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REVIEW ARTICLE

Lysophosphatidic Acid and Sphingosine-1-Phosphate: A Concise Review of Biological Function and Applications for Tissue Engineering

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The presentation and controlled release of bioactive signals to direct cellular growth and differentiation represents a widely used strategy in tissue engineering. Historically, work in this field has primarily focused on the delivery of large cytokines and growth factors, which can be costly to manufacture and difficult to deliver in a sustained manner. There has been a marked increase over the past decade in the pursuit of lipid mediators due to their wide range of effects over multiple cell types, low cost, and ease of scale-up. Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are two bioactive lysophospholipids (LPLs) that have gained attention for use as pharmacological agents in tissue engineering applications. While these lipids can have similar effects on cellular response, they possess distinct chemical backbones, mechanisms of synthesis and degradation, and signaling pathways using a discrete set of G-protein-coupled receptors (GPCRs). LPA and S1P predominantly act extracellularly on their GPCRs and can directly regulate cell survival, differentiation, cytokine secretion, proliferation, and migration—each of the important functions that must be considered in regenerative medicine. In addition to these potent physiological functions, these LPLs play pivotal roles in a number of pathophysiological processes. To capitalize on the promise of these molecules in tissue engineering, these lipids have been incorporated into biomaterials for *in vivo* delivery. Here, we survey the effects of LPA and S1P on both cellular- and tissue-level phenotypes, with an eye toward regulating stem/progenitor cell growth and differentiation. In particular, we examine work that has translational applications for cell-based tissue engineering strategies in promoting cell survival, bone and cartilage engineering, and therapeutic angiogenesis.

Introduction

One of the fundamental tenets of tissue engineering is the presentation and controlled release of bioactive signals to direct cellular growth and differentiation. Historically, work in this field has centered on the delivery of large cytokines and growth factors.[1] These biomacromolecules play critical roles in regulating endogenous tissue growth and maturation and are widely investigated for their therapeutic potential to drive stem cell proliferation and differentiation. However, recombinant proteins are costly to manufacture and difficult to deliver in a sustained manner over time,[1] necessitating the use of supraphysiological doses in clinical applications that can lead to uncontrolled tissue growth. Although there has been less focus on the use of secondary metabolites for tissue engineering applications, there has been a marked increase over the past decade in the pursuit of lipid mediators due to their wide range of effects over multiple cell types,[2,3] low cost, and ease of scale-up.

Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are two bioactive lysophospholipids (LPLs) that have gained attention for use as pharmacological agents in tissue engineering applications. While these lipids can have similar effects on cellular response, they possess distinct chemical backbones, mechanisms of synthesis and degradation, and signaling pathways mediated by a discrete set of G-protein-coupled receptors (GPCRs). LPA and S1P predominantly act extracellularly on their GPCRs and can directly regulate cell survival,[2,4,5] differentiation,[2,6–8] cytokine secretion,[9,10] proliferation,[11–13] and migration[2,12,13]—each of the important functions that must be considered in regenerative medicine.

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In addition to these potent physiological functions, these LPLs play pivotal roles in pathophysiological processes, including autoimmune disease, fibrotic disease, cancer, inflammation, and bone disease.[2,14] These lipids have been incorporated into biomaterials for *in vivo* delivery[4,9,11,15,16] and are several orders of magnitude cheaper than recombinant proteins.[9] Here, the effects of LPA and S1P on both cellular- and tissue-level phenotypes are surveyed, with an eye toward regulating stem/progenitor cell growth and differentiation. In particular, this review examines work that has translational applications for cell-based tissue engineering strategies.

Metabolism and Cellular Signaling

The chemical structures of LPA and S1P have both a phosphate head group and a single fatty acid chain attached to a three-carbon backbone.[14] Their levels in circulation are maintained via tightly regulated metabolic pathways, with plasma levels typically ranging from 500 to 1000 nM.[3,17] While there are multiple routes toward LPA biosynthesis, leading to structural heterogeneity,[18] S1P is solely created through sphingolipid turnover.[10]

The bulk of LPA found in the circulation is generated by the action of autotaxin (ATX), a circulating lysophospholipase D enzyme that cleaves the phosphodiester bonds of LPLs.[2,19] In particular, activated platelets secrete large amounts of lysophosphatidylcholine, lysophosphatidylserine, and lysophosphatidylethanolamine, which are subsequently converted to LPA by ATX.[20] Sphingolipid turnover is initiated when sphingomyelinase converts sphingomyelin into ceramide.[17] In sphingolipid metabolism, ceramide is first converted into sphingosine by enzyme ceramidase and S1P is then produced by the subsequent phosphorylation of sphingosine by sphingosine kinase (SK).[10]

