J Mater Sci: Mater Med (2009) 20:1669–1675
DOI 10.1007/s10856-009-3727-z

Poly (lactic-co-glycolic acid) as a controlled release delivery device

Tee Yong Lim · Chye Khoon Poh · W. Wang

Received: 24 October 2008 / Accepted: 3 March 2009 / Published online: 13 March 2009

Abstract Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer used to make resorbable sutures, and is also used in other applications in tissue engineering. Being an artificial polymer, its degradation rate can be tailored to suit its application. It can be easily moulded into structures with suitable mechanical strength and degrades into relatively harmless products in the body. Its adjustable degradation rate also makes it a potentially excellent controlled release delivery device. However, the functionalization of PLGA with bioactive molecules usually requires extensive chemical modification. Chemical modification may compromise the mechanical strength of PLGA and inactivate the bioactive molecules. In this paper, a study is done to investigate the coating of an angiogenic factor on unmodified PLGA suture substrates for the differentiation of human mesenchymal stem cells (hMSC) into endothelial cells (EC). The results show that the method used to anchor vascular endothelial growth factor (VEGF) onto the PLGA surface can enable the gradual release of VEGF from the substrate into solution to induce the differentiation of hMSCs into ECs. Thus, this method can potentially be used to coat PLGA materials like sutures, meshes and scaffolds, rendering them functional as effective controlled release delivery devices for a wide range of bioactive molecules.

T. Y. Lim (✉) · C. K. Poh · W. Wang
Department of Orthopaedic Surgery, National University of Singapore, Kent Ridge, Singapore 119260, Singapore
e-mail: doslty@nus.edu.sg

1 Introduction

Biodegradable polymers play an important role in providing a temporary support for cell growth and differentiation. Ideally, biodegradable polymers must possess the mechanical properties to suit the application and maintain the in vivo ambient environment. They must also degrade into products that are harmless to the cells and tissues in the body after a suitable length of time. PLGA is one such polymer which has been used to make sutures and other implantable structures, as well as 3-dimensional scaffolds as a support for tissue engineering [1]. In order to enhance initial cell attachment and cell differentiation on PLGA scaffolds, it is usually necessary to functionalize the scaffold surface with bioactive molecules [2, 3]. The adjustable degradation rate of PLGA presents an attractive option to make PLGA-based controlled release delivery devices. PLGA can be grafted with bioactive molecules, to be released gradually to support cell differentiation. Unfortunately, the functionalization of PLGA usually requires extensive chemical modification. Chemical surface modification of PLGA can compromise its mechanical strength [4]. Similarly, chemical modification of bioactive molecules may adversely alter their ability to bind onto their respective cell surface receptors, and thus decrease their biological activity [3]. Thus, a method of anchoring bioactive molecules in their native form to a substrate, which can also control the release of the molecules into solution, would be favoured. In this paper, a study is done to investigate the coating of an angiogenic factor, VEGF, on unmodified PLGA suture substrates to support the differentiation of hMSCs into ECs. The results show that the method of coating VEGF on PLGA substrate surface can enable the gradual release of VEGF from the substrate into solution. Furthermore, the released VEGF retains its biological function and is able to induce the differentiation of

hMSCs into ECs. Thus, this method can potentially be used to coat PLGA materials like sutures, meshes and scaffolds, rendering them functional as effective controlled release delivery devices for a wide range of bioactive molecules.

2 Materials and methods

2.1 Materials

PLGA sutures with a glycolic to lactic acid ratio of 90:10 were purchased from Ethicon. Gelatin was purchased from Sigma-Aldrich Chemical Co. (Singapore). Recombinant human VEGF was obtained from R&D Systems (US). Ultrapure water (>18.2 MΩ cm, Millipore Milli-Q system, Singapore) was used in the experiments. All materials used in cell culture were purchased from Gibco, Invitrogen (Singapore). The degradation of PLGA in solution was assessed by measuring the pH of the solution using a inoLab pH meter. Flow cytometry was done using the CyAn^{TM} ADP Analyzer. PE-conjugated CD133 antibody was purchased from eBioscience (San Diego, US). FITC-conjugated polyclonal goat anti-mouse IgG antibody was purchased from AbD Serotec (UK). Flk1-PE was purchased from R&D Systems (US). Unconjugated monoclonal mouse anti-human von Willebrand Factor (vWF) antibody was obtained from Abcam (UK). Unconjugated mouse-anti-human Tie2 antibody was purchased from Santa Cruz (US). The intensity and stability of VEGF coated onto the suture substrate surface were assessed using a human VEGF ELISA kit purchased from Bender MedSystems (Austria). BD Matrigel^{TM} Basement Membrane Matrix was obtained from BD Biosciences (US). Human mesenchymal stem cells were purchased from Lonza (Lonza Walkersville Inc., USA. Product number: PT-2501).

