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Bioengineering Cell Therapy for Treatment of Peripheral Artery Disease

Ngan F. Huang, Brett Stern, Beu P. Oropeza, Tatiana S. Zaitseva, Michael V. Paukshto, Janet Zoldan

ABSTRACT: Peripheral artery disease is an atherosclerotic disease associated with limb ischemia that necessitates limb amputation in severe cases. Cell therapies comprised of adult mononuclear or stromal cells have been clinically tested and show moderate benefits. Bioengineering strategies can be applied to modify cell behavior and function in a controllable fashion. Using mechanically tunable or spatially controllable biomaterials, we highlight examples in which biomaterials can increase the survival and function of the transplanted cells to improve their revascularization efficacy in preclinical models. Biomaterials can be used in conjunction with soluble factors or genetic approaches to further modulate the behavior of transplanted cells and the locally implanted tissue environment in vivo. We critically assess the advances in bioengineering strategies such as 3-dimensional bioprinting and immunomodulatory biomaterials that can be applied to the treatment of peripheral artery disease and then discuss the current challenges and future directions in the implementation of bioengineering strategies.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: biocompatible materials ■ bioprinting ■ ischemia ■ peripheral arterial disease ■ stem cells ■ stromal cells

Peripheral artery disease (PAD) is an atherosclerotic occlusive disease that is associated with obstructed blood flow to the limb, leading to limb ischemia. PAD accounts for over 6 million patients in the United States and 200 million patients globally.[1] Risk factors for PAD include advanced age, smoking, and diabetes.[2] An advanced form of PAD known as chronic limb-threatening ischemia is associated with gangrene formation, ulceration, and amputation of the limb.[3,4] Surgical and endovascular interventions to restore vascularization to the ischemic limb are effective but not suitable for all patients with PAD. A promising approach to induce revascularization is therapeutic angiogenesis, which aims to induce the formation of new blood vessels from preexisting ones.[5,6] This therapeutic strategy is well suited for patients with PAD, especially patients with diabetes with PAD,[7] who have impaired regeneration capacity. Numerous strategies to augment therapeutic angiogenesis have been tested in clinical studies, including cell, protein, and gene therapies,[8] although the results have only shown minimal-to-moderate therapeutic benefit. Some of the limitations of the cell-based strategies include poor transplant cell survival, short-lived gene/protein delivery, harsh inflammatory host response, and suboptimal therapeutic dosing or frequency. Although cell-based therapies are not off the shelf, compared with protein or gene therapies, autologous cell therapies can be reintroduced into the patient as quickly as on the same day of cell harvest.

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Biomaterials and bioengineering strategies have the potential to improve cell-based therapies through several

Correspondence to: Ngan F. Huang, PhD, Department of Cardiothoracic Surgery, Stanford University, 300 Pasteur Dr, MC 5407, Stanford, CA 94305, Email ngantina@stanford.edu; or Janet Zoldan, PhD, Department of Biomedical Engineering, The University of Texas at Austin, 107 W Dean Keeton St, 1 University Station C0800, Austin, TX 78712, Email zjanet@utexas.edu
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Nonstandard Abbreviations and Acronyms

3D	3 dimensional
ABI	ankle-brachial index
ADSC	adipose-derived stem cell
bFGF	basic fibroblast growth factor
CS	chitosan
CXCR4	C-X-C chemokine receptor 4
EC	endothelial cell
ECM	extracellular matrix
EGFP	enhanced green fluorescent protein
IGF-1	insulin-like growth factor 1
IL	interleukin
iPSC	induced pluripotent stem cell
MSC	mesenchymal stromal cell
OVA	ovalbumin
PACE	Patients With Intermittent Claudication Injected With ALDH Bright Cells
PAD	peripheral artery disease
PEG(PTMC-A)2	poly(trimethylene carbonate)-b-poly(ethylene glycol)-b-poly(trimethylene carbonate) diacrylate
SVF	stromal vascular fraction cell
TACT	Therapeutic Angiogenesis Using Cell Transplantation
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

Highlights

- Bioengineering and biomaterials can improve the survival or efficacy of cell therapy for treatment of peripheral artery disease in the preclinical setting.
- Hydrogels and spatially patterned biomaterials allow for tunable mechanical and biophysical properties, respectively, to exert functional effects on encapsulated cells.
- Biomaterials can be used to genetically modify transplanted cell behavior in the setting of the ischemic limb.
- Emerging technologies include 3-dimensional bioprinting and immunomodulatory materials that can further advance cell therapies in the setting of peripheral artery disease.

mechanisms including (1) sustaining cell viability during implantation, (2) providing ECM (extracellular matrix) signaling cues that augment therapeutic cell efficacy, (3) modulating the local immune or inflammatory environment for improved therapeutic outcomes, and (4) delivering therapeutic factors locally to the affected tissue. Although non-cellular-based strategies involving gene or protein therapies have been extensively reviewed elsewhere,[9,10] we focus on cell-based therapies and the incorporation of bioengineering strategies to advance cell-based therapies. In this review, we give an overview of the state of cell therapy in clinical and preclinical setting of PAD and describe the emerging bioengineering methods to improve the efficacy of cell-based strategies for treatment of PAD.

WHAT WE DO AND DO NOT KNOW FROM CELL THERAPY CLINICAL TRIALS

Clinical trials for treating PAD using cell therapies are based on the reasoning that the transplanted cells may induce angiogenesis through the paracrine secretion of proangiogenic protein growth factors, the release of genetic cargo (ie, extracellular vesicles), the formation of neovasculature, or the incorporation into existing host vasculature. Therapeutic cells that have been tested in clinical trials of PAD include bone marrow–derived mononuclear cells, mesenchymal stromal cells (MSCs), and subpopulations within these cell types based on surface antigen expression.[11] Besides some of the notable clinical trials that are described below, we also summarize examples of ongoing or completed trials in Table 1.

The first cell therapy clinical trial for treatment of PAD was a randomized pilot study of 47 participants called the TACT study (Therapeutic Angiogenesis Using Cell Transplantation).[12] The TACT trial studied the efficacy of autologous bone marrow mononuclear cell and peripheral blood mononuclear blood cell injections (≈10^9 cells per injection) into the ischemic limb of patients with an ankle-brachial index (ABI) below 0.6. Among the 47 participants, 25 were randomized to receive injections of bone marrow mononuclear cells into the gastrocnemius muscle of the relatively more ischemic leg, with the other leg's gastrocnemius muscle receiving saline. The other 22 participants were injected with bone marrow mononuclear cells into one leg's gastrocnemius muscle, whereas peripheral blood mononuclear cells were injected into the other one. Characterization of the bone marrow–derived mononuclear cells showed a subpopulation of $CD45^+$ (cluster of differentiation) cells that expressed proangiogenic factors such as bFGF (basic fibroblast growth factor) and VEGF (vascular endothelial growth factor). Functional measures based on ABI, transcutaneous oxygen pressure, and rest pain were quantified at baseline, 4 weeks, and 24 weeks after cell therapy. Participants treated with bone marrow mononuclear cells or peripheral blood mononuclear cells showed significant improvement in ABI and transcutaneous oxygen pressure between baseline and at 4 weeks, and the results were sustained up to 24 weeks. In contrast, participants treated with saline showed no improvement in ABI and transcutaneous oxygen pressure. In addition,

