS cells and MSCs are transient in the regenerating wound and do not permanently integrate into the tissue. Regenerating skin was harvested at day 1, day 4, day 7, and day 14 in order to determine the

presence of labeled cells. GFP-labeled AFS cells (a) and MSCs (b) are visible in decreasing numbers of the time course of the experiment. *Green*: GFP-expressing AFS or MSC; *Blue*: Nuclear staining, DAPI

Nevertheless, these results suggested that AFS cells have the potential to be an effective therapy for treating skin wounds. Based on the transient nature of the cells, we hypothesized that the augmentation of skin regeneration was due to trophic delivery of cytokines. To further investigate this concept, we first generated conditioned media from these particular populations of AFS cells and MSCs, and analyzed it for a panel of potent growth factors using a proteomics array. As expected, samples tested positive for cytokines such as bFGF, VEGF, hepatocyte growth factor (HGF), and members of the insulin growth factor-binding protein (IGFBP) superfamily (Fig. 2.5). In general, AFS-conditioned media tested positive for more of these cytokines and at higher concentrations. Based on the increased angiogenic activity noted above, we then hypothesized that AFS cells were likely responsible for recruiting endothelial cells via secretion of these factors. We next performed a Transwell migration assay using human umbilical vein endothelial cells as the migrating population. In order to simulate the mechanically soft environment of the *in vivo* wound bed we employed a 2 kPa stiff substrate versus much stiffer traditional plastic substrates on which the AFS cells were seeded as the recruiting population. In addition, a cell-free control was used. A greater number of HUVECs migrated when exposed to the AFS cultures compared to the cell-free control. Interestingly, AFS cells cultured on the softer 2 kPa substrate were significantly more adept at HUVEC recruitment, suggesting that the environment in which they exist plays an important role in their therapeutic effectiveness [13].

The transient nature of this cell therapy combined with the experiments described above certainly argue strongly that the primary mechanism by which AFS cells aid wound healing biology is through secretion of trophic factors. In particular, we demonstrated the ability of AFS cells to recruit endothelial cells, behavior that was conserved *in vivo* in that

| Growth Factor Analysis of Conditioned Media | | |
|---|----------------|-------|
| | (units: pg/mL) | |
| Growth Factor | AFS | MSC |
| bFGF | 459 | - |
| FGF-7 | 1507 | 3106 |
| GDF-15 | 95 | 50 |
| GDNF | 21 | - |
| HGF | 218 | - |
| IGFBP-2 | 1490 | 4167 |
| IGFBP-3 | 8852 | - |
| IGFBP-4 | 65249 | 987 |
| IGFBP-6 | 17470 | - |
| NT-3 | 530 | - |
| OPG | 1882 | 7852 |
| PDGF-AA | - | 176 |
| PIGF | - | 18 |
| VEGF | 5006 | 10953 |

Fig. 2.5 Quantification of growth factors secreted by AFS cells and MSCs *in vitro*. AFS cells secrete more growth factors than MSCs. Presence of growth factor concentrations in AFS- and MSC-conditioned media were determined by proteomic arrays. *Dashes* indicate that growth factors were undetectable

a significantly increased number of blood vessels formed after AFS cell treatment. Indeed, other groups have found that AFS cells themselves and factors from conditioned media derived from AFS cells can induce neovascularization in ischemic skin flap models, restoring viability to the skin [37–39].

Other recruitment mechanisms may be at play as well, as suggested by the high levels of IGFBPs observed in AFS-conditioned media. Members of the IGFBP superfamily have

been shown to modulate survival, migration, and proliferation of cells in the dermis and epidermis [40]. In fact, IGFBP-7 was believed to be responsible for increased migration of both fibroblast and keratinocytes in *in vitro* scratch assays [41]. In a separate study, IGFBP was shown to promote high levels of survival and proliferation of both HaCaT (immortalized keratinocytes) and primary human keratinocytes [42].

6 Maximizing the Effectiveness of AFS Cells in Wound Healing

The potential for AFS cells to be effective as a wound healing treatment is evident from the data presented above. However, the transient behavior observed limits that effectiveness, since when the AFS cells are no longer present, no additional GFs can be secreted. We have been recently exploring alternative hydrogel delivery vehicles to improve the logistics of cell delivery and extend the period of potency of the AFS cells and the cytokines they secrete in the wound.

The fibrin-collagen gel employed above performed adequately, but was perhaps not the most optimal material to be used for bioprinting and wound healing. First, it required two components to be deposited *in situ*, relying on diffusion to mix them together in order for gelation to occur. This resulted in relatively slow gelation that caused some of the gel to pool in lower regions of the wound, and the loss of some gel solution from dripping out of the wound. Additionally, collagen is known to contract during gelation and is the primary component in scarring, potential drawbacks in skin regeneration.

