Title

Strategies for bioengineered scaffolds that support adipose stem cells in regenerative therapies

Journal

Regenerative Medicine, 11(6)

ISSN

1746-0751

Authors

Clevenger, Tracy N
Luna, Gabriel
Fisher, Steven K
et al.

Publication Date

2016-09-01

DOI

10.2217/rme-2016-0064

Peer reviewed

Strategies for bioengineered scaffolds that support adipose stem cells in regenerative therapies

Article *in* Regenerative Medicine · August 2016

DOI: 10.2217/rme-2016-0064

4 authors, including:

Tracy Clevenger
University of California, Santa Barbara
4 PUBLICATIONS **5** CITATIONS
SEE PROFILE

Gabriel Luna
University of California, Santa Barbara
18 PUBLICATIONS **230** CITATIONS
SEE PROFILE

Steven K Fisher
University of California, Santa Barbara
192 PUBLICATIONS **9,310** CITATIONS
SEE PROFILE

Review

For reprint orders, please contact: reprints@futuremedicine.com

Regenerative Medicine

Strategies for bioengineered scaffolds that support adipose stem cells in regenerative therapies

Tracy N Clevenger[1,2,3,4], Gabriel Luna[2,5], Steven K Fisher[2,5] & Dennis O Clegg*[,1,2,3,4]

[1]Center for Stem Cell Biology & Engineering, University of California, Santa Barbara, CA, USA

[2]Neuroscience Research Institute, University of California, Santa Barbara, CA, USA

[3]Department of Molecular, Cellular & Developmental Biology, University of California, Santa Barbara, CA, USA

[4]Institute for Collaborative Biotechnologies, University of California, Santa Barbara, CA, USA

[5]Center for Bio-Image Informatics, University of California, Santa Barbara, CA, USA

*Author for correspondence: dennis.clegg@lifesci.ucsb.edu

Regenerative medicine possesses the potential to ameliorate damage to tissue that results from a vast range of conditions, including traumatic injury, tumor resection and inherited tissue defects. Adult stem cells, while more limited in their potential than pluripotent stem cells, are still capable of differentiating into numerous lineages and provide feasible allogeneic and autologous treatment options for many conditions. Adipose stem cells are one of the most abundant types of stem cell in the adult human. Here, we review recent advances in the development of synthetic scaffolding systems used in concert with adipose stem cells and assess their potential use for clinical applications.

First draft submitted: 27 May 2016; Accepted for publication: 12 July 2016; Published online: 3 August 2016

Keywords: 3D scaffolds • adipose-derived stem cells • ASC • cartilaginous regeneration • osteogenic regeneration • regenerative medicine • soft-tissue regeneration • synthetic scaffold

Stem cells in regenerative medicine

Regenerative medicine is an immense field focused on the replacement, and regeneration of human cells and/or tissues to restore normal functions [1,2]. The replacement of damaged or diseased tissue with functioning healthy cells is the primary goal of this field. The use of stem cells has become fundamental to its rapid expansion and the foundation for developing therapies to treat congenital defects, traumatic injury and disease in a patient-specific manner through the use of autologous tissue [3]. Since the first documented use of the term 'regenerative medicine' and the isolation of human embryonic stem cells, efforts to develop synthetic scaffolds for use in conjunction with stem cells have increased significantly [4–6]. The use of stem cells for regenerative treatments has achieved varying degrees of success with regards to replacing missing or damaged tissue, but progressive improvements have been brought about via recent efforts in tissue engineering [7].

Indeed, a PubMed search for 'regenerative medicine' yields more than 30,000 publications since 1920 [8]. When the search is narrowed to include 'regenerative medicine and stem cells' a list of 11,770 publications is returned. Refining this search still further by using key words such as 'mesenchymal stem cells' yields only 1148 publications. Finally, using the phrase 'regenerative medicine and adipose-derived stem cell' (ASC) produces a total of 156 publications from 2005 to 2016, an indication that research involving ASCs in the field of regenerative medicine remains in its infancy.

Surgeons in the American Society of Plastic Surgeons preformed more than 5.8 million reconstructive surgeries in 2015 alone to repair defects arising from tumor resection, traumatic injury, maxillofacial abnormalities, laceration repair and scar revision [9]. However, even the most common treatments show a significant and unpredictable loss of transplanted tissue volume over time. Volume loss

Future Medicine part of fsg

is a main reason for treatment failure, and therefore a need exists for a microenvironment that produces repeatable, sustainable results over many years. To this end, a biologically inert scaffolding system that can be tailored to the needs of individual patients and presents a means for maintaining tissue volume may prove to be a significant advancement over current treatments.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are a type of adult stem cell commonly used in numerous therapies including liver failure as a result of hepatitis B [10]. BM-MSCs have been approved for use in humans since 1995, and are currently being used in 272 clinical trials according to clinicaltrials.gov. However, unlike pluripotent embryonic stem cells, MSCs are multipotent cells that possess the ability to differentiate into various cell types, including adipogenic, chondrogenic, osteogenic, muscular, cardiac and endothelial lineages [11–15]. The International Society for Cellular Therapy (ISCT) uses three criteria to define MSCs regardless of their source: first, plastic adherence in standard culture conditions; second, expression of nonspecific surface markers CD105, CD90 and CD73 and the absence of CD34, CD45, CD14 or CD11b, CD79α and HLA-DR; and third, differentiation into osteoblasts, adipocytes and chondroblasts under specific stimuli *in vitro* [16,17]. The benefits of using adult MSCs for treatments include the ability for autologous transplants and the absence of ethical controversies surrounding their use. Adipose tissue provides an alternative high-yield source of adult stem cells known as ASCs that are predominately obtained through rudimentary liposuction procedures [18,19].

ASCs share many similarities with BM-MSCs including their potential to develop into similar cell lineages and have no clear distinction between the populations in terms of surface marker or gene expression [16,20–23]. It has been suggested that ASCs can be distinguished from BM-MSCs by their expression of CD36 (F.A.T. – a protein involved in fatty acid metabolism) or CD49d (integrin α4 – a subunit of the integrin receptor for fibronectin and VCAM-1), as well as the lack of CD106 (VCAM1 – a protein involved in the adhesion of vascular cells). However, each of these proteins shows variability in expression patterns between specific ASC populations [24]. Investigations into the gene expression profiles of BM-MSCs and ASCs found that 13.2% of 384 genes examined were differentially expressed between the two populations. Although no identifying markers were specific to each population, genes more highly expressed by ASCs were mainly involved in cellular communication (*FGF9*, *IL1R2*, *CCL3* and *KDR*) while those with higher expression by BM-MSCs were involved in WNT signaling and differentiation pathways (*WNT11*, *WNT7B* and *SOX6*) [25].