G protein-coupled receptors

S1P and LPA elicit pleiotropic cellular effects by activating GPCRs on the cell surface. Both LPLs signal through the endothelial differentiation gene (EDG) family of receptors and are ligands for the P2Y10 receptor (Table 1).[21,22] Extracellular LPA can affect cellular response through at least six GPCRs ($LPA_{1–6}$), while S1P engages at least five ($S1P_{1–5}$). These GPCRs are differentially expressed in various tissues, can change with cellular differentiation state, and have separate coupled subunits that trigger distinct intracellular signaling cascades (Fig. 1).[23–27]

Indeed, the engagement of GPCRs is the most widely accepted primary mechanism by which LPA influences cell behavior.[3,23,24,28,29] LPA_1, LPA_2, and LPA_3 belong to the EDG family of receptors and were the first to be identified and characterized. LPA_4, LPA_5, and LPA_6 were discovered later and classified as purinergic-like (P2Y) receptors, with distinct amino-acid identities and biological functions.[23,24] Three other putative LPA receptors—GPR35,[30] GPR87,[31] and P2Y10[21]—may also be involved in LPA signaling, but their roles are not yet fully characterized.[24,32]

The most important biological role of S1P is to serve as a natural ligand for the EDG family of GPCRs.[26] At least five distinct GPCRs, $S1P_{1–5}$, are known to bind S1P with a high affinity (K_d of 2–30 nM) and each elicits distinct biological actions of which conflicting results have been reported.[25,33,34] To demonstrate the pleiotropic nature of S1P receptors (S1PRs), $S1P_1$ activation enhances barrier integrity and vessel maturation whereas $S1P_3$ conversely promotes vessel permeability and remodeling.[35,36] While $S1P_{1–3}$ are ubiquitously expressed, $S1P_4$ is solely expressed in lymphoid and lung tissues, and $S1P_5$ expression is localized to the brain and spleen.[37]

Intracellular signaling

Intracellular LPA signals primarily through activation of peroxisome proliferator-activated receptor gamma (PPARγ) and may play a key role in regulation of fatty acid metabolism.[29,32] The effects of PPARγ include adipogenic differentiation, lipid metabolism, arterial wall remodeling,[38,39] and dendritic cell differentiation.[40] However, LPA binds strongly at a 3:1 stoichiometric ratio to serum albumin, which serves as a stabilizer and carrier protein.[3] With the exception of cells such as macrophages, vascular smooth muscle cells (SMCs), and platelets that can internalize oxidized low-density lipoprotein associated with LPA,[41,42] the LPA-albumin complex is unable to enter cells in high quantities.[32] This likely limits the effectiveness of strategies seeking to directly stimulate PPARγ signaling when applied to cells cultured in serum-supplemented media *in vitro*.

While capable of eliciting intracellular actions, the intracellular targets of S1P are only recently being discovered.[10,27] S1P produced by SK 2, highly localized in the nucleus, has been reported to bind and inhibit histone deacetylase (HDAC) activity.[27,43] HDAC inhibition is a growing target for cancer

Table 1. G-Protein-Coupled Receptors

Receptor	*Synonyms*	*Receptor family*	*G-protein-coupled subunits*	*References*
LPA_1	LPAR1, EDG2	EDG	G_i, G_q, $G_{12/13}$	23,28,32,122,125
LPA_2	LPAR2, EDG4	EDG	G_i, G_q, $G_{12/13}$	23,28,32,122,125
LPA_3	LPAR3, EDG7	EDG	G_i, G_q	23,28,32,122,125
LPA_4	LPAR4, GPR23, $P2Y_9$	P2Y	G_q, $G_{12/13}$, G_S	23
LPA_5	LPAR5, GPR92	P2Y	G_q, $G_{12/13}$	23
LPA_6	LPAR6, P2RY5	P2Y	$G_{12/13}$	23
$S1P_1$	S1PR1, EDG1	EDG	G_i	122,125
$S1P_2$	S1PR2, EDG5	EDG	G_i, G_q, $G_{12/13}$	122,125
$S1P_3$	S1PR3, EDG3	EDG	G_i, G_q, $G_{12/13}$	122,125
$S1P_4$	S1PR4, EDG6	EDG	G_i, $G_{12/13}$, G_S	122,125
$S1P_5$	S1PR5, EDG8	EDG	G_i, $G_{12/13}$	122,125

LPA, lysophosphatidic acid; S1P, sphingosine-1-phosphate; EDG, endothelial differentiation gene.

