The Incorporation and Release of Bioactive Molecules in Vascular Grafts

Muhammad Shafiq, Hongyu Yan, Adam C. Midgley, Kai Wang, Qiang Zhao, and Deling Kong

Contents

Abstract

Cardiovascular diseases account for massive socioeconomic burden worldwide. Whereas the use of native arteries for vascular grafting is the gold-standard treatment option, donor-site associated infection risk and/or prior use, limit their full therapeutic utilization. Consequently, artificial vascular grafts offer an invaluable solution for vascular grafting. However, their use is often hindered by risk of thromboembolic

M. Shafiq
Key Laboratory of Bioactive Materials of the Ministry of Education, College of Life Sciences, Nankai University, Tianjin, China

Department of Chemistry, Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan
e-mail: muhammad.shafiq917@yahoo.com

H. Yan · A. C. Midgley · K. Wang · Q. Zhao · D. Kong (✉)
Key Laboratory of Bioactive Materials of the Ministry of Education, College of Life Sciences, Nankai University, Tianjin, China
e-mail: yanhongyu85@163.com; midgleyac@nankai.edu.cn; wkcs424@163.com; qiangzhao@nankai.edu.cn; kongdeling@nankai.edu.cn

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complication and intimal hyperplasia (IH). To circumvent these limitations, a wide range of strategies have been pursued, including the use of physico-chemical cues and bioactive molecules to enhance endothelialization, cellularization, and remodeling; as well as to overcome IH, thrombosis, and infection. Various types of bioactive molecules, including extracellular matrix (ECM) proteins, growth factors, growth factor mimetic molecules, peptides, ECM-derived peptides, phage display-derived peptides, and therapeutic molecules have been incorporated into artificial vascular grafts. Through incorporation by various means, such as encapsulation, physical adsorption/absorption, non-covalent conjugation, and covalent conjugation, vascular graft performance and remodeling have shown beneficial outcomes. In this chapter, we discuss the key approaches being pursued in the design of novel polymer types for vascular regeneration applications. Particularly, we provide a framework for the enhancement of endothelialization, the improvement of cellularization, overcoming IH, modulating smooth muscle cell phenotype, and the positive regulation of the inflammatory response towards the polarization of macrophages into anti-inflammatory phenotypes. Moreover, we have identified key biomolecules and approaches, which have potential for improving blood vessel regeneration, but have not yet been comprehensively evaluated. Taken together, the appropriate combination of biomolecules along with fitting scaffold materials, as discussed herein, may greatly benefit vascular reconstruction for healthcare.

1 Introduction

Cardiovascular disease (CVD) is the major cause of death worldwide, and is responsible for over 17.3 million deaths annually (Benjamin et al. 2018; Roth et al. 2015). Autologous vessels, such as saphenous veins and radial or left internal mammary arteries, are gold-standard replacement options for dysfuncitonal veseels in CVD. However, donor-site–associated infection risks, shortage of suitable donors, and high long-term failure rates necessitate the development of alternative options. Artificial blood vessels, including: expanded polytetrafluoroethylene (ePTFE), poly (ethylene terepthalate) (Dacron), and polyurethane, have shown promising potential as alternatives to autologous grafts. Artificial vessels have their own limitations, often failing due to thrombosis, intimal hyperplasia (IH), atherosclerosis, calcification, and infection, when used as substitutes for small-caliber arteries (inner diameter $\leq$ 6 mm) (Zilla et al. 2007). In coronary artery bypass grafting, the use of PTFE conduits resulted in a 1-year patency rate of ~60%, which declined to 32% after 2 years (Hadinata et al. 2009). Patency of ePTFE prostheses was 40–50% when used to bypass the proximal popliteal artery at 5 years and 20% when used for infrapopliteal bypass at 3 years (Klinkert et al. 2004; Kashyap et al. 2002). In above-knee fem-pop bypass, results have shown PTFE graft patency rates of ~59% at 5 years (Ballotta et al. 2003). Klinkert et al. reviewed extensive data on above-knee bypass grafting using saphenous vein and PTFE, reporting that later types of grafts exhibited poor primary and secondary patency in comparison to the vein grafts after 2 and 5 years (Klinkert et al. 2004). Lastly, Greenwald and coworkers reviewed a

substantial data set of implanted ePTFE-based small-diameter vascular grafts (SDVGs) and concluded that most vascular grafts failed due to IH (Greenwald and Berry 2000). In addition, these grafts lacked growth potential and required re-operation, particularly in pediatric patients. Therefore, it is pertinent to find more appropriate biodegradable polymers for the fabrication of SDVGs (Brewster et al. 2007).