ASCs are ten-times more abundant than BM-MSCs in the tissues from which they are isolated. Additionally, ASCs demonstrate a higher proliferative potential, show consistent growth rates in culture and are procured from a minimally invasive procedure by comparison [26,27]. Furthermore, ASCs are robust, capable of self-renewal, can be collected in large quantities and easily expanded in culture. These qualities identify ASCs as a promising source for use in therapeutic regenerative medicine [28–31].

Over the last 18 years, many techniques have been developed to create scaffolding materials that are compatible with stem cells as well as transplantation sites. Scaffolds generated prior to cell seeding allow for the use of reagents typically considered 'harsh,' thereby expanding the potential library of materials for therapeutic use. Topical seeding of cells has been one approach used in the development of synthetic scaffolds in regenerative medicine, but often results in a very low penetration throughout the material, leading to a heterogeneous cellular distribution within the scaffold [32]. Another tactic for the development of scaffolds as regenerative therapies is the use of decellularized extracellular matrix (ECM). These scaffolds are generated from allogenic or xenogenic tissues and are popular for applications involving heart valves, blood vessels, tendons and ligaments. Importantly, this technique most closely mirrors the mechanical and biological properties of human tissue [33]. Complications from using this approach may arise if all cellular components of the donor tissue are not thoroughly removed prior to implantation, increasing the likelihood of immunological rejection, thereby requiring the long-term use of immunosuppressant drugs. Cell encapsulation in natural or synthetic hydrogel matrix is yet another method used in scaffold engineering, since these frameworks can be designed to provide biomimetic environments that polymerize from a liquid to a solid polymer network under specific conditions. By using a one-step procedure to encapsulate stem cells instead of topical cell seeding, a more homogenous cell density with exceptional cell viability is achieved. Here, we provide an overview of the field by examining a collection of synthetic scaffolds currently used in conjunction with ASCs to treat defects of various tissue types.

ASCs in synthetic scaffolds for cartilaginous regeneration

Cartilage is flexible connective tissue located in joints between bones, but regions of cartilaginous tissue also exist in the ear, nose and rib cage. Unlike bone, cartilage is not rigid; however, it is less flexible than muscle or other types of connective tissues, such as fat.

Cartilage is important in providing flexibility to the skeletal system, a critical feature that allows for proper function. Cartilage is primarily composed of chondrocytes, cells responsible for producing the ECM proteins required for the tissue's unique mechanical characteristics [34–37]. Cartilage is unable to self-repair after blunt-force trauma, athletic injury, disease or age-related degeneration. The number of knee surgeries to repair articular cartilage damage each year in the USA increases by 5% annually [38]. The natural lack of vascularization in addition to the minimal cell-to-cell contact restricts cartilage to only minimal spontaneous healing because of the slow dissemination of healing factors to distant cells. Because of these characteristics, treatments frequently consist of surgically removing damaged tissue in order to reduce pain and restore function [35,37,39]. A regenerative approach to cartilage replacement therapy involves the restoration of proper cellular morphology, and the prevention of further deterioration. Current treatments, such as allografting, can carry small, but serious risks of infection and disease transmission while treatments such as autologous chondrocytic transplantations may result in degenerative changes accompanied by pain [36,37,40].

ASCs can be induced into chondrogenic differentiation *in vitro* by the combinatorial influence of growth factors, such as TGF-β1 and TGF-β3, BMP-4 and bFGF. ASCs provide several advantages over autologous chondrocyte treatments because they do not induce an inflammatory response, form new cartilage and possess the potential for restoring long-term cartilage function. The use of 3D scaffolds has gained momentum in the field of cartilage restoration because of their ability to overcome the growth inhibition typically observed in standard *in vitro* cultures [41,42]. Synthetic platforms provide locations for ASCs to adhere, thereby providing an environment conducive for growth and proliferation. Additionally, scaffolds have also been shown to promote differentiation and enable cells to achieve a cartilage-like morphology and express chondro-specific molecules, such as COL2A1 and CSPCP [43].

Poly-lactide-co-glycolide (PLGA) is a copolymer approved for numerous therapeutic uses in humans by the US FDA since 2001. This copolymer may act as a stable or a biodegradable material depending on its formulation, and it possesses a permeable pore network that supports cell adhesion and proliferation. Mehlhorn and Zwingmann showed that these scaffolds were suitable cell carriers for chondrocytes. Furthermore, PLGA networks seeded with ASC-chondrocytes showed excellent volume stability and sufficient elasticity comparable to natural cartilage [44]. These results suggest that PLGA may serve as an effective scaffolding system for chondrocytes derived from ASCs.

Another avenue employs the use of fibrous polyglycolic acid (PGA) stabilized by polylactic acid (PLA). Cui *et al.* demonstrated that this combination of polymers produced promising results during the initial attachment of ASCs, and subsequent proliferation of chondrogenic-induced ASCs. In addition, cells deposited cartilage-specific ECM proteins within the polymer. Degradation times of approximately 2 months *in vivo* appeared to match the natural mechanisms of new cartilage formation. Thus, PGA/PLA in combination with ASCs may also serve as a synthetic scaffold for cartilage regeneration [43].

While PLGA and PGA/PLA comprise the bulk of synthetic polymers used for cartilage regeneration, other synthetic gels incorporate hyaluronic acid (HA), an important component of cartilage, into poly(ethylene)glycol (PEG) polymers. Unterman *et al.* showed that HA-interacting PEG hydrogels improved cartilage tissue formation *in vitro* and *in vivo* in instances where HA was presented at a later stage of differentiation, subsequently resulting in increased chondrogenic phenotypes [45]. Here, carefully considering properties of the native environment resulted in increased success by incorporating HA components that mimic the desired tissue. This is an important case that demonstrated functionalization of a scaffold to more closely replicate a desired environment had a positive effect on graft viability [46].

