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Endothelial progenitor cells overexpressing Grb2-associated binder 1 for in vitro-constructed tissue-engineered heart valves

Aim	Endothelial progenitor cells (EPCs) play important roles in heart valve replacement surgery. Up-regulation of Grb2-associated binder 1 (Gab1) promotes hepatocyte growth factor (HGF) – induced endothelial progenitor cell proliferation and migration. This study aimed to investigate the effects of up-regulation of Gab1 in hepatocyte growth factor-induced EPCs in tissue-engineered heart valves (TEHV).
Material and methods	Fresh porcine aortic valves were placed in 1% Triton X-100 and trypsin buffer for decellularization. EPCs in the control group were cultured normally, whereas those in the experimental group were both HGF stimulated and transfected with adenovirus containing the Gab1 gene. Cells in the two groups were seeded onto the decellularized valve scaffolds and cultured for 3 or 7 days. TEHV were analyzed by HE and AB-PAS staining.
Results	By day 3, the experimental group had formed confluent endothelial monolayers on top of the decellularized valves, on the basis of by HE staining and AB-PAS staining. One week later, the control group showed a imperfect endothelial layer.
Conclusion	HGF-induced EPCs overexpressing Gab1 can endothelialize the decellularized matrix and create functional TEHV, which may then be preconditioned in a bioreactor before clinical implantation.

Keywords	Tissue engineering; heart valve; endothelial progenitor cells; gab1; recellularization
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Introduction

Every year, approximately 290,000 patients worldwide undergo heart valve replacement surgery. By 2050, this number is expected to approach 850,000. Currently, clinically applied models of heart valve replacements include biological valves and mechanical valves. Biological valves exhibit superior hemodynamic behavior with a lower risk of thromboembolism, but they have limited life, owing to tissue calcification and deterioration. Mechanical valves have excellent durability, but recipients require lifelong anticoagulant therapy. An ideal bioprosthesis would overcome these shortcomings and be capable of remodeling, regeneration, and growth [1].

The limitations of currently available heart valve replacements have stimulated investigations of tissue engineered heart valves (TEHVs) constructed by seeding autologous cells onto decellularized tissue or bioabsorbable synthetic scaffolds. In 2006, Serghei reported the use of TEHVs synthesized with autologous endothelial progenitor cells (EPCs) in pediatric cardiac surgery [2]. They demonstrated that such TEHVs are safe and feasible, and they have the potential to be reshaped and grow. In 2010, Sales demonstrated that circulating EPCs have the potential to provide both interstitial and endothelial functions, and thus, they might potentially serve as a single-cell source for construction of autologous heart valves [3]. In 2012, Jordan found that acellular valves conjugated with anti-EPCs antibody (CD133) can achieve rapid reconstruction by host cells after implantation [4]. This approach bypasses the time-consuming and difficult process of seeding cells into valve scaffolds in vitro by collecting host circulating EPCs into the available scaffold matrix in vivo, and it provides a new method for using EPCs to construct TEHVs. EPCs, the main cell source for TEHVs, can adhere, proliferate and cover implants with an endothelial layer. However, autologous EPCs in patients requiring valve replacement are generally inadequate, and covering of the implant with an endothelial layer occurs slowly [5, 6]. The absence of this autologous endothelial layer may cause immunological reactions. If autologous EPCs proliferation and migration could be

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enhanced, then the re-endothelialization time, i.e., the time required for TEHVs to form an endothelial layer, could be decreased, and immunological reactions could be alleviated.

The Grb2-associated binder family of docking proteins are related to the amplification and integration of signal transduction induced by growth factors, antigens, cytokines, and numerous other molecules. Grb2-associated binder 1 (Gab1), a member of the insulin receptor substrate-like, multi-substrate docking protein family, is expressed in various types of cells [7, 8]. Gab1 plays an vitally important role in postnatal angiogenesis and arteriogenesis via hepatocyte growth factor (HGF)/c-Met signaling [9]. Aasrum suggested that Gab1 is involved in mitogenic signaling in response to HGF in hepatocytes [10]. Downregulation of Gab1 inhibits cell proliferation and migration [11]. Furthermore, Gab1 is involved with HGF-induced endothelial cell (EC) proliferation and migration [12]. Fan found that upregulation of Gab1 promotes proliferation and migration of EPCs. Fan also found that, under the same dose of HGF stimulation, Gab1 was upregulated, and the proliferation and migration of human EPCs that was strongly enhanced [13].