Tissue engineering (TE) has been proposed to overcome these challenges through the fabrication of tissue-engineered blood vessels. However, conventional TE approaches comprise lengthy and costly in vitro procedures. An emerging avenue is to design cell-free grafts which can activate the host's own regenerative capabilities, orchestrate cell responses and drive the development of extracellular matrix (ECM) (Mol et al. 2009). By avoiding time-consuming ex vivo cell manipulation procedures, such methodology can deliver cost-effective, yet off-the-shelf available grafts. Recent reports following in situ tissue regeneration have documented remodeling of cell-free grafts into functional blood vessles (Hoerstrup et al. 2006; Koobatian et al. 2015; Muylaert et al. 2016; Shafiq et al. 2018; Wang et al. 2014, 2015a, b, c, 2017a, b; Wu et al. 2012; Yokota et al. 2008).

An inflammation-mediated response has been suggested as an effective approach to drive tissue repair and remodeling, comprising an initial rapid recruitment of immune cells followed by the influx of vascular cell types (Hibino et al. 2011; Roh et al. 2010). In addition, a variety of bioactive molecules such as stromal cell-derived factor-1 alpha (SDF-1α), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factors (BDNFs), and granulocyte colony-stimulating factor (G-CSF) have been proposed to improve the cellularization, remodeling, and/or the patency of prosthetic materials (Cho et al. 2006; Koobatian et al. 2015; Muylaert et al. 2016; Shafiq et al. 2015a; Choi et al. 2016; Chen et al. 2015). Despite obvious advantages of the aforementioned chemokines and growth factors, they possess the disadvantages of being easily degraded by the proteolytic microenvironment and difficulty in synthesis and incorporation into scaffold materials, which overall limits their wider applicability in vascular regeneration applications. Therefore, alternative candidates, such as: cationic peptides, bioactive lipids, and short peptide sequences have more recently been gaining increasing attention from the research community (Muylaert et al. 2016; Shafiq et al. 2017; Grafahrend et al. 2011).

2 Biomolecules to Enhance the Endothelialization of Vascular Grafts

Native arteries consist of the intima, media, and adventitia. The intima is composed of a monolayer of endothelial cells (ECs) and ECM, which forms the internal elastic layer (IEL), the media is composed of smooth muscle cells (SMCs) and associated ECM components, and the adventitia consists of fibroblasts and associated ECM components. Native endothelium acts as a barrier between the peripheral circulation and the blood vessel wall, but also protects blood vessels from thromboembolic complications. To date, various types of strategies have been put forward to establish a luminal layer, which has the capacity to withstand shear pressure and can also provide protection against thrombosis. Accordingly, the luminal side of vascular

grafts have been seeded with various types of adult and progenitor cells, including ECs and endothelial progenitor cells (EPCs), prior to evaluation under both static and dynamic conditions (Kong et al. 2004a; Zhang et al. 2006). While this may help to improve the patency rate of artificial vascular grafts, the scarcity of appropriate number of cell types, as well as their extensive in vitro manipulation, hinders this approach. On the other hand, blood contains endothelial progenitor cells (EPCs), which may be directed to home toward the denuded lumen and promote re-endothelialization (Kong et al. 2004b). Accordingly, different types of biomolecules, antibodies, and aptamers have been shown to selectively enrich EPCs and promote endothelialization of vascular grafts (Li et al. 2013).

The authors' group prepared heparin-releasing polycaprolactone(PCL)/chitosan vascular grafts and assessed their performance in rat abdominal aorta (Yao et al. 2014). Results demonstrated that sustained release of heparin enhanced the growth of ECs, promoted endothelialization, and inhibited the overgrowth of SMCs. This group has also developed VEGF-hydrophobin (HGFI) complexes for surface modification of PCL grafts (Fig. 1). VEGF-HGFI modified grafts showed superior endothelialization, compared to PCL scaffold grafts, as discerned using scanning electron microscopy and anti-CD31 immunofluorescent staining (Wang et al. 2017b). Similarly, VEGF-HGFI functionalized grafts resulted in enhanced vascular SMC (VSMC) regeneration, with the majority exhibiting a contractile phenotype. The enhanced endothelialization and vascular regeneration was ascribed to the incorporation of VEGF, an extensively documented promoter of EPC capture and subsequent differentiation into ECs. This HGFI fusion protein-based system was also used to incorporate EPC-specific peptide, TPS (TPSLEQRTVYAK) (TPS-HGFI).

