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Optimal Methods of Cell Seeding and Cultivation on a Poly(L-lactide) Biodegradable Scaffold

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Abstract—In modern cardiovascular surgery, there remains a problem associated with a lack of venous material for bypass operations. Great interest has emerged in developing small-diameter vascular grafts ensuring stable patency. One possible solution is the creation of a tissue-engineered vascular implant (TEVI) based on a biodegradable polymer scaffold. Being structural and functional features of the scaffold, cell integration and cultivation on the scaffold are the key processes in the TEVI development. The aim of this research is to identify the optimal method of adipose-derived mesenchymal stem cell seeding and cultivation on the tubular biodegradable scaffold from poly(L-lactide). Results of static and dynamic cells cultivation on the scaffold after seeding with the developed filtration method were compared. The proposed method combining filtration seeding and dynamic cultivation has proved its higher efficiency and is suitable for further TEVI development.

Keywords: mesenchymal stem cells, scaffold, poly(L-lactide), tissue engineering
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INTRODUCTION

The application of autologous material for reconstructive operations remains a gold standard in vascular surgery. The number of such procedures is very high (World Health Organization, 2016). Autologous arteries and veins, as well as synthetic vascular prosthesis, are used for these purposes in clinics. However, these materials do not fully satisfy the requirements of modern cardiovascular surgery. The use of synthetic vascular prosthesis is fraught with complications, such as early thrombosis. An absence of endothelial lining fairly often promotes the development of anastomotic stenosis. The risk of infection is high (Kannan et al., 2005). Vascular synthetic grafts are not suitable for reconstruction of small-diameter arteries (coronary and tibial arteries) (Leon and Greisler, 2003; Seifu et al., 2013).

The problems of an insufficient amount of autovenous material and high risk of pathological changes in its wall before and after implantation are also topical (Athanasiou et al., 2011; Harskamp et al., 2013; Kim et al., 2013; Yazdani et al., 2013). In addition, there is problem associated with the need to reconstruct vessels in pediatric surgery with the use of remodeling grafts in a child's growing body (Naito et al., 2011).

Developing tissue-engineered vascular implants (TEVI) that would imitate the structure and function of the natural vessel and exhibit a neurohumoral response should overcome these problems.

Different approaches have been suggested for creating TEVI, such as sheet-based tissue engineering (Peck et al., 2011) and cell cultivation on a biodegradable or decellularized scaffold (L'Heureux et al., 2007). It was proved that a key factor for TEVI development is cell seeding technology (Pei et al., 2002). Cell cultivation improved the patency and durability of TEVI (Pawlowski et al., 2004; Cho et al., 2005; Hashi et al., 2007). However, the mechanisms underlying the formation of a new vascular wall as a result of cell seeding is not quite clear.

The dependence of cultivated cell amount and viability of a vascular graft has not been determined. It is believed that the keys to success are uniform cell distribution within the scaffold and their optimal initial concentration. Consistent cell distribution is plausible for generation of the extracellular matrix in the total matrix volume, resulting in the growth of uniform functional tissue. High plating efficiency, i.e., a high

Abbreviations: AD-MSC—adipose-derived mesenchymal stem cells, A; PLA—poly(L-lactide); TEVI—tissue-engineered vascular implant.

ratio of attached cell number to the number of seeded cells, is critical for rapid tissue regeneration.

Various techniques of cell seeding on the scaffold are known, but most of them are too extended and difficult to apply in the clinic (Parikh and Edelman, 2000; Yow et al., 2006). Large-scale production of small-diameter TEVI requires an inexpensive, reliable, and efficient technique of cell seeding (Ahsan and Nerem, 2005). A static method based on passive distribution of concentrated cell suspension throughout the scaffold remains the most widespread. Its acceptable rate and simplicity are counterbalanced by a number of shortcomings, such as low seeding efficiency and poor cell distribution in the scaffold wall. Alternative methods of cell seeding, such as vacuum, centrifugal, electrostatic, and dynamic methods, were developed to overcome these problems (Solchaga et al., 2006; Soletti et al., 2006; Villalona et al., 2010).

Although cultivation processes have been examined for various cells and scaffolds, all such studies were performed on particular models and cannot be extrapolated on others. The aim of this study was to find optimal methods of seeding and cultivation of adipose-derived mesenchymal stem cells (AD-MSC) on a tubular biodegradable small-diameter scaffold of poly(L-lactide) (PLA). We compared cell seeding methods (static, vacuum) with a filtration technique that we developed. We also compared static and dynamic cell cultivation on the scaffold after filtration seeding.

