REVIEW ARTICLE

Review: Biomimetic nanofibers in the ex vivo expansion of cord blood-derived hematopoietic stem cells (HSCs)

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Abstract

This review summarizes current strategies in the development of advanced nanofibrous polymer-based scaffolds via electrospinning, their applications in mimicking the extracellular matrix, and the use of polymer nanofibers to deliver growth factors or small molecules for ex vivo expansion of HSCs. Hematopoietic stem cell (HSC) transplantation has become the standard of care for patients with hematologic cancers, anemia, and a variety of other malignant and non-malignant disorders. Although mobilized peripheral blood (MPB) has become a preferred source of HSCs for transplants, bone marrow (BM) and umbilical cord blood (UCB) are also frequently utilized. Regardless of source, Regardless of source, HSC transplantation suffers from low cell doses. Therefore, methods to increase the cell dose while maintaining the progenitor phenotype, especially the CD34+ progenitor cells, would have a significant clinical impact. Ex vivo expansion of HSCs prior to transplantation is one approach that offers tremendous promise for increasing cell doses and improving clinical outcomes. Many ex vivo strategies have been developed within the last decade in order to address the issue of low cell dose, with more or less success, mainly determined by the degree of difficulty related with maintaining HSCs self-renewal and stemness properties after long-term expansion. Here, we report the current progress of nanofibrous scaffolds for the ex vivo expansion of hematopoietic stem cells (HSCs). In this review, we present the technique of electrospinning for nanofibrous scaffolds, focusing mainly on preparation methods, materials (synthetic, natural, and hybrid polymers), and surface-structural modifications. The variables of nanofiber processing parameters and its impact on the nanofiber assembly is reported as well as the effect of the solution parameters on the structural morphology of the fabricated nanofibers. Critical features of fabricated nanofibers such as porous structure and high specific surface area are addressed, but more importantly the necessity of mimicking the intrinsic properties of the native in vivo microenvironment of the extracellular matrix (ECM). Researchers have been largely successful in replicating the diverse nature of the ECM through the incorporation of small molecules, growth factors, and signaling molecules into 3D scaffolds to generate biomimetic hierarchical structures. Harnessing the potential of the stem cell niche forms the basis of clinical therapy in the ex vivo expansion of cord blood-derived hematopoietic stem cells. As reviewed in this article, advances in nanofiber ex vivo approaches based upon emerging biomaterials opens new doors for artificial niches. Scientific evidence provided in this review verifies that the nanofiber niche provides an ideal mimic of the physical microenvironment of HSCs thereby offering great potential for clinical applications.

1. Introduction

Electrospun nanofibers have been shown to support growth and proliferation of a wide variety of cell types. Recent efforts have extended this work to afford a favorable approach for stem cell growth and expansion for the ex vivo treatment of hematological diseases like multiple myeloma and leukemia[1]. While increasing efforts have been focused on blood transfusion for these disorders, there is still no genuine therapy for these types of diseases. Recently, Hematopoietic Stem Cells (HSC) have gained tremendous interest in treatment of hematological disorders due to the advancement of gene therapy techniques[2,3]. Hematopoietic stem cells (HSCs) are the stem cells that give rise to other blood cells, a process termed hematopoiesis [4-6]. Hematopoietic stem cells (HSCs) can be found in bone marrow, peripheral blood, and umbilical cord blood, and are critically important in studies of blood disorders and blood-related malignancies. Unfortunately, HSC in the treatment of hematological disorders are still plagued with the limitation of cell dose [7]. Expansion of cord blood progenitor cells offers significant opportunities for improved outcomes for patients who suffer from hematological conditions [8-10]. To overcome the limitation of cord blood cell dose and stem cell number, development of an ex-vivo expansion technology is critically important [11-13]. Recent emerging technology using nanofibers in combination with growth factors, such as cytokines and other biomolecules, has made a significant improvement to the field of regenerative medicine [14].