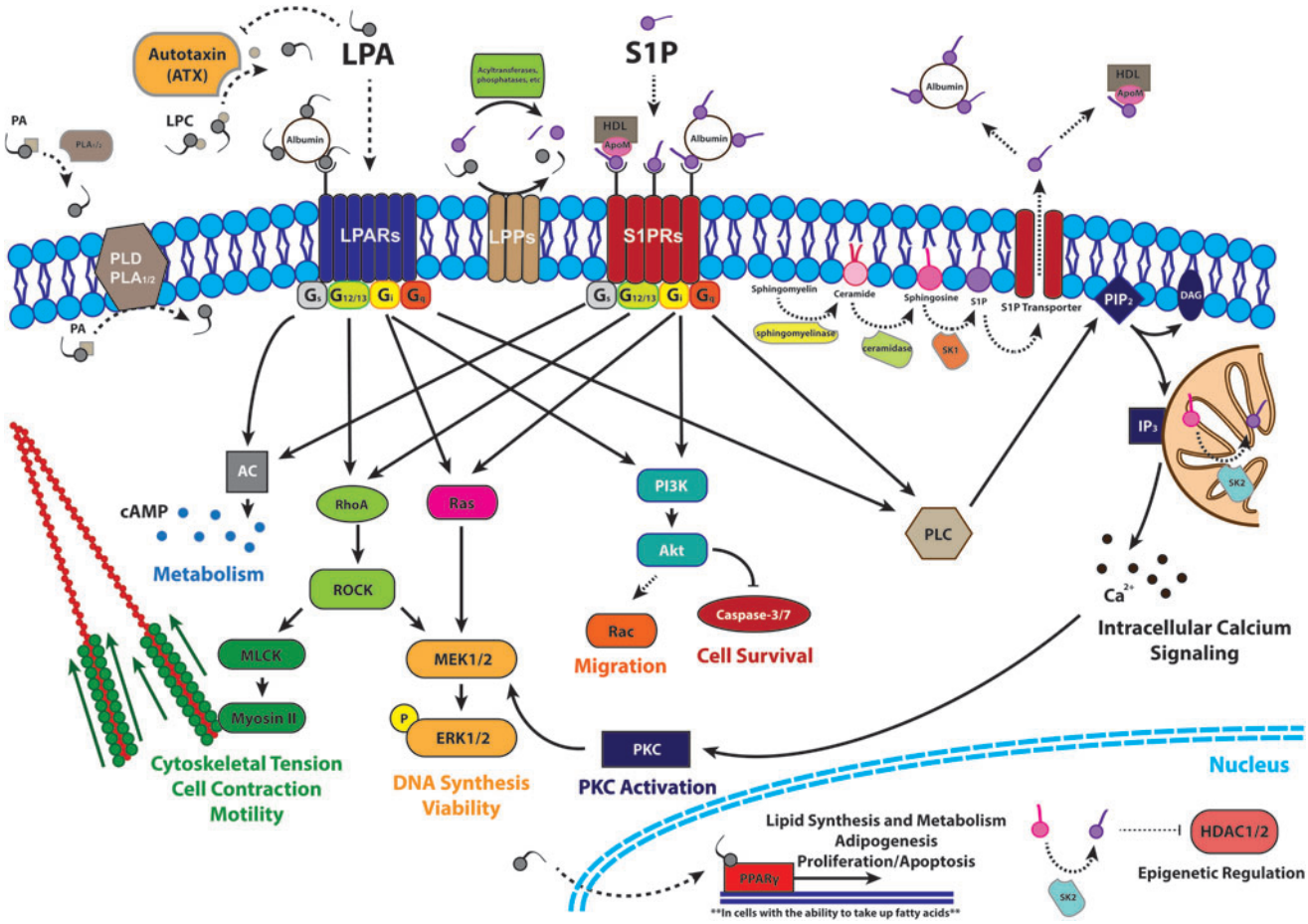


FIG. 1. Examples of the most widely understood mechanisms of LPA synthesis, degradation, and intracellular signaling. LPA, lysophosphatidic acid; PA, phosphatidic acid; PLA, phospholipase A; PLD, phospholipase D; LPP, lipid phosphate phosphatase; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacyl glycerol; AC, adenylyl cyclase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; ROCK, Rho-associated protein kinase; MLCK, myosin light chain kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular-signal-regulated kinases; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor gamma; HDAC, histone deacetylase.[3,15,18,21,23,24,27–29,32,71,73,112,116,118–124] Color images available online at www.liebertpub.com/teb

therapies via reversal of aberrant epigenetic changes associated with human disease. Independent of S1PRs, S1P can activate nuclear factor-κB (NF-κB), a key inflammatory transcription factor.[44] Furthermore, S1P produced in the cell is exported by specific membrane transporters, including spinster 2, which has a role in establishing a vascular gradient of S1P in mice.[17] This release of S1P might also contribute to inside-out signaling by stimulating S1PRs after being transported outside of the cell membrane.[10,45]

Activity of Bioactive Phospholipids in Homeostasis and Disease

LPA and S1P have a wide range of effects on many tissue types and on cells at varying stages of differentiation and development. Many tissue engineering strategies seek to recapitulate or modulate similar cellular responses. Therefore, establishing an understanding of endogenous LPL signaling is critical to the success of such approaches. Furthermore, given the pleiotropic nature of LPL signaling, control over spatiotemporal presentation becomes important for establishing the intended selectivity of receptor activation. We highlight some of these areas and address current studies of LPA and S1P next.