Table 1. Clinical Trials of Biological Therapies for Treatment of PAD

Study title	Study design	Intervention type	Intervention	NCT number
Therapeutic Angiogenesis for Patients With Limb Ischemia by Autologous Transplantation of Bone-Marrow Cells: a pilot study and a randomized controlled trial[12]	Randomized, no masking, multicenter	Biological	BM-MNC	NA
PACE[13]	Randomized, quadruple blind, multicenter	Biological	ALDHbr BMCs	NCT01774097
Safety Study of MultiGeneAngio in Patients With Peripheral Arterial Disease[14]	Single-group assignment, multicenter	Biological	MGA	NCT00390767
ALD-301 for Critical Limb Ischemia, randomized trial[15]	Randomized, triple blind, multicenter	Procedure	ALDHbr BMCs and BM-MNCs	NCT00392509
Autologous Bone Marrow Mononuclear Cell Implantation for Moderate to Severe Peripheral Arterial Disease[16]	Single-group assignment, multicenter	Procedure	BM-MNC	NCT00919516
Granulocyte-Macrophage Stimulating Factor in the Treatment of Peripheral Arterial Disease[17]	Randomized, triple blind, single center	Drug	GM-CSF	NCT01041417
AVANT	Randomized, double blind, single center	Drug	Niacin	NCT02003638
Treatment of Intermittent Claudication by G-CSF-Mobilized PB-MNC	Randomized, single blind, single center	Procedure	PB-MNC	NCT03683628
Treatment of No-Option CLI by G-CSF-Mobilized PB-MNC	Randomized, no masking, single center	Procedure	PB-MNC	NCT03686228
BGC101 (EnEPC) Autologous Cell Therapy From Patient's Own Blood for Treatment of Critical Limb Ischemia	Randomized, double blind, multicenter	Biological	IBGC101 (autologous EnEPC preparation)	NCT02805023
Safety of Intramuscular Injection of Allogeneic PLX-PAD Cells for the Treatment of Critical Limb Ischemia	Single-group assignment, single center	Biological	Placenta-derived adherent stromal cells (PLX-PAD)	NCT00919958
Safety and Preliminary Efficacy of Adipose Derived Stem Cells and Low Frequency Ultrasound in PAD	Randomized, no masking, single center	Biological	LoFU and ADSC	NCT02756884
A Clinical and Histological Analysis of Mesenchymal Stem Cells in Amputation[18]	Nonrandomized, no masking, single center	Biological	Allogeneic bone marrow derived MSC	NCT02685098
Cell Therapy for Peripheral Arterial Disease and Diabetes	Randomized, single blind, single center	Procedure	Cell therapy with a HSC concentrate	NCT03635970

ADSC indicates adipose-derived stem cell; ALDHbr, aldehyde dehydrogenase bright; AVANT, Assessment of Vascular Health After Niacin Therapy; BM-MNC, bone marrow mononuclear cell; BMC, bone marrow cell; EnEPC, endothelial progenitor cell enriched population; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage stimulating factor; HSC, hematopoietic stem cell; LoFU, low-frequency ultrasound; MGA, MultiGeneAngio; MSC, mesenchymal stromal cell; NA, not available; NCT, National Clinical Trial; PACE, Patients With Intermittent Claudication Injected With ALDH Bright Cells; PAD, peripheral artery disease; PB-MNC, peripheral blood mononuclease; and PLX-PAD, placental-derived adherent stromal cells.

the researchers found that bone marrow mononuclear cells exhibited a greater therapeutic effect compared with peripheral blood mononuclear cells, based on ABI assessment after 4 weeks. This pilot clinical study showed promising results that encouraged larger clinical trials by subsequent investigators.

PACE (Patients With Intermittent Claudication Injected With ALDH Bright Cells) was a phase II clinical trial that studied the efficacy of intramuscularly injected autologous bone marrow–derived aldehyde dehydrogenase bright cells into participants with PAD and claudication. Aldehyde dehydrogenase bright cells were used as they were shown to be enriched with stem and progenitor cells that may exert therapeutic benefit.[19] Patients received 10 intramuscular injections of either autologous aldehyde dehydrogenase bright cells (n=38) or a cell-free vehicle control (n=40).[13] The outcome measured included peak walk time, collateral vessel count, peak hyperemic popliteal flow, and limb perfusion. Importantly, these outcome measures revealed no significant benefit at 6 months after cell implantation.

MSCs show promise as a cell source for treating PAD, in part, because they can be sourced from a wide variety of tissues, including bone marrow, adipose tissue, placental tissue, umbilical cord, and Wharton jelly. In addition, MSCs are known to secrete a range of pro-angiogenic factors that make them attractive for clinical studies.[20] Although several dozens of phase I and II clinical trials have tested the efficacy of MSCs in participants with PAD, the patient enrollment size was limited to <100 participants, and the cells were usually injected intramuscularly into the ischemic limb.[21] In addition, there is significant variance in the number of cells used for treatment, as well as what outcomes were measured. Despite this, MSC therapy is generally regarded as safe. With regard to therapeutic efficacy, the outcome measures show promise in these small studies. For example, patients with chronic limb-threatening ischemia originating from Buerger disease benefitted from intramuscularly delivered allogeneic bone marrow–derived MSCs, based on pain at rest, ABI, and walking distance, compared with standard-of-care treatment per month.[22]

Due to the differences in outcomes of individual clinical studies, systematic reviews and meta-analyses of randomized clinical trials have provided additional insights into the overall benefit of stem cell therapy for treatment of PAD. One systematic review of 28 randomized controlled trials showed that autologous stem cell therapy (ie, bone marrow–derived MSCs, bone marrow–derived or peripheral blood–derived mononuclear cells) was associated with significant improvement in ABI and transcutaneous oxygen pressure, along with a decline in limb amputation rate, compared with the control group.[23] Two separate analyses of 28 and 23 studies found that autologous stem cell therapies show to significantly promote wound healing in patients and are both safe and effective for patients with chronic limb-threatening ischemia.[24,25] Another systematic review of 19 randomized controlled trials drew a similar conclusion, with additional findings that local intramuscular cell injection was more effective as a cell delivery modality than intra-arterial injection.[26] However, another meta-analysis of 10 stem cell clinical studies (primarily using bone marrow mononuclear cells of bone marrow–derived MSCs) found no significant benefit in amputation rate, survival, and amputation-free survival, when comparing cell treatment to placebo.[27]

Based on the various meta-analyses, it is well accepted that adult stem cell therapies are generally safe and well tolerated with minimal or transient side effects. However, there is much that we still do not understand. Notably, the efficacy of stem cell therapy remains inconclusive. Contributing to this uncertainty is the fact that the previous clinical studies were relatively small, lacked long-term follow-up, and were not sufficiently powered for detecting statistical significance. It is possible that the follow-up was not performed at an optimal time point that would reveal maximal benefit to vascular function. Additionally, the variance in findings reported by the different meta-analyses may be due to the variability of the transplanted cells, owing to donor-to-donor differences and potential differences in cell harvesting methods. Furthermore, clinical studies do not permit extensive tracking of transplant cell survival, so it is unknown to what extent the transplanted cells survive in the ischemic limb among different donor cells. Such limitations of past clinical studies suggest a need to perform larger clinical studies with more uniform and well-characterized cell populations and longer follow-up times, as well as to develop more standardized approaches to cell to reduce the donor-to-donor differences during cell harvest and to maximize posttransplant survival. Finally, there is still a lack of knowledge of the phenotypic markers that define the optimal stem cell therapeutic, the ideal frequency and dosage of cells, and the variability of autologous cell quality.

Going beyond existing clinical trials of adult stem cell therapies, some of the emerging opportunities include the use of alternative cell types, including induced pluripotent stem cell (iPSC) derivatives or genetically modified cells to boost cell function, as well as the use of bioengineering strategies to improve the survival and efficacy of therapeutic cells upon transplantation. With the use of noninvasive imaging strategies to detect labeled transplanted cells, we can further track the survival of the transplanted cells in preclinical studies, which would not be allowable in clinical studies. These strategies are overviewed below.

