Geometric anisotropy on biomaterials surface for vascular scaffold design: engineering and biological advances

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Abstract
Scaffold designs in combination with drug, growth factor and other bioactive chemicals account for lasting progress of vascular tissue engineering in the past decades. It is a great achievement to adjust tissue matrix composition and cell behaviour effectively. However, regenerating the innate physiologies of a blood vessel still needs its precise architecture to supply the vessel with structural basis for vascular functionality. Recent developments in biomaterial engineering have been explored in designing anisotropic surface geometries, and in turn to direct biological effects for recapitulating vascular tissue architecture. Here, we present current efforts, and propose future perspectives for the guidance on the architectural reconstruction and scaffold design of blood vessel.

1. Introduction
Cardiovascular diseases are the globally leading cause of deaths, which accounted for ∼17.6 million deaths in 2016 (In the United States alone 2 744 248 resident deaths were registered) [1]. In low and middle-income countries, these populations are younger than in the higher income countries [2]. For example, China had the highest rates of diet-related cardiovascular disease deaths (275–324 deaths per 100,000 population) in 2017 [3], and it is estimated that in China by 2030 the deaths related to cardiovascular diseases will increase by 50% [4]. On the other hand, the cardiovascular care cost represents the world’s major disease treatment burden. This is estimated to triple from ∼$272.5 billion in 2010 to ∼$818.1 billion in 2030 based on the projections by the American Heart Association [5]. Current approaches for treating cardiovascular diseases are the best medical therapy, endovascular treatment, and open surgery, of which surgical bypass as the procedure of choice in a number of patients is cost-effective and has recoverable life quality for the patients [6]. However, there are numerous cardiovascular patients worldwide who require surgical bypass procedures who do not have suitable vessels for grafting.

Histologically, vascular substitutes including autograft, allograft, xenograft, synthetic graft and tissue-engineered graft find a wide range of applicability (figure 1) [7]. These substitutes meet at least partial of the characteristics for an ideal vascular graft (safety, function, operation and availability). As the gold standard of vascular substitutes, autograft possesses the most of physiological properties and is biocompatible without either toxicity or immune-rejection. Long-term performance of this graft demonstrates good compliance, with the highest percentage of 5 year patency among existing grafts (e.g. 74% for above-the-knee femoropopliteal arteries), although the patients often suffer from invasive harvesting and limited donor sites to offer healthy vessels [6]. Comparatively, allograft, xenograft and synthetic graft have better availability; however, these grafts, for example the allograft and xenograft have high possibilities of intimal hyperplasia at distal anastomosis, thrombogenicity and immune-rejection [8], and the synthetic graft based on non-biodegradable polymers...
prove to be susceptible to infection [9] and less effective for small-diameter vascular graft due to the occurrence of thrombotic occlusions [7].

Tissue engineering applying biological and engineering principles offers a solution for the patients to have engineered vascular substitutes of ideal properties [10]. This tissue engineered vascular graft not only is living as autologous vessel, immune compatible, and capable of physiological response, but also approaches to an off-the-shelf idea to offer patient-specific design and sufficient availability minimising the waiting time greatly before implantation. In this paradigm, great efforts are underway to resemble the native microenvironment of blood vessel, and incorporate key regulation signals for biomaterials design and engineering. This review will describe recent developments on the physical strategies for recapitulating vascular anisotropy. We shall also discuss the biological relevance of this anisotropy and propose future perspectives. This work has insight of designing vascular scaffold into biomimetic anisotropy, which will advance regenerative therapy for the cardiovascular patients.

2. Physical anisotropy and functionality of blood vessel

2.1. Physiological integration of vascular structure and functions

The circulation system of human body is composed of blood vessels including the arteries, veins and capillaries. Both arteries and veins in large, middle and small diameters possess three distinct layers of tunica intima, media and adventitia from the luminal side outward (figure 2) [11]. Tunica intima is a tight monolayer consisting of endothelial cells (ECs) that organise along blood flow and are able to sense physicochemical and biological changes in the blood, in order to function for antithrombosis, anti-infection/anti-inflammation and delivering signals to regulate cells in other layers [12]. Tunica media has multi-layers of smooth muscle cells (SMCs) embedded in a 3D excellular matrix (ECM) containing abundant collagen type-I/III, elastin and proteoglycan.
This tunica media shows aligned circumference with alternating directions for different layers, to contribute majorly to the integrity and mechanical compliance of blood vessel (e.g. robust strength and vasoactive response) [13]. Tunica adventitia has fibroblasts to deposit loose collagen-enriched ECM, which mainly anchors vessel to the surrounding tissue and ensures vascular integrity.

