Differences in performance of PCL-based vascular grafts as abdominal aorta substitutes in healthy and diabetic rats

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ARTICLE

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Great progress has been made in the field of vascular tissue engineering, with some artificial vascular grafts already exhibiting promising outcomes in animal models. However, these studies were mostly conducted using healthy animals, which are not representative of actual clinical demands. Indeed, patients who require artificial vascular grafts implantation are often accompanied with other comorbidities, such as hyperlipidaemia, hypertension and diabetes which should also be taken into consideration when assessing the potential of vascular grafts that are intended for clinical applications. In the present study, we established a rat model with type 2 diabetes (T2D) for performance evaluation of an electrospun PCL vascular graft. Our data showed that rats with T2D have elevated incidents of adverse event rates, including exacerbated platelet adhesion, inflammation, early calcification and impaired regeneration compared to the non-diabetic controls. Thus, we report that T2D exacerbates the regeneration process after in vivo implantation of vascular grafts. More advanced grafts are in demand for clinical use in patients with clinical complications such as T2D.

1 Introduction

Cardiovascular disease is one of the major disorders with high morbidity and mortality rates. Patients with cardiovascular diseases are often treated by either percutaneous coronary intervention angioplasty (PCI) or surgical bypass [1], the latter of which requires a vascular graft to redirect blood flow around the lesion. As a result, extensive efforts have been devoted into developing artificial vascular grafts in recent years. The strategies include tissue engineered vascular graft (TEVG) in vitro and tissue-engineered blood vessel in vivo, also known as "cell-free" vascular graft [2]. In general, TEVGs are constructed in vitro and surgically implanted in patients. This procedure is extremely time-, labor-intensive, cost-ineffective and greatly limits their clinical potential [1]. As a result, the cell-free vascular graft has become the primary option for vascular graft engineering [3]. A variety of polymers, such as poly(l-lactic acid) (PLLA) [4] and poly(glycerol sebacate) (PGS) [5], have been used to develop vascular grafts with some exhibiting excellent in vivo performance. Yadong Wang's group has reported an inspiring work [6], constructing a cell-free synthetic graft which was successfully integrated as neo-artery in rat abdominal artery. The synthetic graft showed excellent biocompatibility and stability 12 months after implantation [7]. However, despite encouraging preliminary results, these studies were mostly conducted in healthy animals. Few publications have investigated the performance of vascular grafts using disease animal models, which is to the contrary of clinical setting where patients requiring artificial vascular graft implantation are often accompanied with other comorbidities.

Diabetes is now recognized as a major health issue, currently afflicting approximately 415 million people worldwide [8]. Cardiovascular disease is the main cause of death in people with diabetes, accounting for over 50% of all diabetes-incurred fatalities in China. Moreover, in the US, one-third of deaths in elderly patients undergoing percutaneous coronary intervention (PCI) are also attributable to diabetes as reported by World Health Organization (WHO). In fact, long-term diabetes is now considered as a significant contributor to multiple systemic dysfunctions, including reduced level of epithelial progenitor cells (EPCs) [9], chronic inflammation [10], disruption of endothelial cells (ECs) proliferation, migration, function and survival [11, 12], all of which are attributable to impaired vascular regeneration, especially following vascular grafts implantation. Extensive evidence is also supportive of positive correlation of diabetes with long-term adverse events in patients following drug elution stents (DES) and/or bare-metal stents (BMS) implantation [13]. As a result, complications such as diabetes should be taken into

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consideration in research studies assessing the clinical potential of vascular grafts in vivo.

In recent years, poly-ε-caprolactone (PCL) has been implicated in vascular graft engineering owning to its excellent structural integrity, biological compatibility and degradability [14]. Walpoth's group has published a series of studies using PCL as vascular scaffolds [15, 16]. They reported excellent in vivo outcome, including 100% patency, rapid endothelialisation and no thrombosis 18 months after implantation of a PCL vascular graft fabricated by electrospinning in rat abdominal artery [15]. Moreover, PCL could also be fabricated into porous micro and macro-fibrous structures, which would facilitate cell infiltration in vivo. Indeed, we have previously developed a macroporous electrospun PCL vascular graft with thick fibers (5-6 μm) and larger pores (~30 μm). In vivo results demonstrated significantly enhanced cell infiltration, vascularisation and efficient regeneration of functional tunica media after graft implantation, supporting the fact that PCL could be a promising candidate for the development of small diameter vascular substitute [17]. In addition, the arginine-glycine-aspartic acid (RGD) moiety has been widely incorporated in tissue engineering to improve cell compatibility of scaffolds and various biomaterials [18]. RGD modified PCL vascular graft has shown excellent in vivo performance, with rapid endotheliazation and smooth muscle cells regeneration being observed in a rabbit carotid artery model [19]. However, while these data suggested a promising potential of RGD-incorporated PCL vascular graft in clinical application, these works were performed in healthy animal models, which overlook the actual complications of patients who require vascular surgeries. In this study, we established a rat model with Type 2 diabetes (T2D), in which the in vivo performance of an electrospun PCL vascular graft with and without RGD modification was evaluated.