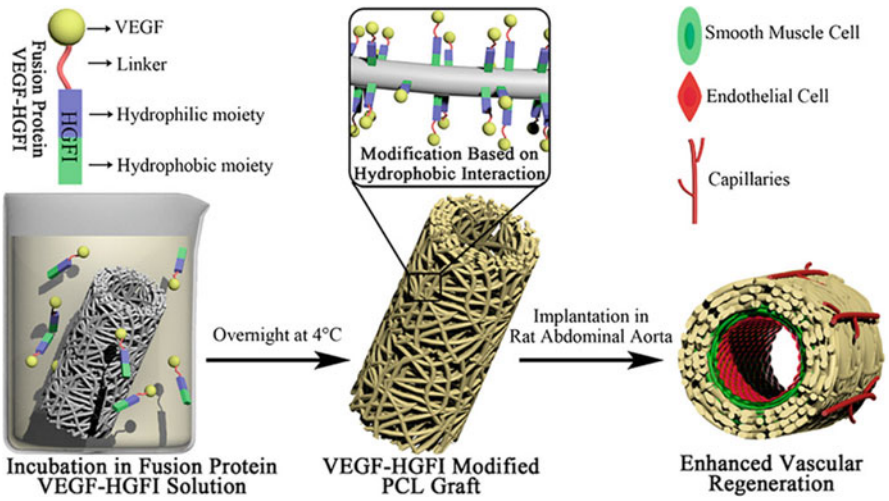

Fig. 1 Graphical representation of the synthesis of VEGF-HGFI complexes and the functionalization of PCL SDVGs with these complexes. Modified vascular grafts were evaluated in vitro and in a rat abdominal aorta implantation model. (Reprinted with permission from Wang et al. (2017b). Copyright (2017) American Chemical Society)

TPS-HGFI was assembled on PCL grafts, thus offering EPC-specific cell engraftment and retention (Ji et al. 2013; Niu et al. 2012).

Tripeptide "*arginine-glycine-aspartic acid*" (RGD) has gathered significant attention from the research community due to its potential to bind various types of cells through interaction with cell surface integrin receptors. Indeed, RGD has been shown to augment the binding of ECs and EPCs in various types of scaffold materials (Kim et al. 2009). The authors' group developed a bioactive coating, *NAP-FFGRGD*, which could be introduced onto PCL SDVGs with ease (Zheng et al. 2012). In vivo implantation of grafts, as carotid artery replacement models in rabbits for up to 2 weeks and 4 weeks, showed an increased degree of endothelialization, cellular infiltration, and SMC regeneration than that of unmodified PCL grafts. This was accompanied by less platelet adhesion and higher overall patency. Mahara and coworkers have been succeeded in developing endothelium-inducing long-bypass grafts (Mahara et al. 2015). Ostrich carotid arteries (ID, 2–4 mm, length, 90 cm) were decellularized by ultra-high hydrostatic pressure (UHP) method and coated with an α4β1 ligand *REDV* as well as a collagen binding peptide. Grafts remained patent when implanted as femoral-femoral by-pass grafts (length, 20–30 cm) in minipigs. Indeed, the luminal surfaces of vascular grafts coated with collagen binding peptide sequences and EC-binding peptide sequences were shown to be confluent with vWF-positive cells. Conversely, all uncoated grafts were occluded and showed thrombosis 3 weeks after implantation.

In another approach to promote endothelialization in synthetic SDVGs, the authors' group has introduced nitric oxide (NO)-generating and cell-adhesive grafts (Wang et al. 2015b, c; Chen et al. 2014). In these studies, selenocystamine was used as a catalyst and *S*-nitrosoglutathione was used as an NO-donor molecule. The in vivo implantation experiments delineated significantly higher endothelialization and luminal coverage with cobblestone-like cells in NO-producing grafts, compared with the control grafts. The improved endothelialization was ascribed to the cooperative effect of cell-adhesive peptide and in situ catalytic NO generation, and may have broader implications for the development of functional vascular grafts with homeostasis-regulating and SMC proliferation-modulating capabilities. Given the important role of endothelium-generated NO in the vascular homeostasis and patency regulation, the authors' group has also introduced enzyme prodrug technique (EPT), wherein an enzyme *galactosidase* was immobilized on PCL grafts, which catalyzed NO generation in the presence of injected NO-producing prodrug (Wang et al. 2015c). In vivo implantation of NO-releasing grafts suppressed platelet adhesion and facilitated vascular regeneration (Fig. 2), suggesting extension of NO-generating scaffolds to other TE applications.

Limited endothelialization and unregulated overgrowth of SMCs is a growing concern in artificial graft generation. The outcome of which not only results in IH, but may also result in in-stent restenosis. To circumvent this issue, the authors' group pioneered novel stents co-eluting endothelialization-promoting VEGF-encoding plasmids and SMC proliferation-inhibiting paclitaxel. Poly(L-lactide-co-glycolide) (PLGA) nanoparticles containing paclitaxel and adorned with VEGF-encoding plasmids on their outer surface (VEGF/PTX NPs) were coated onto stents (Yang et al. 2013).