MATERIALS AND METHODS

Scaffold of PLA microfibers. Tubular scaffolds from PLA fibers were obtained from PLA solution (50 kDa) in chloroform by electrospinning using a Nanon-01A (Japan). PLA solution with a concentration of 15 wt % was pumped through the die in the electrical field ($V = 16$ kV). The distance between the electrodes was 0.15 m. Fiber deposition occurred on cylindrical electrodes. The rotation speed of the cylindrical electrode with a diameter of 1.1 mm was 1500 rpm. Tubular samples with a 1.1-m diameter and 320-μM wall thickness were produced.

Scaffold microstructure. The structures of the tubular scaffolds of poly(L-lactide) were analyzed by scanning electron microscopy. The samples were washed with 0.1 M PBS (pH 7.4), fixed with 4% formaldehyde in PBS for 20 min, washed with PBS, and air-dried. Samples were attached to objective tables, sputtered by platinum using Quorum Q150T ES (Quorum Technologies, United Kingdom), and assessed using a Supra 55VP scanning electron microscope (Carl Zeiss, Germany) in the mode of secondary electron registration at an accelerating voltage of 5 kV.

Isolation and cultivation of AD-MSC. AD-MSC were obtained by cell selection through adhering to plastic (Zhu et al., 2013). Samples of adipose tissue were taken from a Wistar adult male rat (225 g). Animals were treated according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The experiments were approved by the local ethic committee of Pavlov First St. Petersburg State Medical University, Russia. An animal was given inhalation anesthesia (3% isoflurane) to isolate 0.3–0.5 mL of adipose tissue from the anterior abdominal wall. Obtained samples were placed into a Petri dish, mechanically homogenized, and treated with 0.2% collagenase I (Gibco, United States) in Hank's solution using a thermoshaker (Biosan ES-20, Latvia) for 60 min at 37°C. Collagenase activity was blocked with 10% fetal calf serum (Gibco, United States), and suspension was centrifuged at 1500 rpm for 10 min (ELMI CM-6M laboratory centrifuge, Russia). The pellet was suspended in a complete growth medium composed of α-MEM (Paneco, Russia), L-glutamine, 10% fetal calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (Gibco, United States) and cultivated in a CO_2 incubator (Thermo Fisher Scientific, 3423, United States) at 37°C, 5% CO_2, and increased humidity. After 48 h, unattached cells, blood cells, and pieces of connective tissue were removed by PBS washing. The cells were subcultured after 5 days (the first passage) using 0.05% trypsin (Gibco, United States). The cells were cultivated in 175-cm^2 flasks (Eppendorf) and passaged after reaching 70% confluence. Cells from passages 3–8 were used in experiments. Scaffolds were preconditioned in culture medium for 1 h before cell seeding.

Cell seeding on PLA scaffolds was performed by static, vacuum, or developed filtration methods.

AD-MSC static seeding and cultivation under static conditions. AD-MSC seeding was performed by placing cell suspension (1×10^6 AD-MSC in 25 μL) into the scaffold sample (5 mm). Half of the suspension was introduced into the graft lumen, and the second half was uniformly distributed on the outer surface. The scaffold was placed into the CO_2 incubator for 1 h. Every 15 min, it was rotated by 90° along the longitudinal axis and additionally irrigated with the culture medium. Cultivation was then performed in bioreactor tubes in 10 mL medium for 1, 3, and 7 days.

AD-MSC vacuum seeding and cultivation under static conditions. AD-MSC were seeded as described in (Tan et al., 2012) with some modifications. Two 5-mm scaffolds and 3×10^6 cells in 0.2 mL medium were placed in a 3-mL syringe. The syringe was filled with 0.1 mL air and closed with a three-way valve. The syringe piston was pulled back to the 3-mL mark and held there for 10 s. The piston was then returned to the initial position for 10 s. The procedure was repeated ten times. The syringe was closed with a 0.22-μm antibacterial filter (Orange Scientific, Belgium) and placed into the CO_2 incubator for 1 h. Scaffolds were

then placed into bioreactor tubes with 10 mL culture medium for 1, 3, and 7 days.

AD-MSC filtration seeding and cultivation under static conditions. One end of the scaffold was temporary clamped by tweezers. Using a venous catheter connected to an insulin syringe, 2.0×10^6 MSC in 1 mL complete growth medium were introduced into the other end of the scaffold. The filtered medium was collected into a tube, and the procedure of its injection was repeated twice. Thus, the scaffold wall played the role of a filter. The outer diameter of the venous catheter exactly coincided with the internal diameter of the scaffold. The scaffold was then placed into a bioreactor tube with 10 mL culture medium for 1, 3, 7, and 14 days.

