REVIEW

Luminal Surface Engineering, 'Micro and Nanopatterning': Potential for Self Endothelialising Vascular Grafts?

D.S.T. Chong [a,b], B. Lindsey [a], M.J. Dalby [c], N. Gadegaard [d], A.M. Seifalian [b], G. Hamilton [a,b,*]

[a] Department of Vascular Surgery, Royal Free London NHS Foundation Trust, London, UK
[b] Centre for Nanotechnology and Regenerative Medicine, Division of Surgery and Interventional Science, University College London, London, UK
[c] Centre for Cell Engineering, University of Glasgow, Glasgow, UK
[d] Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, UK

WHAT THIS PAPER ADDS

The development of vascular grafts using nanotechnology remains an exciting field. The ability to produce a biomimetic environment in which endothelial cells are able to respond has produced some well-known research. The production of topographical features to enhance endothelialisation within a vascular graft provides another desirable, but perhaps still under-researched, pathway. We hope that further development in this field of research will bring forth future vascular grafts with 'self-endothelialising' potential.

Objective: New technologies are being explored to meet the clinical need for an 'off-the-shelf' small diameter vascular graft with superior or at least equivalent properties to autologous vessel. The field of nanotechnology and fabrication promises major advances in biomaterial design and wall structure to deliver biomimetic grafts. This review brings together recent work on this topic.

Methods: A literature search was conducted of PubMed and ISI Web of Knowledge using relevant keywords. Articles published after January 2005 were given preference. Personal communications and PhD theses were also used as sources.

Results: An evolving focus on surface patterning of biomaterials has been found to carry great potential. Influencing cellular behaviour on prosthetic grafts using graft luminal surface modulation at the micro- and nano-levels is the basis of this recent concept in vascular graft development.

Conclusion: This technology may deliver small diameter grafts with the potential for spontaneous in situ endothelialisation without the need for prior 'seeding', with the potential to open a new chapter in vascular graft development.

Article history: Received 1 July 2013, Accepted 11 February 2014, Available online 15 March 2014

Keywords: Endothelialisation, Surface topography, Vascular grafts

INTRODUCTION

Despite intensive research, the ideal small diameter vascular graft (4—6 mm luminal diameter) with equivalent patency rates to autologous vessels remains to be achieved. The most common clinically used synthetic graft materials remain polytetrafluoroethylene (PTFE) and to a lesser extent Dacron®; however, even with luminal modulation, the patency rates remain poor. These materials are limited by early failure rates secondary to intimal stenosis and thrombosis secondary to low blood flow, compliance mismatch and high shear.[1]

The ideal graft should possess both compliance and antithrombogenicity similar to native vessel. Different strategies have emerged to either improve the graft materials available[2,3] or to produce grafts made of novel materials.[4] Recent improvements of the PTFE graft include attaching heparin to the luminal surface to decrease acute thrombosis rates with encouraging early results. However, when compared with autologous saphenous vein (ASV), PTFE is still inferior, with primary patency rates at 48 months of 61% versus 44.5%.[5,6]

Endothelialisation has long been considered to be the elusive 'gold standard' in vascular graft development. A confluent layer of endothelial cells (ECs) confers essential haemostatic—thrombotic balance, is vasoactive, mediates angiogenesis and inflammation, and prevents intimal hyperplasia.[7] Currently endothelial ingrowth post implantation remains limited to 1—2 cm from the anastomosis, leaving most of the luminal surface uncovered.[8] Biomimetic developments to give enhanced endothelial attachment and retention have varying success, but mostly only in vitro. The Vienna group[9,10] have shown significantly improved long-term patency of PTFE grafts pre-seeded with ECs

* Corresponding author. G. Hamilton, Department of Vascular Surgery, Royal Free London NHS Foundation Trust, Pond Street, London NW3 2QG, UK.

E-mail address: g.hamilton@ucl.ac.uk (G. Hamilton).

1078-5884/$ — see front matter

http://dx.doi.org/10.1016/j.ejvs.2014.02.007

compared with standard (primary patency 69% at 5 years, 61% at 10 years[9] compared with 39–49%).[11]

Interest in the potential for surface topography to influence cellular behaviour began in 1958 when Weiss[12] first reported cellular contact guidance following Harrison's 1911 observation of cells responding to surface shape.[13] Many failed attempts at surface modified topography to modulate cellular behaviour followed until recent developments at the micro and nanoscale. Technological advances in surface modulation at these scales by the electronics industry have led to renewed enthusiasm and a more focussed approach to provide a biomimetic scaffold.[14–16]

In 1959, Richard Feynman[17] first described the process of manipulation at the atomic and molecular level introducing the concept of 'nanotechnology', leading to new biological technologies for surface engineering to control cellular orientation and organisation. In this paper, we will introduce and discuss the emerging techniques of surface 'patterning' to 'endothelialise' vascular grafts.

For endothelialisation within a graft, the cell-surface interface is critically important and therefore surface modulation our most important target. Extracellular matrix (ECM) provides a complex environment of topographical features at the micrometre to nanometre scale, in which vascular cell types can adhere and embed. Being able to 'pattern' the surface so as to recreate these topographical features optimally, increases the potential to 'focus' cellular behaviour including that of stem cells.[18,19] Recent research has used both micro- and nanoscale features in a variety of ways, such as supporting the directed differentiation of stem cells.[20] Although the reality of recreating the ECM in its entire complexity remains remote, some of its topographical features can be reproduced with good effect both at the micro- and nanoscale. New methodologies from the semiconductor industry over the last 10 years have driven the impetus for recreating topographical features able to direct cellular function and migration.

METHODS

An extensive PubMed and ISI Web of Knowledge search was conducted using keywords 'vascular graft', 'endothelial cells', 'endothelialisation', 'nanotopography', 'nanopatterning', 'microtopography', 'micropatterning', 'shear stress', 'haemocompatibility', 'microfabrication', 'nanofabrication' and 'biofunctionalisation'. All articles were reviewed for relevance. Articles published after January 2005 were given preference unless a specific point or method was being introduced. Personal communications and PhD theses were also used as sources.