Vascular health

Both LPA and S1P play significant roles in vascular development and disease, as might be expected from the close relationship with platelet activation. ATX-knockout mice fail to develop a functional vasculature and do not survive embryonic development, at least in part due to the lack of LPA synthesis.[46] LPA (1 μM) also stimulates angiogenesis of developing blood vessels in a chick chorioallantoic membrane (CAM) model,[47] achieving a response quantitatively similar to 50 ng vascular endothelial growth factor (VEGF). LPA-induced vessels were larger in diameter than those induced by VEGF. In mature vessels, LPA can induce endothelial cell mitogenesis[48] and increase the permeability of cell–cell junctions,[49] the latter of which can facilitate metastasis. LPA also induces SMC dedifferentiation[50] and may lower expression of CD36 by endothelial cells.[51,52] Consistent with its role in Rho-ROCK signaling, LPA sensitizes murine aortic endothelial cells to oscillatory shear

stresses by regulating Ca^{2+} transients.[53] Micromolar levels of LPA may induce a vasoconstrictive response[54] in medial SMCs under high shear stresses, but lower doses cause endothelium-dependent vasodilation via endothelial nitric oxide synthase and phospholipase activity.[55]

VEGF is perhaps the most widely studied proangiogenic molecule whose activity has been targeted as a therapeutic for initiating neovascularization and for blocking tumor growth. S1P has also been touted as a complete angiogenic molecule given its contributions at both early stages of angiogenesis and later stages of neovessel stabilization.[8,56–61] S1P plays a pivotal role in regulating angiogenesis and vascular tone.[36,62] $S1P_1$ is essential for vascular development, as $S1P_1$-null mice exhibit lethal embryonic hemorrhage.[8] S1P promotes initial sprouting of capillary-like structures by endothelial cells *in vitro*[13] and synergistically acts with basic fibroblast growth factor for induction of *in vitro* angiogenesis.[63] In addition, S1P plays a crucial role in stabilizing neovessels in arteriogenesis via the recruitment of mural cells and regulation of endothelial cell–cell junctions.[11] While S1PR1 activation has been shown to inhibit VEGF-induced hyperpermeability and aberrant sprouting,[64,65] S1PR3 conversely enhances vessel permeability and remodeling.[35,66] Thus, temporal regulation of S1P signaling is imperative for dictating the overall outcome. In light of its potent activities throughout the process of catalyzing the formation and stabilization of neovessels, S1P has emerged as a promising target for novel therapeutic approaches to treating ischemic diseases.[67] Many studies have also examined the angiogenic activity of LPLs as compared with proangiogenic growth factors, including VEGF, further highlighting their therapeutic potential.[47,68–70] For example, S1P and LPA each induced a similar angiogenic response to VEGF in an *in vitro* chicken CAM assay.[47] S1P has also been shown to surpass VEGF and independently induce sprouting and directed migration of outgrowth endothelial cells to a similar degree as the combination of S1P and VEGF under hypoxia.[70] Thus, given its ''bimodal'' angiogenic ability, one may speculate that it may be more therapeutically effective to deliver S1P with spatiotemporal control rather than combinations of multiple growth factors.

Skeletal biology

LPA signaling influences bone biology and the maintenance of skeletal homeostasis. Developmentally, LPA_1 KO mice exhibit decreased bone density, shorter bone length, and craniofacial dysmorphism. In addition, bone marrow (BM) stem/stromal cells isolated from these animals have reduced osteogenic potential.[71,72] On the contrary, LPA_4 KO mice manifest higher bone mass, with greater trabecular number and thickness.[73]

Mesenchymal stem/stromal cells (MSCs), which participate directly in developmental bone formation by differentiating into osteoblasts,[74] also respond to LPA. In addition to inducing migration,[75] albumin-bound LPA upregulates alkaline phosphatase (ALP) activity, an early marker of osteogenic differentiation, suggesting that LPA directs osteoblastogenesis in MSCs,[7,73,76] likely through LPA_3. However, LPA_4 activation can inhibit osteogenesis,[73] possibly due to its ability to bind intracellular G_s subunits. Therefore, additional regulation of cAMP signaling may be required for optimal bone formation. In bovine endometrial stromal cells, LPA upregulates production of prostaglandin E_2 (PGE_2),[77] which promotes bone formation in osteoblastic cells through suppression of sclerostin expression and a corresponding increase in Wnt signaling.[78] LPA has also been reported to induce MSC differentiation into myofibroblast-like cells.[79] In addition to direct contributions to bone formation, MSCs promote the necessary angiogenic contributions to bone repair and homeostasis by secreting trophic factors that recruit and stabilize endothelial cells.[80] LPA potentiates this behavior by stimulating MSCs to increase secretion of proangiogenic and other cytokines, including VEGF and stromal cell derived factor-1.[81] Furthermore, LPA protects MSCs against apoptosis induced by ischemic conditions, likely through a phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt-mediated pathway.[4,5,82,83]