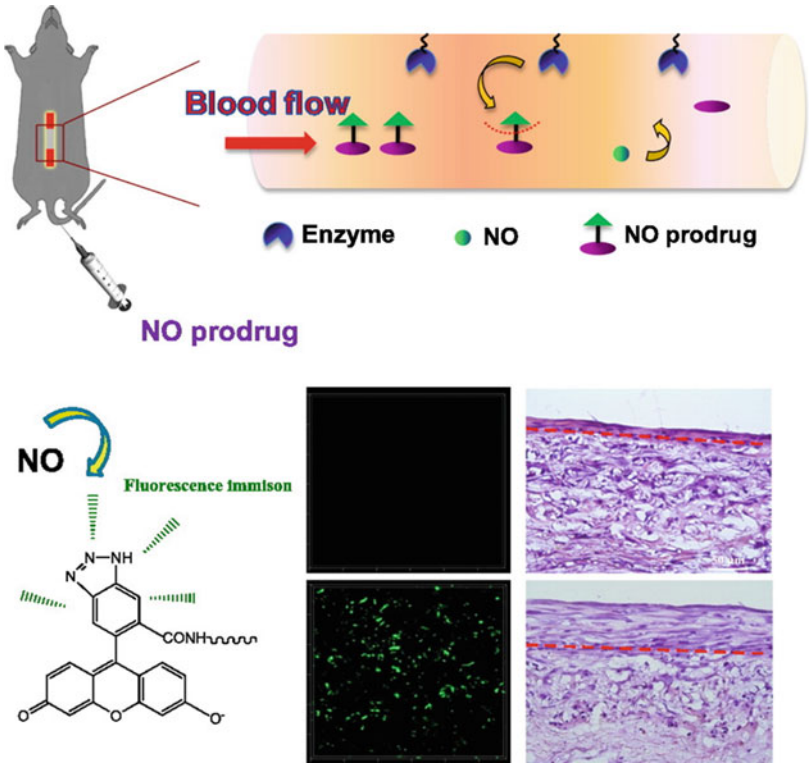

Fig. 2 Graphical abstract: A new concept has been tested in rat abdominal aorta implantation, in which enzyme-immobilized vascular grafts could catalyze exogenously supplied prodrug to release NO locally, which resulted in pro-regenerative physiological functions including the elevated expression of M2 macrophages, enhanced endothelialization, and smooth muscle cell layer regeneration. (Reprinted with permission from Wang et al. (2015c). Copyright © 2015 Elsevier B.V.)

As expected, an earlier expression of VEGF promoted endothelialization, whereas a latent and slow release of paclitaxel inhibited in-stent restenosis. Though we have not yet assessed the potential of this platform in vascular grafts, our current data suggest this approach warrants further investigation and may be helpful for enhancing patency and overcoming thrombosis risk in artificial blood vessels (Fig. 3).

3 Biomolecules to Reduce Intimal Hyperplasia

Thrombosis and restenosis are limiting factors for the success of vascular grafts and stents. Therefore, efforts are being made to incorporate different biomolecules into vascular grafts and stents, which can overcome these problems. As described in the previous section, NO has been shown to enhance endothelialization, inhibit platelet adhesion, and reduce the proliferation of SMCs (Wang et al. 2015). Kushwaha and