3D cell printing, or bioprinting, has become an increasingly attractive option for the treatment of bone lesions as it provides a means to create scaffold structures that alleviate the limitations of the fields due to the complex 3D geometries associated with defects. The use of cells in prepolymer 'bioink' allows a layer-by-layer deposition in a 3D construct that is analogous to tissues and organs [47,48]. This technique provides unique opportunities to develop complexly shaped scaffolds from synthetic material that encapsulate cells as shown by Lee *et al.* [49]. The fabrication of a structure with an ear shape with chondrocytes and adipocytes derived from ASC-derived cells in a polycaprolactone (PCL) hydrogel demonstrated a successful composite tissue. The efficient chondrogenesis and adipogenesis of the cell-printed structure resulted in a step forward for the practicality of 3D printing complex organs for tissue regeneration.

ASCs in synthetic scaffolds for osteogenic therapeutics

In contrast to cartilage, bone has regenerative capacity due to its inherent population of osteoblasts and osteoclasts (bone-forming and bone-resorbing cell types, respectively) [50]. However, these processes are frequently perturbed in cases of trauma, disease or

tumor resection. Bone autografts, in other words, harvesting bone from one anatomic site and grafting into another site in the same subject, is one of the primary approaches currently used for bone augmentation in a variety of orthopedic and maxillofacial procedures. Approximately 800,000 patients receive these grafts annually [51], and while significant skeletal incorporation has been observed in these types of grafts many drawbacks still exist using this approach, such as delayed healing, a complete failure to heal, morbidity at donor sites, quantity restrictions, substantial financial costs due to additional procedures to harvest transplant tissue and discomfort for the patient [52–55].

Collectively, focus has begun to shift toward the development of synthetic systems for use in conjunction with ASCs to replace traditional bone grafts. One study determined that PLGA is a viable scaffold for osteogenic differentiation of ASCs. After 2 weeks of osteogenic induction, mineralized nodular structures were observed by Alzarian Red and von Kossa staining, indicating successful calcification of the ECM [56]. The use of PLGA scaffolds for osteogenic differentiation provides a viable polymer scaffolding option, however, further investigation is needed to determine what external cues may be necessary prior to graft implantation of this particular material, which has shown promise for applications involving chondrogenic and adipogenic lineages; indicating that the basic polymer supports numerous cell fates and must be modified to help direct differentiation.

While polymers have proven to be useful in a variety of other fields, osteogenesis may require unique materials due to the highly specialized mechanical properties of natural bone. Thus, regenerative osteogenic technology has begun to employ the use of titanium metal to create a space that facilitates the migration of implanted cells and their osteogenic differentiation. Titanium is an inert biomaterial that possesses exceptional mechanical strength, is biocompatible and therefore, a prime candidate for use in regenerative applications involving bone. ASCs have shown compatibility with titanium systems, as well as displayed suitable cell adhesion. As a scaffold, titanium enables adhesion and osteoblastic differentiation of ASCs *in vitro*, indicated by an increased deposition of ALP and BGLAP (ECM proteins necessary for matrix mineralization) as well as calcification, confirmed by von Kossa staining [57,58]. The ability of ASCs to acquire the proper phenotypic differentiation as well as produce an ECM and a mineralized matrix suggest titanium as an attractive material as a filler or support structure for bone in growth in regenerative medicine [57].

Calcium phosphate ceramics (CPCs) are another class of scaffolds used for bone regeneration. These are promising synthetic materials due to their resemblance to bone mineral, their malleable bioactive properties and their surface characteristics, which support osteoblast adhesion, proliferation and differentiation *in vivo* [59,60]. Most CPCs examined have been shown to be osteoconductive (growth of bone on a surface) while only certain types exhibit osteoinductive (recruitment and differentiation of immature osteocytes) abilities. There is evidence, however, that increased mircoporosity increases the amount of bone inducing proteins secreted by ASCs *in vitro* [61]. The similarities of CPCs to bone, along with their ability to induce bone growth and promote secretion of important proteins elevate these materials as an intriguing and exciting possibility for osteogenic therapies.

While the similarities of CPC to bone have proven to be beneficial to osteogenic regeneration, the use of decellularized bone (DCB) in combination with PCL shows even greater promise. PCL is a biodegradable polyester polymer used to circumvent the inability of 3D printers to use DCB alone as a printing material. The use of 3D printers to engineer-scaffolding systems using PCL has shown enhanced adhesion of ASCs. These cells exhibited significant upregulation of osteogenic genes such as *BGLAP*, *runx2* and *SPARC*. It was also demonstrated by Alzarian Red staining that ASCs on DCB:PCL materials showed increased calcification. When scaffolds were implanted into calvarial defects in mice, DCB:PCL scaffolds invoked nearly twice the volume of regenerated bone in 12 weeks compared with PCL alone [62].

The use of 3D-printed PCL scaffolding without addition of natural components has also shown by varying the internal pore size of the scaffold, it is possible to influence cell seeding of ASCs. By manipulating this parameter, Temple *et al.* were able to achieve optimal vascular and osteogenic differentiation in 3D-printed scaffolds [63]. This study also showed that maintenance of complex geometrical features such as maxilla and mandible bones maintains this porosity and therefore allows for cell seeding and vascularization similar to previous *in vivo* studies

Similar to PCL, polymers used in other regenerative studies, such PLGA can be blended with natural components to make them more amenable to 3D printing. Lee *et al.* determined that by 3D printing PLGA scaffolds impregnated with BMP-2 and ASCs, it is possible to achieve mandibular regeneration [64]. The use of a small-animal model of mandibular defects allows investigation of the potential for union of transplanted scaffolding with natural bone, within a site of segmental defect. Similarly, Kao *et al.* demonstrated that coating of 3D-printed PLA with bioinspired synthetic coatings increased the adhesion, proliferation, as well as the osteogenic and endothelial differentiation of ASCs in 3D structures [65]. These simple modifications to syn-

thetic 3D-printed scaffolds may serve as the basis for effective delivery carriers in bone tissue engineering.