In mature bone, LPA modulates cytoskeletal organization in osteoblasts and may stimulate extracellular matrix production and organization.[84] Osteoblasts increase ALP production in response to LPA, especially when concomitantly exposed to calcitriol,[7,76] indicating a role in osteoblast maturation. LPA modulates motility in osteoblasts and osteocytes, inducing chemotaxis in murine MC3T3 preosteoblasts[85] and dendrite outgrowth in MLO-Y4 osteoblasts.[86] These findings suggest that LPA may be involved in directing osteoblast migration and morphology during bone remodeling. Recent data provide evidence that osteoclasts produce LPA, which can then act as an additional autocrine signal as well as a paracrine cue to osteoclasts and potentially MSCs.[87,88]

Indeed, osteoclasts, which arise from a hematopoetic rather than mesenchymal lineage, are also responsive to LPA and demonstrate marked increases in Ca^{2+}-mediated intracellular signaling on exposure.[89] LPA participates in regulation of osteoclast number and activity by influencing osteoclastogenesis and enhancing survival of this population through suppression of apoptosis.[88,89] This increase in survival may be due to activation of the extracellular-signal-regulated kinases (ERK)-PI3K/Akt pathway[88] or activation of calmodulin and downstream pro-survival pathways due to increases in cytosolic Ca^{2+}.[89] Consistent with its effect on other cell types, LPA also has cytoskeletal effects on osteoclasts, resulting in retraction of lamellipodia and pseudopodia and a decrease in cell area, although resorptive capacity is largely unaffected.[89]

S1P also promotes osteoblast migration, survival by inhibition of apoptosis,[90,91] and proliferation[92,93] at concentrations similar to those that occur in systemic circulation. Osteoblasts express $S1P_1$, $S1P_2$, and $S1P_5$.[92] As a mitogen, S1P appears to differentially activate the MAPK pathway in both human and rat osteoblasts in a species-specific manner.[94] Primary human osteoblasts also have been shown to increase ALP production after 3 and 5 days of stimulation with S1P *in vitro*.[95] S1P also was reported as a potent serum-derived chemoattractant in inducing MSC mobilization *in vitro*.[25] MSCs express $S1P_{1–3}$, but $S1P_3$ signaling appears to be the predominant regulator of MSC trafficking.

S1P stimulation increases osteoclastogenesis by increasing RANKL in osteoblasts through cyclooxygenase-2 and PGE_2 regulation demonstrated in co-culture studies of BM-derived macrophages and osteoblasts.[90] Furthermore, S1P can contribute to the dynamic control of bone mineral

homeostasis, as it induces migration of osteoclast precursor cells along concentration gradients of S1P both *in vitro* and *in vivo*.[96] The regulation of osteoclast precursor cell trafficking to and from the bone surface is a crucial process in the mediation of bone resorption.

Clinical Applications of Bioactive Lipids in Drug Discovery

Based on the importance of the S1P and LPA signaling axes for various pathologies, a number of drugs are in clinical trials that target these pathways, as reviewed in Kunkel *et al.*[97] FTY720 (fingolimod) is an S1P-based therapeutic with potent immunomodulatory capacity.[14] FTY720 was first clinically studied for its use in improving renal transplantation outcomes and preventing allograft rejection, but it did not reach its clinical end-points.[14,98] However, FTY720 became the first FDA-approved orally bioavailable drug for treating relapsing forms of multiple sclerosis.[14] In a phase I clinical trial (ClinicalTrials.gov Identifier: NCT00661414), sonepcizumab, an S1P-specific monoclonal antibody, was evaluated as an anti-S1P treatment to reduce tumor volume and metastatic potential by inhibiting blood vessel formation.[97,99] Given the pleiotropic actions of S1P and LPA in full, other therapies have used specific receptor targets for therapeutic application. RPC1063 is an $S1P_1$ modulator that has undergone Phase II clinical trials for both relapsing-remitting multiple sclerosis and ulcerative colitis. BMS-986020 (AM152), an antagonist of LPA_1, is also in Phase II clinical trials for treating idiopathic pulmonary fibrosis.[14] In addition, LPA has been studied as a diagnostic for early detection of ovarian cancer (ClinicalTrials.gov Identifier: NCT00986206). While there are several ongoing efforts to target S1P- and LPA-signaling pathways, clinical trials using S1P and/or LPA directly as a therapeutic have not been performed to date.

Applications in Tissue Engineering

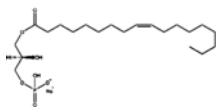
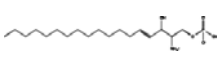
There have been comparatively few efforts to directly use LPLs for clinical treatments or regenerative medicine. Systemic delivery is impractical due to the extremely short circulatory half-life of these lipids,[100] which are eliminated through first-pass hepatic clearance, and LPL quantification can be labor- and cost-intensive (Table 2). Nonetheless, the pleiotropic nature of LPA and S1P, in addition to their ready availability and low cost compared with recombinant proteins, makes these LPLs attractive targets for investigation in many applications. Therefore, these lipids lend themselves particularly well to tissue engineering strategies that utilize material-based approaches to closely regulate the spatiotemporal presentation of signaling molecules. Some of the more promising directions for LPLs in tissue engineering are highlighted next.