2.2 Preparation of substrates

Suture substrates were prepared by stitching sutures onto a square grid 1 cm × 1 cm marked on the base of a plastic weighing boat, to form a suture mat on plastic base. The suture substrates were sterilised by soaking in 70% ethanol for 1 h, rinsed with copious amounts of ultrapure water and allowed to air-dry in a sterile environment. Each substrate was then coated with a solution of 50 ng of VEGF in 50 μl of a mixture comprising 0.1% gelatin and 1% low-melting agarose in the ratio 1:1, and allowed to dry at room temperature overnight. The substrates were then gently rinsed with PBS and allowed to dry. A second thin coating of the gelatine/agarose mixture was then layered over each substrate. The substrates were rinsed with PBS and allowed to dry before use. All the biological assays were done in duplicates, and each assay was done twice.

2.3 Assessment of the degradation rate of PLGA in solution

PLGA suture substrates were prepared as described above. The degradation rate of PLGA in water, PBS and MEM-α supplemented with 10% FBS, was assessed by measuring the change in pH of the respective solutions. One piece of substrate each was placed in 10 ml of the respective solution, and the pH of each solution was measured on days 1, 5, 10, 15, 20 and 25.

2.4 Stability of coated VEGF on suture substrates

For the assessment of the stability of VEGF on the suture substrates, the substrates were trimmed to size and placed individually in a 24-well plate containing 1 ml of PBS per well. The amount of VEGF released from each substrate surface into solution was assessed on days 1, 5, 10, 15, 20 and 25. At each respective point in time, 50 μl of PBS was removed from a well marked with the respective time-point. The amount of VEGF in solution was then measured using the Bender Medsystems human VEGF ELISA kit, following the manufacturer's instructions. The colour intensity of the reaction mixture was measured at 450 nm using an absorbance plate reader. The amount of the VEGF in solution was determined using a series of VEGF solutions of known concentrations as standards which were prepared using a VEGF stock supplied in the ELISA kit. The amount of VEGF at each point in time was then expressed as a percentage of the initial amount of VEGF coated on each suture substrate.

2.5 Cell culture

Human mesenchymal stem cells (hMSC) were seeded into cell culture flasks and cultured in a medium comprising Minimal Essential Medium-alpha (MEM-α), supplemented with 10% foetal bovine serum (FBS), L-glutamine (2 mmol/l), penicillin (100 U/ml) and streptomycin sulphate (100 μg/ml), and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Unattached cells were removed on the following day. Half of the culture medium was replaced by a fresh aliquot of medium once every 2 days. The attached cells were allowed to grow to about 75% confluence. Cells colonies that were formed were identified and individually removed by gentle trypsinization, seeded into a new flask each and allowed to grow to about 75% confluence before being used. Passage 2 hMSCs were used throughout the study. Attached cells were

detached by trypsinization and resuspended in fresh culture medium for subsequent experiments described below.

2.6 Flow cytometry

Cells were detached by trypsinization and then incubated in PBS with 2% FBS for 10 min in an ice bath. 1 µl of the CD133-PE antibody was added to the cells, suspended in 100 µl of PBS with 2% FBS, in separate tubes. After incubation for 10 min in an ice bath in the dark, the cells were washed, resuspended in 1 ml of PBS, and analysed using flow cytometry. A total of 10,000 events were collected for the analysis.

2.7 Preparation of culture medium containing VEGF

For the preparation of VEGF-containing medium, the suture was stitched onto both sides of each plastic substrate and coated with VEGF. In subsequent discussions, these substrates will be termed double-sided substrates. Five pieces of double-sided substrates were placed into a small petri dish, such that the substrates were completely submerged in 3 ml of culture medium containing 2% serum.