Basement membrane is a sheet-like meshwork localised between tunica intima and media. It contains a large amount of insoluble molecules (e.g. heparin-sulfate proteoglycans and collagen type IV) and other minimal components (e.g. collagen type XV and XVIII). Through a process of self-assembly, these constitutes functionalised architecture of orientated fibres and sub-micro/nano scale pores; the large vessels tend to have bigger pores and thicker fibres than those of the small vessels [14]. Basement membrane acts a pivotal role for vascular development and homeostasis, serving as not only a basolateral for ECs and SMCs but also a barrier to separate them from each other [15]. The meshwork of basement membrane also accumulates growth factors for vascular regulation (e.g. von Willebrand factor (VWF) for SMCs recruitment during vascular modeling) [16]. Table 1 summaries the functions of basement membrane in vascular development, structure support and physiological regulation. However, in a diseased vessel migration of SMCs across basement membrane approaches tunica intima to form thrombosis.

### Table 1. Functions of vascular basement membrane.

| Vascular basement membrane | Performance | References |
|----------------------------|-------------|------------|
| Structural support | Compartments to separate vascular endothelium from stroma; Basolateral for ECs and SMCs; Vascular shape maintenance; Mechanical responses to the pulsatile blood flow and vasoconstriction | [15, 17] |
| Vascular development | Direct cell–cell interaction between ECs and stromal cells (e.g. pericytes, SMCs and mesenchymal stem cells); Component and structural alterations; Vascular maturation: SMCs recruitment | [18] |
| Biochemical regulation | Vascular hemostasis; Support for ECs with retained shape, migration, proliferation and differentiation; Support for SMCs with orientated organisation, proliferation, and contractile function; Indirect cell–cell interaction: selective permeability for nutrition and growth factors (e.g. VWF) produced from ECs, SMCs and others | [15, 16, 19] |

2.2. Neovessel formation in developmental and healing processes

2.2.1. Vasculogenesis and angiogenesis

There are two processes for neovessel formation: vasculogenesis (neovascularization in the absence of preexisting blood vessel; figure 3(a)) and angiogenesis (neovascularization from an existing vascular network; figure 3(b)) [10], during which endothelial and perivascular stromal cells make important actions for the neovessel formation. ECs account for the matrix synthesis of endothelial basement membrane, which in the nascent stage is not continuous entirely and exhibits gaps for stromal cell recruitment from the circulation such as SMCs, pericytes and mesenchymal stem cells (MSCs) [18]. This makes transmural contact between myoendothelial components importantly for stabilising neovessels against regression [16], the formation of normal vascular structure (stromal and endothelial cells localised on media and intima, respectively) [20], and signaling pathways related to NO production [21], ECs proliferation [22], and SMCs [23] and MSCs [24] differentiation. Once the nascent endothelium formed, SMCs start to produce an ordered organisation of ECM in tunica media [11] and embed in the ECM adjusting themselves into an unique circumferential alignment at
herringbone helical arrangement [25]. The elastin and collagen of ECM in tunica media account for nearly 50% of the dry weight of a vessel [26]; and elimination of SMCs alters less to static mechanical properties of the vessel [27].

2.2.2. Healing of vascular damage
The repair of damaged vessel is predicated via a direct contribution of seeded cells when scaffold degrades. However, significant cell loss (up to 95%) after the first 24 h exposure to pulsatile flow suggests a less contribution of artificially introduced cells as the direct cell component of neovessel [28]. A further study uncovers the possible mechanism underlying scaffold-assisted vascular healing, through inflammation-mediated tissue remodeling (figures 4(a)–(c)) [29]. This contains several processes in sequence: protein absorption (e.g. inflammatory integrin recognizing and adsorbing to biomaterial surface), complemental activation to mediate inflammation, chemkines to mediate macrophage adhesion towards biomaterials, giant cell aggregation to degrade biomaterial fragments, and finally ECM remodeling into mature vessel (e.g. ECM secretion and degradation by M2 macrophage). Stem cells from seeding contribute mostly to macrophage recruitment to implanted vascular graft, although they may be retained through differentiation into SMCs.

2.3. Tissue engineering strategies for vascular anisotropic reconstruction
Ideally, vascular substitute is an engineered ‘autologous’ and ‘off-the-shelf’ graft through integrating components of cells, signals and biomaterials. Physical anisotropy as a key structural characteristic of mature vessel, is mechanically important for blood supply and influences cells in tunica media and intima with functional morphology. In vitro, when cultured without this regulation the cells will fail to induce organised alignment of cell shape or actin filament, associated with vascular incompliance as observed clinically [30]. In the tissue-engineering field, efforts are directed to mimic vascular microenvironment at different anatomic layers, in order to reconstruct the native-like architecture of vascular anisotropy.