Pharmacological strategies for inhibiting apoptosis and promoting cell persistence

Maintaining cell persistence and survival postimplantation represents one of the biggest hurdles to successful *in vivo* translation of cell therapies. Serum-deprived and hypoxic (SD/H) culture conditions are catastrophic to MSC cultures,[101] and more than 99% of cells delivered to ischemic heart tissue die after 72 h.[102] Although recombinant growth factors such as angiopoietin-1 can confer apoptotic resistance,[103] such proteins are prohibitively expensive, have short half-lives *in vivo*, and are difficult to deliver in a sustained manner. Thus, it is critically important to develop novel methods for enhancing cell survival.

The ability of LPA and LPA receptor agonists to protect multiple cell types against SD/H,[104] combined with the relatively low cost, makes them ideal for many cell delivery applications. Micromolar concentrations of LPA protect neuronal precursors,[105] osteoblasts, osteoclasts,[88] rat and human MSCs,[4,5] and other cell types against SD/H- and endoplasmic reticulum-stress induced apoptosis *in vitro* in a pertussis toxin- and PI3K-dependent manner.[82] Furthermore, these protective effects extend to MSCs injected *in vivo*[83] after preconditioning in medium containing LPA. Similarly, the development of nonlipid agonists of LPA_2 is underway for protecting

Table 2. Chemical, Physical, and Biological Characteristics of Lysophosphatidic Acid and Sphingosine-1-Phosphate

	Chemical and physical characteristics		*Biological characteristics*			
LPL	*Chemical structure*	*MW (g/mol)*	*½ life*	*Physiological levels*	In vivo *production*	*Detection methods*
LPA		436.52	< 1 min; rapidly cleared by first-pass hepatic[100]	~1–20 μM (serum)[3,118] 1–600 nm (plasma)[32,118]	Activated platelets, hair follicles, cancer cells	LC-MS/MS[126,127]; MALDI-TOF[128,129]
S1P		379.47	~15 min (plasma)[130]	~0.1–1.2 μM (plasma)[13,17,130,131] 0.5–75 pmol/mg wet weight (tissues)[130]	Activated platelets, red blood cells, mast cells, cancer cells, endothelial cells	ELISA[70,132]; LC-MS/MS[133]; Radiolabeling[16]; HPLC[130,134]

LPA, lysophospholipid; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight.

gastrointestinal tissue against apoptosis caused by high-dose γ-irradiation.[106]

However, most modern strategies for regenerative medicine utilize cell-instructive biomaterials such as hydrogels or polymer scaffolds to deliver cells to larger defects. Therefore, the ability to deliver signaling molecules such as LPA or LPA receptor agonists in a three-dimensional material is paramount. We have shown that MSCs in peptide-modified Arg-Gly-Asp (RGD)-alginate containing physically entrapped LPA exhibit improved persistence over 4 weeks in a subcutaneous implantation model (Fig. 2A). Furthermore, osteogenically induced MSCs responded differently to ischemic environments and varying doses of LPA compared with undifferentiated cells.[4] Additional work is required to determine optimal concentrations and retention properties in a range of materials that are suited for distinct therapeutic applications.

The sphingolipid rheostat (ratio of S1P to ceramide) can also dictate outcomes of cell survival (S1P-induced) versus cell death (ceramide-induced).[107,108] S1P, generated by overexpression of SK1, protects against apoptosis by activating ERK1/2, Akt, and the NF-κB signaling pathways of cell survival.[26,107] S1P further inhibits release of cytochrome c, activation of caspases, and activation of Jun amino-terminal kinase, a stress-activated protein kinase, in inhibition of apoptosis. S1P has been shown to suppress apoptosis by endothelial cells in SD in a dose-dependent manner.[108] Interestingly, these anti-apoptotic actions appear to be independent of extracellular S1PR signaling.[107]

Therapeutic angiogenesis

Beyond short-term abrogation of SD/H-induced apoptosis, the reestablishment of a vascular supply is critical for successful cell therapies to treat ischemic defects, such as those arising from chronic and acute peripheral vascular disease or tissue loss due to trauma, surgery, or disease. Although surgical interventions for restoration of blood flow are possible, they are both costly and invasive. Growth factor-based approaches have been pursued as treatment options,[1] but limitations related to regulating spatiotemporal release and uncontrolled vessel and tumor growth motivate the development of alternative strategies.