AD-*MSC filtration seeding and cultivation under dynamic conditions.* After filtration seeding as described, the scaffold was placed into a CO_2 incubator for 1 h. The scaffold was then fastened on peripheral venous catheters with a suture thread in a bioreactor camera. Connective tubes were filled with the culture medium (30 mL), preventing air from entering the circuit. The peristaltic pump speed was 1 mL/h for the first 3 h and then was enhanced to 20 mL/h for 12 h and later to 600 mL/h. MSC were cultivated on the scaffold with four (I–IV) regimes: (I) 5 days under dynamic and 2 days under static conditions, (II) 7 days under dynamic conditions, (III) 7 days under dynamic and 7 days under static conditions, and (IV) 14 days under dynamic conditions.

After cultivation scaffolds were washed three times with PBS collecting washing into separate tubes. The number of unattached cells in culture medium and washings were counted with a Countess II automated cell counter (Invitrogen, United States).

Time of AD-MSC adhesion to the PLA scaffold. An amount of 1.3×10^6/mL AD-MSC per 1 cm were seeded into the scaffold by the filtration method and placed into a CO_2 incubator for 1, 3, or 5 h. After that, the scaffold lumen was washed three times with PBS. AD-MSC in the washing solution were stained with 0.4% trypan blue (Invitrogen, United States) and counted with a Countess II automated cell counter (Invitrogen, United States).

Optimal cell number per 1-cm scaffold. An amount of 0.5, 1.5, 2, or 3×10^6 cells were seeded using the filtration method described above. Seeded cells were incubated in the bioreactor tubes with 5 mL complete growth medium for 1 h. Then the scaffold was washed with PBS three times and cells were counted in culture and washed mediums as described above. The number of attached cells was calculated by subtracting from the total number of seeded cells the number of unfixed AD-MSCs in the initial medium and their number in the washing solution. Seeding efficiency was determined as percentage of attached cells to the initial cell number.

Bioreactor device. The bioreactor is constructed as a system with the closed loop (Fig. 1). This allows permanent growth medium perfusion *7* through scaffold lumen *8*. The bioreactor is composed of camera *3*, peristaltic pump *1*, and connecting polyvinyl chloride tubes *5*. The bioreactor camera is fabricated from a transparent plastic container with a hermetically sealed cover possessing gas exchange apertures closed with 0.22-μm antibacterial filters *4* (Orange Scientific, Belgium). A chamber positioned inside the CO_2 incubator served as a reservoir for the culture medium. The scaffold was fixed inside the camera. It was attached to two venous peripheral catheters with a diameter of 1.1 mm. Next to the CO_2 incubator, there is a peristaltic pump (Applix Smart, Germany) with disposable sterile connecting tubes *5* passing through hole of the incubator *6*.

Fluorescence microscopy. After cell cultivation, the scaffold was washed with PBS and fixed with 4% formaldehyde in PBS for 10 min. The fixator was washed out with PBS for 10 min. Samples were incubated with 0.1% triton X-100 (Helicon, Russia) for 20 min for permeabilization of the cell membrane and washed as before. Cell nuclei were stained with DAPI in PBS (1 μg/mL). Preparations were assayed with a Zeiss LSM 5 PASCAL with confocal microscope (Carl Zeiss, Germany).

Statistical analysis. The data were treated with the Microsoft Excel 2010 program. The results are presented as mean ± SD. The significant difference was evaluated with Student's two-tailed test (Student's *t*-test option in Microsoft Excel 2010). The null hypothesis stated that two variables are independent phenomena or there was no association among groups. If the result of the t-test was higher than the significance level ($a = 0.05$), the difference was considered not significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Structure of biodegradable scaffold from poly(L-lactide). A tubular scaffold made of PLA microfibers was obtained by electrospinning with an inner diameter of 1.1 mm and thickness of walls of 320 μm (Fig. 2). Fibers with a diameter of 1.5–4 μm were arranged in the scaffold structure chaotically. The pore size between them is 10–50 μm, which is comparable with MSC size.

Time of AD-MSC adhesion to the PLA-scaffold. AD-MSC counting in the washing solution revealed that most cells attached to the scaffold during the first hour. Further cultivation did not significantly increase the number of adhered cells (Table 1). High cell viability was maintained during the whole cultivation period.