2 Results

2. 1 Establishment of the rat model of type 2 diabetes

After the rats were treated with high glucose and high fat diet, their blood sugar level was above 16.7 mmol/L at the determined time points as shown in Figure 1C, indicating that these rats became diabetic. We also tested the fasting plasma insulin level of these rats. For rats in the high fat diet group, Figure 1A showed that their average fasting plasma insulin level was around 47.4±7.3 μIU/mL, which is significantly higher than what we obtained from the control group (20.1±2.5 μIU/mL). OGTT test results (13.0±0.7 mmol/L) obtained from rats treated by high fat diet are also much higher than the normal level (5.9±0.1 mmol/L) (Figure 1B), and are even higher than the common standard for type 2 diabetes (11.1 mmol/L). All these results demonstrated that we successfully established the rat model of type 2 diabetes. Then we divided these rats into two groups by random.

2.2 Graft fabrication and characterization

The tubular grafts were fabricated under the conditions similar to electrospun mats fabrication. The morphology of PCL-RGD and PCL graft was similar. Therefore, only PCL grafts were shown as representatives (Figure 2). The inner-diameter

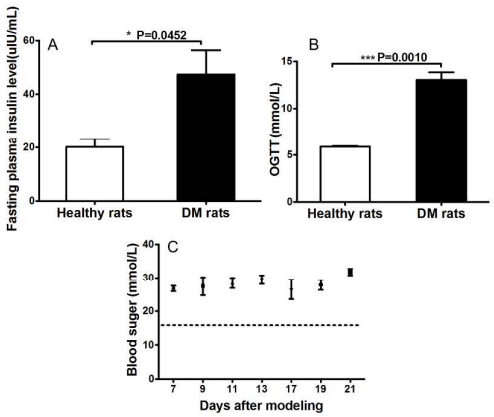

Fig 1. The establishment of the rats model of type 2 diabetics. The fasting plasma insulin level in the diabetic rats group was significantly higher than Non diabetics group (A). OGTT were much higher in diabetics than non-diabetics and also higher than the common standard for type 2 diabetes (11.1mmol/L) (B) blood sugar level all climbed to 16.7 mmol/L and was stable before and after surgery (C).

of the grafts was 2.0 mm and the wall thickness is about 400-500 μm (Figure 2A). The morphology of PCL and PCL-RGD grafts were observed under SEM (Figure 2B, 2C). The averaged diameter was 0.66 ± 0.32 μm for PCL grafts, whereas it was 0.73 ± 0.29 μm for the PCL-RGD grafts. The pore size was 11.51 ± 0.30 μm and 11.35 ± 0.45μm (Table 1).