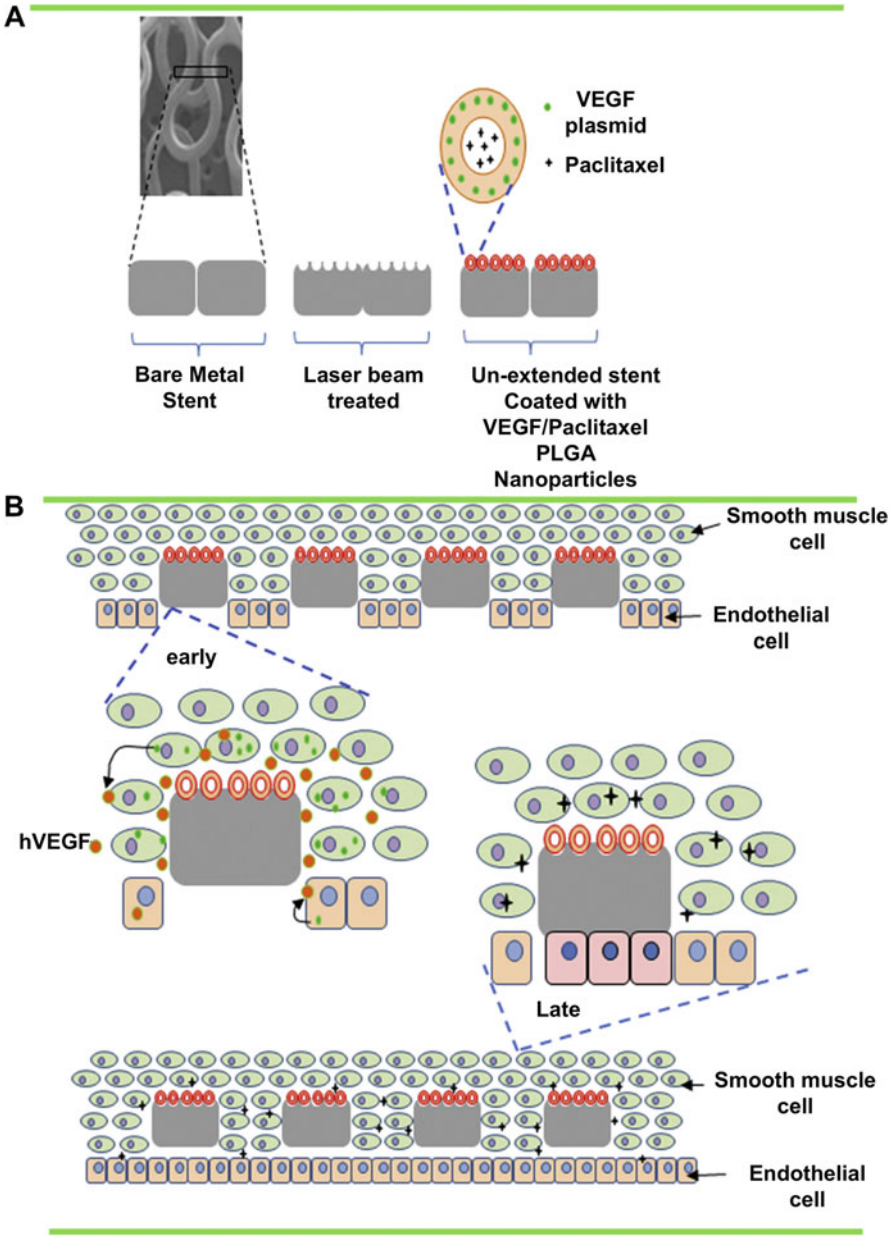

Fig. 3 Schematic diagrams of material design and functional hypothesis. (**a**) The structure of bilayered VEGF/PTX NPs and procedures of stent coating. The bare metal stents underwent nano-poration laser pretreatment, which generates nano-pores to increase the coatable surface area and the overall stability of the VEGF/PTX NP coating. (**b**) Assumed sequential release of VEGF/PTX and the mechanism of their function. The sequential releasing pattern allows rapid re-endothelialization at earlier time points and inhibition of smooth muscle cell proliferation at later time points. (Reprinted with permission from Yang et al. (2013). Copyright © 2012 Elsevier Ltd)

co-workers proposed self-assembling peptide amphiphiles (PAs) containing cell-adhesive and NO-releasing functional moieties (Kushwaha et al. 2010). These PAs were shown to provide burst and sustained release of NO for up to 48 h and 30 days, respectively. The burst release of NO inhibited neointimal formation, whereas the slower and sustained release of NO reduced the proliferation of SMCs, thus circumventing the limitations of current prosthetic materials.

The authors' group developed NO-releasing vascular grafts, which showed potential to enhance endothelialization and patency (Wang et al. 2015b). In other pioneering research work, Acevedo et al. (2004) identified a new role of Nogo-B in vascular homeostasis and identified that this isoform of the Nogo family of proteins was highly expressed in vascular tissues. Genetic loss of this isoform markedly enhanced neointimal formation in injured vessels, whereas adenoviral delivery of the Nogo-B rescued the effect and reduced neointima formation. Furthermore, Nogo-B enhanced the adhesion of vascular cells and blunted the migration of vascular SMCs. Keeping in view these observations, there is importance in investigating the role of Nogo-B in vascular remodeling.