2.8 Effect of VEGF release from suture substrate on MSC differentiation

hMSCs were seeded onto cover slips, each placed in a single well of a 6-well plate at a density of 5,000 cells/cm^2. Unattached cells were removed the following day, and the cells were allowed to grow for another 2 days. The cover slips were then removed from the 6-well plate and individually placed centrally in a petri dish containing five pieces of VEGF-coated double-sided substrates which were lined along the inner circumference of the dish. The negative controls comprise a petri dish with cells in normal medium and another dish with cells grown in medium that had uncoated substrates immersed in it. A dish without any substrate but containing culture medium supplemented with 10 ng/ml of VEGF and 2% serum served as the positive control. The experiment was carried out for 14 days. Half of the medium was removed and replaced with fresh medium every third day.

2.9 Immunofluorescent (IF) microscopy

Cells cultured on the cover slips were fixed in methanol at −20°C for 10 min and then rinsed with PBS. Following which, the cells were incubated with the relevant fluorochrome-conjugated antibody (1:300) for 1 h. For von Willebrand Factor (vWF) staining and Tie2 staining, where unconjugated primary antibodies were used, the cells were rinsed in PBS and further stained with FITC-conjugated goat anti-mouse secondary antibody (1:300) for 1 h. The cells were then viewed using a fluorescent microscope (Olympus IX71), at a magnification of 100× (for VEGFR2) and 200× (for Tie2, vWF and CD31).

2.10 In vitro capillary-like tube formation assay

The assay was performed using 50 µl of BD Matrigel in each well of a 96-well dish at 4°C. The matrigel was left at room temperature for 1 h to allow it to solidify before use. Cells grown on the cover slips were detached with trypsin and the trypsin was removed by centrifugation. The cells were resuspended in normal cell culture medium and 2,000 cells in 50 µl of medium was plated onto the matrigel. The cells were then incubated for 3 h and the capillary-like tube formation was observed under a light microscope.

2.11 Statistical analysis

The results were assessed statistically using one-way analysis of variance. Statistical significance was accepted at $P < 0.05$.

3 Results

3.1 Assessment of the degradation rate of PLGA in solution

The degradation rate of PLGA sutures in water, PBS and cell culture medium was monitored by measuring changes in the pH of the respective solutions over 25 days (Fig. 1).

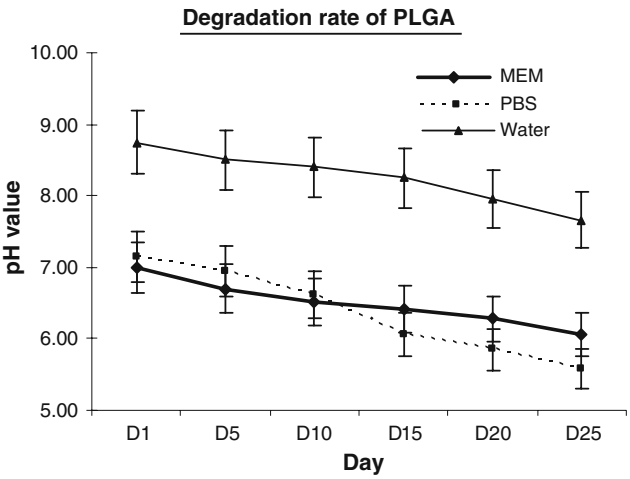

Fig. 1 The degradation rate of PLGA sutures in water, PBS and cell culture medium was monitored by changes in the pH of the respective solutions over 25 days ($n = 2$). Error bars represent the standard deviations. The change in pH is the smallest in MEM, indicating that MEM probably has a higher buffering capacity as compared with the other two media

The pH values show a decrease of about 1.09 (water), 1.56 (PBS) and 0.93 (MEM) after 25 days. The buffering capacity of the cell culture medium has probably contributed to the smaller change in pH as compared with the other two media.

3.2 Stability of coated VEGF on suture substrates

The substrates were coated with a high concentration of VEGF in a VEGF–gelatin–agarose mixture to ensure that the entire substrate surface was covered with the angiogenic factor. A thin layer of gelatin–agarose mixture was layered over the substrate to protect the VEGF from being removed due to abrasion, and to facilitate an even rate of diffusion of VEGF into solution. VEGF was progressively released into solution and its concentration reached a peak at day 15 (Fig. 2). Subsequently up to day 25, VEGF concentration began to decline.