ASCs in synthetic scaffolds for soft tissue regeneration

Soft-tissue defects are relatively common, accounting for nearly 10% of all emergency department visits in addition to causes previously examined (i.e., trauma, tumor resection) [66]. More than 100,000 breast reconstructions after mastectomy, and over 200,000 maxillofacial surgeries were performed in 2015 alone and it is predicted that there will be more than 12,000 new cases of soft-tissue sarcomas in the USA in 2016 [9,67]. The current treatment for many of these conditions is autologous lipotransfer, a procedure involving collecting fat tissue from a patient, minimally manipulating the resultant lipoaspirate and relocating it to the site of reconstruction [68–71]. Although used widely, reports show that there is extensive variability of long-term lipotransfer graft survival, due to unpredictable degrees of resorption and tissue volume loss that can range from 20 to 90% [32,72,73].

Variations in soft-tissue graft survival have been attributed to many causes including a lack of local angiogenesis, sample preparation, as well as innate properties of the transplant site [70]. In a national consensus survey, 92% of physicians stated that their patients experienced some degree of resorption, 52% reported a resorption rate of 50% or greater [74]. Mature adipocytes constitute the majority of the transplant volume, and since these cells are in a terminally differentiated state, they lack the ability for self-renewal and proliferation. The primary cause for transplant death is the lack of revascularization of the transplanted tissue. Success rates are often reported to be as low as 20%, while successful transplants are commonly attributed to the relatively small population of ASCs present in the transplanted fat, and can be enhanced by increasing the number of stem cells transplanted [70,75]. Thus, significant volume loss in these types of transplants provides motivation for finding ways to decrease the loss and thereby increase the likelihood of a successful transplant. The recent development of cell-assisted lipotransfer using concentrated ASCs as a lipoaspirate additive before transplantation leads to significantly improved results, specifically in terms of thickness gains observed during the first 6 months, and a reduction in thickness loss at 1 year [76]. However, even cases using stem cell enrichment, marginal volume losses were still documented, and in most cases no gain of regenerated tissue was reported [70]. Therefore, engineering synthetic scaffolds that can support the survival of the transplanted stem cell population while simultaneously promoting adipogenic differentiation has been proposed as a novel avenue for improving the success of these types of transplants.

Various synthetic scaffolding materials have been examined to determine structural viability for stem cell survival and adipose tissue reconstruction. Patrick *et al.* demonstrated that ASCs seeded into a PLGA scaffold and implanted subcutaneously into rats showed maximum adipose tissue formation after 2 months, but noted that between 3 and 12 months, a complete loss of reconstructed adipose tissue and degeneration of the PLGA scaffold occurred [77]. This loss of tissue may arise from the degradation of the scaffolding, especially since signs of PLGA degradation were apparent as early as 1 month post-transplantation. Thus, successful scaffolds for adipose tissue transplantation may require prolonged degradation times in order to allow for the maturation of regenerating tissue.

Cho *et al.* demonstrated that implanting a support made of PGA and PLA before injecting pre-adipocytes provided enough support to maintain the volume of the implants and showed regeneration of the adipose tissue after 6 weeks in athymic mice [78]. However, this approach utilizes an implant primarily acting as structural support for ASCs that are injected in a solution. Although stability of the transplanted volume was reported, there was no systematic method to measure pre-implantation volume, leading to difficulties in determining whether adipose growth was due to the implanted cells. Additionally, no conclusive evidence was shown to indicate that the regenerated cells originated from transplanted ASCs. In clinical applications, it will be imperative to determine that the incorporation and differentiation of implanted cells replaces missing tissue.

The use of blended copolymers has recently become increasingly popular for applications in therapeutic treatments. The blending of poly(glycerol sebacate) (PGS), a biodegradable and biocompatible synthetic elastomer specifically designed to imitate the mechanical behavior of soft tissue, with PLA (to overcome the quality and flexibility concerns of using PGS alone) has shown promise. Frydrych *et al.* showed that ASCs seeded onto the surface of a PGS/PLA scaffold exhibited significant amounts of cellular penetration and substantial collagen accumulation over 21 days [79]. However, *in vitro* degradation assays determined that degradation appeared to progress too rapidly (50% loss after ~30 days) for this scaffold to support the growth of target tissue, a phenomenon also observed by Patrick *et al.* [77].

Blending different scaffolding polymers provides the advantage of utilizing the positive attributes of each material. Lin *et al.* used mixtures of gelatin sponges and polyglycolic meshes encased in microfilament polypropylene mesh to support adipose tissue regeneration using predifferentiated ASCs [80]. The

Table 1. Synthetic scaffolds used in conjunction with adipose-derived stem cells

Scaffold material	Cartilage	Bone	Adipose
PLGA	Mehlhorn *et al.* (2009) [44]	Lee *et al.* (2008) [56] Lee *et al.* (2015) [64]	Patrick *et al.* (2002) [77]
PGA/PLA	Cui *et al.* (2009) [43]	–	Cho *et al.* (2005) [78]
HA-PEG	Unterman *et al.* (2012) [45]	–	–
PCL	Lee *et al.* (2014) [49]	Temple *et al.* (2014) [63]	–
Titanium	–	Gastaldi *et al.* (2010) [57] Marycz *et al.* (2015) [58]	–
CPC	–	Samavedi *et al.* (2013) [59] Barrere *et al.* (2006) [60] Li *et al.* (2011) [61]	–
DCB/PCL	–	Hung *et al.* (2016) [62]	–
PLA	–	Kao *et al.* (2015) [65]	–
PGS/PLA	–	–	Frydrych *et al.* (2015) [79]
Gelatin/PGA/PP	–	–	Lin *et al.* (2008) [80]
PEG	–	–	Clevenger *et al.* (2016) [88]

CPC: Calcium phosphate ceramic; DCB: Decellularized bone; HA: Hyaluronic acid; PCL: Polycaprolactone; PEG: Poly(ethylene)glycol; PGA: Polyglycolic acid; PGS: Poly(glycerol sebacate); PLA: Polylactic acid; PLGA: Poly-lactide-co-glycolide; PP: Polypropylene.

gelatin–polyglycolic mesh was observed to degrade completely within 60 days; however, the polypropylene mesh is biostable and remains as a permanent resident of the transplant procedure. In fact, it was demonstrated that after 6 months *in vivo* these scaffolds retained their shape, a trait attributed to the nondegradable mesh, while the newly formed adipose tissue occupied the space within the scaffold [80]. This avenue is an improvement in terms of the longevity of engineered adipose tissue; however, this system is complex and requires lengthy *in vitro* cultures, thus it may prove too difficult to translate to clinical applications.