In this study, we evaluated whether HGF-induce EPCs overexpressing Gab1 could be used as seed cells to form a complete endodermis on decellularized valve scaffolds in order to construct TEHVs.

Material and methods

Isolation and cultivation of EPCs

Human umbilical cord blood was taken from the placental cords of volunteers who had undergone a Cesarean section delivery. To avoid thrombosis, the umbilical cord blood was collected immediately after delivery of the placenta. EPCs were isolated from 50 ml of fresh umbilical cord blood, as previously described [14]. Isolated mononuclear cells were seeded in a 25-cm^2 tissue culture flask pre-coated with human fibronectin (Sigma, USA). The mononuclear cells were cultured in Endothelial Cell Growth Medium-2 (EGM-2 Lonza, Cologne, Germany) containing 2% fetal bovine serum, hydrocortisone, human fibroblast growth factor-B, vascular endothelial growth factor, human recombinant long-insulin-like growth factor-1, ascorbic acid, human epidermal growth factor, gentamicin, amphotericin, and heparin. After 3 days, non-adherent cells were removed, and the remaining cells were maintained in EGM-2 in a 37°C, 5% CO_2 incubator.

Characterization of EPCs

To determine the endothelial phenotype of EPCs, coverslips were blocked with 1% blocking solution (BSA) for 30 min at room temperature and then incubated with primary antibodies against CD31 (1:1000, Abcam, Cambridge, MA, USA), von Willebrand factor (1:500, Abcam, Cambridge, MA, USA) at 4°C overnight. Next, the cells were incubated with fluorescein-conjugated secondary antibodies for 1 hr at room temperature. Photographs were taken with a fluorescence microscope.

Transfection and analysis of EPCs genes

The adenovirus vector Ad-Gab1-GFP (containing the Gab1 gene) was purchased from Biowit Technologies (Shenzhen, China). EPCs were incubated in six-well culture plates, digested, and counted. EPCs were then transfected at a multiplicity of infection of 20, as previously described [13]. EPCs treated with Ad-Gab1-GFP were used for the AD-Gab1 group, and non-treated EPCs were used for the control group. After cell transfection, the cells were maintained in an incubator at 37°C.

Western blotting

The cell protein concentration was estimated with the Bradford method, and the proteins were transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk, probed with anti-Gab1 (Abcam, Cambridge, MA, USA), and stained with horseradish peroxidase-coupled secondary antibodies. The protein was detected with ECL Western Blotting Substrate (Solarbio, Beijing, China) and a cooled CCD camera system (Vilber Fusion Solo 4S; Paris, France).