3.3 Flow cytometry

Passage 2 hMSCs were subjected to flow cytometry analysis to check for the presence of endothelial cell progenitors. No detectable CD133+ cells was found.

3.4 In vitro differentiation of hMSCs into endothelial cells

The hMSCs express the endothelial cell markers, Flk-1 (VEGFR-2), Tie2 and vWF after a 7-day culture in medium

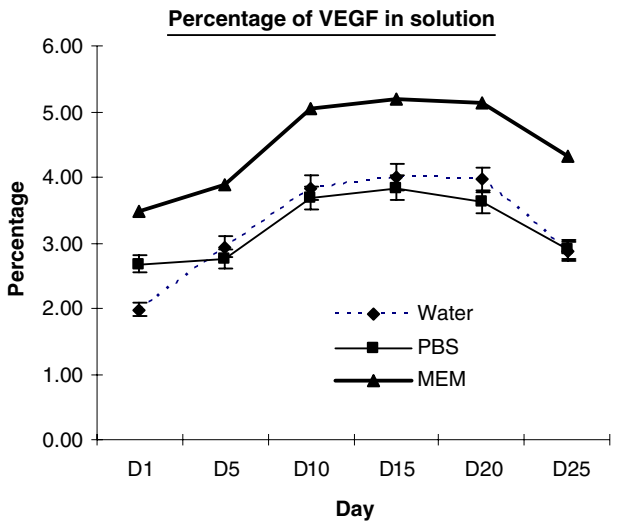

Fig. 2 Concentration of VEGF in solution, expressed as percentage of the initial coating concentration of VEGF on the suture substrate ($n = 2$). Error bars represent the standard deviations. VEGF is progressively released and its concentration in solution reaches a peak at about day 15, which corresponds to approxiamately 2.6 ng/ml (MEM), 2 ng/ml (PBS) and 2 ng/ml (water) per substrate

with VEGF released from the VEGF suture substrates (Fig. 3a, c). After a 14-day differentiation, the differentiated cells also show a weak expression of CD31 (Fig. 3d). The ability of the differentiated cells to form a capillary network on semi-solid medium was demonstrated in an in vitro angiogenesis assay using Matrigel (Fig. 4a). A visual count revealed that about 90% of the cells exposed to VEGF form tube-like structures. hMSCs in the negative controls did not form any capillary network and remained as single cells on the Matrigel (Fig. 4b).

4 Discussion

PLGA is a synthetic biodegradable polymeric material which has been used as a material for sutures, and for the fabrication of scaffolds in tissue engineering to provide temporary support for cellular survival and tissue development. It has a controllable degradation rate, possesses suitable mechanical strength, and is also an easy material to mould into different shapes [2, 3]. Its adjustable degradation rate also makes it suitable to be used as a controlled release delivery device. Unfortunately, PLGA usually needs to be chemically modified so that other molecules may be covalently grafted onto its surface. Chemical modification may consequently weaken the mechanical strength of PLGA [1, 4]. Similarly, most bioactive molecules which are intended to be grafted onto PLGA usually also need to be chemically modified [5, 6]. For example, VEGF can be oxidized using periodate oxidation for subsequent grafting onto dihydrazide modified PLGA [3]. However, chemical modification of a bioactive molecule may inactivate it or alter its effective biological function. The oxidized forms of two human VEGF isoforms, $VEGF_{165}$ and $VEGF_{121}$, have been reported to lose their ability to bind to the VEGF receptor, VEGFR-2 [2]. While cell-surface heparan-sulphate proteoglycans can restore the VEGFR-2 binding ability of oxidized $VEGF_{165}$ by playing a chaperone-like role, the same process cannot restore the VEGFR-2 binding ability of oxidized $VEGF_{121}$. Thus, the reported decrease in activity of $VEGF_{121}$ could probably be due to oxidative damage. Hence, a method to functionalize a biodegradable material without compromising its mechanical strength and degradation properties would be an advantage. Similarly, bioactive molecules should be attached to a matrix without any alteration to their chemical structure which may consequently compromise their biological activity. In this study, a simple and effective method of coating PLGA sutures with VEGF was used. PLGA degrades by hydrolysis into its constituent lactic and glycolic acids [7]. As degradation proceeds, the presence of more acidic groups may thus autocatalyze the hydrolysis process. For this reason, it can be expected that the release