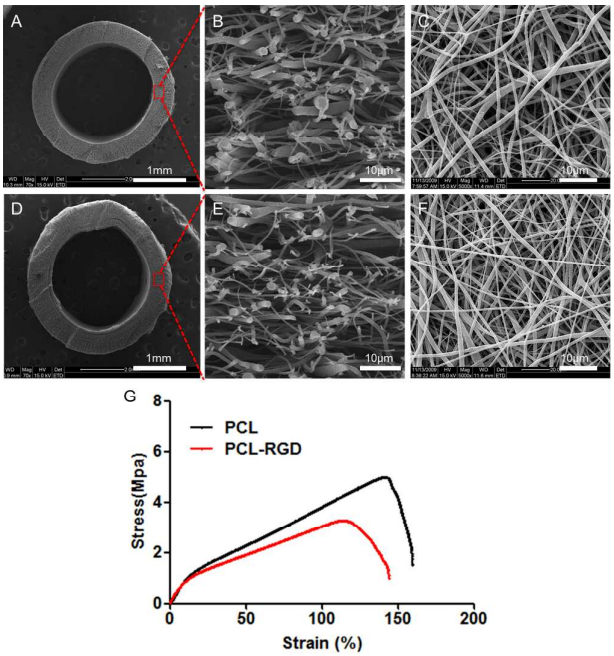

Fig 2. Morphology of PCL and PCL-RGD vascular grafts observed by SEM (ID=2mm). Cross-sections were shown as A and D. B and E was the magnification of A and D. C and F were the inner surface of the grafts and G was the representative stress-strain curve. A, B, C were PCL graft and D, E, F were PCL-RGD graft.

Table1. Quantitative measurement of the structural parameters of the electrospun PCL grafts. (N=20)

	PCL grafts	PCL-RGD grafts
Fiber diameter (μm)	0.66±0.32	0.73±0.29
Pore size(μm)	11.51±0.30	11.35±0.45

The mechanical properties of the vascular grafts were evaluated by tensile test. In general, the stress-strain curve of RGD-functionalized PCL was similar to that of pure PCL (figure 2G), both of which demonstrating high level of toughness. The mechanical properties (including tensile strength, Young's modulus and elongation at break) were shown (Table 2), which were still sufficient to satisfy the requirement of artificial blood vessel.

Table2. Mechanical properties measured by tensile test. (N=4)

	PCL graft	PCL-RGD grafts
Young's modulus (MPa)	7.759 ± 0.95	6.43 ± 0.82
Stress at max (MPa)	4.07 ± 0.15	4.32 ± 0.48
Strain at break (%)	152.20 ± 14.98	115.70 ± 32.82

2.3 Blood compatibility

The blood compatibility was tested in the arteriovenous shunt model (AV-shunt). There was no visible thrombus in either PCL or PCL-RGD grafts after in contact with whole blood for 2 hours under the physiological condition. Subsequently, SEM images showed that platelet adhesion on PCL-RGD (Figure 3A) surface was less than that on PCL surface (Figure 3B). However, in diabetic rats, lots of protein and platelets covered on the surface of the graft lumen in both PCL (Figure 3C) and PCL-RGD group (Figure 3D). In addition, mepacrine staining, a specific staining approach for platelet, was performed and examined by confocal laser microscopy. As shown in Figure 3, PCL and PCL-RGD grafts retrieved from diabetic animals showed substantial increase of platelets adhesion (Figure 3C' and D') compared to grafts from the non-diabetic controls (Figure 3A' and B').

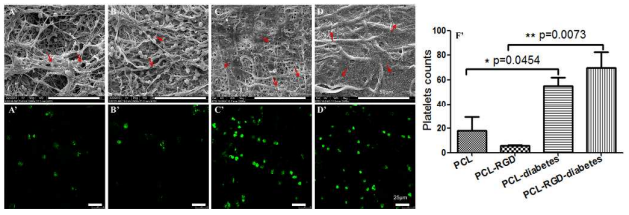

Fig 3. Blood compatibility of PCL and PCL-RGD in Arteriovenous shunt assessment. Images were representatives from at least three independent samples. SEM characterization of the luminal surface of PCL (A, C) and PCL-RGD (B, D) graft implanted for 2 h. Red arrows indicated platelets in SEM image. Images from Mepacrine staining were shown as A', C' (PCL) and B', D'(PCL-RGD). The quantitative data from mepacrine staining was shown as F'. Image A, B and A', B' represented in Non-diabetic rats, while C, D and C', D' in diabetic rats.

2. 4 Patency of the grafts

Grafts were implanted into the abdominal aorta of rats in both the diabetic and the non-diabetic group for 1 month (Figure 4A). Throughout the entire period, there was no bleeding, infection or ischemia of legs observed in any of the rats (Figure 4C). Angiographies were carried out at the time of sacrifice to confirm the patency of grafts (Figure. 4B). As a result, all grafts in the two groups were patent. After exposure (Figure 4D) grafts were clear and no aneurysm was observed.