EXPERIMENTAL ENDOTHELIAL CELL THERAPIES IN PRECLINICAL TESTING

A cell type that has shown promise in preclinical setting is endothelial cells (ECs) derived from human pluripotent stem cells, including embryonic stem cells and iPSCs. Pluripotent stem cell–derived ECs can be derived reproducibly using well-established differentiation protocols.[28–33] Compared with primary human ECs, pluripotent stem cell–derived ECs have been shown to recapitulate many of the phenotypic, transcriptional, and functional properties of bonafide ECs.[34] Although iPSCs and embryonic stem cells both have theoretically long-term expansion capability, autologous ECs can only be derived from iPSCs. We and others have demonstrated the therapeutic efficacy of pluripotent stem cell–derived EC injection in murine model of experimental PAD.[30,35–37] We previously showed that injections of human iPSC-ECs in mice with induced hind limb ischemia could improve blood perfusion and vascular density in ischemic limbs for up to 28 days, whereas delivery of nontherapeutic cells such as fibroblasts did not exert any therapeutic benefit.[30] Besides pluripotent stem cell–derived ECs, ECs have been successfully generated directly from somatic cells by the transient activation of the Yamanaka factors (*Oct3/4*, *Sox2*, *KLF4*, and *C-MYC*) in conjunction with endothelial-inducing soluble factors[38] or by direct endothelial reprogramming of ETS (E26 transformation-specific) transcription factor *ETV2*[39,40] or miR-208b-3p.[41] These experimental cell types serve as alternative sources for therapeutic ECs that may have clinical translational potential.

An important consideration in the preclinical assessment of stem cell therapies is the animal model of PAD. A commonly used model of PAD involves surgical ligation or excision of the murine femoral artery, which induces acute impairment in blood flow to the lower limb.[42] Although this model induces limb ischemia that is experienced in clinical subjects, it does not recapitulate other pathological aspects of PAD, including endothelial dysfunction or fewer endogenous stem cells, which are further exacerbated by concurrent diabetes.[8,43] Published studies further demonstrate variability of induced ischemia to varying muscle groups, with the distal anterior hind limb muscles showing the greatest consistency of

ischemia induction.[44] Alternative animal models involve ameroid constructors to gradually induce ischemia, but it can result in distinctively different molecular signaling processes, compared with an acute ligation model.[45] Additionally, considerations of strain, age, and sex differences can further influence the severity of the animal model, as reviewed previously.[46] These technical constraints should be considered when interpreting the data derived from preclinical models.

BIOENGINEERING APPROACHES TO ENHANCE POSTTRANSPLANT CELL SURVIVAL

Since non-cell-based experimental bioengineering approaches to treat PAD have been reviewed elsewhere,[9,10] here, we focus on bioengineering strategies to enhance cell-based therapies. Despite the therapeutic potential of cell therapies, the viability of cells during and after cell implantation is a bottleneck that limits the efficacy of the cells. In recent decades, a number of biomaterials and bioengineering strategies have sought to mimic the physiological tissue microenvironment, to promote cell survival in the ischemic limb and augments the therapeutic efficacy of the implanted cells. These strategies use controllable hydrogels, spatially nanopatterned biomaterials, and bioengineering strategies to genetically prime the therapeutic cells for transplantation.

Cell-Encapsulated Hydrogels

Cell therapies have emerged as promising treatments for PAD by exerting paracrine effects that induce angiogenesis or reduce inflammation, as well as by directly promoting new vasculature formation.[47,48] Nevertheless, direct cell injection faces significant limitations, primarily due to poor cell retention at the site of injury and poor viability in the ischemic environment[49]; encapsulation of cells in hydrogels, which are polymer scaffolds that hold a high degree of water content, has proven beneficial to address these challenges. To this end, our research group has been actively developing angiogenic hydrogels designed for encapsulating human iPSC-derived endothelial progenitors and driving their self-assembly into vascular networks. We have shown that matrix elastic modulus and degradability play crucial roles in plexus formation.[33,50,51] However, these properties are often interchangeable in most angiogenic hydrogels.[52] Therefore, we developed interpenetrating networks of collagen and hyaluronic acid hydrogel that allow independent tunability of elastic modus and degradability to better stimulate angiogenesis.[50] These systems underscore the importance of the role of mechanoregulation on vascularization so that angiogenic biomaterials can be effectively deployed in the clinic and ultimately improve vascular health.

Furthermore, for successful clinical deployment, it is imperative to develop injectable hydrogels, which have shown promise as a minimally invasive method for cell delivery to alleviate PAD. One commonly used technique for developing injectable hydrogels involves the use of a polymer with temperature-controlled gelation. For example, Foster et al[53] developed shear-thinning injectable hydrogels, termed SHIELD, to improve cell viability of encapsulated iPSC-ECs in vivo. SHIELD consists of 2 polymers: a multiarm poly(ethylene glycol) attached to cell adhesion peptides and poly(N-isopropyl-acrylamide), a thermally responsive polymer with a lower critical solution temperature of 32 °C (Figure 1A). The SHIELD system forms an injectable gel below 32 °C with a storage modulus under 50 Pa but can stiffen in vivo up to 1000 Pa by adjusting the poly(N-isopropyl-acrylamide) concentration (Figure 1B). In vitro, SHIELD hydrogels demonstrated improved cell viability (Figure 1C) and proliferation of iPSC-ECs. In vivo in hind limb ischemia models, iPSC-ECs delivered through the hydrogel with ≈400 Pa storage modulus exhibited prolonged retention, with 25% of the cells delivered using the SHIELD hydrogel remaining after 3 days compared with only 7% of the cells when delivered through bolus injection. iPSC-ECs encapsulated in the SHIELD hydrogel also significantly enhanced arteriole density (100 arterioles/mm^2) compared with the PBS control group (26 arterioles/mm^2) and iPSC-ECs delivered without the hydrogel (34 arterioles/mm^2). These findings suggest that the SHIELD system promotes transplant cell survival and the formation of larger diameter vessels, compared with delivering the cells in saline.

Cell-based therapies for PAD can exert their effects through paracrine signaling rather than directly participate in forming new vasculature.[55] Zhao et al[56] developed an injectable hydrogel to enhance retention and viability of MSCs using a chitosan (CS) hydrogel. CS was chosen for its thermal responsivity and in vivo degradability. CS was further modified through covalent attachment of the C domain of IGF-1 (insulin-like growth factor 1). The MSCs used in this study were genetically modified to express red fluorescence protein for detection in vivo and luciferase to measure cell viability, since luciferase activity in these cells is directly proportional to MSC viable cell count. For in vivo testing, the researchers induced hind limb ischemia in mice engineered to express luciferase downstream of the VEGFR2 promoter, thereby allowing for the quantification of the proangiogenic effects of the delivered MSCs. Although for all tested groups, the MSC count significantly decreased by up to 1000-fold 8 days after injection, the IGF-1-CS hydrogel had a significantly higher signal compared with the unmodified CS hydrogel and when MSCs were delivered using PBS. Despite the low retention of MSCs in vivo, the delivered MSCs stimulated angiogenesis, as evidenced by increased