The direct mitogenic[48] and proangiogenic[47] effects of LPA on endothelial cells suggest that controlled release of this molecule may stimulate an angiogenic response *in vivo*. However, the challenges of accurately modeling release in *in vitro* systems, including artificial synthesis/degradation, make it difficult to effectively tailor material properties before *in vivo* implementation. An alternative strategy for using LPA in therapeutic angiogenesis is to take advantage of the ability of stromal cell populations to function as pericytes that promote and stabilize blood vessel formation.[109] Such pericytes naturally secrete growth factors such as VEGF and have been investigated as a vehicle to continuously supply local angiogenic cues.[110,111] Since LPA induces the secretion of proangiogenic and inflammatory cytokines from MSCs,[75,81] entrapment of stromal cells in LPA-containing constructs could result in elevated secretion of angiogenic growth factors. Indeed, human adipose-derived stromal cells (ASCs) entrapped in fibrin gels containing LPA significantly improved recovery and functional outcome in a mouse model of critical limb ischemia.[9] Two

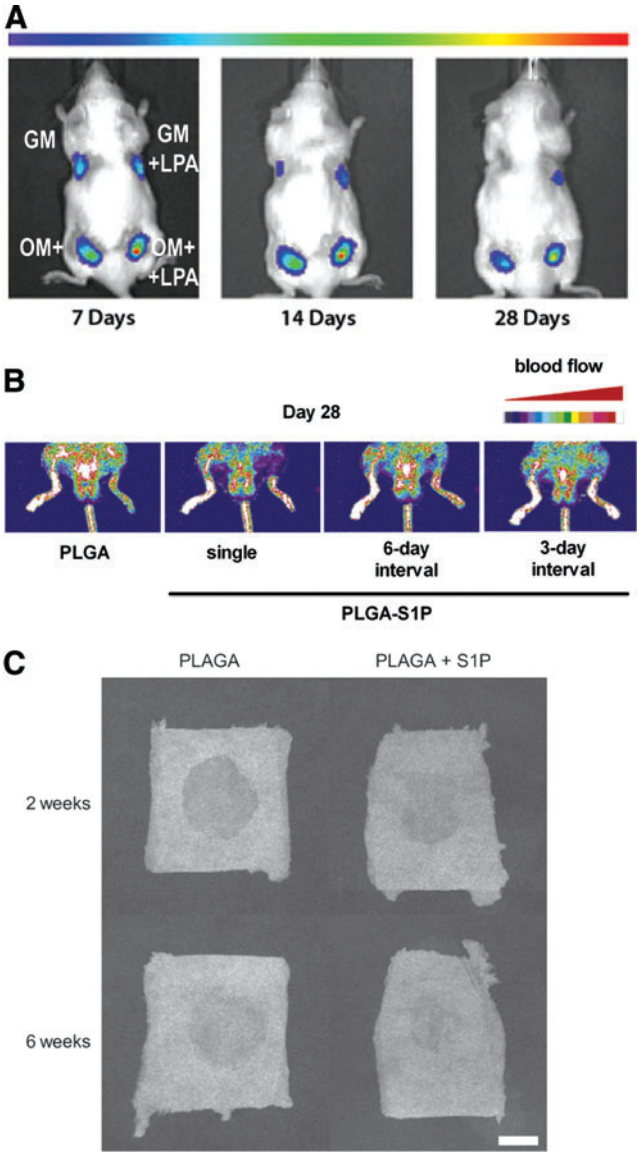


FIG. 2. Delivery of lysophospholipids for tissue engineering applications. Delivery of LPA enhanced survival of transplanted human mesenchymal stem/stromal cells, preconditioned with either growth medium (GM) or osteogenic medium (OM), within alginate hydrogels after 28 days *in vivo* as assessed by bioluminescence imaging (**A**). Figure reproduced with permission from Mary Ann Liebert.[4] Intermittently repeated, local injections of sphingosine-1-phosphate (S1P)-loaded poly(lactic-*co*-glycolic acid) (PLGA) microparticles (PLGA-S1P) resulted in enhanced blood flow recovery in murine ischemic hindlimbs after 28 days *in vivo* when examined by Laser Doppler blood flow analysis (**B**). Figure reprinted with permission from Elsevier.[57] Sustained delivery of S1P from PLGA scaffolds within critical-sized, rat cranial defects led to greater cranial bone healing after 2 and 6 weeks *in vivo* when evaluated by X-ray imaging analysis (**C**). Figure reprinted with permission from Elsevier.[11] Color images available online at www.liebertpub.com/teb

weeks after femoral artery ligation and resection, animals treated with both fibrin-entrapped ASCs and LPA showed significantly increased blood vessel formation compared with mice treated with only ASCs or LPA, while all groups receiving LPA exhibited reduced limb necrosis and loss.

Improved tissue preservation without concomitant increases in vascularization in mice treated with LPA alone[9] also supports mounting evidence that LPA can modulate local inflammation and the immune response.[112] Given the close interplay between the angiogenic and inflammatory axes in wound healing, effective LPA presentation merits further investigation for applications involving ischemic defects or vascular injury.