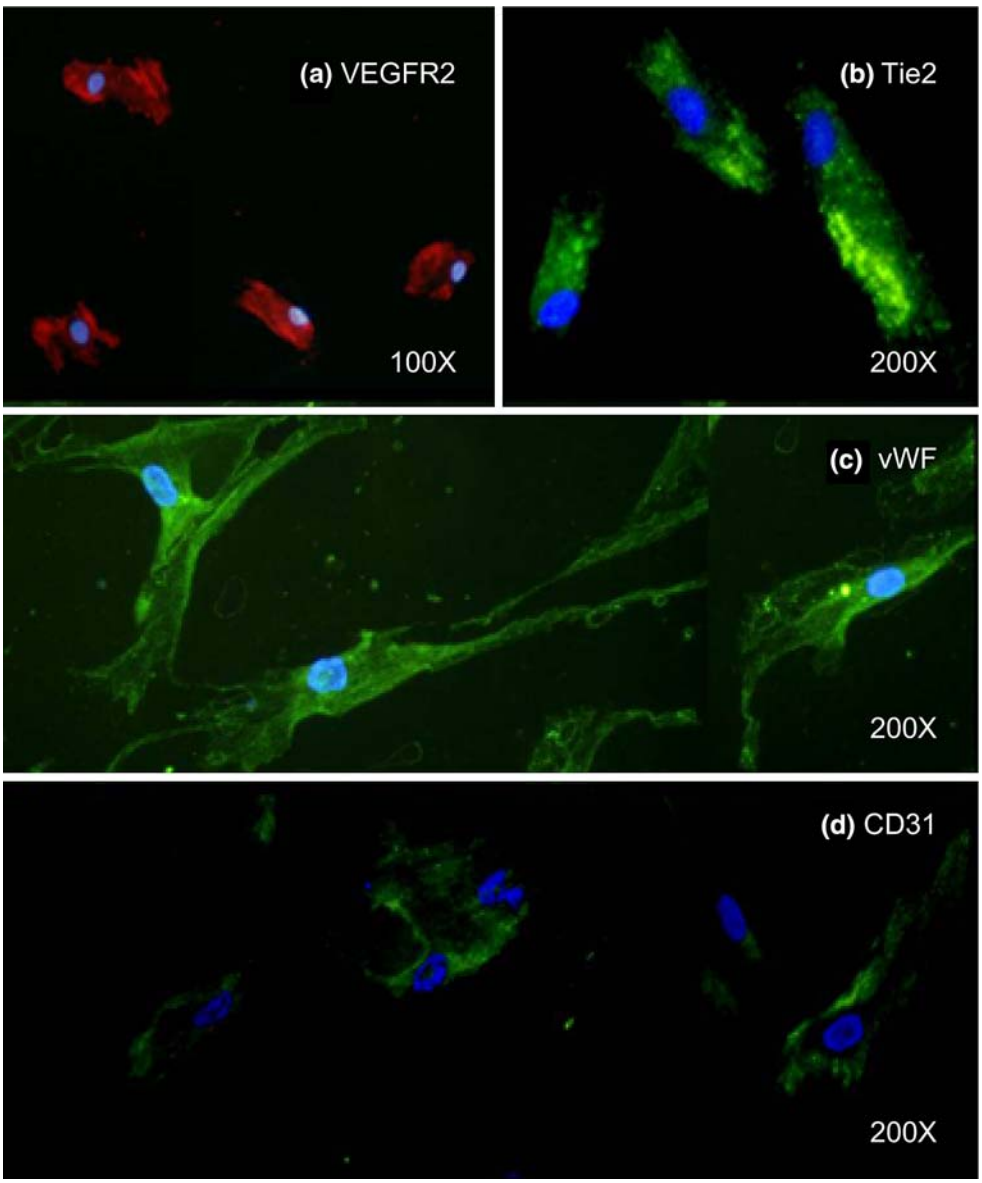

Fig. 3 Immunofluorescent staining reveals that the hMSC-derived cells show expression of EC markers, VEGFR2 (Flk-1), Tie2 and vWF (**a**–**c**) after a 7-day differentiation. The weak expression of the late EC marker, CD31, was detected after a further 7 days of differentiation (**d**)