In tunica intima, endothelium is subjected to a continuous fluid shear stress of 10–20 dynes cm$^{-2}$ [31]. In vitro, subjected to this mechanical stimulus ECs retrieve cobblestone-like morphology and aligned organisation.
similar to what occurs under blood flow. In another case, when exposure to shear flow fibroblast-like stromal cells express enhanced SMCs-specific markers [32], while embryonic MSCs differentiate towards ECs-like cells [33]. The cells exhibit aligned organisation parallel to the flow direction regardless differences at cell origination. On the other hand, in the native vessel ECs and SMCs both experience a cyclic strain from pulsatile stress (5%–30% strain and 30–90 cycles min⁻¹ [34]). Stem cells tend to express elevated SMCs markers when a strain is applied parallel to micropatterns; but the cells decline SMCs marker expression when the applied strain direction is perpendicular [35]. Unfortunately, modeling of a cyclic strain induces cellular alignment perpendicular to the strain axis [36], which makes it inappropriate for triggering the aligned circumference of SMCs as in the native vessels. Other strategies such as biochemical and electrical cues are also developed for engineering anisotropic cell organisation. For example, the aligned organisation of SMCs occurs with stimuli of glial cell line-derived neurotrophic factor and fibronectin. However, these strategies are reported less for tissue-engineering reconstruction of alignment, probably due to their limited effectiveness and controllability.

Geometric structure on biomaterials that mimics the architectural anisotropy of blood vessel (e.g. endothelial basement membrane) obtains extensive interests. In blood vessel, basement membrane constitutes more complexity to vascular microenvironment, which interacts directly with SMCs and ECs, and mediates both direct and indirect myoendothelial communications. On tunica media side, the multi-layers of SMCs organise into alignment towards the fibre direction of basement membrane [19]. This organised alignment and accordingly the morphology of SMCs are crucial for maintaining cellular contractile phenotype [37]; and due to lack of organised contractile apparatus, SMCs culturing in vitro tend to lose contractile phenotype and shift into a synthetic status with ECM over-secretion [38]. On tunica media side, the topography of basement membrane can influence numerous behaviours of ECs such as the cell morphology, migration, proliferation and differentiation [14]. In comparisons to the other tissue-engineering strategies using mechanical, electrical and biochemical stimuli, geometric structure as the bioactive cues require no external stimuli, and are effective and more controllable in reconstructing vascular anisotropy. With the biomimetic structure of anisotropy, vascular scaffolds can retrieve the physiological morphology and function of stromal and endothelial cells, to build the engineered, idea vascular substitutes.

3. Engineering biomaterials with anisotropic geometry

The geometries of biomaterials for tissue anisotropy reconstruction can be formed as orientated fibre and ridge/groove array. The fibres can be highly porous and routinely produced from electrospinning and flow shear, while the ridge/groove arrays are often fabricated on substrate biomaterials, through methods such as lithography, soft lithography, direct laser writing and abrasion wear. Table 2 summarises typical engineering strategies for biomaterials with anisotropic geometry.

3.1. Electrohydrodynamic fabrication

Based on the electrohydrodynamic principle, electrospinning applying traditional flat collector often deposits randomly orientated and nonwoven fibre mats, due to bending instability of the jet during spinning process [39]. To fabricate orientated fibres, electrospinning can use the rotating drum and disk collectors (figures 5(a) and (b))

Figure 4. Neovessel formation mediated by cells and biomaterials. (a) Recruitment of monocytes and macrophages, and MCP-1 release by BMCs after graft implantation. (b) Vascular graft degradation to induce infiltration of monocytes and macrophages which release multiple angiogenic cytokines and growth factors (e.g. VEGF), thereby recruiting SMCs and ECs onto the graft. (c) Migration of monocytes and macrophages away from the implant position after complete graft degradation and neovessel formation. (BMC: bone marrow stem cells; MCP-1: monocyte chemoattractant protein-1; VEGF: vascular endothelial growth factor). Adapted with permission from Roh et al 2010 [29].
Table 2. Fabrication methods for engineering geometric anisotropy on biomaterials.