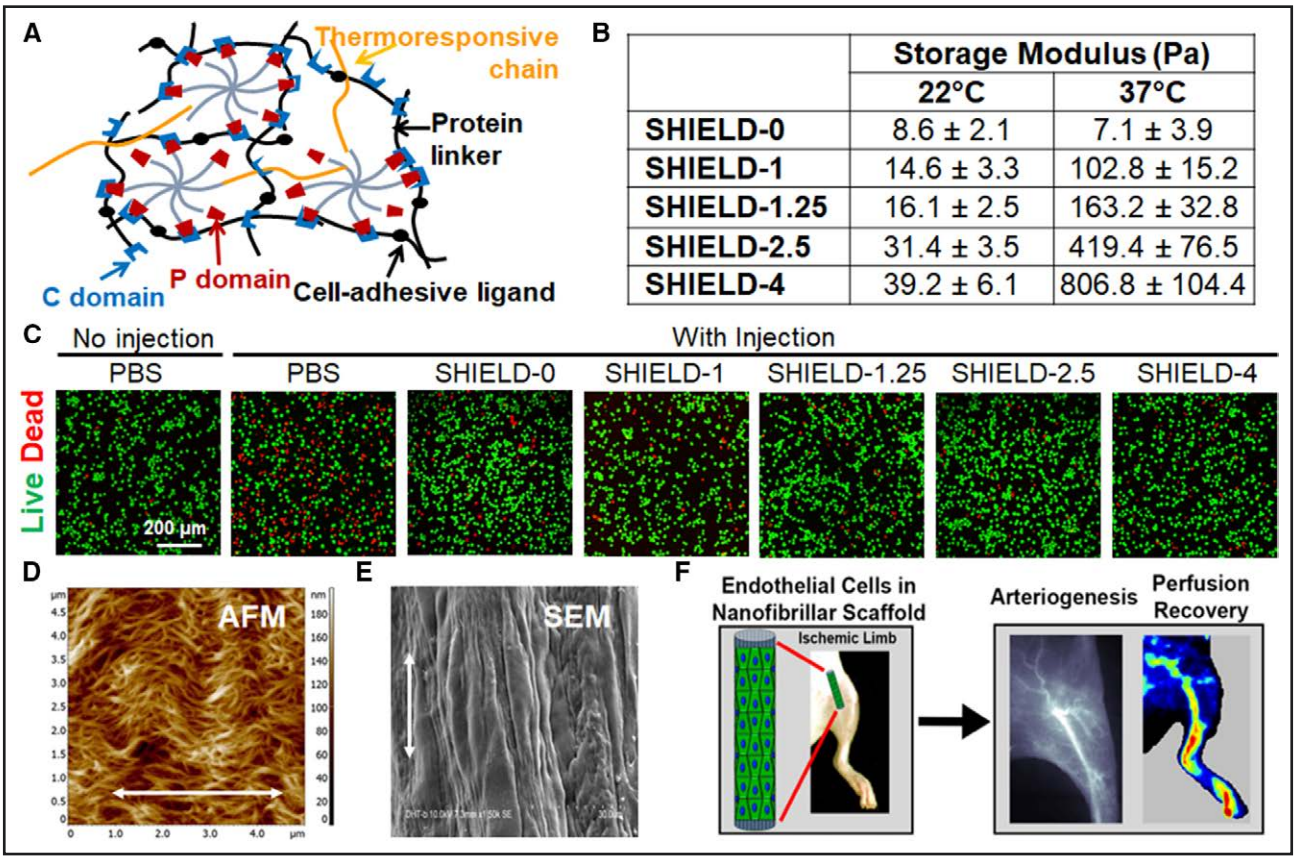

Figure 1. Engineered hydrogels and scaffolds can be engineered to have mechanically or biophysically tunable properties. **A**, Schematic of engineered SHIELD (shear-thinning injectable hydrogel) is formed by mixing together 2 components: C7-engineered protein and 8-arm PEG-P1 (P1 peptide-containing poly[ethylene glycol]) with or without thermoresponsive poly(N-isopropyl-acrylamide; PNIPAM) to form a gel. **B**, By changing the PNIPAM content, the storage (G′) moduli of SHIELD hydrogels can vary at 37 °C among physiologically relevant levels. **C**, By tuning the mechanical properties of SHIELD, the survival of encapsulated iPSC-ECs within hydrogel formulations after syringe injection is affected. **D**, Spatially nanopatterned collagen scaffold structure is visualized by atomic force microscopy (AFM) imaging. **E**, ECs cultured on the scaffold form elongated cells along the direction of the collagen nanofibril orientation. **F**, Nanopatterned scaffolds seeded with ECs induce revascularization and reperfusion in a mouse model of PAD. SEM indicates scanning electron microscopy. **A** through **C**, Adapted from Foster et al.[53] **D** through **F**, Reprinted from Nakayama et al.[54] Copyright ©2015, the American Chemical Society.

luciferase signal from mouse ECs expressing VEGFR2. The highest signal was observed with the IGF-1-CS hydrogel, peaking at day 10. This increase in VEGFR2 signal correlated with a ≈3-fold increase in capillary density in mice receiving MSCs encapsulated in IGF-1-CS compared with MSCs delivered without a hydrogel. Moreover, the IGF-1-CS group showed the highest percentage of limb salvage (70%), with lower rates of necrosis (30%) and no amputations required. In contrast, when MSCs were delivered alone, only 30% achieved limb salvage, with higher rates of necrosis (50%) and limb amputation (20%). Overall, the IGF-1-CS hydrogel improved MSC retention, stimulated angiogenesis, increased capillary density, and enhanced limb salvage rates in the hind limb ischemia model compared with MSCs delivered without a hydrogel.

Similarly, Young et al developed an injectable hydrogel system for intramuscular delivery of adipose-derived stem cells (ADSCs). ADSCs have well-studied proangiogenic effects through paracrine signaling, much like MSCs.[57–59] The researchers utilized a hydrogel composed of methacrylated CS with covalently bound RGD (arginine-glycine-aspartic acid) for cell attachment, alongside poly(trimethylene carbonate)-b-poly(ethylene glycol)-b-poly(trimethylene carbonate) diacrylate (PEG[PTMC-A]$_2$). The PEG(PTMC-A)$_2$ component enhanced the mechanical properties of CS and reduced the hydrogel's swelling ratio, enabling it to withstand cyclic forces in muscle tissue without causing damage to the surrounding tissue. By modulating the PEG(PTMC-A)$_2$ content, the target compressive modulus (5–15 kPa) and compressive strength at failure (0.5) were achieved. The polymers were cross-linked via a temperature-sensitive free radical polymerization technique using ammonium persulfate and tetramethylethylenediamine. This polymerization process exhibits a slow cross-linking rate of 9 minutes at room temperature, facilitating comprehensive mixing of the components without premature gelation. However, it enables rapid cross-linking (3 minutes) upon in vivo injection. ADSCs showed high viability under

both normoxic (98%) and hypoxic (90%) conditions, with hypoxic conditions promoting increased release of proangiogenic factors, including a 10-fold higher VEGF release and 3-fold higher angiopoietin-1 release after 14 days in culture, supporting their intended use for inducing angiogenesis in ischemic tissue. In a hind limb ischemia model, the cell-laden hydrogels maintained ADSC density similar to that after 1 week of in vitro culture, equivalent to 30% cell retention, resulting in a significant increase in the number of endothelial $CD31^+$ cells within the muscle tissue when ADSCs were delivered in the hydrogel (170 cells/mm^2) compared with bolus injection (140 cells/mm^2). Although functional recovery was not reported, these findings hold promise for future applications in PAD treatment. Together, these studies highlight the utility of injectable hydrogels as carriers of the implanted cells that can promote cell survival and improve the angiogenic outcomes.

Some of the advantages of injectable hydrogels for the encapsulation and codelivery of stem cells include the provision of a biomimetic ECM environment that may improve cell survival and function within the harsh ischemic limb, the tunability of the hydrogel's mechanical and biochemical properties, and the minimally invasive nature of direct intramuscular injection of the cell-encapsulated hydrogels. These advantages are countered by limitations, including the scalability and safety of the hydrogels for clinical use, the retention of the hydrogels over time, and potential concerns of inflammatory or immune response to the hydrogels. With the notable exception of alginate, most hydrogels are not currently Food and Drug Administration approved. Hydrogels that involve complex chemistries or recombinant DNA technology may be subjected to additional concerns of scalability or permissible endotoxin levels, respectively.