kinetics of any bioactive molecule, in this case, VEGF, will be affected. The degradation of the PLGA sutures was studied by monitoring the changes in the pH of each of the solutions in which the sutures are submerged. The results show that the PLGA suture substrates underwent a gradual degradation process. The degradation in cell culture medium had the smallest change in pH. This could be due to the greater buffering capacity of the culture medium. The control of pH in vivo could be an important consideration if PLGA were to be used as a delivery device, as large pH changes may affect the degradation rate of PLGA. The in vivo environment is dynamic: as PLGA degrades, blood flow and tissue fluid surrounding the implant site would probably remove the products of degradation, and thus prevent the accumulation of the acidic break-down products. This would reduce the rate of autocatalytic degradation of the PLGA implant, and consequently prevent a massive release of the attached VEGF. In this study, the degradation rate of PLGA was correlated with the release profile of VEGF from the PLGA substrate over a similar length of time. VEGF is chosen because it plays a key role in vasculogenesis and angiogenesis. Due to this important role, VEGF is often used in the in vitro differentiation of haematopoietic stem cells or endothelial progenitor cells into endothelial cells [8–10]. VEGF is known to have a short half-life in solution [11]. Hence, it is paramount that VEGF must be continually released at an optimal rate so that there are sufficient biologically active quantities of it

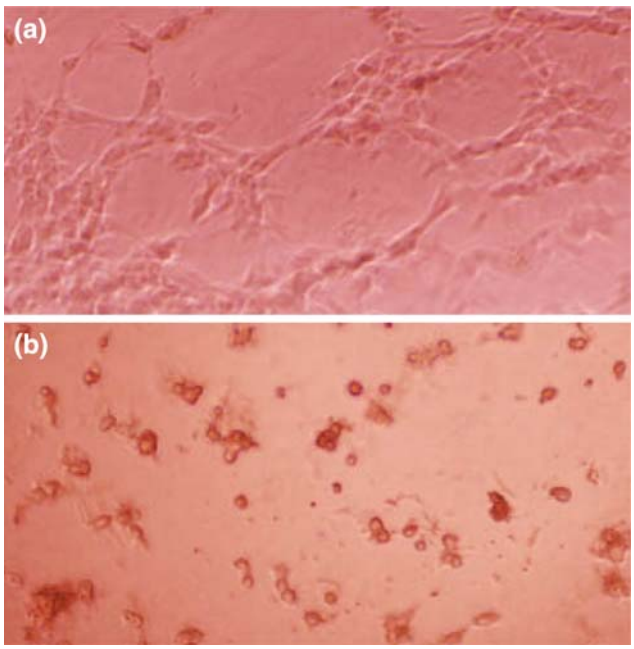

Fig. 4 Endothelial cells differentiated from hMSCs display a capillary network formation on Matrigel. A visual count reveals that about 90% of the cells form the network (**a**). Untreated hMSCs remain as isolated, single cells on the Matrigel surface (**b**)

in solution to mediate EC differentiation. The results show that VEGF is progressively released from the substrate surface and reached a peak on day 15. This indicates that our PLGA suture substrate is capable of the sustained release of amounts of biologically active VEGF.

The hMSCs have been characterized by flow cytometry and confirmed by the supplier to express MSC markers including CD105. Using flow cytometry, a further check was done to ensure that there were no endothelial progenitors present among the hMSCs. The presence of CD133+ cells was not detected. The aim of this study is to test if the VEGF released from the suture substrates can induce the differentiation of the CD105+ hMSCs into ECs. The results show that the hMSC-derived cells expressed VEGFR2 (Flk-1), Tie2 and vWF after 7 days of VEGF-mediated differentiation. However, CD31, which has been implicated in various biological phenomena, including cell migration, angiogenesis, transendothelial migration and modulation of integrin-mediated cell adhesion, was not detected on the differentiated cells after a 7-day VEGF treatment. This is consistent with the observation similarly reported by others [12, 13]. After a further 7 days of differentiation, the weak expression of CD31 was detected, indicating that this marker is expressed late in EC differentiation. Based on this finding, an in vitro angiogenesis assay was carried out. hMSC-derived cells differentiated for 14 days in medium with VEGF released from the suture substrates were plated onto a Matrigel surface. The formation of a capillary network was observed 3 h later. A visual count showed that about 90% of the treated cells form the tube-like network. Similarly, about 90% of hMSCs cultured in normal medium supplemented with 10 ng/ml (positive control) form a capillary network on Matrigel. Undifferentiated hMSCs in medium without VEGF (negative control) remained as single cells on the Matrigel. As the hMSCs had been ascertained to be non-CD133+, the possibility that the differentiated cells originated from a small population of endothelial progenitors can be excluded. Thus, the results indicate that the method used in this study to coat the PLGA substrate with VEGF can retain the biological activity of this growth factor, and progressively release it to mediate EC differentiation.