| Method          | Example          | Advantage                                      | Limitation                                      | References |
|-----------------|------------------|-----------------------------------------------|-------------------------------------------------|------------|
| EHD fabrication | I. ES with disk rotating | P(LLA-CL) fibre  | • Highly orientated fibre;                      | Potential toxic residue due to solvent usage; | [62]       |
|                 | II. ES with mandrel rotating | PCL fibre          | • Scalable and reproducible fabrication;        | Poor mechanical properties;                  | [63]       |
|                 | III. ES with two frames | Carbon fibre      | • Applicable for various polymers.             | Safety concern due to the high voltage and rotating speed; | [39]       |
|                 | IV. EHD writing   | PCL fibre          |                                                 | Low efficiency of fibre fabrication (EHD writing). | [55]       |
| Lithography     | I. Direct lithography | PUA array         | • Precise parameter control;                   | Complex set of fabrication tools and procedures; | [64]       |
|                 | II. Soft lithography | DMA array         | • Capable for complex geometries;              | Usage of toxic solvent;                      | [65]       |
|                 |                  |                  | • Improved applicability for materials (Soft lithography). | Limited applicable materials (Direct lithography). |           |
| Direct laser writing |              | P(LLA-CL) array   | • Precise parameter control;                   | High cost and specific technique require.    | [50]       |
|                 |                  |                  | • Capable for complex geometries.              |                                                 |           |
| Flow shear      |                  | Collagen fibre    | • Easy and safe operation.                     | Low efficiency of fibre alignment;           | [66]       |
|                 |                  |                  |                                                 | Solvent usage and residual;                 |           |
|                 |                  |                  |                                                 | Limited scalable fabrication.               |           |
| Single-axial drawing |              | PLGA fibre        | • Highly orientated wave-like fibre;           | Low scalable fabrication.                    | [53]       |
|                 | I. Drawing of polymeric fibre | PLGA fibre     | • Improved porosity (Cycle drawing);           |                                                 |           |
|                 |                  |                  | • Mechanical enhancement;                      |                                                 |           |
|                 | II. Drawing of polymeric film | PCL film        | • Highly orientated ridge/groove array;        | Dense film bulk.                             | [42]       |
| Method                       | Example            | Advantage                                      | Limitation                      | References |
|------------------------------|--------------------|------------------------------------------------|---------------------------------|------------|
| Abrasive                     | PDMS array         | ■ Great mechanical enhancement;                | ■ Irregular geometries;         | [58]       |
|                              |                    | ■ Applicable for semi-crystalline polymers.    |                                 |            |
|                              |                    | ■ Highly orientated feature.                   | ■ Limited to stiff materials.   |            |
| Top-down freezing procedure  | Collagen wall      | ■ High porosity.                               | ■ Specific technique require.   | [59]       |
|                              |                    | ■ Non-connected and orientated wave.           | ■ Poor cell alignment.          | [61]       |
| Self-forming ion beam        | Silicon wave       | ■ Non-connected and orientated wave.           | ■ Poor cell alignment.          |            |

* a EHD, electrohydrodynamic; ES, electrospinning.  
* b PLA, poly(lactic acid); PCL, poly(ε-caprolactone); P(LLA-CL): PLA and PCL copolymer; PLGA, poly(lactic-co-glycolic acid); PUA, polyurethane acrylate; DMA, dimethacrylate; and PDMS, polydimethylsiloxane.
Electrospinning using a disc can generate highly orientated fibres at a relatively smaller rotating speed, while a mandrel requires generally a rotating speed larger than 1000 rpm. Several mechanisms that contribute to the fibre orientation include the dramatic increase in field strength at disc edge and the rapid-rotating induced uniaxial pulling and air flow. However, due to the large pulling force these two methods are easy to make fibre discontinuous and difficult to peeling off. An alternative method applying two conductive frames (separated by a void gap) as the collector also forms orientated fibres effectively. This method utilises the electrostatic forces that are in opposite directions and stretch the fibres between the two frames to align perpendicular to gap edges. This method facilitates aligned fibre transfer to other substrates, and layer-by-layer stacking of the fibres at different angles in alternative layers. Width of the two-frame gap is up to several centimeters, which hinders a scale fabrication of aligned fibres using this method.

Limitations related to electrospinning to produce highly orientated fibres include: (i) the reduced porosity due to significant fibre packaging in fibrogenesis process, (ii) the chemical solvent usage that gives potential risk of residuals and raises problems with regard to the safety considerations, (iii) the use of a high voltage and complex setup with low efficiencies of aligned fibre yield, and (iv) the poor mechanical properties of electrospun fibres that remain a big concern for applications in mechano-active tissue regeneration (e.g. vascular substitute to bear a robust burst strength).

Different from electrospinning, electrohydrodynamic writing applies a three-dimensional micro/nano moving stage that provides precise control on the spatial deposition of fibres. This method generates single continuous fibre, thereby allowing for printing straight fibre lines. The mechanism underlying this straight and single fibre fabrication is through adjusting tip-to-collector distance into the straight range of a jet flow, in combination with the dragging force from moving stage. As such, this method is able to fabricate highly aligned fibres with pre-determined directions, with better control of fibre parameters than that of electrospinning.