Biophysical Patterning Cues in Biomaterials

Besides mechanically tunable hydrogels, another strategy to improve the survival of therapeutic cells is by the presentation of biophysical patterning cues from biomimetic scaffolds. Native ECMs such as collagen confer various topographical patterns within blood vessels. To mimic the woven spiral structure of collagen bundles in relaxed blood vessels[60] that have high mechanical strength, aligned collagen fibrillar scaffolds with the woven-like helical and crimped configurations were developed, as shown by atomic force microscopy (Figure 1D).[54] These configurations mimic the aligned nanoscale patterning of collagen-based fibrous tissue under reduced load. When these scaffolds were then seeded with primary human ECs, the cells responded to the nanoscale pattern by organizing their cytoskeleton along the axis of the collagen fibrils, as shown by scanning electron microscopy (Figure 1E).[54,61,62] When human iPSC-EC–seeded aligned scaffolds were implanted into ischemic hind limbs of mice, the transplant cell viability was significantly higher, compared with cell viability on nonpatterned scaffolds. Importantly, human iPSC-ECs cultured on aligned scaffolds also persisted for over 28 days, when implanted into ischemic tissue, whereas iPSC-derived endothelial progenitors implanted on scaffolds without nanopatterning persisted for only 4 days. Along with improvement in cell viability, ECs seeded on aligned nanofibrillar scaffolds also enhanced vascular perfusion recovery (Figure 1F).[54]

Additionally, these EC studies demonstrated that aligned nanofibrillar collagen scaffolds guide EC cellular organization, modulate endothelial inflammatory response, and enhance cell survival after implantation in normal and ischemic tissues.[61,62] The alignment of the ECs on these scaffolds directly influenced their biology, where the aligned ECs were 50% less adhesive for monocytes than the ECs grown on randomly oriented fibrillar scaffolds. The finding of increased cell viability after delivery on aligned scaffolds into ischemic tissue suggested that such nanofibrillar scaffolds may be beneficial as a delivery vehicle for cell therapy. Further studies revealed that the aligned nanofibril pattern promoted greater endothelial outgrowth in vitro than nonpatterned scaffolds, in part, by integrin-α1 activation, and enhanced blood perfusion recovery and arteriogenesis in the murine ischemic hind limb, compared with cell delivery or scaffold delivery alone.[54]

With the goal to explore more clinically accessible and abundant therapeutic cell sources, stromal vascular fraction cells (SVFs) from adipose tissue were tested for seeding into the cell-seeded aligned nanofibrillar scaffold. In vitro studies showed that SVF cells cultured on the scaffold had a 6-fold higher level of VEGF secretion, compared with that of SVF cells cultured in suspension.[63] Importantly, when SVF-seeded scaffolds were transplanted into immunodeficient mice with induced hind limb ischemia, the cell-seeded scaffolds induced a significant higher mean perfusion ratio after 14 days, compared with cells delivered in saline.[63] These findings show that both ECs and SVF cells delivered on aligned nanofibrillar scaffolds into ischemic tissue stimulated blood perfusion recovery. The recovery mechanisms underlying this therapeutic effect may include both angiogenesis and arteriogenesis, which could be mediated by patterned scaffold-induced activation of integrin-α1 and increased VEGF secretion.[54,63,64] These studies demonstrate an important role of nanopatterning cues in directing cell behavior, which can be applied toward improving the survival and angiogenic function of implanted cells in the setting of limb ischemia.

The advantages of biophysical patterning include the activation of topography-mediated molecular signaling in adherent therapeutic cells that can induce cell survival or therapeutic function, as well as the induction of cellular reorganization and alignment that can lead to

further effects on cell migration and immunomodulation to cell types such as ECs.[64] Compared with injectable hydrogels, however, biophysically patterned scaffolds require more invasive delivery techniques, such as a trocar for intramuscular delivery, and should be mechanically strong enough to withstand surgical manipulation. Another disadvantage of spatially patterned scaffolds is the lack of knowledge of how cells will respond to topographical patterning upon partial degradation when the topographical cues may not be as evident.

Biomaterial-Based Genetic Cell Modification

The process of angiogenesis in PAD treatment necessitates the sustained presentation of numerous growth factors at different time points to facilitate revascularization treatment.[65,66] However, achieving consistent and spatiotemporal release kinetics for multiple growth factors over an extended period presents a significant challenge in biomaterial engineering. Gene delivery emerges as a promising solution by combining the advantages of cell therapy and growth factor delivery systems. Through viral or plasmid transfection, cells can be genetically modified to overexpress specific growth factors, which are then introduced to the ischemic tissue. This innovative approach allows for tunable and long-term release of growth factors, surpassing the limitations of conventional drug delivery systems. Moreover, these genetically modified cells leverage their inherent capacity to stimulate vascularization, thereby presenting an additional advantage for enhancing therapeutic outcomes.[67] Some representative examples of using genetic engineering to treat PAD can be found in Table 2, containing a variety of target genes and delivery methods. VEGFA is a widely studied growth factor known for its potent proangiogenic effects.[78] Previous studies have demonstrated the beneficial outcomes of VEGFA delivery in murine hind limb ischemia models, leading to functional recovery.[79–81] Similarly, the overexpression of VEGFA in transplanted cells has shown promising results, as demonstrated by Park et al.[68] In their work, they successfully transfected MSCs with VEGFA and EGFP (enhanced green fluorescent protein). Upon injection of the transduced MSCs into the ischemic hind limbs of mice, the authors observed notable improvements in blood perfusion in the affected limbs, with perfusion reaching 79% of normal levels in mice receiving transduced MSCs, compared with 53% and 26% for mice receiving nontransduced MSCs and no treatment, respectively. In addition, they observed a 4.5-fold reduction in fibrosis in mice treated with transduced MSCs compared with nontransduced MSCs. This work exemplifies the utility of genetic modification techniques to enhance the proangiogenic characteristics of implanted cells.

Besides the use of viral transduction for genetic modification of cells, polymer nanoparticles are also effective for cellular transfection. Nanoparticles composed of poly(β-amino ester) can modify the DNA into the nanoparticle structure and thereby be transfected into cells.[82] The poly(β-amino ester) nanoparticles had a 1-fold to 2-fold higher transfection efficiency compared with lipofectamine with reduced cytotoxicity. Using this nanoparticle technology, Deveza et al[69] modified ADSCs with plasmids encoding either the G-protein–coupled receptor CXCR4 (C-X-C chemokine receptor 4) or VEGF, as demonstrated by the gene and protein expression analysis. When the genetically modified ADSCs were injected into the murine ischemic limb, the cells that were genetically modified with CXCR4 alone or together with VEGF showed the highest degree of cell survival at 10 days post-transplantation. Furthermore, CXCR4-modified ADSCs had 100% limb salvage, compared with the negative control group that only had ≈40% limb salvage. Along with the improvement in limb salvage, there was a significant improvement in blood perfusion recovery in the group treated with CXCR4-modified ADSCs, compared with cells modified with an irrelevant gene. This study highlights the potential benefit of a nanoparticle-based genetic modification strategy that could encounter less safety concerns toward clinical translation. An additional example of using nanoparticles to deliver genetic material is the research by Lamin et al.[72] The researchers targeted *ADAM12* (a disintegrin and metalloproteinase domain-containing protein 12) and miR-29a-INH, which are normally upregulated and downregulated in ischemic tissues, respectively. Given the disruption of these regulatory mechanisms in PAD, they used lipid nanoparticles with ultrasound-triggered release to deliver plasmids containing the *ADAM12* gene or a miR-29a-INH inhibitor to the ischemic limb. They observed that while both *ADAM12* upregulation or miR-29a-INH inhibition improved limb perfusion and muscle twitch force, indicating functional recovery, inhibiting the miRNA had a more significant positive effect on ischemic recovery. Together, these examples highlight the utility of various bioengineering and biomaterial strategies to improve the survival or therapeutic efficacy of transplanted cells for treatment of limb ischemia.