Various other methods of immobilizing bioactive molecules onto substrates have been reported, with most of them requiring extensive modification to the substrate surface and to the bioactive molecules. Despite the potential risks that chemical and genetic modifications pose to the performance of both the substrates and the bioactive molecules, there have been some encouraging results. Basic fibroblast growth factor (bFGF) loaded within heparin conjugated PLGA microspheres has been shown to be released gradually in vivo to support angiogenesis [14]. Heparin is a highly sulphated glycosaminoglycan that has binding affinities to various growth factors. Heparin also helps to mitigate the mitogenic activity of growth factors through the growth regulation of fibroblast and endothelial cells [15]. Thus, heparin is commonly used in the fabrication of matrices for the controlled release of growth factors [16, 17]. Others have combined condensed plasmid DNA encoding osteogenic growth factors and angiogenic growth factors, together with bone marrow stromal cells for loading into PLGA scaffolds. The scaffolds have been used for the in vivo induction of bone tissue regeneration and blood vessel formation [18]. Although not as potentially risky as cellular transplantation of virus-mediated genetically modified cells, substrate-immobilized plasmid DNA may result in the continuous secretion of a high level of a growth factor. Thus, plasmid DNA encoding an angiogenic factor like bFGF may accelerate the growth of pre-existing tumours or occult cancer, although bFGF is only weakly oncogenic [19–24]. Furthermore, continuous delivery of a relatively high concentration of bFGF can possibly worsen diabetic retinopathy [25]. A growth factor can also be anchored to a substrate using a cysteine tag [26]. However, this method involves the genetic modification of VEGF and its expression in *Escherichia coli*. The modified VEGF carries a cysteine tag that allows it to be conjugated to fibronectin using a cross-linking agent. Although fusion tag conjugates may be an attractive alternative to immobilize bioactive molecules onto implantable substrates, it involves

extensive genetic modification and relies on the use of micro-organisms to express the modified protein.

Each additional step in a modification procedure can potentially compromise the performance of an implantable device, as well as increase the risk potential associated with its application. It is therefore reasonable to be concerned that extensively modified implants and bioactive molecules can be potentially hazardous in vivo. Thus, simple functionalization procedures should be as widely adopted as possible. The method of immobilizing VEGF onto PLGA substrates reported here possesses the advantage of ease of fabrication and poses a relatively minimal risk in vivo. Thus, this method can serve as a model for the attachment of other bioactive molecules onto various types of substrates, especially for the fabrication of controlled release devices.

Acknowledgement This study is funded with a grant from the Singapore A*STAR/BMRC.