While each of these synthetic scaffolds possesses positive attributes, each neglects to consider important interactions of cells with their surroundings. Immediately following seeding into a synthetic scaffold, cells must be afforded sites of adhesion from which they are able to receive signals for survival, proliferation and differentiation. Since these polymers are biologically inert, it is critical to engineer attachment sites that provide favorable interactions between the ASCs and their surroundings. PEG is a polymer that was approved for use in humans by the FDA in 1979, and is currently used in a myriad of applications ranging from food additives to pharmaceutical products and drug delivery systems [81,82]. Recently, it was demonstrated that incorporation of Arg-Gly-Asp (RGD) variant peptides (linear RGD, cyclic RGD and vitronectin-derived RGD) into PEG-based gels is a feasible approach to functionalizing an inert biomaterial [83–87]. These peptides provide sites for ASC attachment at the time of cell incorporation. It was demonstrated that various adhesion peptides provided transplanted ASCs with enhanced directed adipogenic differentiation by comparison to systems that contained no attachment peptide. Peptides considered to be highly adhesive lead to smaller lipid vacuoles and thus immature adipocytes. However, peptides containing RGD derived from vitronectin (less adhesive) allowed ASCs to attach when incorporated into the hydrogel, while remaining rounded morphologically [88]. This approach demonstrates that the initial environment encountered by ASCs may influence their ability to differentiate in 3D scaffolds.

Conclusion

Despite its relative youth, the field of regenerative medicine is expanding quickly, encompassing exciting developments in the area of bioengineering, stem cell biology and materials research. ASCs hold enormous potential in this field. The multipotency of ASCs provides the potential building blocks for the treatment and regeneration of damaged tissue. Their relative abundance, and their ease of access, suggests that ASCs may provide an improvement over other stem cells used in therapeutic treatments.

The design of complex and smart materials able to interact with cells to direct their biological response and differentiation has been on the rise since the advent of tissue engineering. It has been shown that the interactions of cells with their environment plays a critical role in their health and development [89]. In order to regenerate and restore healthy tissue after an insult, disease or defect the ability to direct implanted cells along specific pathways may prove to be paramount. There are

many avenues currently under investigation to determine the best method of combining ASCs and synthetic scaffolds to obtain an optimal graft or implant for the desired application. Table 1 summarizes various synthetic matrices that have been evaluated in the last 15 years for treatment of cartilage, bone and adipose tissue defects. One of the most common polymers, PLGA, has been used for all three purposes, showing positive results when used in osteogenic applications, neutral results when used in chongrogenic repair and a notable loss of volume when applied to cases of adipogenic tissue growth. This is a prime example of the diverse capability of synthetic polymers used with stem cell populations. It is critical to consider the downstream consequences for all proposed scaffolding materials; specifically, scaffolding systems successfully used in one tissue type may not be yield similar results in another. Mimicking the native environment of the target tissue is likely to play a significant role for the long-term survival of virtually all transplant scaffolds.

Indeed, it was recently demonstrated that various adhesion peptides provided transplanted ASCs with enhanced directed adipogenic differentiation by comparison to systems that contained no attachment peptide [88]. The use of vitronectin-derived attachment peptides promoted the development of larger lipid vacuoles, further suggesting that the interaction of the ASCs with their scaffolding may have a significance impact on the desired differentiation and health of implanted cells [90,91]. Biomimetic PEG hydrogels may prove to be superior synthetic scaffolds for use in tissue reconstruction [92–94].

Future perspective

Applications of adipose-derived stem cell therapies have enormous potential for expanding the field of regenerative medicine. Their ability to differentiate into numerous cell types, as well as their abundance, places ASCs at the forefront in the development of next-generation therapeutic treatments. Vascularization of grafts and implants is another chief concern with respect to cell viability. This is a critical issue in current approaches for regeneration and treatment, and thus an important factor to address when developing new synthetic scaffold systems. The ability of ASCs to differentiate into endothelial cells along with a scaffold that supports a desired differentiation may increase the chances of achieving a scaffold that produces viable, long-lasting, vascularized tissue [95–98].

Transitioning scaffolding materials from the laboratory to clinical applications poses challenges that require further investigation. For example, additional demonstration of long-term safety in preclinical animal models will be necessary prior to their use in clinical applications. This process is timely, labor-intensive and expensive; however, the treatment benefits will outweigh the initial hurdles encountered in the exploration and development of synthetic scaffold for use as regenerative therapeutics.

Disclaimer

The content within does not necessarily reflect the position or policy of the government, and endorsement should not be inferred.

Financial & competing interests disclosure

This work was supported by the California Institute for Regenerative Medicine (CIRM; DR1-01444, CL1-00521, TB1-01177, TG2-01151 and FA1-00616) to DO Clegg, the National Science Foundation (IIS-0808772 and ITR-0331697) to SK Fisher and a grant to the University of California Santa Barbara Institute for Collaborative Biotechnologies from the US Army Research Office (W911NF-09-0001) to DO Clegg. TN Clevenger was a predoctoral fellow of the California Institute for Regenerative Medicine. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Adipose stem cells in synthetic scaffolds for cartilaginous regeneration

- Synthetic scaffolds made from polymers such as poly-lactide-co-glycolide (PLGA), polyglycolic acid/polylactic acid (PLA) and poly(ethylene)glycol show the ability for adipose-derived stem cells (ASCs) to survive and differentiate along a chondrogenic lineage.

ASCs in synthetic scaffolds for osteogenic therapies

- Titanium, calcium phosphate ceramics and PLGA promote the mineralization of extracellular matrix secreted by ASC-derived osteocytes.

ASCs in synthetic scaffolds for adipogenic replacement

- PLGA, polyglycolic acid/PLA and polyglycerol sebacate/PLA are all blends of synthetic polymers that have been used in the adipogenic differentiation of ASCs.
- Poly(ethylene)glycol scaffolds containing different adhesive peptides have shown the *in vivo* influence the adipogenic differentiation of ASCs.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Atala A, Lanza RP. *Methods of Tissue Engineering.* Gulf Professional Publishing, San Diego, CA, USA (2002).