RESULTS

Our first finding was that although many of the complex topographical features of the ECM are 3-dimensional (3D), most of the research so far has been carried out on predominantly 2-dimensional substrates (2D).[21]

Surface topographical engineering

There is continuing debate regarding whether micro- or nanoscale surface modulation is of more importance in influencing cellular behaviour. Most believe[22] that nanoscale topography is more important because of its similar scale to all cell receptors, which direct changes in cytoskeletal organisation, motility, differentiation, gene expression and cell shape. However, others have challenged this emphasis, mostly on practical grounds.[23,24] A further important factor is whether the luminal surface can be constructed in 'ordered' patterns facilitating cellular alignment and adhesion, or where the methodology results in a 'disordered' surface less conducive to cellular attachment.

Fabrication methods employed for the production of ordered topographical features (Table 1) are photolithography and electron beam lithography (EBL). Photolithography involves the transfer of a pattern using a light source onto substrates coated with light-sensitive polymeric photoresistant material, then followed by selective chemical removal of the resist (a radiation-sensitive compound used

Table 1. Common fabrication techniques employed to create surface topographical features.

Technique	Method
Photolithography	Method in which light is used to generate structures on a surface. Requires a layer of light-sensitive polymer, a photoresist, which is exposed to ultraviolet light (UV) through a mask layout. The exposure causes crosslinking, polymerisation or degradation of the resist. 2-dimensional (2D) topographical features may be constructed using synthetic polymers. However, the limitation is the resolution of the topographical features, dependent on the wavelength of light used
Electron beam lithography (EBL)	Extension of photolithography in which high-energy electrons are used to expose an electron-sensitive resist. There are both positive and negative resists. EBL has mostly been used to develop nanoscale surfaces
Soft lithography	Utilises elastomeric polymers to develop patterns based on embossing, moulding, and printing methods. The three main processes are micro-stamping, stencil patterning, and microfluidic patterning. Advantages are low costs, ease of use, and high throughput without the requirement of a clean room
Electrospinning	Used to create ultra-fine fibre topographies down to the nanoscale using an electrically charged droplet of polymer melt or solution. This is usually employed to produce 'unordered' surfaces, although 'ordered' surfaces also can be produced but this is limited

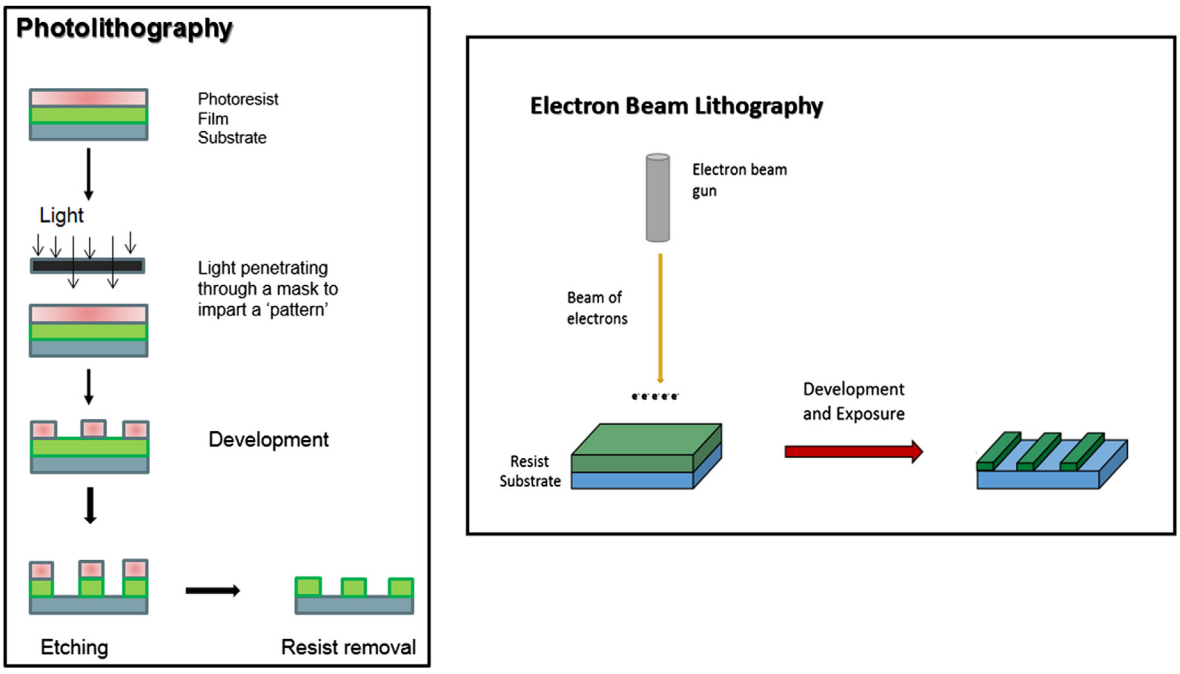

Figure 1. Diagrams to show the popular micro and nanofabrication techniques of patterns.

to transfer a pattern to a substrate) (Fig. 1). The resultant pattern is then used to direct either etching or material deposition. The diffraction limit of light governs the resolution of possible topographical features, usually only at the microscale level.