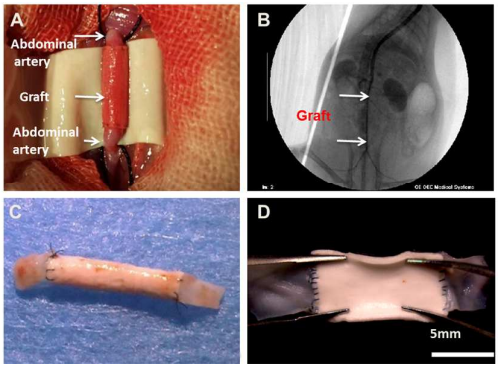

Fig 4. 2.0 mm inner diameter vascular grafts made of electrospun PCL micro and nano-fibers were used for infrarenal abdominal aorta replacements in the rat model (A); All grafts were patent by angiography at 1month (B); Implanted grafts had no bleeding, leakage or aneurysmal dilatation (C); and grafts were free of any macroscopic thrombi visualized by stereomicroscope (D).

2. 5 Platelet adhesion

Data from SEM (Figure 5) showed that at the suture site, the grafts were covered by endothelial cells, which grew in continuity to the host artery and were elongated in the direction of the blood flow, in both Non diabetics and diabetics groups (upper panel). However, in the middle part of grafts, there were almost no platelet (PCL and PCL-RGD) in the Non diabetics whereas there were plenty of platelet aggregates in the diabetics group (lower panels).

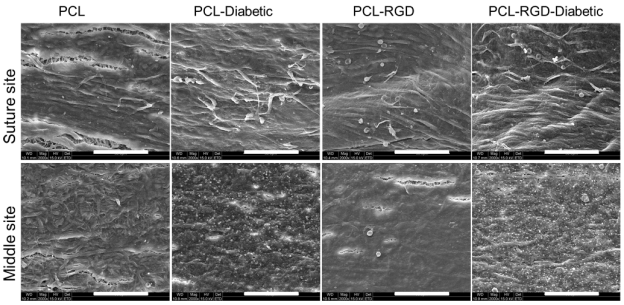

Fig 5. Luminal surfaces of explanted grafts at 1 month. At the suture site, the grafts were covered by endothelial cells, which grew in continuity to the host artery and were elongated in the direction of the blood flow, in both Non-diabetic and diabetics group (upper panel). However, in the middle part of grafts, there are almost no platelets deposit (PCL and PCL-RGD) in the Non-diabetics whereas there are plenty of platelet aggregates in the diabetics group (lower panels), scale bar: 50 μm.

2. 6 Inflammatory and calcification

From H&E staining (Figure 6), we observed many macrophages in the vascular wall beneath the lumen in the diabetic group, whereas few were found in the normal group. These data indicated that inflammatory response in diabetics group was much stronger than minimal in the non-diabetics group. In addition, calcification was observed in one rat of the diabetic group one month after implantation (Figure 6E).

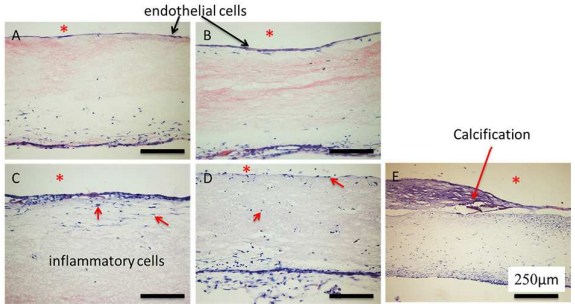

Fig 6. H&E staining. Longitudinal sections of PCL in Non-diabetic rats (A), PCL graft in Diabetic rats (C), PCL-RGD graft in Non-diabetic rats (B) and PCL-RGD graft in diabetic rats (D) were cut from the grafts implanted for 1 month. Graft lumen was indicated by asterisk. Calcification was observed on one PCL grafts in diabetic rats group (E) .PCL and PCL-RGD grafts in diabetic rats (n=3); in non-diabetics rats (n=5).

2. 7 Neo-tissue regeneration

To test the regeneration of grafts after implantation, we performed immunohistology staining. Anti-vWF antibody and anti- αSMA antibody were respectively used for endothelial cells and smooth muscle cells detection in the grafts. Endothelialization on the luminal surface is important to maintain the long-term patency of vascular grafts. As figure 7 showed, the luminal surfaces of the PCL grafts were covered by ECs (24.12 ± 2.15%, n=4 v.s 70.96 ± 12.88) in the diabetics group comparing with the non diabetics group. And in PCL-RGD grafts, 21.96± 1.86 %, n=4, v.s 74.84 ± 10.57 %. It inferred that diabetes dramatically limited the endotheliazation process.