The advantages of biomaterial-based genetic cell modification include the tunability of cell transfection kinetics, thereby potentially enabling more sustained duration of transfection, compared with traditional cell transfection methods in solution. Furthermore, biomaterials are well suited for localized cell transfection, thereby limiting transfection only to the transplanted cells or other cells in the immediate vicinity of the biomaterial. In contrast, one of the limitations of biomaterial-based genetic cell modification include the possibility of genomic integration, thereby raising concerns of the safety of this strategy for clinical use. However, recent developments in modified mRNA-based transfection technologies may circumvent this obstacle.[83]

Table 2. Overview of Preclinical Testing of Genetic Engineering for Peripheral Artery Disease Treatment

Genes of interest	In vitro or in vivo	Cell type	Delivery method	Observations
VEGFA	In vitro	MSCs	Nonviral plasmid	Improvements in blood perfusion
				Reduction in fibrosis[68]
VEGFA and CXCR4	In vitro	ADSCs	PBAE nanoparticles	Improvements in blood perfusion
				Improved cell survival
				No necrosis[69]
HIF-1a	In vitro	ADSCs	Nonviral plasmid	Increase in VEGF expression
				Increase in vessel length and density[70]
Nestin-1	In vitro	ADSCs	Adenovirus	Reduction in inflammatory cytokines
				Increase in autoinflammatory markers[71]
ADAM12 and miR-29a-INH	In vivo	NA	Lipid nanoparticles with F-triggered release	Improvements in blood perfusion
				Increased capillary density
				Increased muscle contractile force[72]
MiR-140-3p	In vivo	NA	Lentivirus	Reduction in smooth muscle proliferation causing decrease in restenosis following angioplasty[73]
HIF-1a	In vivo	NA	Baculovirus	Increase in capillary and arteriole density
				Increase in angiogenesis-related genes at day 14[74]
bFGF	In vivo	NA	Gene gun (pyro-driven jet injector)	Increased skeletal muscle density
				Increased CD31$^+$ cell count[75]
PTGIS	In vivo	NA	Glutaraldehyde-polyethyleneimine nanoparticles	Improvements in blood perfusion, CD31$^+$ cell count, and number of blood vessels 14 and 21 d after treatment[76]
HGF	In vivo	NA	Modified mRNA	Release of HGF mRNA for 4 wk post-implantation
				Increase in microvascular density within 50 μm of the scaffold[77]

Column 2 differentiates between treatments in which cells were modified before implantation in the host (in vitro) and treatments in which factors for upregulating the gene of interest were delivered directly to the host (in vivo). ADAM12 indicates a disintegrin and metalloproteinase domain-containing protein 12; ADSC, adipose-derived stem cell; bFGF, basic fibroblast growth factor; CD31$^+$, cluster of differentiation 31; CXCR4, C-X-C chemokine receptor 4; HGF, hepatocyte growth factor; HIF-1a, hypoxia inducible factor 1 subunit alpha; MSC, mesenchymal stromal cell; NA, not available; PBAE, poly(β-amino ester); PTGIS, prostaglandin-I synthase; and VEGF, vascular endothelial growth factor.

EMERGING CELL-BASED BIOENGINEERING TECHNOLOGIES FOR PAD TREATMENT

Three-Dimensional Bioprinting

Three-dimensional (3D) bioprinting is an emerging technology that enables the precise positioning of cells and biomaterials in defined spatial geometries. Among the different types of 3D bioprinting, thermal inkjet and extrusion-based bioprinting are the most common (Figure 2). Thermal inkjet bioprinting utilizes heat, laser, or piezoelectric energy sources to deposit cells and bio-inks in defined geometries (Figure 2A). The orifice of the inkjet print head allows single cells to pass through after being subjected to high-energy sources (Figure 2B).[84] Thermal inkjet printing was the first technology that was used for bioengineering applications with protein arrays and DNA chips as the first bioprinting products.[87–89] Following these successes, printing bacteria and mammalian cells with high viability rates paved the way for a wider range of applications.[90] For example, Oropeza et al[84] demonstrated the ability to print human microvascular ECs using a modified inkjet bioprinter and successfully implanted the constructs into a murine model, resulting in a significant increase in the vascular formations in the implant area (Figure 2C).[91]

Advances in 3D printing have led to the development of new approaches to treat PAD, specifically in generating large tissue-engineered vascular graft replacements.[92] For example, Mirabella et al[93] used 3D printing to create parallel polysaccharide filaments, each surrounded by fibrin gel seeds with ECs. Subsequently, the polysaccharide filaments were dissolved to create small-diameter channels. Upon implantation in a hind limb ischemia model, they found that different channel geometries resulted in varying degrees of perfusion recovery. Specifically, endothelialized channels oriented in parallel exhibited nearly complete recovery 5 days post-surgery, whereas gels with ECs but lacking channels showed significantly less recovery. Freeman et al[94] developed a method for 3D bioprinting vascular grafts directly onto a rotating surface. Unlike previous methods where scaffolds were printed on a flat surface requiring support material, this approach eliminates that need by printing on a cylindrical surface, enabling the creation of arbitrarily large vessels. The bio-ink utilized contains human dermal fibroblasts, gelatin, and fibrinogen. Cooling the bio-ink to room temperature allows