At the nanoscale, EBL uses a focused beam of electrons which is then raster scanned (pattern of parallel lines) across the substrate (Fig. 1). This is in contrast to photolithography where the entire substrate is simultaneously exposed. Importantly, as the diffraction limit of electrons is much smaller, sub-micron scale features can be created.[25]

These techniques use a silicon wafer mould for polymeric substrates such as polyurethane and polycaprolactone, which in turn can fabricate vascular grafts with the desired surface topography. Both photolithography and EBL are powerful techniques but limited by low throughput, high cost, and the need for trained staff and specialist facilities. In summary, ordered topographies are generally expensive,

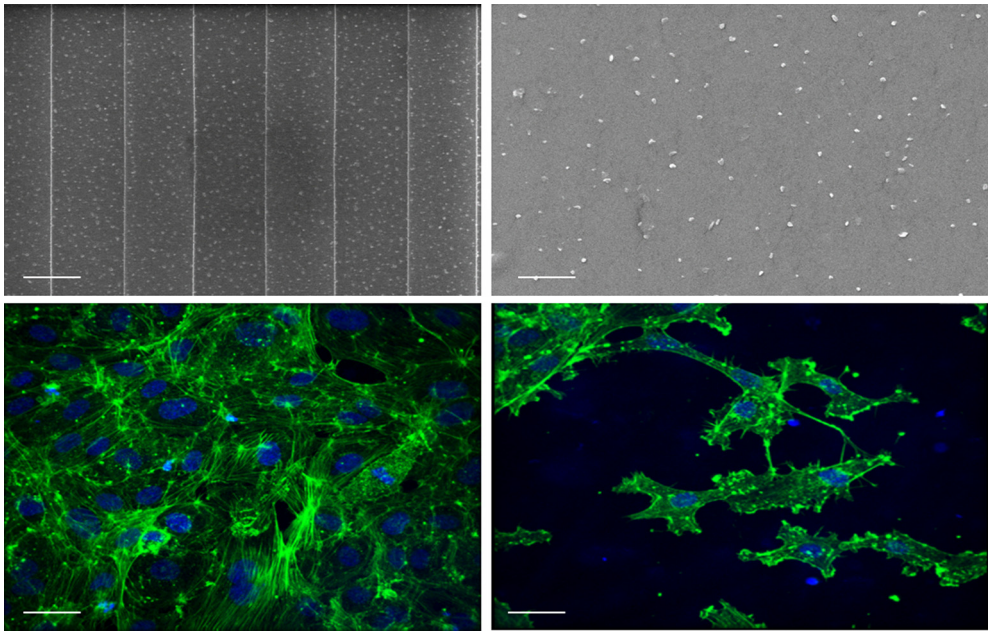

Figure 2. Human umbilical vein endothelial cells (HUVECs) grown on micro-grooved (pitch 25 μm) and non-patterned nanocomposite polycarbonate urea urethane over 5 days. Nuclei (blue) and actin (green) of the cells are shown using confocal microscopy (authors' unpublished data). Scale bar 10 μm.

Table 2. Some of the in vitro work undertaken to illustrate EC behaviour on different micro- and nanoscale grooves and channels to show EC alignment and migration under both stress and static conditions.

Cell type	Substrate	Pattern	Dimensions and pitch	Static/shear flow culture?	Description	Year/ref.
Human aortic endothelial cells (HAECs)	PDMS	Ridges and grooves	Pitch: 400—4000 nm Feature depth: at least 300 nm	Shear flow	EC were exposed to both flow (20 dyne/cm^2) and topographical stress. EC exhibited greater alignment than shown when exposed to either stimulus alone	2012[58]
HUVEC (Human umbilical vein endothelial cells) and HAECs	Polyurethane	Ridges and grooves	Pitch: 400—4000 nm	Static	Cell types exhibit orientation and elongation on anisotropically ordered ridges >800 nm and migrate parallel to the long axis of the ridges. However, the heterogeneity of ECs response to topographical cues were seen	2010[40]
Human coronary artery endothelial cells (HCAECs), smooth muscle cells (HCASMCs) and human fibroblasts	PDMS substrates	Grooves	Groove depth: 50—200 nm. Groove widths: 2, 3, 5 and 10 μm	Static	Grooves with smaller width or greater depth induce significant directed migration and partial orientation	2010[38]
Human microvascular endothelial cells (HMECs)	Type I collagen films	Microgrooves	Parallel channels with groove and ridge widths of 650 nm with 300 nm depth, 500 nm with 250 nm depth, and 332.5 nm with 200 nm depth	Shear flow	Cell culture studies show that nanopattern did not affect endothelial cell proliferation and had minimal effect on cell alignment, but significantly enhanced cell retention under shear flow conditions	2009[37]

Continued

Table 2-*continued*

Cell type	Substrate	Pattern	Dimensions and pitch	Static/shear flow culture?	Description	Year/ref.
Bovine aortic endothelial cells (BAECs)	PDMS	Microgrooves	Symmetric patterns: 5 × 5, 3 × 3, 2 × 2 Asymmetric patterns: 5 × 2. Widths of ridges and channels 5 and 2 μm. Separation 500 μm	Shear flow	Width dimension of 3D microgroove guides direction of endothelial cell migration in absence of flow. Critical groove width for cell migration is 2 μm. Microgrooves guide orientation of actin stress fibres parallel to grooves after exposure to flow (moderate and high shear) for at least 4 hours	2008[39]
BAECs	PDMS	Micro- and nanochannels	Channel widths of 4 and 5 μm with different depths 200 nm, 500 nm, 1 μm, and 5 μm	Static	Cells on deepest channel depth show loss of alignment	2005[68]
BAECs	Micro-patterned ECM by injection of collagen-1 into PDMS mould	Microchannels	Widths 15, 30, and 60 μm	Static	ECs on 15 μm collagen strips had 30% less adhesion area and lower shape index, had fewer but polarised focal adhesions, and migrated faster	2001[36]

and labour-intensive, but have the advantage of ultimate control over surface geometries and high reproducibility.

Disordered topographical features are produced spontaneously by cheaper, quicker, and easier methods than for ordered surfaces. Unfortunately, this results in topography which is random, imprecise, and with little geometrical symmetry throughout the graft. Currently, electro-spinning is the main fabrication technique for vascular grafts with disordered features[26,27] employed to produce functioning synthetic and biodegradable grafts. With the development of more sophisticated methods of electrospinning, thin layers of ordered electrospun nanofibres can be made in the laboratory but produced in bulk, the fibre ordering remains very much random. Thus, current disordered electrospun grafts lack directionality to promote the desirable contact guidance of ECs into the construct to achieve endothelial coverage without pre-seeding.[28] Other fabrication methods for random surface topography such as polymer demixing[29] and colloidal lithography[30] have encouraging results with certain cell types including ECs.[29]

Recently, lithographic techniques for producing the more desirable ordered topography have been developed with higher resolution[31] and throughput, and lower production costs.[32] For example, micro- and nanoscale structures can be replicated with high fidelity either using silicon masters or nickel masks via hot embossing and injection moulding techniques at industrial scale as seen with the mass production of Blu-Ray DVDs.[33,34] Direct-write of patterns ('maskless lithography') onto polymeric surfaces[32,35] is a further promising methodology, but with some concerns of possible toxicity from the high energy beams generated damaging the polymeric surfaces and changing the surface chemistry.