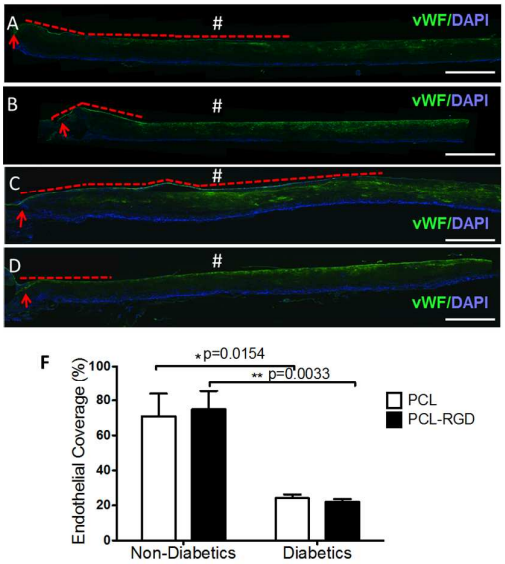

Fig 7. Endothelial coverage of the graft lumen by immunofluorescence staining using vWF antibody. Longitudinal sections of PCL in Non-diabetic rats (A), PCL graft in Diabetic rats (B), PCL-RGD graft in Non-diabetic rats (C) and PCL-RGD graft in diabetic rats (D) were cut from the grafts implanted for 1 month. Layer of regenerated endothelial cells is marked by red dotted line. The ratio of endothelialization was calculated based on the staining of longitudinal sections (F). Data were expressed as mean ± SEM. * P<0.05, ** P<0.01. PCL and PCL-RGD grafts in diabetic rats (n=3); in non-diabetic rats (n=5). # inferred as lumen and the red arrow indicated the suture site. Scale bar: 500 μm.

Smooth muscle cells (SMCs) presence and organization are also important for the long-term stability of vascular grafts. Interestingly, SMCs staining showed that SMCs were mostly underneath the lumen in the PCL group, however, in the PCL-RGD group SMCs were mostly in the neo-tissue surrounding the grafts at 1 month. And compared with the non-diabetic group, the grafts in the diabetics group were much slowly (Figure 8).

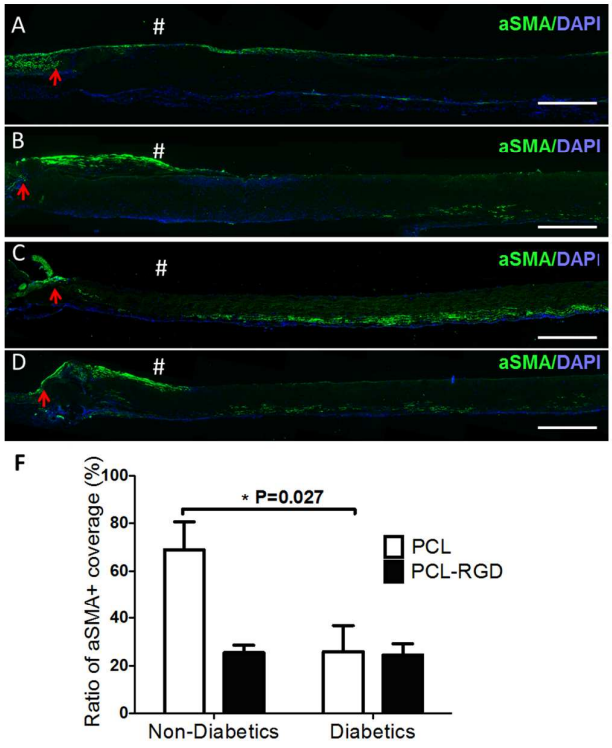

Fig 8. SMCs coverage of the graft lumen by immunofluorescence staining using aSMA antibody. Longitudinal sections of PCL in Non-diabetic rats (A), PCL graft in Diabetic rats (B), PCL-RGD graft in Non-diabetic rats (C) and PCL-RGD graft in diabetic rats (D) were cut from the grafts implanted for 1 month. The ratio of SMCs was calculated based on the staining of longitudinal sections (F). Data were expressed as mean ± SEM. * P<0.05. PCL and PCL-RGD grafts in diabetic rats (n=3); in non-diabetic rats (n=5). # inferred as lumen and the red arrow indicated the suture site. Scale bar: 500 μm.