### 3.2. Flow shear

Flow shear produces orientated fibres using a hydrodynamic flow (figures 6(a)–(c)). Lanfer et al. developed this method based on a microfluidic system using collagen solution, to orientate collagen fibres at adjustable densities and morphologies. The mechanism behind this fibre fabrication involves collagen fibrillogenesis under flow shear force. If one end of the fibril attaches to substrate surface, the streaming fluid aligns remaining
fibril part at a position of low hydrodynamic resistance. When compared to electronspinning, this method is easier and safer for operation, with potential for scalable production. However, this method remains limited due to the inhomogenous morphology and low alignment efficiency of induced fibres. In addition, how to peel off the fibres from substrates for either further processing or applications is still awaiting investigation.

3.3. Lithography and soft lithography

Direct lithography includes a wide range of techniques allowing for the fabrication of specific and well-defined structural geometries, to recapture biological patterns of cells, ECM fibres and proteins [48, 49]. Laser, x-ray, electron beam and ionic beam are applicable for lithography, which transfers pattern from a master to substrate biomaterials. Procedures of lithography involve surface coating of master, exposure to the irradiation, development, and washing (figure 7(a)). The applied masters can be negative and positive resists that experience cross-links or deteriorates after irradiation exposure, respectively. This method is capable of fabricating designed geometries, and allows for the precise control of structure parameters (e.g. groove width and depth). However, direct lithography requires a complex set of patterning tools and procedures involving the usage of toxic solvents. Furthermore, direct lithography is compatible with limited materials; and these materials should be photo-resistant, such as silicon and polyurethane acrylate which often have little value for the translational research due to non-biodegradability.

Soft lithography is one of the most common and widely used methods for structural fabrication (from hundreds of nano to micrometers). In the process, an elastomeric stamp of patterned relief structures is applied to replicate the structures via self-assembly and replica modeling (figure 7(b)). Comparatively, soft lithography extends the applicability of direct lithography to more biodegradable materials, such as collagen, poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(methyl methacrylate) (PMMA) and poly(lactic-co-glycolic acid) (PLGA). However, soft lithography needs a pre-fabricated model and an additional procedure of peeling off,
which results in structural defects particularly for a large-scale fabrication. Similar to direct lithography, the structural fabrication using soft lithography relies on solvent usage, and the structures are often in dense.

3.4. Direct laser writing
Direct laser writing offers an excellent strategy for biomaterials to form surface structural geometries at precise parameters (figure 8). This method avoids the usage of toxic solvents, and is able to fabricate structures in a rapid, non-contact and single-step manner when compared to electrospinning and soft lithography [50]. Currently, direct laser writing has been investigated for various biomaterials such as PCL, PLC and poly(D-lacticacid) (PDLA). The lasers can be with long (>10 ns) and short (<10^{-15} s) pulses. CO2 and UV lasers are often the long pulses which breakdown polymers through high-energy photon absorption; and they generate pulses longer than thermal waves to propagate in polymers and lead to thermal-induced material changes [51]. Differently, the ultra-short pulse lasers (e.g. Ti:Sapphire femtosecond laser) breakdown polymers through a multi-photon absorption [51], which is able to remove almost any target materials due to their high peak energy (>1012 w cm^{-2}). The mechanism underlying femtosecond laser ablation are related to several phenomena: (i) ionizing the targeted materials at early phase of the laser pulse time, (ii) absorption of free electrons, (iii) formation of a strong electric field due to the free electrons breaking away from material surface, (iv) ions breaking away from the material bulk if electron energy is larger than binding energy, and (v) materials remove [43].

3.5. Single-axial drawing of polymers
Single-axial drawing of polymers is versatile for the fabrication of orientated fibres and ridges and grooves. This method is reported early for natural tissue ECM (e.g. small intestinal submucosa [32]) and electrospinning fibres (figure 9(a)) (e.g. PLGA fibres [33]). However, single-axial drawing of fibres directly often results in side shrinking effect due to Poisson’s ratio, thereby leading to a significant decline in the porosity of fibrous mats. The difficult for scalable fabrication and handling issues also relate to this method for the orientated fibre fabrication and future tissue-engineering application. A recent development to draw electrospun mats cyclically is reported to introduce orientated wave-like fibres, which show enlarged porosity capable of inducing cell infiltration and tissue ingrowth [54].