Endothelial cells and surface topography

Several studies have looked at the behaviour of ECs on different substrates with different patterning themes (see Fig. 2). The patterns generated have mainly been grooves, pillars, pits and ridges in the nano- and micrometre ranges. Li et al.[36] conducted an important initial study looking at EC behaviour on micro-channels made with different widths of collagen-1 strips. Collagen strips 15 μm wide yielded 30% less overall adhesion area, but resulted in more polarised focal adhesion and faster EC migration. Another study showed that differing size and depth of channels had minimal effect on cell alignment; however, confirmed that nanoscale patterns significantly enhanced cell retention under shear flow conditions.[37] Table 2 demonstrates a recent body of in vitro work on the importance of grooves on alignment of ECs promoting parallel migration and cell alignment, especially under low and normal stress conditions. These findings bring us closer to defining both optimal surface topography and stress flow also in a graft design to promote EC function as close to that in the native environment. ECs exhibit different behaviours on different polymeric surfaces. For example, on polydimethylsiloxane (PDMS) polymers ECs exhibit behaviour sensitive to the width and depth of the micro- and nanochannels.[38] One group quotes a critical groove width of 2 μm for EC migration,[39] whereas another has shown preferential orientation and elongation of ECs on anisotropically ordered ridges larger than 800 nm on a polyurethane substrate.[40] Both of these studies were conducted in vitro and illustrate the importance of the scaffold as well as the surface in the behaviour of ECs. It is beyond the scope of this review to further discuss scaffolds as our focus is cell behaviour on the surface–biomaterial interface, but several excellent reviews address this issue.[41,42]

Recent research has shown the importance of topography in terms of cellular interaction with the surface with regard to morphology, adhesion, gene expression, and proliferation.[15,20,43] The ability to harness and influence cellular interactions using surface topography provides the potential to stimulate inward migration of ECs from the anastomoses, thus eliminating 'pre-seeding', allowing 'self-endothelialisation' and achieving the goal of a prosthetic graft with equivalent function to autologous.

Protein and peptide patterning

Protein adsorption plays a pivotal role in determining the biodurability and biocompatibility of synthetic materials. The role of peptides and proteins in patterning can be considered in two main ways:

1. 'immediate' effect — preferential protein or peptide adsorption onto the graft surface to either induce or decrease certain effects;
2. 'later' effect — using a protein or peptide 'patterned' or enhanced surface to influence cellular behaviour.

Transmission of chemical and mechanical signals from the ECM to the cell is primarily mediated by integrins, a family of cell-surface transmembrane receptors. Integrin–ECM interactions govern cell survival, growth, migration, and differentiation, and are currently central to many biomimetic tissue engineering strategies. Furthermore, intimately coordinated clusterings of ECM ligands, integrins, and cytoskeletal components form macromolecular aggregates known as focal adhesions both inside and outside the cell membrane. Focal adhesions develop on micro- and nanometre scales with integrins in the 10 nm diameter range and 20 nm long extracellular domains.[44,45] As a result, coatings of ECM macromolecules such as collagen, laminin, or recognition peptides such as RGD, can form biofunctionalised surfaces to control specific cell responses.[46] These coatings act like a 'glue', attracting and encouraging cellular adhesion.

EC adhesion and migration require that cell-surface integrin receptors recognise and bind to the ligands in the ECM. The RGD ligand, a component of fibronectin (Fn) and other matrix proteins attached to the graft surface will influence endothelial cell behaviour. Indeed, a relatively low RGD density of 6×10^6 RGD ligands/mm^2 (equivalent to spacing of 440 nm) promotes cellular adhesion and

spreading, whereas higher densities of 6×10^7 RGD ligands/mm^2 (spacing of 140 nm) are needed for the formation of focal adhesions and stress fibres in ECs. Therefore, incorporation of RGD in the nanometre range is instrumental in promoting cellular adhesion. However, the complexity of the interplay among RGD, surface topography, and chemistry remains a multifaceted problem requiring further development before its incorporation in graft topography manufacture.[44,47]

Surface patterning with these key adhesion molecules such as Fn, collagen, and talin to increase adhesion strength shows promise. Increasing surface Fn concentration accelerates both the extent of cell spreading and proliferation rates. This effect is further amplified with greater cell proliferation onto nano-patterned Fn surfaces.[39,48] This work was taken further by Dickinson et al.[49] who found that Fn microgrooves of 50 μm width promoted alignment and elongation of HUVECs. This work is highly relevant to improving graft function as ensuring optimal endothelial adhesion once exposed to the shear stress flows within the vascular graft after implantation is a vitally important factor. The current role of proteins and peptides to promote adhesion is limited, however, because of the problems of short half-lives, sourcing, and stability, in addition to possible systemic side-effects.[50]

Haemocompatibility

Surface modification, especially topographical changes, can have effects on the haemocompatibility of the synthetic graft, in particular causing platelet aggregation, which may contribute to premature vascular graft thrombosis. However, the literature presents rather conflicting information as to the exact role of topographical manipulation to platelet response, with some authors reporting no significant difference between platelet activation on smooth or nanoscale topographical surfaces.[51] The latest studies have shown that platelet activation may be dependent on the dimension of surface features as well as their aspect ratio (ratio of feature width to its height — 'hill and valley'). Certain nanostructured materials can have inherent antithrombogenic properties and if, in addition, high aspect ratio features are incorporated in their design then the platelet contact will be mainly confined to the tips of the nanopillars (or 'hilltops') and therefore activation is minimised.[52,53]

Initial protein adsorption on the material surfaces plays an important part in eliciting a platelet response.[52,54] Fibrinogen (Fg) and albumin (Alb) were identified as the crucial proteins that determine and induce a platelet adhesion response.[46,55] Most importantly the conformational state of both adsorbed Fg and Alb can be instrumental in mediating platelet response. Sivaraman et al.[46] showed that platelet adhesion was correlated to concentration of fibrinogen. However, the correlation is greater with the degree of adsorption-induced unfolding of Fg, with this unfolding leading to two distinctly different types of platelet binding sites being exposed, one that induces platelet adhesion and one that both activates as well as induces binding. These findings are important, with protein adsorption and protein behaviour on nanostructured materials being crucial areas of investigation. Although there is no general consensus, there is recognition that platelet—surface interaction is dependent on topography and protein adsorption. Modulation of unfolding of the proteins remains an ongoing aim in the development of surface-modulated grafts.