2 Mao AS, Mooney DJ. Regenerative medicine: current therapies and future directions. *Proc. Natl Acad. Sci. USA* 112(47), 14452–14459 (2015).

3 Mironov V, Visconti RP, Markwald RR. What is regenerative medicine? Emergence of applied stem cell and developmental biology. *Expert Opin. Biol. Ther.* 4(6), 773–781 (2004).

4 Kaiser LR. The future of multihospital systems. *Top Health Care Financ.* 18(4), 32–45 (1992).

5 Thomson JA. Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391), 1145–1147 (1998).

6 Tibbitt MW, Rodell CB, Burdick JA, Anseth KS. Progress in material design for biomedical applications. *Proc. Natl Acad. Sci. USA* 112(47), 1444–14451 (2015).

7 Harrison RH, St-Pierre J-P, Stevens MM. Tissue engineering and regenerative medicine: a year in review. *Tissue Eng. Part B Rev.* 20(1), 1–16 (2014).

8 Marine D, Manley OT. Homeotransplantation and autotransplantation of the spleen in rabbits: III. Further data on growth, permanence, effect of age, and partial or complete removal of the spleen. *J. Exp. Med.* 32(1), 113–133 (1920).

9 Surgeons ASOP. 2015 Cosmetic Plastic Surgery Statistics. 1–3 (2016). www.plasticsurgery.org/Documents

10 Peng L, Xie D-Y, Lin B-L *et al.* Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. *Hepatology* 54(3), 820–828 (2011).

11 De Francesco F, Ricci G, D'Andrea F, Nicoletti GF, Ferraro GA. Human adipose stem cells: from bench to bedside. *Tissue Eng. Part B Rev.* 21(6), 572–584 (2015).

12 Pittenger MF, Mackay AM, Beck SC *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411), 143–147 (1999).

13 Sung HJ, Hong SC, Yoo JH *et al.* Stemness evaluation of mesenchymal stem cells from placentas according to developmental stage: comparison to those from adult bone marrow. *J. Korean Med. Sci.* 25(10), 1418 (2010).

14 Baer PC, Griesche N, Luttmann W, Schubert R, Luttmann A, Geiger H. Human adipose-derived mesenchymal stem cells *in vitro*: evaluation of an optimal expansion medium preserving stemness. *Cytotherapy* 12(1), 96–106 (2012).

15 Yu J, Tu Y-K, Tang Y-B, Cheng N-C. Stemness and transdifferentiation of adipose-derived stem cells using L-ascorbic acid 2-phosphate-induced cell sheet formation. *Biomaterials* 35(11), 3516–3526 (2014).

16 Bourin P, Bunnell BA, Casteilla L *et al.* Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 15(6), 641–648 (2013).

•• **Seminal publication on the definition of adipose stem cells.**

17 MD MD, Le Blanc K, Mueller I *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4), 315–317 (2012).

18 Zuk PA, Zhu M, Ashjian P *et al.* Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell* 13(12), 4279–4295 (2002).

19 Bernacki SH, Wall ME, Loboa EG. Isolation of human mesenchymal stem cells from bone and adipose tissue. In: *Methods in Cell Biology.* Elsevier, San Diego, CA, USA, 257–278 (2008).

20 Baer PC. Adipose-derived mesenchymal stromal/stem cells: an update on their phenotype *in vivo* and *in vitro*. *WJSC* 6(3), 256 (2014).

•• **Excellent review on the current state of the adipose stem cell field.**

21 Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells Int.* 2012(3), 1–11 (2012).

22 Fraser JK, Zhu M, Wulur I, Alfonso Z. Adipose-derived stem cells. *Methods Mol. Biol.* 449, 59–67 (2008).

23 Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev.* 21(14), 2724–2752 (2012).

24 Baer PC, Kuçi S, Krause M *et al.* Comprehensive phenotypic characterization of human adipose-derived stromal/stem cells and their subsets by a high throughput technology. *Stem Cells Dev.* 22(2), 330–339 (2013).

25 Noël D, Caton D, Roche S, Bony C, Lehmann S. Cell specific differences between human adipose-derived and mesenchymal-stromal cells despite similar differentiation potentials. *Exp. Cell Res.* 314(7), 1575–1584 (2008).

26 Zhu X, Shi W, Tai W, Liu F. The comparition of biological characteristics and multilineage differentiation of bone marrow and adipose derived mesenchymal stem cells. *Cell Tissue Res.* 350(2), 277–287 (2012).

27 Peng L, Jia Z, Yin X *et al.* Comparative analysis of mesenchymal stem cells from bone marrow, cartilage, and adipose tissue. *Stem Cells Dev.* 17(4), 761–774 (2008).

28 Wankhade UD, Shen M, Kolhe R, Fulzele S. Advances in adipose-derived stem cells isolation, characterization, and application in regenerative tissue engineering. *Stem Cells Int.* 2016(5), 1–9 (2016).

29 Lim M-H, Kim HW, Paik K-C, Cho SC, Yoon DY, Lee H-J. Association of the DAT1 polymorphism with attention deficit hyperactivity disorder (ADHD): a family-based approach. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 141B(3), 309–311 (2006).

30 Lim MH, Ong WK, Sugii S. The current landscape of adipose-derived stem cells in clinical applications. *Expert Rev. Mol. Med.* 16, e8 (2014).

31 Minteer DM, Marra KG, Rubin JP. Adipose stem cells. *Clin. Plast. Surg.* 42(2), 169–179 (2015).

32 Chan CW, McCulley SJ, Macmillan RD. Autologous fat transfer – a review of the literature with a focus on breast cancer surgery. *Br. J. Plast. Surg.* 61(12), 1438–1448 (2008).

33 Petit F, Minns AB, Dubernard J-M, Hettiaratchy S, Lee WPA. Composite tissue allotransplantation and reconstructive surgery: first clinical applications. *Ann. Surg.* 237(1), 19–25 (2003).

34 Behonick DJ, Werb Z. A bit of give and take: the relationship between the extracellular matrix and the developing chondrocyte. 120(11), 1327–1336 (2003).