Endothelial-to-mesenchymal transition (EndoMT) has been reported to be the major culprit of stenosis and is widely thought to be promoted by transforming growth factor β1 (TGF-β1) (Kitao et al. 2009). While SDVGs have been shown to heal in clinical investigations, stenosis risk hampers their clinical utilization. Breuer and co-workers addressed this issue by using TGF-β1 receptor (TGFβ1R) inhibitor-eluting vascular grafts (Lee et al. 2016). In vivo evaluation showed the promise of this approach and abolished stenosis resulting from EndoMT, which may have long-term implications for the success of SDVGs in clinical applications. Moreover, SDVGs eluting heparin or heparin along with sirolimus were implanted into the abdominal aortas of rabbits and were reported to decrease IH, compared to drug-free or ePTFE grafts (Ishii et al. 2008). Heat shock protein 27 (HSP27; MAPKAP kinase II) was also shown to be a regulator of intimal thickening. Lopes and co-workers identified the role of a cell-permeant peptide inhibitor (MK2i) of the kinase, in down-regulating the phosphorylation of HSP27 (Lopes et al. 2010). Treatment of saphenous vein grafts with MK2i reduced intimal thickening and connective tissue growth factor expression. IH primarily results from the phenotypic modulation of SMCs from quiescent "contractile" to proliferative "synthetic" phenotypes, and infiltration of SMCs from medial to the luminal side. Thus, inhibition of SMCs synthetic phenotypes holds great promise in the inhibition of IH. These studies also demonstrated decreased accumulation of ECM components, including collagen and fibronectin, which are often found in the intimal hyperplastic lesions.

The ECM protein, elastin, plays a key role in the maintenance of vascular structure and control of SMCs phenotypic modulation. Recently, Shinoka's group reported significant contribution of tropoelastin in halting IH (Sugiura et al. 2017). A dip-coating of tropoelastin on the luminal side of grafts not only lowered the numbers of α-SMA^{+} cells, but also lowered the thickness of the neointimal layer. Since the recruitment and proliferation of SMCs leads to IH and subsequent stenosis and graft failure, inhibiting SMC recruitment may a viable solution. Williams et al. (2016) reported that Wnt2-enhanced SMC migration, which was due to increases in

the Wnt1-inducible signaling pathway protein-1 (WISP-1). Abolishing Wnt2 or WISP-1 reduced the numbers of α-SMA^{+} cells and the thickness of the neointimal layer. It was shown that resolvin D1 attenuated neointimal formation in vivo, which was due to its role in resolving inflammation. Wu et al. (2017) also documented significant decreases in neointimal hyperplasia, as mediated by resolvin D1 either loaded into poly(L-lactide-co-glycolide) wraps or incorporated into Pluronic hydrogels, in wire-induced carotid injury models.

4 Biomolecules for SMCs Regeneration and Phenotypic Modulation

The universal and common pathological process of cardiovascular disease is IH, characterized by the augmented proliferation and migration of VSMCs and increased synthesis of ECM, which co-committedly and progressively obliterates the vessel lumen. These cellular events are the consequence of dedifferentiation processes of VSMCs, which can lose their contractile function and regaining undifferentiated synthetic genotype and phenotype. VSMCs plasticity is incurred by growth factors, cytokines, cell-cell contacts, and ECM components. Furthermore, VSMCs exhibit hyperpolarized mitochondria and an imbalance replication/apoptosis ratio, which render them highly proliferative. Prevention of this VSMC phenotype has the potential for alleviating IH and subsequent obliterative vascular diseases, such as thrombosis and in-stent restenosis.

MicroRNAs (miRNAs) are small, endogenous antisense RNAs of approximately 22 nucleotides in length that regulate gene expression typically via mRNA degradation or translation repression. Previous studies have suggested that miRNAs are critically involved in the progression of IH. Modulation of various miRNAs could inhibit IH, suggesting a therapeutic approach for vascular disease (Hibino et al. 2016). In this regard, miR-145 has been reported to promote the contractile phenotype of VSMCs, thus lowering the IH risk in native arteries (Hutcheson et al. 2013).

5 Biomolecules for Enhancing Cell Infiltration and Endogenous Cell Recruitment into Vascular Grafts

Besides developing functional grafts with endothelialization and vascular remodeling abilities, different solutions have been put forward to promote vascularization and/or cellular infiltration into vascular grafts. Heparin has affinity for a myriad of cytokines and chemokines and therefore may be used as a platform to develop novel scaffold materials. We and others have developed heparin-blended scaffold materials and vascular grafts, which were later adorned with bioactive moieties including VEGF and monocyte chemoattractant protein 1 (MCP-1) (Zhang et al. 2014). In vivo evaluation of these scaffolds facilitated tissue repair by polarizing macrophages toward an alternatively-activated phenotype and by directly influencing the angiogenesis process. Similarly, our group has achieved