This review seeks to describe the advances in research centering the use of nanofiber scaffolds in stem cell therapy for the growth and expansion of hematopoietic stem cells. Ex vivo expansion of cord blood-derived hematopoietic stem cells using biomaterials and/or growth factors continues to stimulate increasing interest as well as pose significant challenges [15-17]. One of the greatest obstacles presented lies in mimicking the architecture and properties of the extracellular matrix (ECM). The ECM is composed of biomolecules such as proteins and polysaccharides that form a complex microenvironment for cells [18-24]. ECM can be envisioned as a diverse fibrous structure responsible for the cellular processes of adhesion, mechanical support, migration, signaling, and cell proliferation [18-24]. Thus, it is imperative to design scaffolds that can mimic niche of the ECM as well as the perform functions of the ECM in an attempt to accelerate cell adhesion, proliferation and regulate cell fate [25]. Such an analogue would require topographical characteristics and geometry on the macro-, micro- and nanoscales due to the diverse nature of the ECM matrix [25].

In many traditional culture systems HSCs are cultured as suspension cells in the presence of ECM components or growth factors that act to enhance cell proliferation while regulating differentiation. In the ex vivo expansion of cells on nanofibers it has been shown that the interaction of HSCs with the substrate becomes a highly influential factor in cell fate [26]. In the natural bone marrow microenvironment, termed 'niche,' HSCs maintain close contact with a complex network of cell-cell and cell-matrix interactions which plays a significant role in stem cell phenotype [27-30]. A key challenge for researchers in the ex vivo expansion of HPSCs researchers continues to be the creation of multidimensional fibrous structures that comprise all the components of nanoscale architecture and topographical features, micro- and macroscale gradient structures, as well as biological domains to interact with target growth factors and cells [25,31-34] . An often-overlooked factor influencing fate decisions is the interaction of HSCs with a substrate. In the natural bone

marrow microenvironment, HSCs maintain close contact with a complex network of stromal cells and extracellular matrix, likely indicating that cell-cell and cell-matrix interactions play an important role in maintaining their stem cell phenotype. With a goal of mimicking the cell niche, many researchers have developed 3D nanofiber based cell culture substrates that provide topographical and substrate immobilized biochemical cues that act in synergy with growth factors to enhance HSC proliferation while minimizing differentiation [35].

2. Fabrication of Nanofibers

Fibrous scaffolds are important three-dimensional substrates that provide an inducive likeness to the ECM, increasing cell proliferation and expansion of HPSCs due to their topological similarities, large surface to volume ratio, flexibility, porosity, and versatility. [36-39] The most well-known techniques for producing nanofibrous structures are self-assembly, phase separation, and electrospinning. [25] Electrospinning has gained increasing interest due to its ability to generate the optimal cell environment for a wide variety of applications including tissue regeneration, drug delivery, cancer and stem cell study, and neurite cell growth. [35-42] Depending on the desired application and characteristics needed, scaffolds can be customized to reflect appropriate structure-function properties. [43] A nanofiber can range anywhere from submicron to nanometers in diameter and can easily be designed to have specific structural, mechanical, and chemical properties depending on its purpose. [43-45]

Electrospinning uses electrostatic forces to generate nanofibers that are inherent with structural properties that play a crucial role in controlling cell behavior. [38-41] A typical electrospinning setup consists of three primary components: 1) a high voltage power supply (usually in the kV range); 2) a syringe with a metallic needle; and 3) a grounded collector (solid substrate or liquid media) which generates an electric field between a counter electrode and a positively charged spinneret filled with a polymer solution. [25] A polymer jet is formed at the metal needle tip that moves through the charged spinneret to the counter electrode, allowing the solvent to evaporate and forming continuous polymer fibers with diameters of tens of nanometers to a few micrometers (depending on the polymer solution and electrospinning parameters) that gather on the collector. [25] Polymer viscosity may be manipulated through increasing polymer molecular weight or solution concentration, thereby increasing fiber diameter. Significant amounts of work have been carried out to study the relationships among fiber characteristics, processing and material parameters. The electrospinning variables that affect fiber formation and structure morphology of fabricated nanofibers include solution parameters (polymer concentration, solvent volatility, and solution conductivity) and processing parameters (flow rate, needle to collector distance, and applied voltage). [25,46]