Single-axial drawing of polymers obtains further development in fabricating orientated ridges and grooves for biomedical application (figure 9(b)) [42]. Distinct from invariant geometry of the structures from lithography, soft-lithography and direct laser writing, this method provides a more flexibility in shapes of the ridges and grooves while retaining a well consistency in orientation. This represents physiologically relevant to heterogeneity of the natural tissue matrix, and probably accounts for the reported high efficiency of cell alignment (e.g. larger than 85% of cells aligned in ±10° [42]), rapid cell reorientation [42], and a wide applicability for different cells (e.g. mesenchymal stem cells [42], endothelial cells [18, 43] and tenocytes [54, 55]). This method advances the fabrication of ridges and grooves on freestanding and flexible thin film over the whole surface, providing simple, solvent-free, and reproducible produce of cell alignment.

Ridges and grooves are formed because of the differences in deformation of polymeric crystals and amorphous regions during single-axial drawing [42], thereby this method is applicable for the semi-crystalline polymers (e.g. PCL and polyethylene). This method is also compatible with different pre-fabrications such as heat press, solvent cast and electrospinning [56]. These ridges and grooves are distinct in the resistances against degradation, which has potential for a lasting and more prolonged regulation on cellular alignment when compared to structural geometries from other methods [57]. In addition, polymeric single-axial stretching fabricates ridges and grooves without damage to surface integrity. Not only that, this method due to the strain-related reinforcement to the film, results in the potential application of anisotropic geometry in

![Figure 8](image.png)
load-bearing tissue regeneration such as blood vessel [56]. With incorporation of post-perforation using lasers, engineering of basement membrane with the dual-structures of anisotropic and through-hole geometries is possible to mimic the functions as in the natural vessel such as cell alignment, mass transportation and direct cell–cell contact [18, 43].

3.6. Other fabrication methods for engineering biomaterials with anisotropic geometries

Shimizu et al reported an alternative method for simple and low-cost fabrication of orientated ridges and grooves using abrasives [58]. The micropatterned iron model obtaining from one directional grinding on sandpapers provides a fabrication principle similar to that of soft-lithography, to transfer structural anisotropy to polymer substrates (figure 10). The structural parameters can be varied when different sandpapers are applied. However, structural anisotropy from this method accompanies with surface destruction and contamination. The direct usage of abrasives also requires substrates to be rigid and having enough thickness, which may not be suitable for thin films the extensively applied materials for tissue-engineering vascular grafts. Directional top-down freezing [59, 60] and self-forming via ion beam [61] are also capable of fabricating anisotropic biomaterials. However, these methods are less reported for vascular tissue engineering and still waiting for further development to have effective cell alignment.

4. Reconstructing vascular anisotropy with biomaterials surface geometry

Cells recognise the orientation of fibres, ridges and grooves on engineered biomaterials surface, and distinguish them from isotropic biomaterials with adjusted biological response. In vascular tissue engineering, these cells including fibroblasts [67], ECs [68] and stromal cells (e.g. SMCs [30] and MSCs [69, 70]) when interact with the biomaterials surface, obtain the innate organisation of self-alignment and morphology of elongation, triggering downstream functional events [71].
4.1. Cellular alignment and elongation

4.1.1. Orientated ridge/groove arrays

Cellular response to biomaterials surface is intuitive, and since 1964 this has been extensively studied for controlling cell behaviour [72]. Cells to align and elongate towards geometric anisotropy rely on a mechanism of ‘contact guidance’, of which focal adhesion and actin filament are confined on intervening ridges, leading to orientation of the long axis of focal adhesions along surface anisotropy (figure 11(a)) [73, 74]. Using an engineered ridge/groove gradient, further exploration uncovers the mysterious veil that the alignment of focal adhesion is because of structural orientation converting to the anisotropy in topographical rigidity and cellular deformation resistance, thereby stabilising focal adhesion enriched on ridge edges and co-orientating finally with the ridges [75]. Cell marginal expansion preferred along the ridge direction with inhibited lateral extension across grooves, also contribute to the elongation of cellular morphology as evidenced by invading of cell protrusion easily along a direction parallel to the ridges while rapid retraction of protrusion in lateral direction [76].

Structural parameters are crucial for biomaterials geometric anisotropy to elicit cell response. Ridge depth dictates the extent to which cells respond to given lateral dimensions. For example, human corneal epithelial cells (HCECs) do not align along the underlying arrays when ridge depth is less than 150 nm [77]. When the ridge depth increases from 150 to 800 nm, morphological adaption such as cellular alignment and elongation are available. Similarly, a minimal ridge depth has also been reported for osteoblasts as small as 33 nm [78]. These may suggest a threshold of ridge depth which is minimal for eliciting cell alignment. In addition, this dimensional threshold is cell specific, in agreement with a law of nature that the cells of different tissues interact with ECM at different scales such as HCECs ECM in sub-micron/micron scales and bone ECM in nanometers.