sustained release of VEGF through its interaction with heparin-grafted PCL grafts (Wang et al. 2012). Heparin-grafted PCL grafts provided sustained release of VEGF, which improved the anticoagulant properties of vascular grafts as assessed using in vitro hemocompatibility assays. Such methodology can be extended to vascular grafts to speed up the regenerative process. While several bioactive molecules including G-CSF, VEGF, MCP-1, and SDF-1 have been used for enhancing cellularization in vascular grafts, the high molecular weight of these biomolecules and their processing difficulty hinders the full therapeutic utilization of these biomolecules. Consequently, several research groups are pursuing phage display-derived peptides and short bioactive motives to facilitate cellularization for enhancing remodeling in vascular grafts. In this regard, our group and Soo Hyun Kim's group have used neuropeptide substance P (SP), which not only facilitated endogenous stem/progenitor cell recruitment but also affected inflammatory responses and induced macrophages polarization. Vascular grafts containing SP showed improved neovascularization, SMCs regeneration, and cellularization (Shafiq et al. 2016a). Similarly, Muylaert et al. (2016) and our group (Shafiq et al. 2018) have developed vascular grafts modified with SDF-1α peptide, which showed enhanced cellularization than that of the control grafts, which were devoid of SDF-1α peptide. Given that these short bioactive molecules can be easily synthesized and incorporated into artificial vascular grafts, they may afford the fabrication of off-the-shelf available vascular grafts. Moreover, polymeric materials containing these peptides can be synthesized and used for the fabrication of vascular grafts, which could promote remodeling and healing in vivo.

6 Biomolecules for Induction of Inflammatory Responses to Polarize Macrophages and Enhance Vascularization

It is well known that inflammatory responses play key roles in the remodeling of artificial vascular grafts into functional neo-arteries. Macrophages exhibit different phenotypic polarization, among them are the proatherogenic M1 macrophages and anti-inflammatory M2 macrophages. It has been found that alternatively activated M2 macrophages promoted blood vessel formation by affecting the local inflammatory microenvironment (Hibino et al. 2011; Roh et al. 2010). Moreover, macrophage-derived tumor necrosis factor-α (TNF-α) has been reported to promote endothelial differentiation of vascular stem/progenitor cells and inhibit SMC differentiation (Wong et al. 2014). Accordingly, several strategies utilizing physicochemical cues and bioactive molecules capable of affecting inflammatory responses have been implemented to promote macrophage polarization and neo-vessel formation. Our group and others have reported the effect of physical cues on macrophage polarization, and we observed that electrospun fibers with thick fibers and large pores facilitated macrophage polarization toward the M2 phenotype, which subsequently assisted tissue repair (Fig. 4) (Garg et al. 2013; Wang et al. 2014; Sun 2017).

Similarly, our group has developed vascular grafts containing organoselenium-modified polyethyleneimine (sePEI), which catalyzed the regeneration of NO

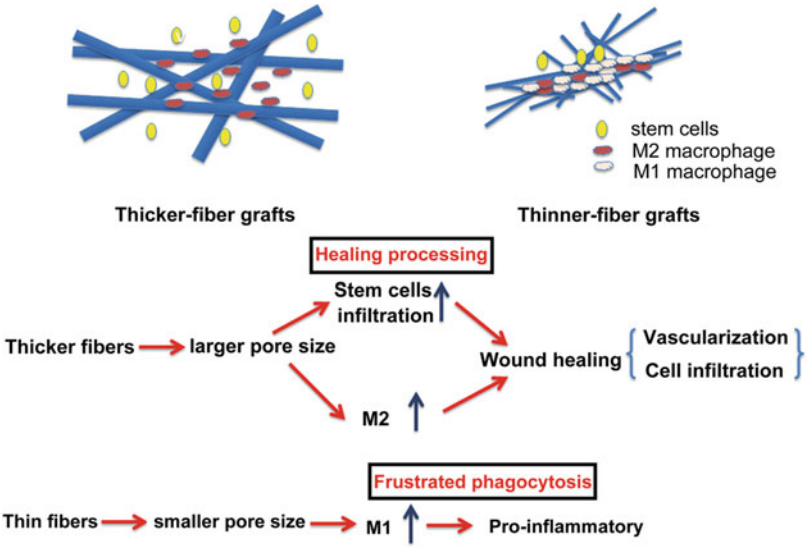

Fig. 4 This schematic illustrates the hypothesis of the reported study on the influence of fiber diameter over macrophage polarization. Thicker-fiber grafts with larger pores may modulate the polarization of macrophages into M2 phenotype, which secrete wound-healing cytokines and enhance cell infiltration, vascularization, and tissue remodeling. However, the thinner-fiber grafts with smaller pores maintain the macrophages in M1 phenotype, which secrete proinflammatory factors and play a negative role in tissue regeneration. (Reprinted with permission from Wang et al. (2014). Copyright © 2014 Elsevier Ltd)

production in vivo, modulating macrophage polarization (Tang et al. 2018). In addition, SP has been reported to induce macrophage polarization toward the M2 phenotype and thus promoted wound healing and neo-tissue formation (Leal et al. 2015). Our group has designed star-shaped poly(L-lactide-co-ε-caprolactone) (PLCL) co-polymers, which were further conjugated with SP and fabricated into SDVGs. SP-containing grafts showed macrophage M2 polarization and enhanced vascular regeneration, when compared to the control grafts (Shafiq et al. 2015a, b, 2016a). Likewise, interleukin 4 (IL-4) effectively polarized macrophages toward an anti-inflammatory phenotype. Qian et al. (2018) introduced IL-4 onto silk fibroin using heparin disaccharides, which facilitated macrophage polarization in vitro and in vivo, as well as reducing the foreign body reaction to scaffolds in vivo.