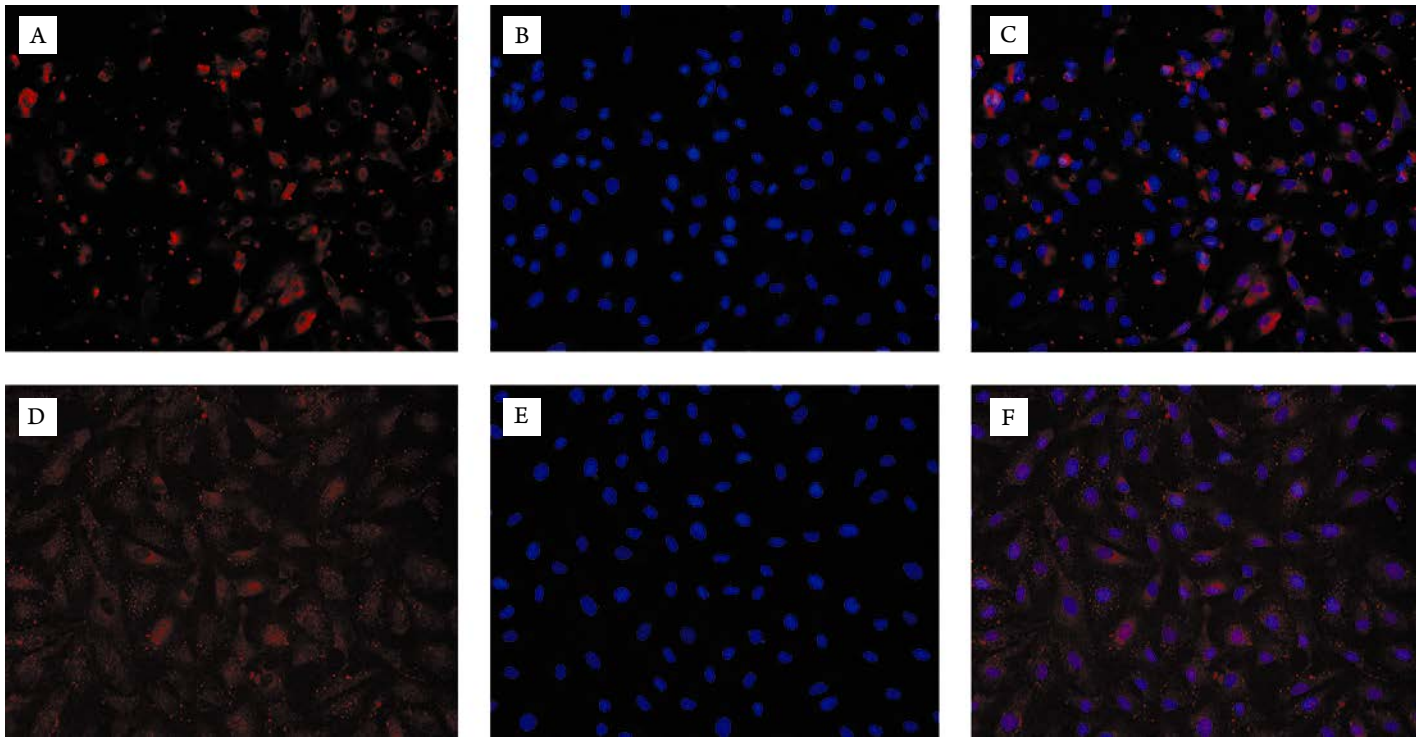
MTT assay for cell proliferation

The transfected Gab1 cells were collected and seeded in 96-well plates at a density of 6×10^3 cells/well in EGM-2 with HGF at 20 ng/ml. After 12, 24, 36, 48, and 72 hr of culture, 20 μl MTT solution (0.5% MTT) was added to each well and incubated for 4 hr at 37°C. Then the culture medium was removed, and 150 μl of dimethyl sulfoxide (Sigma-Aldrich, St.Louis, MO, USA) were added. The optical density was measured at 570 nm with a spectrophotometer.

Heart valve scaffold fabrication

Fresh porcine aortic valves were obtained from the local slaughterhouse, and the leaflets were brought to the laboratory in phosphate buffer saline (PBS) with 100 U/ml penicillin and 10 μg/ml streptomycin at 4°C. The leaflets were placed in 5 mM Tris buffer (pH=8) for 24 hr at 4°C. Next, they were placed in new 5 mM Tris buffer (pH=8) with 1% Triton X-100 (Sigma, USA) for 24 hr at 4°C. Subsequently, the valves were washed three times with PBS (15 min, 4°C) and transferred to PBS supplemented with DNase (100 mg/ml), RNase (20 mg/ml; Beyotime, Shanghai, China), and trypsin (100 mg/ml) for 90 min at 37°C. Finally, the cells were transferred to new 5 mM Tris buffer (pH=8) with 1% Triton X-100 for 24 hr at 4°C, washed several times with PBS, and stored in PBS at 4°C until further processing.

Figure 1. Expression of endothelial cell antigens by EPC. Immunofluorescence was performed after blood isolation and before seeding with antibodies to CD31 (**A**), vWF (**D**), nuclei 4,6-diamidino-2-phenylindole (DAPI) staining (**B**, **E**). Shown are antigen staining (red) and DAPI staining (blue). CD31 staining and DAPI staining double positive cells(**C**), vWF staining and DAPI staining double positive cells (**F**). Original magnification: ×200



Histological examination

The valve leaflets were stained with 4;6-diamidino-2-phenylindole (DAPI) to observe nuclei. Alcian blue-periodic acid schiff (AB-PAS) and Masson staining were performed to visualize glycosaminoglycan (GAG) and collagen fibers, respectively.

Total DNA extraction and quantification

DNA was extracted from the valve leaflets with a Genomic DNA Mini Preparation Kit with Spin Column (Beyotime, Shanghai, China), according to the manufacturer's protocol. The DNA concentration was measured with a NanoDrop spectrophotometer at 260 nm, and the total DNA concentration was expressed as ng/mg dry weight.

Cell seeding and in vitro maintenance

Cells in the experimental and control groups were separately digested, re-suspended, and counted. Decellularized valve leaflets were placed at the bottoms of culture dishes, and the EPCs were dribble-seeded at an approximate cell density of 5×10^6 cells/cm^2.