Shear stress

In addition to surface topography, shear response and cell retention are other factors that will affect EC attachment

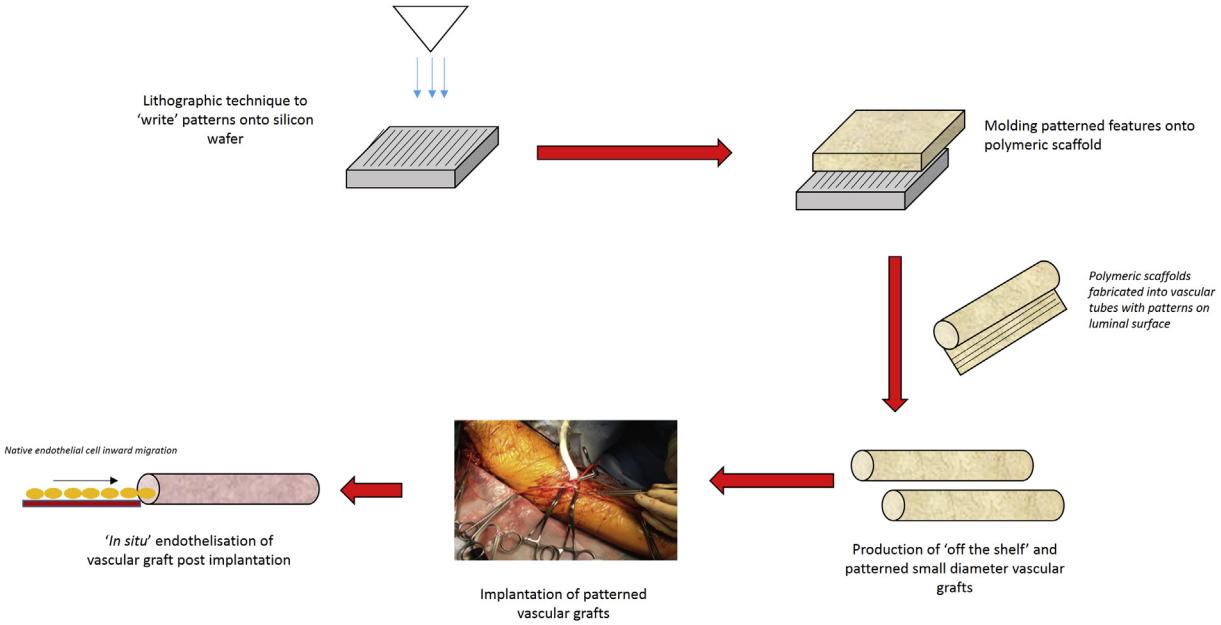

Figure 3. Flow chart to show the fabrication of patterned small diameter vascular grafts.

within a dynamic circulation.[56] In general, experiments conducted in vitro fail when exposed to physiological circulatory pressures and stress because of delamination of the EC layer.[57] Therefore, a confluent layer of EC within the luminal surface of the graft would only be successful if it was able to withstand these pressures and stresses. Furthermore, fluid shear stress experienced by vascular ECs in vivo provides an important mechanical cue that can direct cell migration and induce the activation of biochemical processes. Morgan et al.[58] found that when ECs were exposed to both flow stress and topographical guidance, they exhibited greater alignment than was shown when the cells were exposed to either stimulus alone, demonstrating a synergistic interaction. Other cell culture studies have shown that patterning can significantly enhance cell retention under shear flow conditions,[37,59] especially when the flow is parallel rather than perpendicular to cell alignment,[39] indicating that shear flow conditions play an important part in EC adhesion. These are important findings that support the concept of graft luminal patterning resulting not only in EC alignment but also improved retention and resistance to shear stress.

Vascular graft development using topographical features

Using a variety of surface patterning techniques, vascular grafts made of either synthetic or biodegradable polymers have been tested. So far the materials which have been tested are polyurethane, poly-L-lactic acid, and poly-DL-lactic acid, using high fidelity transfer micropatterns.[40,60]

One of the first prototypes of a 'patterned' vascular graft was developed by Zorlutuna et al.[61] and this was a double-sided nanopatterned tubular collagen graft in which both sides were seeded with vascular smooth muscle cells (VSMCs) and ECs. Nanopatterns on the outside were shown to successfully orientate the VSMCs circumferentially as in natural vessels enhancing the tensile strength. The luminal nanopatterns increased EC retention under haemodynamic conditions. Both ECs and VSMCs retained their phenotypes and proliferative capacity, showing great potential as a vascular graft prototype. Other vascular constructs using both controlled micro- and nano topography on biodegradable scaffolds have been developed with successful outcomes,[62] confirming the reality and promise of using patterning for the development of vascular grafts (see Fig. 3).

The use of electrospinning to produce 3D scaffolds has captured the imaginations of many researchers.[63] The mechanical geometry and composition of the scaffolds can be controlled to a high degree, and a variety of substrates can be chosen such as collagen, polyurethane, or polycaprolactone. The luminal surface provides an interactive topography for the adhesion of cells in a stress flow situation. However, the main disadvantages of electrospun scaffolds are inability to reliably define an ordered surface topography, the resultant need for seeding prior to implantation, in addition to poor reproducibility of ideal fibre and pore sizes.[64] However, electrospinning can deliver a final structure which can behave mechanically like native vessel but with a long-term risk of calcification developing in electrospun polycaprolactone vascular grafts in a long-term animal model.[65] This may prove to be an important complication as recent data[66] have shown calcification in all vascular grafts to be more common than previously recognised, with 68% of clinically implanted standard e-PTFE vascular grafts developing some degree of calcification as early as 1 month post-implantation. Calcification can stiffen the graft, reduce compliance altering haemodynamic flow patterns, and exacerbate endothelial dysfunction leading to premature graft failure. It is important to recognise that research conducted so far has mainly been in vitro, with limited in vivo work.