Hydrogel biomaterials have been widely explored for bioprinting applications. We previously developed a series of hyaluronic acid (HA)-based hydrogels with different crosslinking techniques to facilitate deposition from bioprinting devices. The base materials of the HA-based hydrogels we implemented consist of thiolated HA, thiolated gelatin, and a polyethylene glycol diacrylate (PEGDA) crosslinker [43, 44]. This hydrogel has been implemented in numerous regenerative medicine applications, including 3-D cell culture [45], postsurgical adhesion prevention [46], tumor xenografts [47, 48], and wound healing [49]. However, in their native form, these materials require 15–30 min to crosslink at physiological pH, which is unsuitable for the fast deposition nature of bioprinting. To overcome that limitation, we employed variations of the hydrogel and different crosslinking approaches to improve bioprinting procedures. First, we developed a 4-arm PEG-based crosslinker which resulted in a stiffer hydrogel, allowing stacking of cellular and noncellular extruded rods in an increased aspect ratio into a tubular structure [50]. Second, we implemented methacrylate-based photopolymerization to employ a 2-step photocrosslinking protocol to print a cellular tube [51]. Next, we employed the thiophilic nature of gold nanoparticles and used them in place of the PEGDA crosslinker to make slow-forming gels.

This property allowed extrusion of partially gelled materials into 3-D orientations that would later fuse together in a single seamless structure [52]. Recently, we discovered that by adding the Irgacure 2959 photoinitiator a solution comprises the HA and gelatin base materials mentioned above, we could use UV irradiation to achieve nearly instantaneous photopolymerization. We tested this hydrogel against a panel of other commonly used materials for characteristics such as gelation times, ease of use, biocompatibility, and immunogenicity and found that it was suitable for use in our bioprinting devices and likely a good choice for implementation as a cell delivery vehicle for a wound healing treatment [53].

Preliminary studies have demonstrated that the properties of the photocrosslinkable HA hydrogel, including the ability to incorporate heparin for growth factor release, pair well with the pro-angiogenic and secretory properties of AFS. *In vitro* testing confirmed that encapsulation of AFS cells in the heparinized HA hydrogel (Extracel-HP, commercially available from ESI-BIO) supported daily release of AFS-secreted protein for 2 weeks. Furthermore, AFS-secreted VEGF release was maintained for same length of time. Notably, in fibrin-collagen gels, VEGF release was not detectable after day 7. We tested the combination of AFS cells delivered in Extracel-HP in a similar mouse model of full thickness wound healing as described above in which we observed similar wound closing and angiogenic activity. However, we also noted an improvement in the extracellular matrix composition. Specifically, there was a lower ratio of fibrotic collagen Type I to collagen Type III, which is associated with healthy healing. Additionally, there was greater staining for elastin, proteoglycans, and GAGs in HA-AFS tissues than other groups. These *in vitro* and *in vivo* data indicate that the choice of delivery vehicle for cell therapy matters greatly and can effectively improve cell therapies. By employing a less inflammatory and contractile hydrogel with dynamic growth factor release capabilities, we believe we maintained growth factor activity in the wound for a longer period of time and improved the ECM in the regenerated skin.

7 Limitations and Future Directions

In the work in our laboratory, the primary limitation we have come across in our results is the inability to limit contraction during the wound healing process. In both of our studies described above, contraction contributed significantly to the overall wound closure. However, we believe that by incorporating the optimal delivery vehicle we can minimize healing by contraction, and rather induce re-epithelialization to be primarily responsible. Interestingly, when we employed HA above, the overall level of contraction did not decrease dramatically. However, the aspect ratio of the healing wound changed significantly. Specifically, wounds treated with HA

healed uniformly resulting in roughly a square-shaped area of re-epithelialized skin. In contrast, control wounds and fibrin-collagen-treated wounds healed with a high aspect ratio, contracting more in the medial-lateral direction. This difference may be significant for limiting contraction and scar formation, but will require further investigation.

More general limitations, but limitations nonetheless, are those of regulatory hurdles associated with bringing any cell-based therapy or product to market and to patients in the clinic. The time and financial cost required to generate a simple drug molecule that is commercially available is staggering. Products that incorporate living cells are inherently more complex, resulting in a regulatory pathway that is therefore more complex, and not fully developed at this time. To that end, we have been exploring cell-free, but bioactive biomaterials that can mimic the biological behavior of AFS cell therapy in wounds. By using a cell-free, but AFS-like, approach we hope to address these issues.

8 Conclusions

Extensive skin wounds including severe burns can be devastating to patients, even when treated, and have a huge financial burden on the medical establishment. These patients require a treatment that results in protection of the wound during the healing process and closure of the wound in as short a time as possible. Although many improvements have been made in treatments for such wounds, the autologous split-thickness skin graft is still the best clinically available treatment despite its limitations. The studies presented here suggest that AFS cells may have the potential to address the clinical need for more effective treatment of burns and skin wounds. AFS cells seem to be able to induce accelerated wound healing through mechanisms associated with secreted cytokines, rather than integrating in the regenerating tissue. Because of this transient nature, the delivery vehicle in which the cells are deposited is important for maximizing their effect. Combinations of effective and versatile cell populations such as AFS cells in tandem with easy-to-use biomaterials complete with their own beneficial characteristics may lead to cell therapy products that will make a substantial impact in medicine.

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