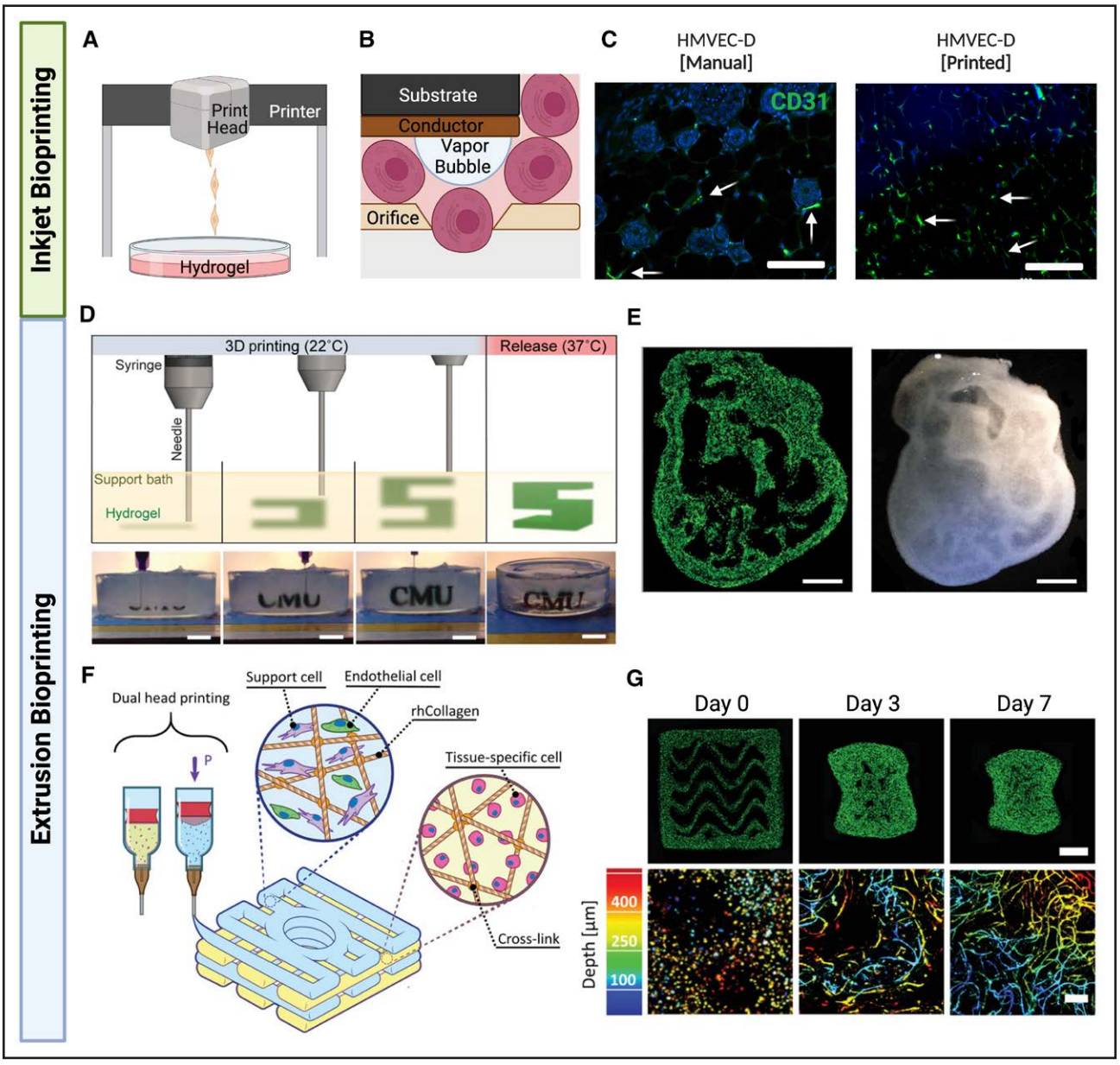

Figure 2. Advancements in 3-dimensional (3D) bioprinting for vascular graft replacements.
A and **B**, Thermal inkjet printers use heat to force droplets through the small nozzle. The small orifice of the inkjet print head allows single cells to pass through after being subjected to high temperatures and pressures, which in turn cause activation of angiogenic factors. **C**, In vitro studies demonstrate the increase in vascular formation for inkjet-printed constructs, compared with those with manually seeded cells (scale bar, 100 μm). **D**, A gelatin slurry is used as a support bath throughout the 3D bioprinting process, allowing for precise printing of alginate before cross-linking. **E**, The cross section of a 3D printed embryonic chick heart (scaled 10×) made using fluorescent (green) alginate, the internal structures can be seen through the translucent wall (scale bar, 1 mm). **F**, Collagen, endothelial cells, and support materials are extruded using a dual print head to form vascularized constructs. **G**, Vessel network images at days 0, 3, and 7. **Top**, The full construct (scale bar, 1 mm). **Bottom**, The vascular depth using color-coded projection (scale bar, 200 μm). CD31 indicates cluster of differentiation 31; CMU, Carnegie Mellon University; HMVEC-D, human dermal microvascular endothelial cells; P, pressure; and rhCOllagen, recombinant human collagen. **B** and **C**, Adapted from Oropeza et al.[84] **D** and **E**, Adapted from Hinton et al.[85] **F** and **G**, Adapted from Szklanny et al[86] with permission. Copyright ©2021, John Wiley and Sons.

partial solidification of gelatin, facilitating printing on the rotating cylinder. Following printing, the vascular graft is immersed in thrombin to cross-link the dissolved fibrinogen. By subsequently raising the temperature to 37 °C, the gelatin melts and clears from the construct. Control over the viscosity and stiffness of the printed graft was achieved by altering the gelatin concentration in the bio-ink and adjusting the gelatin heat treatment time. The researchers successfully cultured fibroblasts within the construct for up to 2 months. Notably, this approach for generating large-diameter vessels is highly scalable, and further incorporation of endothelial or smooth muscle cells could serve as a valuable treatment for patients with PAD.

Another significant advancement involved the use of freeform reversible embedding of suspended hydrogel bioprinting, in which a support bath consisting of gelatin slurry enables precise printing of bio-inks in defined geometries such as a 3D printed embryonic chick heart cross section (Figure 2D and 2E).[85] Szklanny et al[86] applied freeform reversible embedding of suspended hydrogel printing technology to create a large-diameter vessel surrounded by a dense capillary bed (Figure 2F and 2G). In this work, a biocompatible poly(lactide-co-glycolide)–poly(l-lactide) polymer with fenestrations was used to construct the large-diameter vessel, enabling capillary sprouting. Endothelialization of the vessel involved coating it with fibrinogen and flowing ECs through the tube. Surrounding the large-diameter vessel, a methacrylated collagen bio-ink was printed, consisting of a combination of human adipose–derived ECs and dental pulp stem cells as support cells. These cells were chosen because they are both easy to isolate and can form vascular networks.[95–97] In vivo experiments were performed in rats by resecting and clamping the femoral artery and using the large-diameter vessel as a replacement. The authors first implanted a defenestrated large-diameter vessel without the surrounding collagen gel to demonstrate that the large-diameter vessel could anastomose with other vessels. While using a nonendothelialized vessel showed some improvement in blood flow (65% compared with a ligated femoral artery), significantly greater improvements were observed when an endothelialized vessel was used (85% of normal blood flow). The authors then repeated this experiment using both the large-diameter vessel and the surrounding collagen to verify integration of the collagen hydrogel's capillary bed with the host vasculature and with the large-diameter vessel. Both approaches demonstrate promising possibilities for the future of vascular grafting using 3D printing technology.

The primary advantage of 3D bioprinting lies in its capacity to create intricate macroscale structures, particularly beneficial for treating PAD, where large-diameter vessels are essential. Additionally, 3D bioprinting facilitates the production of personalized vascular grafts and tissues tailored to meet the specific needs of individual patients, enhancing compatibility and integration upon implantation. The incorporation of different bio-inks allows for the introduction of multiple cell types, contributing to a more accurate recreation of native tissue organization. However, a significant drawback of this approach is the challenge of identifying suitable bio-inks for printing—ones that are both biocompatible and possess the necessary mechanical properties. Ensuring that these bio-inks support cell survival and function while maintaining structural integrity poses a key hurdle. Maintaining cell viability during the printing process and ensuring that the printed cells continue to function as intended after implantation are crucial considerations, especially given the specificity required for each cell and polymer type. In addition, while bioprinting enables the creation of large-diameter vessels, current technologies lack the resolution to print smaller scale structures, such as capillaries. Further research and clinical trials are imperative to validate the effectiveness, safety, and long-term outcomes of 3D bioprinting in PAD treatment. Additionally, ongoing exploration and development are needed to address regulatory considerations and enhance the scalability of the technology for widespread clinical use.