Optimal AD-MSC seeding density. Table 2 and Fig. 3 show that augmented AD-MSC seeding density linearly increases the number of attached cells but to a

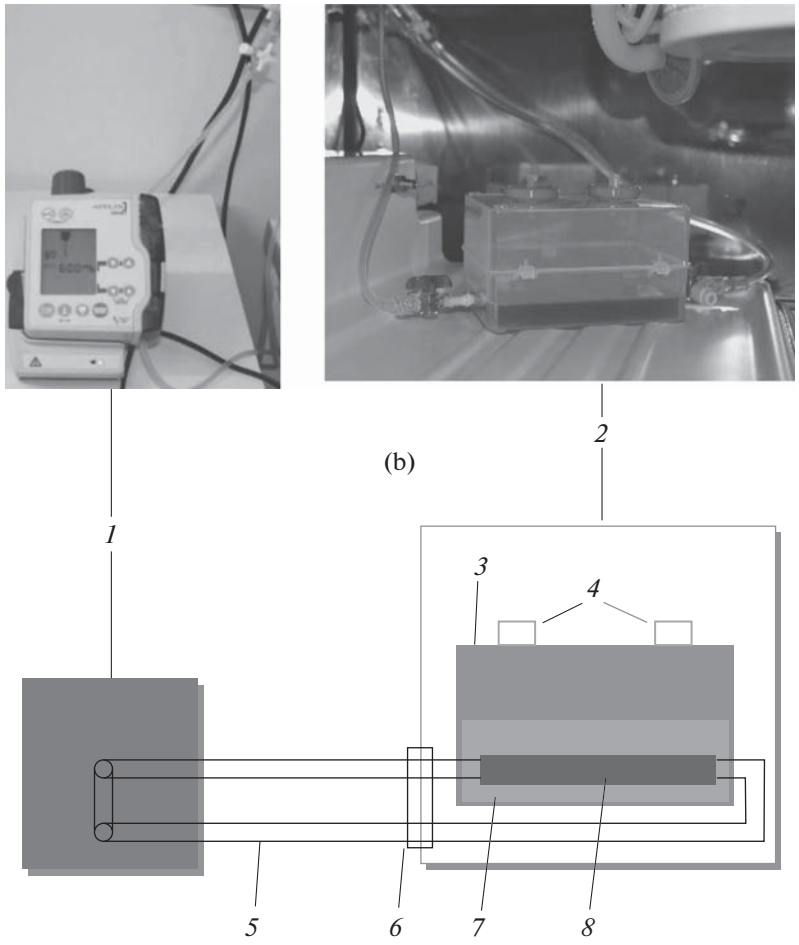

Fig. 1. (a) Photo and (b) scheme of the constructed bioreactor. (*1*) Peristaltic pump, (*2*) main part of the bioreactor in a CO_2 incubator, (*3*) bioreactor camera, (*4*) antibacterial filters, (*5*) connective tubes, (*6*) technological opening in a CO_2 incubator, (*7*) growth medium, and (*8*) fixed scaffold.

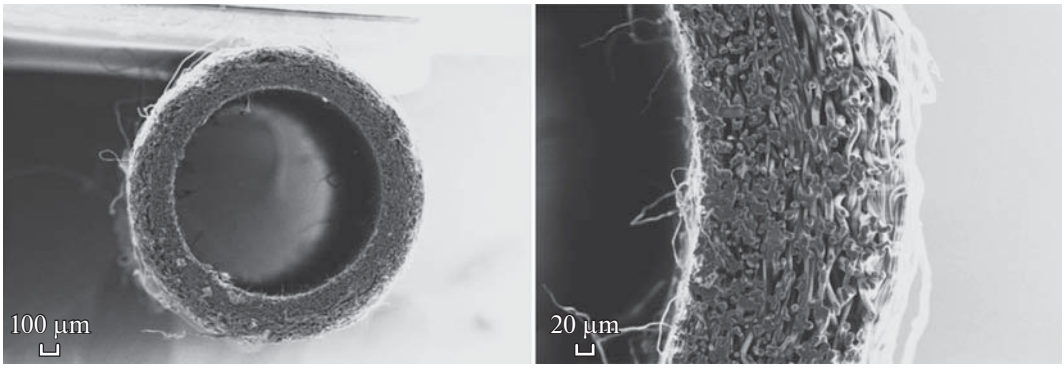

Fig. 2. Electron microscopy of PLA graft end: (a) cross section and (b) graft wall.