35 Kölliker A. *Manual of Human Histology.* Syndenham Society, London, UK (1853).

36 Breinan HA, Minas T, Hsu HP, Nehrer S, Sledge CB, Spector M. Effect of cultured autologous chondrocytes on repair of chondral defects in a canine model. *J. Bone Joint Surg. Am.* 79(10), 1439–1451 (1997).

37 Mobasheri A, Csaki C, Clutterbuck AL, Rahmanzadeh M, Shakibaei M. Mesenchymal stem cells in connective tissue engineering and regenerative medicine: applications in cartilage repair and osteoarthritis therapy. *Histol. Histopathol.* 24(3), 347–366 (2009).

38 McCormick F, Harris JD, Abram GD *et al.* Trends in the surgical treatment of articular cartilage lesions in the United States: an analysis of a large private-payer database over a period of 8 years. *Arthroscopy* 30(2), 222–226 (2014).

39 Campbell CJ. The healing of cartilage defects. *Clin. Orthop. Relat. Res.* 64, 45–63 (1969).

40 Centers for Disease Control and Prevention (CDC). Update: allograft-associated bacterial infections – United States, 2002. *MMWR Morb. Mortal Wkly. Rep.* 51(10), 207–210 (2002).

41 Xu J, Wang W, Ludeman M *et al.* Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. *Tissue Eng. Part A* 14(5), 667–680 (2008).

42 Sun AX, Lin H, Beck AM, Kilroy EJ, Tuan RS. Projection stereolithographic fabrication of human adipose stem cell-incorporated biodegradable scaffolds for cartilage tissue engineering. *Front Bioeng. Biotechnol.* 3, 115 (2015).

43 Cui L, Wu Y, Cen L *et al.* Repair of articular cartilage defect in non-weight bearing areas using adipose derived stem cells loaded polyglycolic acid mesh. *Biomaterials* 30(14), 2683–2693 (2009).

44 Mehlhorn AT, Zwingmann J. Chondrogenesis of adipose-derived adult stem cells in a poly-lactide-co-glycolide scaffold. *Tissue Eng. Part A* 15(5), 1159–1167 (2009).

45 Unterman SA, Gibson M, Lee JH *et al.* Hyaluronic acid-binding scaffold for articular cartilage repair. *Tissue Eng. Part A* 18(23–24), 2497–2506 (2012).

46 DeForest CA, Anseth KS. Advances in bioactive hydrogels to probe and direct cell fate. *Annu. Rev. Chem. Biomol. Eng.* 3(1), 421–444 (2012).

47 Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. *Biotechnol. Adv.* 34(4), 422–434 (2016).

48 Bajaj P, Schweller RM, Khademhosseini A, West JL, Bashir R. 3D biofabrication strategies for tissue engineering and regenerative medicine. *Annu. Rev. Biomed. Eng.* 16(1), 247–276 (2014).

49 Lee J-S, Hong JM, Jung JW, Shim J-H, Oh J-H, Cho D-W. 3D printing of composite tissue with complex shape applied to ear regeneration. *Biofabrication* 6(2), 024103 (2014).

50 Rodan GA, Martin TJ. Role of osteoblasts in hormonal control of bone resorption – a hypothesis. *Calcif. Tissue Int.* 33(4), 349–351 (1981).

51 Laurencin CT, Ambrosio AM, Borden MD, Cooper JA. Tissue engineering: orthopedic applications. *Annu. Rev. Biomed. Eng.* 1, 19–46 (1999).

52 Prolo DJ, Rodrigo JJ. Contemporary bone graft physiology and surgery. *Clin. Orthop. Relat. Res.* (200), 322–342 (1985).

53 Couture J, Cabana F. Irradiated allograft bone in spine surgery. *Spine* 38(7), 558–563 (2013).

54 Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. *J. Bone Joint Surg. Am.* 84-A(5), 716–720 (2002).

55 St John TA, Vaccaro AR, Sah AP *et al.* Physical and monetary costs associated with autogenous bone graft harvesting. *Am J. Orthop.* 32(1), 18–23 (2003).

56 Lee JH, Rhie J-W, Oh DY, Ahn ST. Osteogenic differentiation of human adipose tissue-derived stromal cells (hASCs) in a porous three-dimensional scaffold. *Biochem. Biophys. Res. Commun.* 370(3), 456–460 (2008).

57 Gastaldi G, Asti A, Scaffino MF *et al.* Human adipose-derived stem cells (hASCs) proliferate and differentiate in osteoblast-like cells on trabecular titanium scaffolds. *J. Biomed. Mater. Res. A* 94(3), 790–799 (2010).

58 Marycz K, Śmieszek A, Grzesiak J *et al.* The osteogenic properties of multipotent mesenchymal stromal cells in cultures on TiO_2 sol-gel-derived biomaterial. *BioMed. Res. Int.* 2015(1), 1–11 (2015).

59 Samavedi S, Whittington AR, Goldstein AS. Calcium phosphate ceramics in bone tissue engineering: a review of properties and their influence on cell behavior. *Acta Biomater.* 9(9), 8037–8045 (2013).

60 Barrère F, van Blitterswijk CA, de Groot K. Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. *Int. J. Nanomedicine* 1(3), 317–332 (2006).

61 Li X, Liu H, Niu X *et al.* Osteogenic differentiation of human adipose-derived stem cells induced by osteoinductive calcium phosphate ceramics. *J. Biomed. Mater. Res.* 97B(1), 10–19 (2011).

62 Hung BP, Naved BA, Nyberg EL *et al.* Three-dimensional printing of bone extracellular matrix for craniofacial regeneration. *ACS Biomater. Sci. Eng.* doi:10.1021/acsbiomaterials.6b00101 (2016) (Epub ahead of print).

•• **Innovative study combining 3D printing and stem cells for regenerative therapy.**

63 Temple JP, Hutton DL, Hung BP *et al.* Engineering anatomically shaped vascularized bone grafts with hASCs and 3D-printed PCL scaffolds. *J. Biomed. Mater. Res. A* 102(12), 4317–4325 (2014).

64 Lee MK, DeConde AS, Lee M *et al.* Biomimetic scaffolds facilitate healing of critical-sized segmental mandibular defects. *Am. J. Otolaryngol.* 36(1), 1–6 (2015).