3 Discussion

Therapeutic intervention for cardiovascular disorders is limited by the availability of small-diameter autologous blood vessels. This limitation has since fuelled the development of alternative vascular prostheses that are made of biodegradable polymers [20]. Due to its biocompatibility and mechanical strength, PCL has been widely used in tissue engineering and promising results have been extensively reported in vivo. However, due to the relatively slow degradation rate and bio-inert property, functional modification of the PCL grafts is still required [21]. The arginine-glycine-aspartic acid (RGD) moiety is a signature sequence of the cell adhesion segment of fibronectin [22, 23], which binds to nearly half of all known human integrins [24]. Modification of vascular graft with RGD could facilitate regeneration of functional blood vessels by physically attracting cell adhesion, particularly the adhesion of the endothelial progenitor cells (EPC). Indeed, Hersel and coworkers have previously demonstrated enhanced cell infiltration in the RGD modified group [25]. We have also reported positive performance of RGD modified PCL graft (PCL-RGD), demonstrating improved patency and accelerated endotheliazation of RGD-PCL in rabbit carotid artery [19]. However, one limitation of these earlier investigations is that most of these studies were performed in healthy animal models without considering disease complications that are common under clinical settings. To evaluate the performance of the PCL-RGD vascular graft under clinical conditions where patients are often accompanied with other comorbidities, we have established a rat model with T2D and used for RGD-PCL evaluation. Our results showed major adverse events, with impaired endotheliazation in particular, from diabetic animals following graft implantation. In addition, no significant difference was detectable between the PCL and the PCL-RGD implanted groups in diabetic rats, in contrast to our previous work [19]. Several factors may be attributable to the lack of significance of the RGD-modified PCL graft in diabetic rats shown in this study, one of which may be reduced availability of circulating ECs and EPCs caused by diabetes.

Rapid endotheliazation is crucial to prevent acute thrombus formation and a decisive factor for long-time performance of vascular grafts. Our data showed that, one month after graft implantation, the lumen side was nearly all covered with ECs in healthy rats, in comparison to diabetic rats of which only 20% coverage was observed. It has already been established that the EPCs play an important role in vascular regeneration after injury [26] by enhancing endotheliazation after vascular graft implantation in vivo [27]. Our data showed significant reduction in the number of circulating $CD34^+$ cells in peripheral blood that were collected from diabetic rats compared to healthy animals (T2D/control: 0.65%/1.16%).This observation is consistent with data from clinical trials, where EPCs extracted from individuals with T2D exhibit impaired properties in adhesion, proliferation and vasculature formation [28]. These abnormalities in EPCs function could be responsible for the impaired neovascularization observed in diabetic patients, which may be improved by improving EPCs activities. Indeed, it has been shown that lifestyle alteration or anti-diabetic intervention is able to improve EPC activity. In addition, some commonly prescribed anti-diabetic agents including statin, insulin, ace-inhibitors and PPARγ-agonists have all exhibited significant EPC-modulating effects [29-32], which could ameliorate diabetes-associated vascular disorders. Since restoration of EPCs population and function could compensate for diabetes-induced vascular impairment, cell therapy may be adopted as a plausible alternative approach for vascular regeneration in diabetic individuals.

We also observed substantial local inflammation shortly after graft implantation, which is also accountable for compromised vascular regeneration in diabetic rats. Previous studies have suggested that endothelial dysfunction is associated with increased arterial wall inflammation and cytokine production. Elevated expression of vascular inflammatory mediators was also observed, including the vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-Selectin (SELE) and nuclear factor-B (NF-kB). These data are consistent with epidemiological analyses which have long-established a causal association between diabetes and cardiovascular diseases.

In summary, in contrast to earlier studies where RGD modified PCL vascular graft implantation exhibited excellent regenerative properties, we observed no beneficial impact of the PCL-RGD vascular graft in rats with diabetes. This is partly caused by the substantial detrimental effect of diabetes on endothelial reformation that is essential for vascular regeneration. Thus, given the apparent lack of performance of the vascular grafts in diabetic animals, we propose that further studies for vascular graft evaluation should include diseased models such as diabetes for better pre-clinical evaluation.