DISCUSSION

Small diameter grafts have been the focus of much research because of low patency rates. With current materials in clinical use, the lack of endothelialisation is a real problem. These grafts probably suffer thrombosis caused by low blood flow and thrombogenicity rather than defects in the material.

There is a great deal of interest in the potential for decellularised vascular grafts. Olausson et al.[67] recently reported the use of decellularised deceased donor vein graft seeded with autologous stem cells as a vascular bypass graft for a child with extrahepatic portal vein obstruction. However, this process took 4 weeks from acquisition of the vein to implantation because of the decellularisation and recellularisation procedures. This illustrates the major logistic problem with current approaches, which are all highly labour-intensive, costly, cumbersome in terms of process and specialist facilities, and furthermore will have limited usage outside of major academic vascular centres. Other vascular graft materials, especially those which are biodegradable, are limited by the need for pre-seeding, culture and despite major improvements in technology, are still a limited option for 'off-the-shelf' products. Biodegradable polymers for the use of vascular grafts outside of some limited paediatric indications are still viewed by many surgeons with scepticism as early mechanical failure can lead to disastrous consequences.

The importance of the luminal surface of the graft has long been recognised, but until the recent advances in heparin bonding this had been a problem which has seen little advance in its resolution. Heparin bonded grafts show improved patency when used in both vascular and endovascular procedures but this is only in the short term, the improvement remains limited and does not approach that of saphenous vein. The goal of an off-the-shelf prosthetic small diameter graft which performs as well as saphenous vein or indeed artery, has not yet been achieved.

However, the advances delivered by nanotechnology in realisation of this goal are now real, with a few novel engineered graft materials coming to translational clinical studies. As yet, these advances have largely focused on wall and scaffold structure to deliver compatibility with native

artery in compliance and mechanical properties, and reduced thrombogenicity. Our understanding of the importance but complexity of the luminal and cellular interaction of blood vessels has also advanced significantly. This is at the point where graft design and structure can incorporate these specialised surface features to begin to realise the concept of a 'biomimetic' graft. Vascular grafts can now be tailormade for different vascular uses based on novel and existing materials to encourage the desired cellular behaviour post-implantation, to therefore encourage long-term patency. This review describes current approaches and advances on engineering of the graft luminal surface using the principles of nano-engineering. The creation of micro- and nanoscale surface patterns for prosthetic grafts, both biodegradable and non-biodegradable, holds much promise for achieving the goal of spontaneous endothelialisation either by attachment of circulating endothelial progenitor cells or by migration. The technology to construct a graft surface in three dimensions with patterning and incorporation of important vasoactive proteins has been realised in the laboratory and now is proceeding to in vivo assessment. With current and imminent advances in micro- and nanoscale fabrication, the reality of creating synthetic vascular grafts using this technology promises to open a new chapter in vascular research, and with the realistic possibility that patterning of current grafts such as PTFE can deliver improved performance in the near future. Much further work is needed, is ongoing in several centres and the vascular surgeon's dream of a small diameter 'off-the-shelf' graft or stent graft with the potential of function equivalent at least to saphenous vein, and possibly to that of native artery, has come much closer to reality.

CONFLICT OF INTEREST

None.

FUNDING

The authors would like to acknowledge research grants from The Wellcome Trust, Royal Free Charity and European Society of Vascular Surgery.