Comparatively, cells align less on the ridge/groove arrays with nanodimensions and more on micron geometries. From nanoscale to microdimension, the increase in groove width accounts for increased cell alignment and elongation. However, a too big groove width typically larger than 50 μm, results in delayed alignment that requires cellular confluence before alignment occurring and gives a lower efficiency [42]. Ohara and Buck hypothesised that cells would fail to alignment if cellular focal adhesions could not sense surface features with an excessive pitch [73]. The delayed cell alignment with wider grooves may suggest that if groove width goes beyond singular cell dimension, the focal adhesions of the cell will sense underlying biomaterials as an isotropic rigid surface, resulting in cell morphology as observed on the flat surface.

It is valuable to note that cell alignment is sensitive to interfering factors. The presence of structures across ridges and grooves may influence cells to align along the geometric anisotropy [18]. Too small an aspect ratio of rectangle between parallel and perpendicular directions reduces significantly cell alignment. Mechanical stimuli such as a cyclic strain in a perpendicular direction to the ridges and grooves impact cell alignment negatively [79, 80]. Cell seeding density and serum in medium also influence cell alignment to surface geometric anisotropy; the increase in seeding density results in better initial cell alignment [81], while the remove of serum dramatically inhibits cell alignment [82].
4.1.2. Orientated fibre mats
Fibrous mats may provide spatial guidance for cell extension following the fibre direction. A ‘Janus feature’ is observed for cells cultured at the boundary between random and aligned fibres, which indicates a radial organisation at random fibre side and an orientated growth at the aligned fibre side [83]. The cells cultured on double-layered mats are able to sense both layers of fibres. Orientated fibres are also reported to biomimic ECM anisotropy at tunica media, and elicit SMCs to align into a spindle-like contractile phenotype [84].

4.2. Cellular migration
Cell migration is critical for vascular physiological and pathological statuses. The migration of FBs, SMCs, ECs and MSCs contributes to vascular wound healing and tissue regeneration. It is reported that compared to isotropic surface, cells culturing on orientated ridges and grooves have considerable increase in migration along the anisotropy [85]. The presence of crossing ridges further increases cell mobility, particularly for grids having a smaller size than individual cell, and a lower ridge depth tends to fast cellular migration along the ridges [67]. On a ridge/groove gradient (groove width: 1–9.1 μm), cells migrate depending on ridge density; the cell speeds fastest at an intermediate density of ridge pattern while the lower speeds on denser or sparse ridge patterns [75]. These findings suggest the potential of geometric anisotropy to guide cell migration for vascular regeneration.

4.3. Cellular adhesion and proliferation
Cell adhesion and proliferation are other vital properties that may affect vascular regeneration. SMCs show rapid adhesion and proliferation when culturing with aligned fibres of an average diameter of 500 nm [62]. In contrast, Thakar et al observed SMCs culturing on orientated ridges and grooves reduced cell proliferation and DNA synthesis (10 and 2.8 μm in groove width and depth, respectively) [86]. Although geometric anisotropy to interfere cell adhesion and growth is confirmed, other factors may regulate how the cells to response to anisotropic biomaterials surface, as evidenced by different cell behaviours to the orientated fibre mats and ridge/groove arrays. Agreed with this, ECs enhance proliferation on a titanium nano-/micro patterned surface [87], while the cells from human umbilical veins tend to decline proliferation on the ridge/groove pitches of 400 and 80 nm [88]. Culturing on flat and micropatterned surfaces, C2C12 myoblasts have similar anisotropy proliferation [58]. Probably, there exists heterogeneity in factors that regulate cell proliferation including geometric anisotropy and cell source. In agreement with this, nanoscale biomaterials surface (e.g. orientated ridges and grooves at 400 nm pitch) may benefit vascular tissue engineering through enhancing ECs adhesion strength, and thereby stabilising endothelialisation against flow shear.

4.4. Cellular differentiation
To increase in vivo adaptation, vascular grafts are expected to maintain an appropriate phenotype of cells as in the native healthy blood vessels, and it is becoming clearer that geometric anisotropy acts a crucial role in regulating cell morphology and function for vascular tissue-engineering application. Mechanism behind these regulations is known as a mecano-transduction pathway that can be direct or indirect [18, 54, 71, 89]. Direct mechanotransduction relies on the links between a cell shape and nucleus through the cytoskeleton and nuclear lamins. Cellular elongation couples with the orientated organisation of cytoskeleton, which up-regulates and moves nuclear lamins onto the nucleus periphery to induce nucleus shape deformation and finally genomic adaptation (figures 11(b) and (c)) [90]. Indirect mechanotransduction involves chemical signaling cascades that can be initiated by focal adhesion-associated proteins [91], and probably includes the integrin-mediated Rho GTPase and the FAK signaling network [92].