Moreover, certain lipid mediators such as Resolvin D1 have shown great potential for resolving inflammatory response and promoting anti-inflammatory macrophages, suggesting their potential benefits in lowering IH incidence and promoting blood vessel regeneration (Sok et al. 2017; Wu et al. 2017; Shi et al. 2019). Indeed, our group showed that modulation of ECs and macrophages by the sustained release of resveratrol from PCL-based SDVGs improved the macrophage polarization to M2 phenotype in vivo, and facilitated endothelialization and vascularization in vivo (Fig. 5) (Wang et al. 2017a). In addition, other modulators for promoting

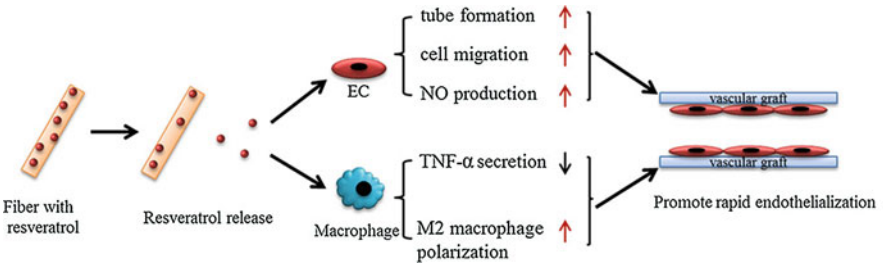

Fig. 5 Schematic diagrams of material design and functional hypothesis. Resveratrol was incorporated into PCL graft during electrospinning. Resveratrol could be released from the scaffolds in a sustained and controlled manner. Cell culture results indicated that the migration of ECs, NO production, and the ability of tube formation increased in the resveratrol-containing PCL. Meanwhile, culture of macrophages on resveratol-containing PCL demonstrated reduced secretion of TNF-α, a major proinflammatory factor, and increased mRNA expression of M2 macrophage-related genes. In vivo implantation in rat abdominal aorta demonstrated fast endothelialization and enhanced vascular regeneration. (Reprinted with permission from Wang et al. (2017a). Copyright © 2017, American Chemical Society)

pro-regenerative macrophages have been pursued, which could be positively evaluated for their potential for the regeneration of artificial blood vessels. Such modulators include: galectin 1, interleukin 10 (IL-10), dexamethsone, and the secreted ectodomain of sialic acid binding Ig-like lectin-9 (Abebayehu et al. 2017; Potas et al. 2015; Jiang et al. 2017; Kano et al. 2017; Shafiq et al. 2016b).

7 Conclusion

Vascular tissue regeneration has great potential for vascular reconstruction. A considerable amount of work has been carried out on the design of suitable scaffold materials for the design of SDVGs. However, associated risks including thromboembolic complications, intimal hyperplasia, and infection ultimately impede the full clinical implementation of SDVGs. Incorporation of biomolecules aimed at overcoming such limitations and favoring graft remodeling through endogenous stem/progenitor cell recruitment are viable solutions and may afford the design and fabrication of off-the-shelf available vascular grafts for vascular reconstruction. Bioactive molecules may also be incorporated into vascular grafts to facilitate endothelialization through driving the recruited progenitor cell differentiation into desired lineages, to favor remodeling, and to inhibit IH. Likewise, cell-free grafts capable of delivering biomolecules in a sustained and controlled fashion may further assist the remodeling process of vascular grafts for neoartery regeneration by enhancing endogenous cell recruitment and/or their differentiation as well as the deposition of the ECM components necessary for vascular graft functionalizing. Various methodologies have been developed for the incorporation of bioactive molecules into vascular grafts, including the direct encapsulation into vascular grafts, decoration on the luminal side, or conjugation by using physico-chemical

methods. However, care must be taken such that the bioactivity of the incorporated biomolecules is preserved. Keeping in view the simultaneous role of endothelialization, SMC regeneration, and ECM deposition, simultaneous use of multiple growth factors or biomolecules may be helpful for vascular regeneration. Taken together, judicious selection of biomolecule as well their incorporation method may lead to the design of off-the-shelf available vascular grafts for vascular tissue reconstruction.

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