65 Kao CT, Lin CC, Chen YW, Yeh CH, Fang HY, Shie MY. Poly(dopamine) coating of 3D printed poly(lactic acid) scaffolds for bone tissue engineering. *Mater. Sci. Eng. C Mater. Biol. Appl.* 56, 165–173 (2015).

66 Ong TK, Dudley M. Craniofacial trauma presenting at an adult accident and emergency department with an emphasis on soft tissue injuries. *Injury* 30(5), 357–363 (1999).

67 Cancer Society A. Cancer facts. 1–72 (2015). www.cancer.org/acs/groups/content

68 Kantanen DJ, Closmann JJ, Rowshan HH. Abdominal fat harvest technique and its uses in maxillofacial surgery. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 109(3), 367–371 (2010).

69 Yoshimura K, Asano Y, Aoi N *et al.* Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications. *Breast J.* 16(2), 169–175 (2010).

• **Currently, the most efficient treatment of soft tissue defects.**

70 Domenis R, Lazzaro L, Calabrese S *et al.* Adipose tissue derived stem cells: *in vitro* and *in vivo* analysis of a standard and three commercially available cell-assisted lipotransfer techniques. *Stem Cell Res. Ther.* 6, 2 (2015).

71 Hammer-Hansen N, Akram J, Damsgaard TE. The versatility of autologous fat transplantation in correction of facial deformities: a single-center experience. *Plast. Surg. Int.* 2015, 703535 (2015).

72 Toyserkani NM, Quaade ML, Sørensen JA. Cell-assisted lipotransfer: a systematic review of its efficacy. *Aesth. Plast. Surg.* 40(2), 309–318 (2016).

73 Parrish JN, Metzinger SE. Autogenous fat grafting and breast augmentation: a review of the literature. *Aesthet. Surg. J.* 30(4), 549–556 (2010).

74 Kaufman MR, Bradley JP, Dickinson B *et al.* Autologous fat transfer national consensus survey: trends in techniques for harvest, preparation, and application, and perception of short- and long-term results. *Plast. Reconstr. Surg.* 119(1), 323–331 (2007).

75 Coleman SR. Facial augmentation with structural fat grafting. *Clin. Plast. Surg.* 33(4), 567–577 (2006).

76 Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesth. Plast. Surg.* 32(1), 48–55 (2007).

77 Patrick CW Jr, Zheng B, Johnston C. Long-term implantation of preadipocyte-seeded PLGA scaffolds. *Tissue Eng.* 8(2), 283–293 (2002).

78 Cho SW, Kim SS, Rhie JW, Cho HM, Choi CY, Kim BS. Engineering of volume-stable adipose tissues. *Biomaterials* 26(17), 3577–3585 (2005).

79 Frydrych M, Román S, MacNeil S, Chen B. Biomimetic poly(glycerol sebacate)/poly(l-lactic acid) blend scaffolds for adipose tissue engineering. *Acta Biomater.* 18, 40–49 (2015).

80 Lin S-D, Wang K-H, Kao A-P. Engineered adipose tissue of predefined shape and dimensions from human adipose-derived mesenchymal stem cells. *Tissue Eng. Part A* 14(5), 571–581 (2008).

81 FIP E. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to an application on the use of polyethylene glycol (PEG) as a film coating agent for use in food supplement products. 1–22 (2007). www.efsa.europa.eu/sites/default

82 Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew. Chem. Int. Ed. Engl.* 49(36), 6288–6308 (2010).

83 Hwang NS, Varghese S, Lee HJ, Zhang Z, Elisseeff J. Biomaterials directed *in vivo* osteogenic differentiation of mesenchymal cells derived from human embryonic stem cells. *Tissue Eng. Part A* 19(15–16), 1723–1732 (2013).

84 Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. *Biomaterials* 22(22), 3045–3051 (2001).

85 Peyton SR, Raub CB, Keschrumrus VP, Putnam AJ. The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells. *Biomaterials* 27(28), 4881–4893 (2006).

86 Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat. Biotechnol.* 23(1), 47–55 (2005).

87 Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 24(24), 4385–4415 (2003).

88 Clevenger TN, Hinman CR, Ashley Rubin RK *et al.* Vitronectin-based, biomimetic encapsulating hydrogel scaffolds support adipogenesis of adipose stem cells. *Tissue Eng. Part A* 22(7–8), 597–609 (2016).

89 Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 126(4), 677–689 (2006).

•• **Critical paper on the effects the matrix has on stem cell differentiation.**

90 Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. *Science* 294(5547), 1708–1712 (2001).

91 Konttinen YT, Kaivosoja E, Stegaev V *et al. Extracellular Matrix and Tissue Regeneration.* Springer, Dordrecht, The Netherlands, 21–80 (2010).

92 Amer LD, Holtzinger A, Keller G, Mahoney MJ, Bryant SJ. Enzymatically degradable poly(ethylene glycol) hydrogels for the 3D culture and release of human embryonic stem cell derived pancreatic precursor cell aggregates. *Acta Biomater.* 22, 103–110 (2015).

93 Sridhar BV, Brock JL, Silver JS, Leight JL, Randolph MA, Anseth KS. Development of a cellularly degradable PEG hydrogel to promote articular cartilage extracellular matrix deposition. *Adv. Healthc. Mater.* 4(5), 702–713 (2015).

94 Stevens KR, Miller JS, Blakely BL, Chen CS, Bhatia SN. Degradable hydrogels derived from PEG-diacrylamide for hepatic tissue engineering. *J. Biomed. Mater. Res. A* 103(10), 3331–3338 (2015).

95 Szöke K, Beckstrøm KJ, Brinchmann JE. Human adipose tissue as a source of cells with angiogenic potential. *Cell Transplant.* 21(1), 235–250 (2012).

96 Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells *in vitro* and improve postnatal neovascularization *in vivo*. *Biochem. Biophys. Res. Commun.* 332(2), 370–379 (2005).

97 Rehman J. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109(10), 1292–1298 (2004).

98 Zanotelli MR, Ardalani H, Zhang J *et al.* Stable engineered vascular networks from human induced pluripotent stem cell-derived endothelial cells cultured in synthetic hydrogels. *Acta Biomater.* 35, 32–41 (2016).