The material in which the nanofibers are composed are just as critically important as the three-dimensional design of the generated scaffolds. Electrospun polymeric nanofibers and scaffolds have been fabricated using copolymers, homopolymers, polymeric blends, and organic-inorganic hybrid materials. Amongst these categories, synthetic and natural polymers are the most often used in the design of nanofibrous scaffolds with various structural properties. [25] Researchers have generated nanofibers from a large variety of materials ranging from natural macromolecules such as collagen, chitosan and silk fibroin, to synthetic biodegradable polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers (PLGA). The

main advantages of synthetic polymers are their spinnability, excellent mechanical integrity, and cost-effectiveness [25] Polyesters such as poly (e-caprolactone) (PCL) continue to be the most frequently used synthetic material owing to its high tensile strength making it an ideal candidate for applications in which mechanical strength is important. [25]

Synthetic polymers generally provide high flexibility in synthesis, processing, and modification and tend to more cost-effective versus natural polymers. More importantly, their mechanical properties can be effectively and preferentially tuned. [25] However, the backbone of synthetic polymers is typically inert, lack bioactivity and thus require additional modifications. In contrast, natural polymers are inherently bioactive, presenting cell-interactive domains on their backbones, and offer better adhesion, proliferation, and differentiation of cells than is available with synthetic polymers.[25] To exploit the advantages of both synthetic and natural polymers, researchers have fabricated hybrid scaffolds, a blend of both types of polymers, to tune the functionality of the nanofibers enabling the physical properties and high bioactivity more favorable for ex vivo expansion of HSCs.

To further exploit the tunability of nanofibers, these substrates can also be functionalized by anchoring mechanical and biochemical signals as well as cell-recognizable ligands to improve cellular interactions. To this end, growth factors have been incorporated onto nanofiber matrices and shown to be important signals in the microenvironment, whose concentration, temporal and spatial distributions play important roles in cell function. It has been an area of recent interest that the introduction of biological materials on the surface of nanofibers, such as growth factors and cell signaling mechanisms may provide an even more effective substrate for cell survival. [48-49] The surface properties of polymeric nanofibers can also be manipulated to enhance wettability, hydrophilicity, and cell adhesion.[25]

Developing scaffolds that mimic the architecture of natural human tissues at the nanoscale is a major challenge, but a well-defined structure is essential for the scaffold to properly imitate native ECM in guiding cell growth or tissue regeneration. [25] In this effort, the electrospinning device has the capabilities to adopt various configurations to advantageously organize fiber deposition. For example, a rotating collector can achieve an aligned electrospun fiber structure assisting cell migration. Random or aligned nanofiber meshes with an average diameter ranging from 100 nm to over 1 mm have been prepared through control of various electrospinning parameters. [50] Aligned electrospun nanofibers can easily be produced by orienting the fibers in one direction through injection onto a collector at a high speed (more than 1500 rpm). [50] As expected, these electrospun nanofibrous scaffolds with suitable alignments have been found very effective in shaping cell morphology, guiding cell migration, and controlling various levels of cell behavior. [25,51] For example, HSCs cultured on aligned electrospun PCL nanofibers showed cellular alignment and elongation within the scaffold, which enhance their growth and expansion. [51,52]

3. Nanofiber Ex-Vivo Expansion of Cord Blood Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) can be found in bone marrow, peripheral blood, and umbilical cord blood, and are important in studies of blood disorders and blood-related malignancies. CD34+ stem cells are finding more importance in gene therapy due to their ability to differentiate into multiple blood cell lineages. [53-56] Due to their behavioral and

structural similarities, there is great difficulty in distinguishing hematopoietic progenitor stem cells from normal white blood cells. [53-56] Cell surface biomarkers, such as CD34 antigen, are most known for its expression on hematopoietic stem cells which help to distinguish between the cell types. [53] CD34 is a glycosylated transmembrane protein, which is present on the surface of some progenitor cells including hematopoietic stem cells[53]. The identification of CD34 on hematopoietic cells has opened the door to their development as a novel therapeutic strategy to treat hematopoietic conditions. [53-56] However, CD34+ stem cells can only be extracted in low quantities and are unable to proliferate once extracted, making them expensive and ineffective for studies that require a large proportion of cells.