4 Experimental

4.1 Materials

Poly (ε-caprolactone) (Mw=80,000) purchased from Solvay Interox (England), NapFFGRGD synthesis in our lab. Methanol, chloroform, alcohol and anhydrous sodium carbonate were obtained from Tianjin Chemical Reagent Company (Tianjin, China), Streptozotocin (STZ) were bought from Sigma. Sprague Dawley (SD) rats were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). Procedures involved in animal experiments were in compliance with the regulations of the Tianjin Committee of Use and Care of Laboratory Animals and the overall project protocol was approved by the Animal Ethics Committee of the Chinese Academy of Medical Science.

4.2 Fabrication of vascular grafts

Grafts were fabricated by the electrospinning method as previously described in detail [19]. Briefly, PCL (10%, w/v) was dissolved in 5:1 mixture of chloroform ⁄ methanol by stirring at room temperature overnight. The electrospinning set-up includes a syringe pump (New Era Pump Systems, Inc.), a high-

voltage supply (Gamma High Voltage Research, Inc.), and a rotating mandrel to collect the fibers. A positive voltage (18 kV) was applied to the polymer solution by the power supply. PCL solution (10%, w/v) was delivered through a 22-gauge blunt tip syringe needle at a constant flow rate of 3 ml/h using the syringe pump. To construct a small-diameter tubular graft, a rotating stainless steel rod (2.0 mm diameter) was used as the collector. The grafts were vacuum-dried in a desiccator at room temperature for 48 h. PCL-RGD grafts were prepared by the method reported as in the literature [19].

4.3 Characterization of the vascular grafts

The surface morphology images of electrospun PCL and PCL-RGD grafts were taken by using scanning electron microscope (SEM, Quanta 200, Czech) with an accelerating voltage of 15 kV. Gold coated samples before SEM analysis.

The average fiber diameter and pore size were measured based on scanning electron microscopy (SEM) images and analyzed using ImageJ software (NIH USA, 2008). Results are expressed as mean ± standard error of the mean.

Tensile stress–strain curves for the electrospun mesh grafts were obtained by using an Instron universal tensile tester (model 3345, Norwood, MA). The electrospun mesh grafts with 20 mm in length were utilized for the measurement. The distance between the two grips was set as 10 mm. Tension test was performed at ambient temperature with crosshead speed of 10 mm/min. Each test was repeated on 3 specimens.

4.4 The rat model of type 2 diabetes

Briefly, female Sprague Dawley (SD) rats weighing 50g were fed with high glucose and high fat diet. After 2 months, the fast plasma insulin level of the rats was measured by Tianjin Medical University Metabolic Diseases Hospital using Insulin Elisa Kits. And they were given an intraperitoneal injection of streptozotocin (30mg/kg body weight). Whole blood was obtained 7 days later from the tail vein, and the glucose level was monitored using a complete blood glucose monitor for oral glucose tolerance test (OGTT). After 12-hour fasting, the STZ-treated rats were taken 30% glucose solution by intragastric administration (0.67mL/100g) and with glucose levels higher than 11.1 mmol/L were considered diabetic. Another standard: STZ-treated rats with glucose levels by random higher than 16.7 mmol/L were considered diabetic. The glucose level was monitored before and after the surgery. After induction of diabetes, the rats had been maintained for 2 weeks before they were subject to any experiments.

4.5 Blood compatibility tested by arteriovenous shunt

Arteriovenous shunt model in rats was used to further evaluate the hemacompatibility of PCL-RGD. The tubular grafts (n=4) were assembled into a loop and connected to the abdominal artery at one end and to the abdominal vein at the other end with 24-G indwelling needles under the standard surgical procedure applied to live animals. After circulation for 2 hour, the circuit was perfused with physiological saline, and then cut into two segments. One segment was fixed in 2.5% glutaraldehyde for SEM analysis. The other segment was stained with mepacrine solution (10 mM) for 90 min [33-34], and examined by confocal laser scanning microscopy. The amount of adhered platelets was counted from 3 randomly selected fields for statistical analysis.