Table 1. Dependence of MSC adhesion on cultivation time

Parameter	Cultivation time, h		
	1	2	3
MSC number in the washing solution ($\times 10^3$)	314 ± 3	356 ± 4	348 ± 3
Cell viability, %	95 ± 2	93 ± 3	96 ± 2

certain extent. With a seeding density of more than 2.0×10^6, no increase in the number of adhered cells was observed. This shows that the optimal seeding density in AD-MSC suspension is 2.0×10^6 cells per 1 cm of scaffold length. Under these conditions, the plating efficiency was 63%.

AD-MSC integration with the scaffold under static seeding and cultivation. AD-MSC suspension passively seeded into the scaffold was distributed in a nonuniform way. One day after seeding, most cells were located in clusters and did not penetrate deeply into the graft wall (Fig. 4a). Further cultivation did not facilitate cell distribution within the scaffold volume (Fig. 4b). After 7 days, the inner and mostly outer graft surface were covered with MSC unevenly without cell penetration deep into the scaffold wall (Fig. 4c).

AD-MSC integration with the scaffold under vacuum seeding and static cultivation. With vacuum seeding, MSC were mostly distributed on the graft outer surface as clusters without deep penetration (Fig. 4f). For 3 days, the cells were gradually distributed along the outer matrix surface with insignificant deep invasion (Fig. 4h). No uniform cell distribution and penetration into the graft volume were observed after 7-day cultivation.

AD-MSC integration with the scaffold under filtration seeding and static cultivation. With filtration seeding, the scaffold wall plays the role of a kind of filter for cells. Under these conditions, MSC are distributed mostly in the graft. One day after seeding, a cell density gradient was formed from the maximum on the inner surface to the minimum on the outer graft surface (Fig. 4d). The difference in cell density inside the graft declined for 3 days (Fig. 4e), and, after 7 days, the initial gradient in cell density was practically evened out by an increased number on the graft well periphery (Fig. 4f).

AD-MSC integration with the scaffold under filtration seeding and dynamic cultivation. Under dynamic cultivation, MSC were uniformly distributed in the graft wall. Seven days of exposure to a pulse wave in regimes I and II resulted in uniform cell distribution and formation of monolayer on the inner graft wall. The cell-produced external contour had a smooth structure. Regimes I and II did not differ in cell distribution (Figs. 5a, 5b). The uniform cell distribution after prolonged cultivation under regime III was accompanied with increased cell number (Fig. 5c). Uniform cell distribution and high density in the graft volume were also observed after 14 days of cultivation with a pulse wave (regime (IV). The maximum cell number was examined on the inner scaffold wall (Fig. 6a). The graft incubated under static conditions after filtration seeding had a smaller cell density, and its inner layer was not saturated with cells (Fig. 6b).

All in all, the study demonstrated biological compatibility of tubular scaffold from PLA. Most AD-MSC attached to the graft during the first hour. The optimal number of cells for seeding was 2.0×10^6 per 1 cm of the graft length. Passive seeding did not provide uniform cell distribution throughout the scaffold volume. Most cells were distributed on the outer graft surface with vacuum seeding. The optimal technological solution for cell integration with the scaffold is filtration seeding, as the graft wall plays the role of a filter. This method is feasible for uniform cell distribution in the scaffold volume. AD-MSC distribution in the scaffold was facilitated by exposure to a continuous pulse wave in the constructed bioreactor. The density of the cell layer depended on cultivation time. A combination of cell filtration seeding and dynamic cultivation proved to be the most effective and suitable for further TEVI development.

Table 2. MSC plating efficiency in dependence of the number of cells seeded in grafts

Parameter	Number of seeded MSC, $\times 10^6$				
	0.5 ± 0.01	1.5 ± 0.02	2.0 ± 0.02	3.0 ± 0.03	4.0 ± 0.03
Efficiency, %	17	35	63	48	36
Viable cell number in the washing solution, %	75	58	54	85	90

Plating efficiency was determined 1 h after cell seeding as the ratio of attached cells to the initial cell number, %.

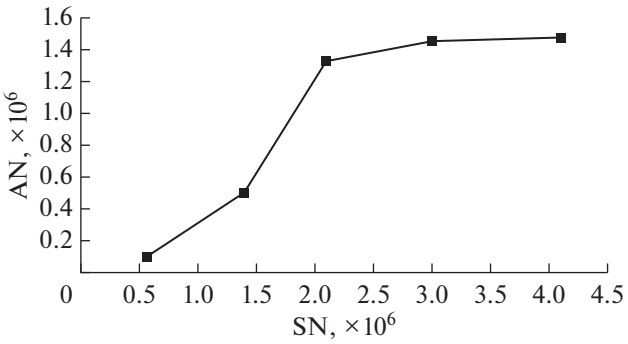

Fig. 3. Dependence of adhered MSC number (AN) to number of seeded cells (SN). AN was calculated by subtracting the cell number in washing solution and unattached cells in the medium from the total number of seeded cells.