In the treatment of a number of blood disorders, bone marrow transplantation is generally considered the most efficacious, but these methods of harvesting are invasive to patients and are plagued with infection transmission. Umbilical cord stem cell transplantation is an even better choice due to lower risk of infection but suffers from delayed engraftment.[57] Ex vivo expansion of HSCs shows great promise in the treatment of hematopoietic diseases in the large-scale amplification of live, whole cells or the maturation of a specific lineage of cells to the patient. [57-60] Use of nanofiber scaffolds enables HSCs to expand more extensively versus standard expansion protocols [67-77]; this is important because, although umbilical cord blood is often richer in CD34+ stem cells than is the bone marrow, the number of cells still tends to be insufficient for transplantation. Ex vivo expansion of CD34+ stem cells on nanofibers are a promising alternative to bone marrow and/or umbilical transplantation for the treatment of leukemia and other hematopoietic disorders. [57-66] CD34 was the first cell-surface marker used to enrich human HSCs for study of hematopoiesis. CD34+ HSCs are capable of differentiation into all cells of the hematopoietic lineage and have a high proliferative capacity.

Recent advances in the expansion of HSCs have been reported with umbilical cord-derived stem cells grown in serum-free conditions in nanofiber meshes that were expanded 225-fold ex vivo without cell differentiation. [63] In this specific case, CD133+ stem cells were seeded onto nanofiber scaffolds glued to the bottom of wells in a tissue culture plate and cultured in serum-free expansion medium containing several signaling factors essential to the maintenance of multipotency. Phenotype analysis of the expanded cells indicated that 24% of the cells retained their CD133 expression and 94% retained CD34 expression. Expression increased of the proadhesive molecule LFA-1. Mild to moderate expression of other myeloid markers indicated that expanded cells retained progenitor characteristics. [63] Furthermore, later experiments under similar conditions yielded 5 million-fold expansion of CD34+ stem cells on a nanofiber matrix. These cells maintained stemness and potential for differentiation into vascular endothelial cells despite their extensive expansion. [63] As the experiments sought to assess the efficacy of umbilical cord derived stem cells for their translational potential to promote angiogenesis and regenerate tissues in various degenerative diseases, the expanded cells were differentiated *in vitro* into various cell lineages. [63] [63]

Kang *et al* reported significant 164-fold expansion of hematopoietic stem/progenitor cells (HSPCs) from umbilical cord blood using extracellular matrix (ECM) protein-coated three-dimensional hierarchical scaffolds without change in phenotype.[71] Fischer *et al*. presented their recent work in development of a closed, nanofiber-based platform for large-scale clinical expansions of cord blood-derived CD34+ cells and

showed their nanofiber substrate expands CD34+ cells from CB an average of more than 150-fold in 10 day culture, which is at least 2-fold higher than that obtained in standard tissue culture plates. [68] Denchent *et al.* studied four different 3D biomaterial scaffolds (PCL, PLGA, fibrin and collagen) for cord blood (CB)-CD34(+) cell expansion in presence of cytokine supplementation and discovered that CB-CD34(+) cells cultured on human-derived 3D fibrin scaffolds reached 5×10^8-fold expansion after fourteen (14) days. Cells maintained their primitive phenotype and exhibited superior morphological, migratory and adhesive properties.[72] Feng *et al.* presented an expansion strategy using a three-dimensional (3D) scaffold conjugated with an extracellular matrix molecule, fibronectin (FN), to partially mimic the hematopoietic stem cell niche. [77] After 10 days of culture in serum-free medium, human umbilical cord blood CD34+ cells cultured in FN-conjugated scaffold yielded the highest expansion of CD34+ cells (~100 fold).The expanded human CD34+ cells were found to successfully reconstitute hematopoiesis in NOD/SCID mice. [77]