REFERENCES

1 Byrom MJ, Bannon PG, White GH, Ng MKC. Animal models for the assessment of novel vascular conduits. *J Vasc Surg* 2010;**52**(1):176—95.
2 Battaglia G, Tringale R, Monaca V. Retrospective comparison of a heparin bonded ePTFE graft and saphenous vein for infra-genicular bypass: implications for standard treatment protocol. *J Cardiovasc Surg (Torino)* 2006;**47**(1):41—7.
3 Schleicher M, Hansmann J, Elkin B, Kluger PJ, Liebscher S, Huber AJ, et al. Oligonucleotide and parylene surface coating of polystyrene and ePTFE for improved endothelial cell attachment and hemocompatibility. *Int J Biomater* 2012;**2012**: 397813.
4 Ahmed M, Ghanbari H, Cousins BG, Hamilton G, Seifalian AM. Small calibre polyhedral oligomeric silsesquioxane nano-composite cardiovascular grafts: influence of porosity on the structure, haemocompatibility and mechanical properties. *Acta Biomater* 2011;**7**(11):3857—67.
5 Dorigo W, Di CF, Troisi N, Pratesi G, Innocenti AA, Pulli R, et al. Lower limb revascularization with a new bioactive prosthetic graft: early and late results. *Ann Vasc Surg* 2008;**22**(1):79—87.
6 Dorigo W, Pulli R, Piffaretti G, Castelli P, Griselli F, Dorrucci V, et al. Results from an Italian multicentric registry comparing heparin-bonded ePTFE graft and autologous saphenous vein in below-knee femoro-popliteal bypasses. *J Cardiovasc Surg (Torino)* 2012;**53**(2):187—94.
7 Ranjan AK, Kumar U, Hardikar AA, Poddar P, Nair PD, Hardikar AA. Human blood vessel-derived endothelial progenitors for endothelialization of small diameter vascular prosthesis. *PLoS One* 2009;**4**(11):e7718.
8 Zilla P, Bezuidenhout D, Human P. Prosthetic vascular grafts: wrong models, wrong questions and no healing. *Biomaterials* 2007;**28**(34):5009—27.
9 Deutsch M, Meinhart J, Zilla P, Howanietz N, Gorlitzer M, Froeschl A, et al. Long-term experience in autologous in vitro endothelialization of infrainguinal ePTFE grafts. *J Vasc Surg* 2009;**49**(2):352—62 [discussion 62].
10 Deutsch M, Meinhart J, Fischlein T, Preiss P, Zilla P. Clinical autologous in vitro endothelialization of infrainguinal ePTFE grafts in 100 patients: a 9-year experience. *Surgery* 1999;**126**(5):847—55.
11 Klinkert P, Post P, Breslau P, Van Bockel J. Saphenous vein versus PTFE for above-knee femoropopliteal bypass. A review of the literature. *Eur J Vasc Endovasc Surg* 2004;**27**(4):357—62.
12 Weiss P. Cell contact. *Int Rev Cytol* 1958;**7**:391—423.
13 Harrison RG. On the stereotropism of embryonic cells. *Science* 1911;**34**(1):279—81.
14 McNamara LE, Burchmore R, Riehle MO, Herzyk P, Biggs MJ, Wilkinson CD, et al. The role of microtopography in cellular mechanotransduction. *Biomaterials* 2012;**33**(10):2835—47.
15 Biggs MJ, Richards RG, Gadegaard N, McMurray RJ, Affrossman S, Wilkinson CD, et al. Interactions with nanoscale topography: adhesion quantification and signal transduction in cells of osteogenic and multipotent lineage. *J Biomed Mater Res A* 2009;**91**(1):195—208.
16 Csaderova L, Martines E, Seunarine K, Gadegaard N, Wilkinson CD, Riehle MO. A biodegradable and biocompatible regular nanopattern for large-scale selective cell growth. *Small* 2010;**6**(23):2755—61.
17 Feynman R. There's plenty of room at the bottom. *J Microelectromech Syst* 1992;**1**(1):60—6.
18 Curtis ASG, Dalby MJ, Gadegaard N. Nanoprinting onto cells. *J R Soc Interface* 2006;**3**(8):393—8.
19 Curtis ASG, Dalby M, Gadegaard N. Cell signaling arising from nanotopography: implications for nanomedical devices. *Nanomedicine — UK* 2006;**1**(1):67—72.
20 Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, et al. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat Mater* 2007;**6**(12): 997—1003.
21 Kim DH, Provenzano PP, Smith CL, Levchenko A. Matrix nanotopography as a regulator of cell function. *J Cell Biol* 2012;**197**(3):351—60.
22 Curtis AS, Gadegaard N, Dalby MJ, Riehle MO, Wilkinson CD, Aitchison G. Cells react to nanoscale order and symmetry in their surroundings. *IEEE Trans Nanobiosci* 2004;**3**(1):61—5.
23 Vartanian KB, Kirkpatrick SJ, Hanson SR, Hinds MT. Endothelial cell cytoskeletal alignment independent of fluid shear stress on