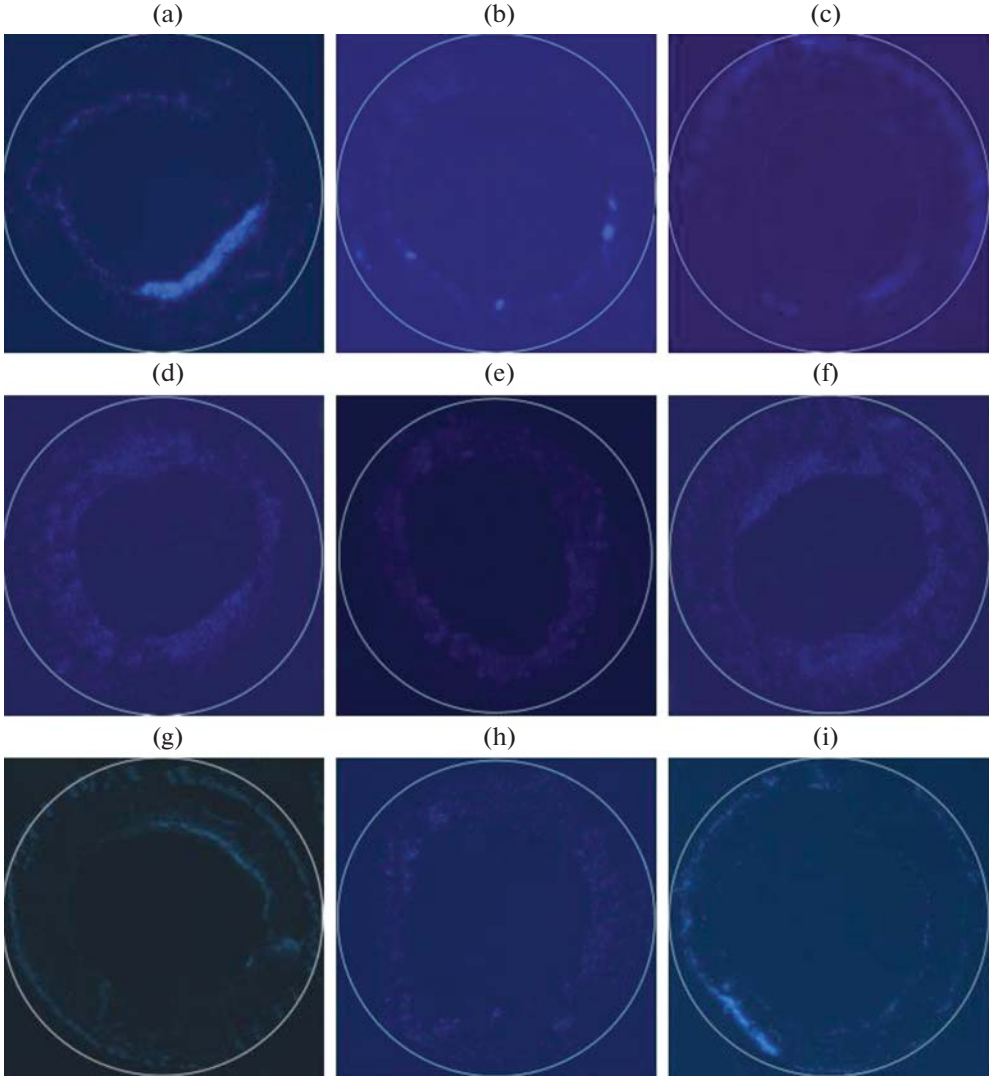

Fig. 4. Cell distribution in grafts with different seeding methods. (a, b, c) Passive, (d, e, f) filtration, and (g, h, i) vacuum. MSC distribution after (a, d, g) 1, (b, e, h) 3, and (c, f, i) 7 days. Fluorescent microscopy. Nuclei are stained with DAPI. Ob. 2.5×.