Cord blood derived adult stem cells have been shown to be advantageous in treating various degenerative disorders because of their multipotent nature and reduced immunogenicity. However, the amount of stem cells procured from a single cord blood unit is insufficient for any clinical or preclinical applications. Kanji *et al.* expanded and isolated CD133+ cells from freshly collected human umbilical cord blood on aminated polyethersulfone nanofiber scaffolds. After 10 days of culture of 20,000 CD133+ cells per plate, the group obtained almost a total of 5 million expanded cells (~250-fold amplification). [78] Flowcytometric analysis revealed that around 87% cells were positive for CD34+ after nanofiber-mediated expansion. Moderate expressions of other hematopoietic markers were also observed in nanofiber-expanded cells, such as, CD14, CD86, vWF, CD31, indicating that these expanded cells retained their hematopoietic progenitor characteristics. [78] This research demonstrated the efficacy of CD34+ cell therapy for healing of cutaneous wounds in mice with diabetes. Therefore, umbilical cord blood-derived CD34+ cells expanded on nanofiber scaffold showed great potential in this work as a promising stem cell source for future cell-based therapy for diabetic wounds. Aminated PES nanofiber coated plates provided three dimensional (3D) configurations mimicking bone marrow micro structures and were significantly successful in expanding human umbilical cord blood-derived CD34+ cells while preserving their stemness and their biological functionality.[78]

Chua *et al.* studied nanofiber scaffolds with amino groups conjugated to the fiber surface through different spacers (ethylene, butylenes, and hexylene groups, respectively) and investigated the effect of spacer length on adhesion and expansion of umbilical cord blood hematopoietic stem/progenitor cells (HSPCs). Electrospun polymer nanofiber scaffolds were functionalized with poly(acrylic acid) grafting, followed by conjugation of amino groups with different spacers. HSPCs were expanded on aminated scaffolds for 10 days. [80] Aminated nanofiber scaffolds with ethylene and butylene spacers showed high-expansion efficiencies (773- and 805-fold expansion of total cells, 200- and 235-fold expansion of CD34+CD45+ cells, respectively). [80] HSPC proliferation on the aminated scaffold with hexylene spacer was reported significantly lower (210-fold expansion of total cells and 86-fold expansion of CD34+CD45+ cells), but maintained the highest CD34+CD45+ cell fraction (41.1%). [80] Colony-forming unit granulocyte-erythrocyte-monocyte-megakaryocyte and long-term culture-initiating cell maintenance was similar for

HSPCs expanded on all three aminated nanofiber scaffolds.[80]

Given the positively charged nature of the fiber surface and that CD34 antigen is a highly sialylated and negatively charged glycophosphoprotein, Chua *et al.* hypothesized that the initial adhesion of HSPCs on aminated nanofibers is likely mediated by CD34 antigen via electrostatic charge-to-charge interaction. It was found that due to negatively charged sialic acid, CD34 antigen functions as anti-adhesin, preventing cell-to-cell adhesion between HSPCs. [80] However, when CD34 is bound to antibodies or to its extracellular ligand, cell-to-cell adhesion is enhanced, either through concentration of CD34 to a cap region, and/or through antibody-CD34 (or ligand-CD34) mediated intracellular signaling, which may also result in an upregulation of cell-adhesive molecules in HSPCs. [80] Therefore, it is likely that HSCs interact with surface amino groups, either directly mediating or indirectly facilitating HSC adhesion to the substrate. This work demonstrated that aminated nanofibers enhanced cell-substrate adhesion and ex vivo expansion of HSPCs; and the spacer, through which amino groups were conjugated to nanofiber surface, significantly influenced HSC adhesion and expansion outcome. The results of this work highlighted the importance of scaffold topography and cell-substrate interaction to regulating HSPC proliferation and self-renewal in cytokine-supplemented expansion. [80]