micropatterned surfaces. *Biochem Biophys Res Commun* 2008;**371**(4):787—92.
24 Anderson DEJ, Hinds MT. Endothelial cell micropatterning: methods, effects, and applications. *Ann Biomed Eng* 2011;**39**(9):2329—45.
25 Biswas A, Bayer IS, Biris AS, Wang T, Dervishi E, Faupel F. Advances in top-down and bottom-up surface nanofabrication: techniques, applications and future prospects. *Adv Colloid Interface Sci* 2012;**170**:2—27.
26 Garg K, Sell SA, Madurantakam P, Bowlin GL. Angiogenic potential of human macrophages on electrospun bioresorbable vascular grafts. *Biomed Mater* 2009;**4**(3):031001.
27 McClure MJ, Sell SA, Simpson DG, Walpoth BH, Bowlin GL. Tri-layered electrospinning to mimic native arterial architecture using polycaprolactone, elastin, and collagen: a preliminary study. *J Vis Exp* 2011;(47).
28 Sill TJ, von Recum HA. Electro spinning: applications in drug delivery and tissue engineering. *Biomaterials* 2008;**29**(13): 1989—2006.
29 Dalby MJ, Riehle MO, Johnstone H, Affrossman S, Curtis AS. In vitro reaction of endothelial cells to polymer demixed nanotopography. *Biomaterials* 2002;**23**(14):2945—54.
30 Wood MA, Riehle M, Wilkinson CDW. Patterning colloidal nanotopographies. *Nanotechnology* 2002;**13**(5):605—9.
31 Farsari M, Chichkov B. Materials processing: two-photon fabrication. *Nat Photon* 2009;**3**(8):450—2.
32 Liao X, Brown KA, Schmucker AL, Liu G, He S, Shim W, et al. Desktop nanofabrication with massively multiplexed beam pen lithography. *Nat Commun* 2013;**4**.
33 Pranov H, Rasmussen HK, Larsen NB, Gadegaard N. On the injection molding of nanostructured polymer surfaces. *Polym Eng Sci* 2006;**46**(2):160—71.
34 Stormonth-Darling JM, Gadegaard N. Injection moulding difficult nanopatterns with hybrid polymer inlays. *Macromol Mater Eng* 2012;**297**(11):1075—80.
35 Huo F, Zheng Z, Zheng G, Giam LR, Zhang H, Mirkin CA. Polymer pen lithography. *Science* 2008;**321**(5896):1658—60.
36 Li S, Bhatia S, Hu YL, Shiu YT, Li YS, Usami S, et al. Effects of morphological patterning on endothelial cell migration. *Biorheology* 2001;**38**:101—8.
37 Zorlutuna P, Rong Z, Vadgama P, Hasirci V. Influence of nanopatterns on endothelial cell adhesion: enhanced cell retention under shear stress. *Acta Biomater* 2009;**5**(7):2451—9.
38 Biela SA, Su Y, Spatz JP, Kemkemer R. Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano-micro range. *Acta Biomater* 2009;**5**(7):2460—6.
39 Uttayarat P, Chen M, Li M, Allen FD, Composto RJ, Lelkes PI. Microtopography and flow modulate the direction of endothelial cell migration. *Am J Physiol Heart Circ Physiol* 2008;**294**(2):H1027—35.
40 Liliensiek SJ, Wood JA, Yong J, Auerbach R, Nealey PF, Murphy CJ. Modulation of human vascular endothelial cell behaviors by nanotopographic cues. *Biomaterials* 2010;**31**(20):5418—26.
41 Ayres CE, Jha BS, Sell SA, Bowlin GL, Simpson DG. Nanotechnology in the design of soft tissue scaffolds: innovations in structure and function. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2010;**2**(1):20—34.
42 Freed LE, Engelmayr GC, Borenstein JT, Moutos FT, Guilak F. Advanced material strategies for tissue engineering scaffolds. *Adv Mater* 2009;**21**:3410—8.
43 Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew Chem Int Ed Engl* 2009;**48**(30):5406—15.
44 Le Saux G, Magenau A, Bocking T, Gaus K, Gooding JJ. The relative importance of topography and RGD ligand density for endothelial cell adhesion. *PLoS One* 2011;**6**(7):e21869.
45 Hylton DM, Shalaby SW, Latour Jr RA. Direct correlation between adsorption-induced changes in protein structure and platelet adhesion. *J Biomed Mater Res A* 2005;**73**(3):349—58.
46 Sivaraman B, Latour RA. The relationship between platelet adhesion on surfaces and the structure versus the amount of adsorbed fibrinogen. *Biomaterials* 2010;**31**(5):832—9.
47 Le Saux G, Magenau A, Gunaratnam K, Kilian KA, Bocking T, Gooding JJ, et al. Spacing of integrin ligands influences signal transduction in endothelial cells. *Biophys J* 2011;**101**(4):764—73.
48 Shiu YT, Li S, Marganski WA, Usami S, Schwartz MA, Wang YL, et al. Rho mediates the shear-enhancement of endothelial cell migration and traction force generation. *Biophys J* 2004;**86**(4): 2558—65.
49 Dickinson LE, Rand DR, Tsao J, Eberle W, Gerecht S. Endothelial cell responses to micropillar substrates of varying dimensions and stiffness. *J Biomed Mater Res A* 2012;**100**(6):1457—66.
50 Jay SM, Lee RT. Protein engineering for cardiovascular therapeutics: untapped potential for cardiac repair. *Circ Res* 2013;**113**(7):933—43.
51 Dalby MJ, Marshall GE, Johnstone HJ, Affrossman S, Riehle MO. Interactions of human blood and tissue cell types with 95-nm-high nanotopography. *IEEE Trans Nanobiosci* 2002;**1**(1):18—23.
52 Koh L, Rodriguez I, Venkatraman S. The effect of topography of polymer surfaces on platelet adhesion. *Biomaterials* 2010;**31**(7):1533—45.
53 Chen L, Han D, Jiang L. On improving blood compatibility: from bioinspired to synthetic design and fabrication of biointerfacial topography at micro/nano scales. *Colloids Surf B* 2011;**85**(1):2—7.
54 Roach P, Farrar D, Perry CC. Interpretation of protein adsorption: surface-induced conformational changes. *J Am Chem Soc* 2005;**127**(22):8168—73.
55 Sivaraman B, Latour RA. Time-dependent conformational changes in adsorbed albumin and its effect on platelet adhesion. *Langmuir* 2012;**28**(5):2745—52.
56 McCracken K, Tran P, You D, Slepian M, Yoon J-Y. Shear- vs. nanotopography-guided control of growth of endothelial cells on RGD-nanoparticle-nanowell arrays. *J Biol Eng* 2013;**7**(1):11.
57 Walpoth BH, Bowlin GL. The daunting quest for a small diameter vascular graft. *Exp Rev Med Dev* 2005;**2**(6):647—51.
58 Morgan JT, Wood JA, Shah NM, Hughbanks ML, Russell P, Barakat AI, et al. Integration of basal topographic cues and apical shear stress in vascular endothelial cells. *Biomaterials* 2012;**33**(16):4126—35.
59 Hwang S, Kwon K, Jang K, Park M, Lee J, Suh K. Adhesion assays of endothelial cells on nanopatterned surfaces within a microfluidic channel. *Anal Chem* 2010;**82**:3016—22.
60 Lin C-C, Co CC, Ho C-C. Micropatterning proteins and cells on polylactic acid and poly(lactide-co-glycolide). *Biomaterials* 2005;**26**(17):3655—62.
61 Zorlutuna P, Vadgama P, Hasirci V. Both sides nanopatterned tubular collagen scaffolds as tissue-engineered vascular grafts. *J Tissue Eng Regen Med* 2010;**4**(8):628—37.
62 Seunarine K, Meredith DO, Riehle MO, Wilkinson CDW, Gadegaard N. Biodegradable polymer tubes with lithographically controlled 3D micro- and nanotopography. *Microelectron Eng* 2008;**85**:1350—4.
63 Hasan A, Memic A, Annabi N, Hossain M, Paul A, Dokmeci MR, et al. Electrospun scaffolds for tissue engineering of vascular grafts. *Acta Biomater* 2014;**10**(1):11—25.

64 Boland ED, Matthews JA, Pawlowski KJ, Simpson DG, Wnek GE, Bowlin GL. Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front Biosci* 2004;**9**:1422—32.

65 de Valence S, Tille JC, Mugnai D, Mrowczynski W, Gurny R, Moller M, et al. Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model. *Biomaterials* 2012;**33**(1):38—47.

66 Mehta RI, Mukherjee AK, Patterson TD, Fishbein MC. Pathology of explanted polytetrafluoroethylene vascular grafts. *Cardiovasc Pathol* 2011;**20**(4):213—21.

67 Olausson M, Patil PB, Kuna VK, Chougule P, Hernandez N, Methe K, et al. Transplantation of an allogeneic vein bioengineered with autologous stem cells: a proof-of-concept study. *Lancet* 2012;**9838**:230—7.

68 Uttayarat P, Toworfe GK, Dietrich F, Lelkes PI, Composto RJ. Topographic guidance of endothelial cells on silicone surfaces with micro- to nanogrooves: orientation of actin filaments and focal adhesions. *J Biomed Mater Res A* 2005;**75**(3):668—80.