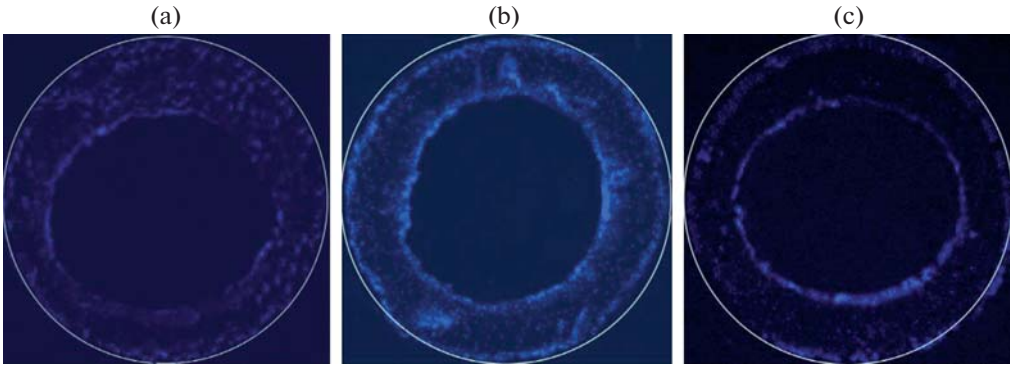

Fig. 5. MSC distribution in grafts under various cultivation regimes. (a) Cultivation for 5 days under dynamic and 2 days under static conditions, regime I; (b) 7 days under dynamic conditions (regime II); and (c) 7 days under dynamic and 7 days under static conditions (regime III). Ob. 2.5×.

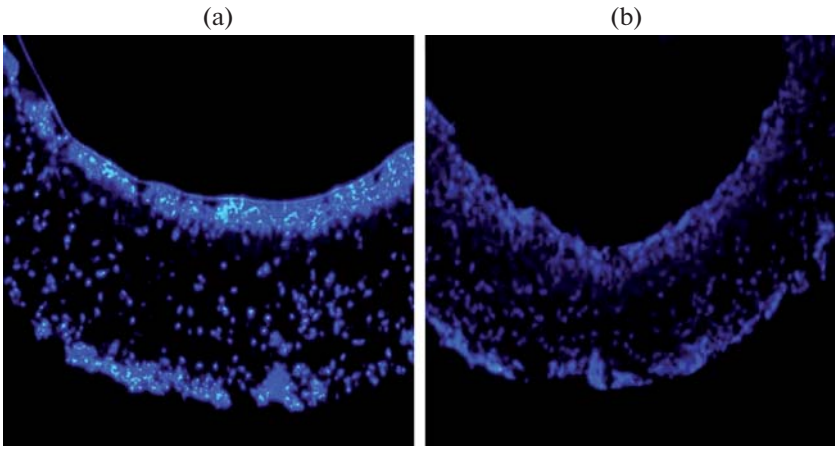

Fig. 6. Cell distribution in the graft after MSC cultivation for 14 days under (a) dynamic (regime IV) or (b) static conditions. Ob. 10×.

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REFERENCES

Ahsan, T., and Nerem, R.M., Bioengineered tissues: the science, the technology, and the industry, *Orthod. Craniofac. Res. Vol.*, 2005, vol. 8, pp. 134–140.

Athanasiou, T., Saso, S., Rao, C., Vecht, J., Grapsa, J., Dunning, J., Lemma, M., and Casula, R., Radial artery versus saphenous vein conduits for coronary artery bypass surgery: forty years of competition—which conduit offers better patency? A systematic review and meta-analysis, *Rev. Article. Eur. J. Cardio-Thoracic Surg.*, 2011, vol. 40, pp. 208–220.

Cho, S.W., Lim, S.H., Kim, I.K., Hong, Y.S., Kim, S.S., Yoo, K.J., Park, H.Y., Jang, Y., Chang, B.C., Choi, C.Y., Hwang, K.C., and Kim, B.S., Small-diameter blood vessels engineered with bone marrow-derived cells, *Ann. Surg.*, 2005, vol. 241, pp. 506–515.

Harskamp, R.E., Lopes, R.D., Baisden, C.E., de Winter, R.J., and Alexander, J.H., Saphenous vein graft failure after coronary artery bypass surgery: pathophysiology, management, and future directions, *Ann. Surg.*, 2013, vol. 257, pp. 824–833.

Hashi, C.K., Zhu, Y., Yang, G.Y., Young, W.L., Hsiao, B.S., Wang, K., Chu, B., and Li, S., Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, vol. 104, pp. 11915–11920.