Mousavi *et al.* developed a biomimetic nanofiber scaffold coated with collagen for *ex vivo* expansion of cord blood-derived CD34+ cells. This scaffold provided an appropriate substrate for cell entrapment, 3D cell-cell interaction, cell-ECM interactions, and proliferation. The culture system revealed improved maintenance and expansion of HSCs within 3D scaffolds that mimic the bone marrow microenvironment. Bone marrow ECM proteins (including collagens, fibronectin, and laminin) were studied in the preparation of anchorage sites that are recognized by cells through specific cell adhesion molecules. [81] It was determined that the ECM plays a critical function in the maintenance of stem cells in their niches as well as their mobilization from the niche.[81] Thus, the superiority of 3D PCL nanofiber scaffolds coated with collagen, compared to 2D cultures, expanding HSC, is predominantly based on the scaffold architecture and ECM protein. Quantitatively, the addition of collagen in the nanofiber 3D culture method was found to be statistically significant compared to the 2D standard culture method. It was determined that the addition of a growth factors cocktail (TPO, Flt-3, and SCF) to the standard culture medium amplified the number of CD34+ cells and total nucleated cells to 2.66-fold and 38-fold, respectively. [81] In contrast, after 10 days of expansion in a 3D PCL-collagen culture medium, the group detected 20-fold increases in the number of CD34+ progenitors and 50-fold in the number of total nucleated cells. In comparison to the conventional CD34+ cell culture, nanofiber PCL scaffold coated with collagen could effectively promote the maintenance of CD34+ phenotype and primitive progenitor cells. Thus *ex vivo* expansion of CD34+ cells was found to be effectively promoted in nanofiber PCL-based coating with collagen. [81]

Thrombocytopenia is one of the major draw backs after CB transplantation. The usage of umbilical cord blood is limited in adults because of the low content of HSCs and megakaryocytes (Mk) progenitors, even in one or two units, resulting in delayed platelet recovery in grafts of CB cells. To overcome this limitation, Fatemeh *et. al* studied the effect of 3-D culture environments using aminated PES nanofibers to study the proliferation and differentiation of $CD34^{+}$ progenitors toward Mk progenitors. Aminated PES nanofiber was investigated as

the 3-D culture, accompanied by a serum-free StemSpan and cytokine cocktail. [82] All experimentation was conducted in parallel with the conventional system, which was carried out in 2-D culture. Two-dimensional cultures were found to have restricted efficiency for the growth of hematopoietic progenitors. By contrast, 3-D cultures could function like the *in vivo* hematopoietic microenvironment by regulating cell fate. The number of expanded $CD34^+$ cells was 1.8 times higher on the aminated PES nanofiber than those cultured on the conventional system in a 7-day expansion. [82] HSCs were found to proliferate more rapidly on the PES scaffold compared with 2-D culture. In similar research, the expansion of $CD34^+$ cells was reported to be 3.5-fold higher on the aminated PES substrate than that on the commercial tissue culture polystyrene surface (TCPS) in a 10-day expansion. [82] One feasible mechanism may be the trapping and enriching of certain protein ingredients of the medium by aminated nanofibers, and these components could affect the cell expansion. Aminated PES nanofibers could possibly catch cytokines in the culture medium, fix to the supplemented growth factors as hypothesized by Chua *et. al*, and act as a niche for hematopoietic stem cells. This study indicated increased $CD34^+$ cell population in aminated PES compared to the conventional system. After differentiation, the amount of CD41/CD61-expressing cells and the quantity of NF-E2 expression level increased in the aminated PES versus the 2-D system. [81] The quantity of GATA-1 expression level was reduced on $CD41/CD61^+$ cells compared to $CD34^+$ cells, with no difference between the aminated PES and the conventional system. Aminated PES nanofiber could have more effect on the proliferation of $CD34^+$ cells and Mk differentiation than the conventional culture. Thus, injection of the expanded cells and differentiated Mk progenitors, along with the transplantation of umbilical cord stem cells might accelerate recovery of platelets and decrease the period of thrombocytopenia after transplantation.[82]