4.6 In vivo implantation

Tubular grafts (inner diameter: 2.0 mm; length: 10 mm) were implanted into the abdominal aorta of SD rats (male 280-350g). Rats were anesthetized with an intraperitoneal injection of chloral hydrate (0.3mg/kg body weight). Heparin (100 IU/kg) was administrated with intravenous injection as the anticoagulant. After the abdominal aorta was isolated and clamped, a 5-mm segment of aorta just between the renal arteries and the aortoiliac bifurcation was removed. PCL-RGD (n=5) or PCL (n=5) grafts were implanted by end-to-end anastomosis using interrupted 8-0 monofilament nylon sutures (BEAR, Japan) under an operative microscope. The number of stitches used for each anastomosis ranged from 8 to 10. The distal clamp was removed firstly, and then was the proximal clamp to restore the blood flow. The total ischemia time was between 30 to 45 minutes. No anticoagulant was administered postoperatively. Rats were followed up to 1 month. Signs of thrombosis and aneurysm formation were carefully checked. Angiography was performed before sacrifice by injection of 1 ml contrast material (Hexabrix 320, Guerbet, France), and the blood flow was assessed using a C-arm digital fluoroscopy system (Sirus Power/C, Hitachi Medico, Tokyo, Japan). PCL (n=4) and PCL-RGD (n=4) were implanted in diabetic rats, PCL (n=5) and PCL-RGD (n=5) in healthy rats.

4.7 Histology and immunology staining

For histological investigations, explanted grafts were cut into 2 longitudinal halves. Then one part of the grafts was fixed in 4% formaldehyde for histological section and the others were fixed in 2.5% glutaraldehyde for SEM assay. For histological analysis, cryosections (5 μm) were stained with hematoxylin and eostin (H&E). Immunochemical staining were performed to identify the vascular endothelial cells and smooth muscle cells using anti-vWF antibody (mono, DAKO, Japan) and anti-αSMA antibody (mono, Boster, China), respectively following the procedures reported previously (Ref. try our papers).

4.8 Statistical analysis

All quantitative data were obtained from at least three samples for analysis. Results were expressed as the mean ± standard error of the mean (SEM). A two-tailed unpaired Student's t-test was used to compare the differences. Difference with $p < 0.05$ was considered to be statistically significant

5 Conclusions

We evaluated the performance of two types of vascular grafts in diabetic rats and non-diabetic controls. Our data showed that rats with diabetes have higher major adverse events rates, including slow endotheliazation, severe inflammation, early calcification and platelet adhesion. Since

long-term diabetes is associated with impaired EC/EPC function and compromised endothelization during vascular regeneration, development of vascular graft designed to improve endotheliazation for diabetic patients would be of significant importance in clinical application of vascular grafts.

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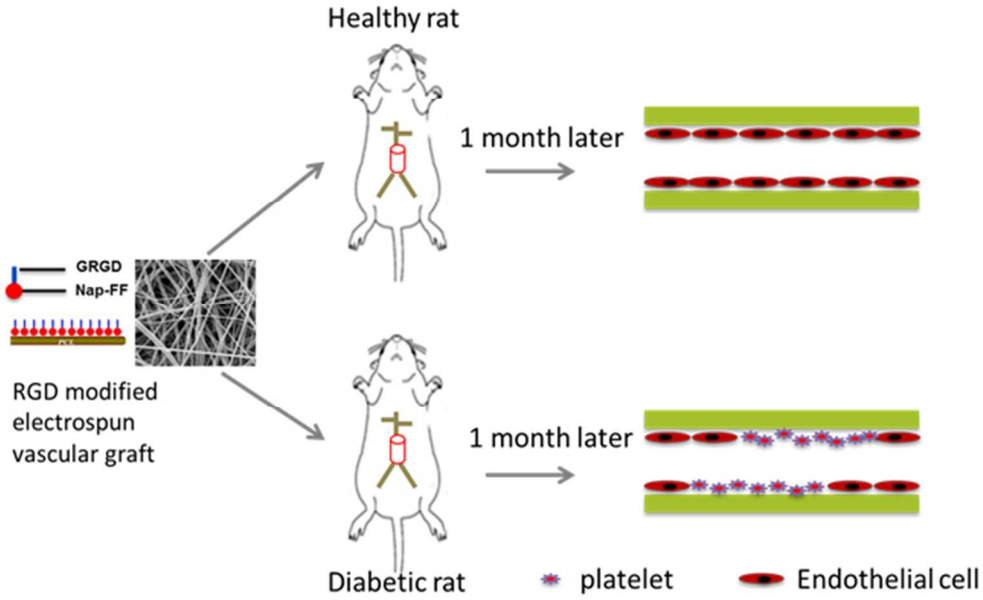

Highlight

Diabetes exacerbates the regeneration process after in vivo implantation of vascular graft.

55x39mm (300 x 300 DPI)