Immunomodulatory Biomaterials

In the context of PAD, the inflammatory microenvironment in ischemic tissue poses a significant challenge to angiogenesis. Genetic engineering approaches have been used to tackle this issue. For example, Jiang et al[71] genetically engineered ADSCs to overexpress Nestin-1, a protein normally associated with neuronal development[98] that has also been shown to improve EC viability[99] and promote an anti-inflammatory macrophage M2 phenotype.[100] The objective of their research was to reduce restenosis following angioplasty, and the retention of M2 macrophages is crucial in achieving this goal, as they aid in the clearance of senescent cells, known to contribute to restenosis.[101] Additionally, the Nestin-1–overexpressing ADSCs were encapsulated in a hydrogel composed of graphene oxide, polydopamine, and polyacrylamide. This composite hydrogel facilitated the wrapping of the affected arteries and provided support to the cells. Implanting the hydrogel in mice resulted in a >50% reduction in the expression of the proinflammatory markers TNF-α (tumor necrosis factor alpha) and IL (interleukin)-6, associated with M1 macrophages, and a 4-fold increase in the expression of the autoinflammatory markers Arg-1 (arginase 1) and IL-10, associated with M2 macrophages. This study highlights the role of hydrogels, in conjunction with genetically modified stem cells, to regulate inflammation.

Even in the absence of transplanted cells, biomaterials can exert immunomodulation of the ischemic muscle tissue through the release of anti-inflammatory factors. This strategy can potentially obviate the genetic modification of cells. One potential benefit of delivering anti-inflammatory factors using biomaterials is the higher degree of control in the release kinetics, compared with conventional bolus delivery of soluble factors in saline. For example, when IL-4–releasing nanoparticles were injected to the ischemic limbs of mice for 15 days, the IL-4–releasing nanoparticles were able to significantly improve muscle contraction force by 40%, compared with the group receiving only PBS treatment.[102] The contractile velocity also showed a similar degree of improvement, although vascular perfusion was not significantly different among the treatment groups. Mechanistically, flow cytometry analysis of the hind limb tissue demonstrated twice as many M2 macrophages and half as many M1 macrophages in the group receiving IL-4–releasing nanoparticles, compared with the saline control group. An alternative approach was developed by

Kwee et al[103] who implanted an antigen-releasing scaffold in animals previously vaccinated with the same antigen. Mice with hind limb ischemia that were previously vaccinated with OVA (ovalbumin) and aluminum then received OVA-releasing scaffolds. This implantation led to the accumulation of antigen-specific T helper 2 cells at the site of limb ischemia, thereby promoting blood perfusion recovery of ischemic tissue. These examples illustrate examples in which biomaterial-based delivery of immunomodulatory factors can influence functional recovery of the ischemic limb muscle.

The primary advantage of immunomodulation for treating PAD is that it leverages a patient's innate ability for repair and regeneration. This approach eliminates the need for cell encapsulation, streamlining both the development of the treatment and the regulatory approval process. One notable disadvantage is the lack of clarity regarding the optimal timing for introducing these materials and their long-term effects. Additionally, individual patient responses to immunomodulatory biomaterials may vary, posing challenges in predicting and ensuring consistent treatment outcomes. Factors such as the patient's overall health, immune system status, and genetic variability could influence the effectiveness of the treatment. It is important to note that while immunomodulatory biomaterials show promise, further research and clinical trials are needed to fully understand their efficacy and safety in the context of PAD treatment. Table 3 summarizes the pros and cons of the various biomaterial-based strategies to treat PAD.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Despite the progress made in the advancements in bioengineering technology, the application of bioengineering-assisted cell therapy has been largely limited to preclinical PAD studies. Some of the challenges in adapting these technologies for clinical translation include the scalability of the biomaterials to clinical sizes, along with the safety of the biomaterials, especially when prepared with recombinant DNA technology or xenoproteins. From the commercialization perspective, the incorporation of bioengineered elements can lead to a more complicated regulatory pathway, compared with cell therapy alone. Engineered tissues and other biomaterial-based biologics can fall into multiple categories of therapeutic agents (ie, tissues, biological products, drugs, and medical devices) that can prolong or complicate the regulatory process for Food and Drug Administration approval.[104] Some biomaterials already have Food and Drug Administration approval, such as poly(lactide-co-glycolide) and collagen. Therefore, as more biomaterials become Food and Drug Administration cleared, biomaterials will be more widely adopted for clinical use.

In addition, the mouse hind limb ischemia model for preclinical testing fails to recapitulate conditions seen in clinical settings. A recent review by Krishna et al concluded that the acute ischemia induced from ligating the femoral artery results in significantly different cell responses compared with the chronic ischemia that occurs in PAD. In addition, success in hind limb ischemia models is often measured based on the presence or absence of limb necrosis rather than metrics more commonly used in clinical settings such as blood perfusion rate, treadmill walking, and pain at rest.[105]

Besides the regulatory hurdles associated with bioengineering-related devices, further advancements in bioengineering technology are necessary. For example, the geometric complexity of 3D bioprinted constructs has improved in the past decade, but the resolution of bioprinted structures using conventional extrusion-based bioprinting is limited to 200 to 500 μm.[87,106,107] The recent development of lithography-based bioprinting can produce larger bioprinted constructs within minutes at a resolution approaching 25 μm.[108,109] It is, therefore, anticipated that

Table 3. Benefits and Limitations of Bioengineering Strategies for Cell Delivery

Bioengineering strategy for cell delivery	Pros	Cons
Cell-encapsulated hydrogels	Injectable system for minimally invasive delivery	Safety and potential immune response of synthetic or engineered hydrogels
	Mechanically tunable hydrogels for optimal cell retention	Clinical scalability of synthetic or engineered hydrogels
Cells on spatially patterned scaffolds	Topography-mediated cellular organization modulates cellular function	Scaffolds require more relative, more invasive procedures for implantation, compared with injection
	Topography-mediated molecular signaling being beneficial for cell survival or function	Uncertainty of topography-mediated effects upon partial degradation of scaffold
Biomaterial-based genetic cell modification	Long-term release of angiogenic factors	Risk of immune response
	Biomaterials limit off-target transfer of genetic material	Potential issues of genomic integration
3D bioprinting of cells	Can create large-scale scaffolds with complex geometries	Bio-inks can be challenging to develop
	Patterning of multiple cell types	Poor resolution at small (capillary) scale
Immunomodulatory biomaterials	Leverages patient's own regenerative potential	Best time line for administration is unknown
	Potential for acellular treatment	Patient response can vary

3D indicates 3 dimensional.

these technologies will continue to break the boundaries to create more physiologically relevant tissue geometries.

With the increasing popularization of personalized medicine, we envision that cell therapy and biomaterials will become increasingly tailored to address patient-specific disease conditions. For example, emerging research in preclinical models suggests that lifestyle choices such as tobacco exposure can negatively affect the efficacy of stem cell therapy for treatment of PAD.[110,111] Therefore, autologous stem cells from some patients may need preconditioning to reverse or reprogram the cells into a functional state before transplantation. In this regard, biomaterials can become a tool to modulate cellular function.

In summary, cell therapies have been tested in a limited number of clinical trials with results that suggest minor to moderate improvement. Bioengineering and biomaterials approaches have the potential to improve the effectiveness of cell-based therapies for treatment of PAD through the modulation of cell survival and function of the transplanted cells and the cells in the recipient tissue. As technical and regulatory hurdles become overcome, we anticipate that bioengineering strategies will become more widely used in conjunction with therapeutic cells for treatment of PAD.

ARTICLE INFORMATION

Affiliations

Department of Cardiothoracic Surgery (N.F.H., B.P.O.), Stanford Cardiovascular Institute (N.F.H., B.P.O.), and Department of Chemical Engineering (N.F.H.), Stanford University, CA. Center for Tissue Regeneration, Repair and Restoration, Veterans Affairs Palo Alto Health Care System, CA (N.F.H., B.P.O.). Department of Biomedical Engineering, The University of Texas at Austin (B.S., J.Z.). Fibralign Corporation, Union City, CA (T.S.Z., M.V.P.).

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Disclosures

T.S. Zaitseva and M.V. Paukshto are employees of Fibralign Corporation that manufactures a nanopatterned collagen medical device. The other authors report no conflicts.

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