Jiang *et al.* explored an ex vivo HSC expansion model to overcome the insufficient number of primitive cells from cord blood, to minimize the delay of engraftment and the risk of neutropenia or thrombocytopenia. Early acting cytokines, such as stem cell factor (SCF), Flt3- ligand, thrombopoietin (TPO) and lineage-specific growth factors, such as interleukin (IL)-3 and IL-6 were studied in various combinations in serum or serum free culture systems. [83] These growth factors are known to play key roles in the regulation of early stages of hematopoiesis by maintaining the self-renewal capability and proliferation of HSPCs. Cryopreserved human umbilical cord blood CD133+ cells were expanded in cytokine-supplemented medium on ethylenediamine (EDA)- or 2-aminoethyl methacrylate hydrochloride (AEMA)-grafted polyethersulphone (PES) nanofibre scaffolds for 10 days.[83] Previous studies by this group indicated that in addition to the signaling molecules supplemented in the medium, substrate surface properties including topographical, mechanical and biochemical properties can significantly influence the proliferation and differentiation of CD34 cells. [81] Thus, the effect of nanofiber surface amine density on the ex vivo expansion efficiency of cryopreserved human UCB CD133+ cells in serum-free culture, and the effect of conjugate chemistry on cell adhesion and proliferation were investigated. Despite the fact that only a small fraction of surface carboxylic groups were converted to primary amino groups, the surface amine density greatly influenced hematopoietic progenitor expansion. [83] The percentage of CD34+ cells among expanded cells increased gradually with the surface amine density, but the overall expansion fold was found to have decreased. Therefore, the highest CD34+ cell expansion was obtained with intermediate

surface amine densities (20 – 80 nmol cm^2). [83] The findings from this work highlighted the importance of surface conjugation chemistry and cell– substrate interaction in promoting hematopoietic progenitor adhesion and proliferation. Increased progenitor adhesion and proliferation on the aminated fiber matrices are surmised to originate from medium components being selectively absorbed onto the positively charged surface. The surface amino groups on the nanofibers are theorized to have the potential to selectively enrich hematopoietic cytokines from the culture medium thus enhancing the expansion of HSCs for future clinical appliations. [83]

4. Conclusions

This review summarizes current strategies in the development of advanced nanofibrous polymer-based scaffolds via electrospinning, their applications in mimicking the extracellular matrix, and the use of polymer nanofibers to deliver growth factors or small molecules for ex vivo expansion of HSCs. Hematopoietic stem cell transplantation has traditionally been used as a standard treatment for various hematological disorders. CD34+ stem cells have rapidly become a promising alternative to bone marrow transplantation for the treatment of leukemia and other hematopoietic disorders. Research has shown that the use of umbilical cord blood as well as bone marrow and peripheral blood suffer from low count of stem cells which is insufficient for transplantation into adults. 3D conditions such as nanofibers are used to increase the surface to volume ratio in order to increase cellular interactions thus enabling HSCs to expand more extensively vs standard expansion protocols. Previously, in 2D environments, such as standard culture conditions, the importance of cell-cell interaction and stem cell microenvironment cues were overlooked. The ECM niche is a complex network where provide topographical, mechanical, and biochemical signals regulate stem cell function such as self-renewal, differentiation, migration, and homing. Furthermore, growth in a nanofiber scaffold minimizes stem cell differentiation and maintains HSC multipotency. Differentiation, if desired, can be induced by the introduction of various signaling factors, both intrinsic and external, that drive hematopoiesis. CD34+ HSCs can be directed to differentiate towards cells of hematopoietic lineages. The ability to control the viability of the stem cells as well as their phenotypic expressions, in the use of nanofiber scaffolds as cell carriers and/or culture substrates is one of promising potential. In this review, *ex vivo* expansion of HSCs using nanofibers shas become increasingly important in the treatment of hematopoietic disorders due to the fact that chemotherapeutic drugs and radiation are commonly used to eliminate aberrant hematopoietic cells often leaving a void in the bone marrow and circulating system. Ex vivo expansion using nanofibers enables the delivery of healthy, stem cells to repopulate and produce the blood cells needed by the body to transport oxygen, maintain immune functions, and promote clotting. As reviewed in this article, advances in nanofiber ex vivo approaches based upon emerging biomaterials open new doors for artificial niches. Scientific evidence verifies that nanofiber niche engineering provides an ideal mimic of the physical microenvironment of HSCs thereby offering great potential for future clinical applications.

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