Kannan, R.Y., Salacinski, H.J., Butler, P.E., Hamilton, G., and Seifalian, A.M., Current status of prosthetic bypass grafts: a review, *J. Biomed. Mater. Res. B Appl. Biomater.*, 2005, vol. 74, pp. 570–581.

Kim, F.Y., Marhefka, G., Ruggiero, N.J., Adams, S., and Whellan, D.J., Saphenous vein graft disease: review of pathophysiology, prevention, and treatment, *Cardiol. Rev.*, 2013, vol. 21, pp. 101–119.

L'Heureux, N., Dusserre, N., Marini, A., Garrido, S., de la Fuente, L., and McAllister, T., Technology insight: the evolution of tissue-engineered vascular grafts-from research

to clinical practice, *Nat. Clin. Pract. Cardiovasc. Med.*, 2007, vol. 4, pp. 389–395.

Leon, L. and Greisler, H.P., Vascular grafts, *Expert Rev. Cardiovasc. Ther.*, 2003, vol. 1, pp. 581–594.

Naito, Y., Shinoka, T., Duncan, D., Hibino, N., Solomon, D., Cleary, M., Rathore, A., Fein, C., Church, S., and Breuer, C., Vascular tissue engineering: towards the next generation vascular grafts, *Adv. Drug Deliv. Rev.*, 2011, vol. 63, pp. 312–323.

Parikh, S.A. and Edelman, E.R., Endothelial cell delivery for cardiovascular therapy, *Adv. Drug Deliv. Rev.*, 2000, vol. 42, pp. 139–161.

Pawlowski, K.J., Rittgers, S.E., Schmidt, S.P., Bowlin, and G.L., Endothelial cell seeding of polymeric vascular grafts, *Front. Biosci.*, 2004, vol. 1, pp. 1412–1421.

Peck, M., Gebhart, D., Dusserre, N., McAllister, T.N., and L'Heureux, N., The evolution of vascular tissue engineering and current state of the art, *Cells Tissues Organs*, 2011, vol. 195, pp. 144–158.

Pei, M., Solchaga, L.A., Seidel, J., Zeng, L., Vunjak-Novakovic, G., Caplan, A.I., and Freed, L.E., Bioreactors mediate the effectiveness of tissue engineering scaffolds, *FASEB J.*, 2002, vol. 16, pp. 1691–1694.

Seifu, D.G., Purnama, A., Mequanint, K., and Mantovani, D., Small-diameter vascular tissue engineering, *Nat. Rev. Cardiol.*, 2013, vol. 10, pp. 410–421.

Solchaga, L.A., Tognana, E., Penick, K., Baskaran, H., Goldberg, V.M., Caplan, A.I., and Welter, J.F., A rapid seeding technique for the assembly of large cell/scaffold composite constructs, *Tissue Eng.*, 2006, vol. 12, pp. 1851–1863.

Soletti, L., Nieponice, A., Guan, J., Stankus, J.J., Wagner, W.R., and Vorp, D.A., A seeding device for tissue engineered tubular structures, *Biomaterials*, 2006, vol. 27, pp. 4863–4870.

Tan, L., Ren, Y., and Kuijer, R., A 1-min method for homogenous cell seeding in porous scaffolds, *J. Biomat. Appl.*, 2012, vol. 26, pp. 877–889.

Villalona, G.A., Udelsman, B., Duncan, D.R., McGillicuddy, E., Sawh-Martinez, R.F., Hibino, N., Painter, Ch., Mirensky, T., Erickson, B., Shinoka, T., and Breuer, Ch.K., Cell-seeding techniques in vascular tissue engineering, *Tiss. Eng. B: Rev.*, 2010, vol. 16, pp. 341–350.

World Health Statistics, Monitoring Health for the SDGs, Sustainable Development Goals, World Health Organization, Geneva: WHO Press, 2016, pp. 64–121.

Yazdani, S.K., Otsuka, F., Nakano, M., Ladich, E., and Virmani, R., Pathology of saphenous vein grafts, *Interv. Cardiol. Clin.*, 2013, vol. 2, pp. 241–249.

Yow, K.H., Ingram, J., Korossis, S.A., Ingham, E., and Homer-Vanniasinkam, S., Tissue engineering of vascular conduits, *Br. J. Surg.*, 2006, vol. 93, pp. 652–661.

Zhu, M., Heydarkhan-Hagvall, S., Hedrick, M., Benhaim, P., and Zuk, P., Manual isolation of adipose-derived stem cells from human lipoaspirates, *J. Vis. Exp.*, 2013, vol. 79, pp. 50585.

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