The manipulation of local S1P gradients represents a novel and exciting approach to recruit endogenously or exogenously supplied stem/progenitor and effector immune cells for regenerative medicine in recent research.[16] S1P has also been recognized as an attractive therapeutic agent for delivery based on its key involvement in both angiogenesis and arteriogenesis.[11,58] S1P stimulates the proliferation and migration of endothelial cells and promotes vascular stabilization through recruitment of pericytes and SMCs to surround neovessels. S1P further supports luminal expansion of arterioles and venules by stimulating SMC proliferation, migration, and differentiation into a more contractile phenotype. S1P may also recruit BM-derived circulating endothelial precursor cells to ischemic tissues, potentially mediated through $S1P_3$, to stimulate angiogenesis.[25] Daily intramuscular injections of S1P increased capillary density and promoted blood flow recovery in murine ischemic hindlimbs.[56] However, material-controlled sustained release would be a more clinically relevant approach toward therapeutic angiogenesis without the need for daily injections. The deployment of S1P-loaded poly(lactic-*co*-glycolic acid) (PLGA) microparticles improved blood flow recovery and reduced VEGF-induced edema (Fig. 2B).[57] S1P encapsulated within PLGA thin films promoted short-term enlargement of arteriolar and venular diameters.[11] Furthermore, temporally separated delivery of VEGF and S1P from porous hollow cellulose fibers has been shown to enhance cellular infiltration in a modified murine Matrigel plug assay.[65] However, biomaterial-based gradients of S1P are short-lived in the tissue due to degradation by S1P lyase,[16] thereby motivating the investigation of new methods to locally sustain these signals. One such method involving co-delivery of S1P and 4-deoxy-pyridoxine, an S1P lyase inhibitor, from PLGA films substantially increased local tissue S1P and sphingolipid concentrations over time.[16]

Orthopedic applications

LPA holds promise for successful use in bone repair applications, even though the LPA-directed signaling axis in osteoblast and osteoclast differentiation has not yet been fully elucidated. In addition to the pro-survival implications for cells delivered to ischemic defect sites, LPA may be targeted for use in directing differentiation or maturation of MSCs, preosteoblasts, or osteoblasts through the pathways previously described. Although the hydrogel-based systems that have been investigated for cell survival and therapeutic angiogenesis are only mildly osteoinductive, we have shown that the addition of mineralized PLGA microspheres to fibrin gels further enhances osteogenic differentiation[113]; such a system would allow for similar physical entrapment of LPA and presentation. Alternately, other groups are pursuing the covalent attachment of LPA to titanium constructs with the goal of stimulating osteoblast maturation and osseointegration[114] on scaffolds better suited to load-bearing applications.

Given the pro-angiogenic and pro-arteriogenic nature of S1P, exogenous delivery has also been used in approaches for enhancing tissue-engineered bone regeneration.[11,115] In a rat cranial defect model, defects filled with S1P-loaded PLGA macroporous scaffolds exhibited significantly increased bone volume after 2 and 6 weeks of healing versus empty scaffolds alone (Fig. 2C). The substantial bone healing correlated with an increased number of blood vessels. This functional involvement of S1P in the formation of new bone is hypothesized to be due to its ability to both remodel the microvasculature and stimulate recruitment and proliferation of osteoblast precursor cells.[11] However, future studies are required to fully understand S1P signaling in bone formation.

Orthopedic applications of LPA are not limited to treatment of bony defects. LPA treatment of self-assembled fibrocartilage constructs synthesized by articular chondrocytes and meniscal cells resulted in tissue with improved tensile properties and superiorly aligned collagen structures.[6] LPA is produced *in situ* by resting zone growth plate chondrocytes in response to 24R,25-dihydroxyvitamin D_3 and inhibits apoptosis induced by inorganic phosphate.[116] Similarly, LPA stimulates proliferation of rat chondrocytes *in vitro*.[117] Since this cell type is notoriously difficult to expand in culture, successful induction of a proliferative response could have significant implications for scale-up of tissue engineered cartilages. These studies emphasize the feasibility of using bioactive lipids to enhance the physical and biochemical properties of tissue engineering strategies that call for *ex vivo* expansion or generation of tissue constructs for subsequent implantation into *in vivo* defect sites.

Conclusion

LPA and S1P are inexpensive lipid mediators that have pleiotropic effects in many different cell and tissue types. To date, these lipids have been primarily studied in biological and mechanistic contexts. However, if careful consideration is given to controlling presentation, release, and degradation in conjunction with established biomaterials-based delivery vehicles, these molecules hold great promise for enhancing the efficacy of tissue engineering solutions for a wide range of pathologies and defects. Given the diverse and pleiotropic nature of these LPLs, delivery of S1P or LPA may provide an attractive alternative to the delivery of multiple growth factors. Thus, further studies must be performed to compare the efficacy of these bioactive molecules for specific therapeutic applications.

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Disclosure Statement

No competing financial interests exist.

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