References

1. Wen F, Chang S, Toh YC, Teoh SH, Yu H. Development of poly (lactic-co-glycolic acid)-collagen scaffolds for tissue engineering. Mater Sci Eng C. 2006;27:285–92. doi:10.1016/j.msec.2006.05.007.
2. Nakamura M, Abe Y, Tokunaga T. Pathological significance of vascular endothelial growth factor A isoform expression in human cancer. Pathol Int. 2002;52:331–9. doi:10.1046/j.1440-1827.2002.01367.x.
3. Sharon JL, Puleo DA. Immobilization of glycoproteins, such as VEGF, on biodegradable substrates. Acta Biomater. 2008;4:1016–23. doi:10.1016/j.actbio.2008.02.017.
4. Chu CC. Hydrolytic degradation of polyglycolic acid: tensile strength and crystallinity study. J Appl Polym Sci. 1980;26:1727–34. doi:10.1002/app.1981.070260527.
5. Lee ES, Na K, Bae YH. Polymeric micelle for tumor pH and folate-mediated targeting. J Control Release. 2003;91:103–13. doi:10.1016/S0168-3659(03)00239-6.
6. Stella B, Arpicco S, Peracchia MT, Desmaele D, Hoebeke J, Renoir M, et al. Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci. 2000;89:1452–64. doi:10.1002/1520-6017(200011)89:11<1452::AID-JPS8>3.0.CO;2-P.
7. Catiker E, Guner A, Gumusderelioglu M. Degradation of PLA, PLGA homo- and copolymers in the presence of serum albumin: a spectroscopic investigation. Polym Int. 2000;49:728–34. doi:10.1002/1097-0126(200007)49:7<728::AID-PI443>3.0.CO;2-3.
8. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood. 2000;95:3106–12.
9. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. Br J Haematol. 2001;115:186–94. doi:10.1046/j.1365-2141.2001.03077.x.
10. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood. 2001;98:2615–25. doi:10.1182/blood.V98.9.2615.
11. Ennett AB, Kaigler D, Mooney DJ. Temporally regulated delivery of VEGF in vitro and in vivo. J Biomed Mater Res. 2006;79:176–84. doi:10.1002/jbm.a.30771.
12. Jackson DE. The unfolding tale of PECAM-1. FEBS Lett. 2003;540:7–14. doi:10.1016/S0014-5793(03)00224-2.
13. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells. 2004;22:377–84. doi:10.1634/stemcells.22-3-377.
14. Chung HJ, Kim HK, Yoon JJ, Park TG. Heparin immobilized porous PLGA microspheres for angiogenic growth factor delivery. Pharm Res. 2006;23:1835–41. doi:10.1007/s11095-006-9039-9.
15. Lee H, Cusick RA, Browne F, Ho Kim T, Ma PX, Utsunomiya H, et al. Local delivery of basic fibroblast growth factor increases both angiogenesis and engraftment of hepatocytes in tissue-engineered polymer devices. Transplantation. 2002;73:1589–93. doi:10.1097/00007890-200205270-00011.
16. Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res. 2003;65:489–97. doi:10.1002/jbm.a.10542.
17. Steffens GC, Yao C, Prevel P, Markowicz M, Schenck P, Noah EM, et al. Modulation of angiogenic potential of collagen matrices by covalent incorporation of heparin and loading with vascular endothelial growth factor. Tissue Eng. 2004;10:1502–9.
18. Huang YC, Kaigler D, Rice KG, Krebsbach PH, Mooney DJ. Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. J Bone Miner Res. 2005;20:848–57. doi:10.1359/JBMR.041226.
19. Cohen H, Levy RJ, Gao J, Fishbein I, Kousaev V, Sosnowski S, et al. Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. Gene Ther. 2000;7:1896–905. doi:10.1038/sj.gt.3301318.
20. Cornetta K, Smith FO. Regulatory issues for clinical gene therapy trials. Hum Gene Ther. 2002;13:1143–9. doi:10.1089/104303402320138925.
21. Krebsbach PH, Gu K, Franceschi RT, Rutherford RB. Gene therapy-directed osteogenesis: BMP-7-transduced human fibroblasts form bone in vivo. Hum Gene Ther. 2000;11:1201–10. doi:10.1089/10430340050015248.
22. Peters MC, Polverini PJ, Mooney DJ. Engineering vascular networks in porous polymer matrices. J Biomed Mater Res. 2002;60:668–78. doi:10.1002/jbm.10134.
23. Sheridan MH, Shea LD, Peters MC, Mooney DJ. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. J Control Release. 2000;64:91–102. doi:10.1016/S0168-3659(99)00138-8.
24. Simari RD, O'Brien T. Clinical trials in vascular gene therapy: the missing piece of the puzzle. Arch Pathol Lab Med. 2002;126:317–9.
25. Bensaid M, Malecaze F, Prats H, Bayard F, Tauber JP. Autocrine regulation of bovine retinal capillary endothelial cell (BREC) proliferation by BREC-derived basic fibroblast growth factor. Exp Eye Res. 1989;48:801–13. doi:10.1016/0014-4835(89)90065-1.
26. Backer MV, Patel V, Jehning BT, Claffey KP, Backer JM. Surface immobilization of active vascular endothelial growth factor via a cysteine-containing tag. Biomaterials. 2006;27:5452–8. doi:10.1016/j.biomaterials.2006.06.025.