During vascular regeneration, SMCs often lose contractile apparatus and shift to a synthetic status that accompanies with ECM over-secretion, excessive cell proliferation, and trans-myointernal migration leading to intimal hyperplasia [37, 38]. These synthetic SMCs with the typical ‘hill and valley’ morphology have well spreading and random organisation during culture expansion in vitro to high passages [30]. Recently, geometric anisotropy is proposed for regulating SMCs status similar to the effects of TGF-β1 [86]. Williams et al suggest that geometric anisotropy as the cues on up-regulating expression of SM-α-actin and SM-MHC could be as effective as TGF-β1 particularly in the low passage of SMCs [30]. Similarly, primary SMCs culturing on an orientated microchannel surface up-regulate the expression of SMCs contractile markers including SM-α-actin, calponin and SM-MHC [93]. This differentiation of SMCs couples with declined deposition of the total amount of elastin and collagen when the cells become aligned and elongated.

The effects of geometric anisotropy on MSCs differentiation are complex. Human embryonic MSCs culturing on orientated fibres and ridge/groove arrays seem to differentiate towards a neural lineage, in the conditions regardless retinoic treatment of [64]. However, this lineage differentiation appears to be dependent on the sources of stem cells. For example, MSCs raised from bone marrow source can obtain myogenic differentiation when culturing on orientated fibrous mats and ridge/groove arrays, which associates with
5. Concluding remarks and future prospects

There is growing interest for tissue-engineering idea vascular substitutes which develops scaffolds to guide cell behaviour, thereby regenerating the natural architecture and function of blood vessel. This concept of ‘tissue-engineered, idea vascular substitute’ represents an innovation to generate graft that is living and immune compatible as an autologous vessel, and simultaneously approaches to an off-the-shelf idea minimising greatly the waiting time of patients before implantation. The basis of this innovation follows the general principle of tissue engineering, of which a scaffold is designed in combination with drugs, growth factors and other bioactive chemicals to regulate cellular behaviour and in turn the ECM composition. Along this research direction, great achievements on the bench top research account for lasting progresses for vascular tissue engineering in the past decades, and hatch out probably next focusing point towards vascular structure regeneration. In this paradigm, correct ECM and cell components are assembling into the native vessel architecture, approaching to the aim of functional tissue regeneration.

In circulatory system, the unique anatomic structure of blood vessel makes up its innate physiologies, which provide the bases of mechanical support against blood pressure, vasoactive response, and cell functionality. These have a close relation to the physical anisotropy of vascular structure, although the underlying mechanisms of anisotropy established in processes of vasculogenesis, angiogenesis, and healing remain unclear in detail. Benefiting from the concept of tissue engineering, vascular scaffold can be engineered to reconstruct the physical anisotropy in blood vessels, through designing biomaterial-cell interface with geometries. In this field, great developments are achieved including the principles and techniques of fabrication compatible for biodegradable/resorbable materials, and the biological understandings on effects of the biomaterials with geometric anisotropy as reviewed in detail in this paper.

On the other hand, scaffold design for tissue engineering is necessary of considering the mechanisms of neotissue formation during development and physiological healing. As such, the engineering of vascular scaffold is needed to support those processes of neotissue formation, which often requires biomaterials to be biodegradable/resorbable, biocompatible and hemocompatible. Not only that, the scaffold for vascular regeneration should be with robust mechanical properties and a large porosity enough for cell infiltration, cell-cell communication and mass transportation; this two aspects of a scaffold are often opposite to each other for the fabrication. For instance, a dual-phase separation is developed recently for vascular scaffold fabrication of biomimicked nanofibrous and interconnected porous architecture with adjustable mechanical properties, which provides favorable performance for the potential reconstruction of small diameter blood vessel [95]. Future work targeting on scaffolding an integration of properties related to the basic tissue-engineering requirements, directing ECM and cell component formation, and reconstructing correct tissue architecture is expected for the advanced development of vascular scaffold. For instances, a translation of cellular alignment from 2D to 3D is necessary to meet at least the dimensional demand of vascular wall thickness. Furthermore, how to tailor the cell and ECM alignment following orthogonal directions as in tunica intima and media, and to build the circumferential alignment following herringbone helical arrangement as in tunica media, are still challenging towards a precise reconstruction of the vascular architecture. Future advances in vascular scaffolding will depend on multidisciplinary developments that combine not only biological and material science, but also engineering principles and fabrication such as 3D cell alignment by integrating laser perforation and single-axial drawing.

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Author contributions

ZW conceived the original ideas of this manuscript. All authors participated in the literature review, discussion and writing of the manuscript. ZW, WZ and CX executed the figure drawings. ZW and XW directed the work.

Conflicts of interest

The authors declare no competing financial interest.

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