Then the experimental group cells were cultured in EGM-2 supplemented with 20 ng/ml HGF. The control group cells were cultured in EGM-2. The cell medium was changed once every 2 days, and TEHVs were cultured in vitro for 3 or 7 days.

Histomorphological analysis

The specimens that were cultured for 3 or 7 days in vitro were fixed in 4% paraformaldehyde for 15 min and then embedded in paraffin for HE and AB-PAS staining.

Statistical analysis

All results are expressed as mean±standard error of the mean. Comparisons between groups with normally distributed data were made with Student's t-tests. If measurements failed the normality test, the nonparametric Mann–Whitney rank sum tests were used. These analyses were performed with Sigma Stat (SPSS, Chicago, IL, USA). P-values < 0.05 were considered significant.

Results

Characterization of EPCs

Mononuclear cells isolated from umbilical vein blood appeared small and round after culture in EGM-2. The cells showed numerous endothelial cell-surface antigens, including CD31, vWF (Figure 1).

Overexpression of Gab-1 in transfected EPCs and western blotting

EPCs were transfected with Ad-Gab1-GFP. After 24 hr of transfection, the cells that had been transfected successfully showed green fluorescence under a fluorescence

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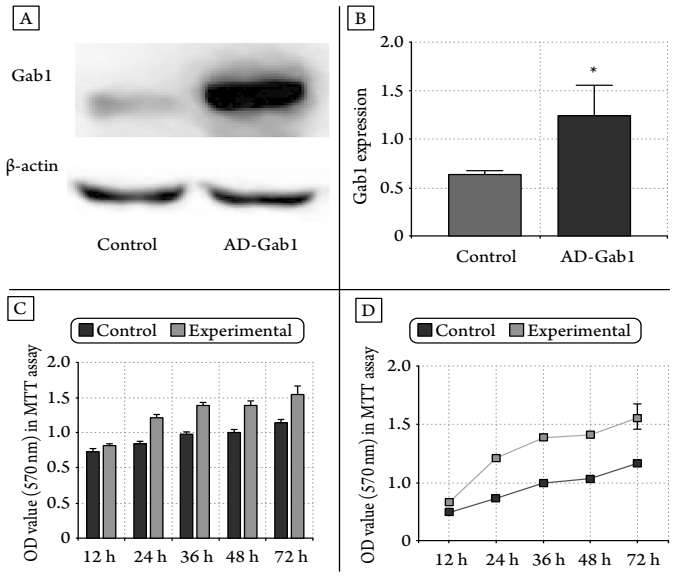
Figure 2. Gab1 expression in EPCs transfected with adenovirus. (**A, B**) Expressions of Gab1 protein in endothelial progenitor cells after transfected



* p<0.05. (C, D) MTT assay was performed to assess cell proliferation between control group and experimental group at 12 hr, 24 hr, 36 hr, 48 hr, and 72 hr. ** p<0.01, **** p<0.0001.

Table 1. DNA Content (ng/mg) in Heart Valves

Content	Cellular	Decells
Total DNA (ng/mg)	306.2±14.9	12.8±5.6

microscope. Western blotting indicated that the expression of Gab1 protein in adenovirus-transfected endothelial cells was significantly higher than that in untransfected endothelial cells (Figure 2 A, B). In the MTT experiment, the optical density was measured at 570 nm. Thus, the larger the optical density value, the more cells. Assays at different times showed that the cell proliferation ability was greater in the experimental group than the control group (Figure 2 C, D).

Histological assessment of cellular and decellularized tissues

AB-PAS staining indicated the amounts of GAG in the heart valves. Masson's trichrome indicated maintenance of fibers, and DAPI staining indicated cell removal through a decellularization process. (Figure 3 A-F). The DNA content of the decellularized heart valves was visibly lower than that in the native heart valves. (Figure 3, Table 1).

Histomorphology of TEHVs

At 3 days, HE staining and AB-PAS staining confirmed the contiguity of the endothelial cell monolayer of the neotissue. The control group did not form a complete endothelial layer until day 7 (Figure 4).

Figure 3. Light and fluorescent microscopy of native (**A, B, C**) and decellularized (**D, E, F**) heart valves. AB-PAS, Alcian blue-periodic acid shiff staining; Masson, Masson staining; DAPI, DAPI staining. Decellularized heart valve showed no remaining nuclear material. Original magnification: AB-PAS and Masson ×100, scale bar, 100 μm; DAPI ×200. Table 1 shows DNA content of native and decellularized heart valves

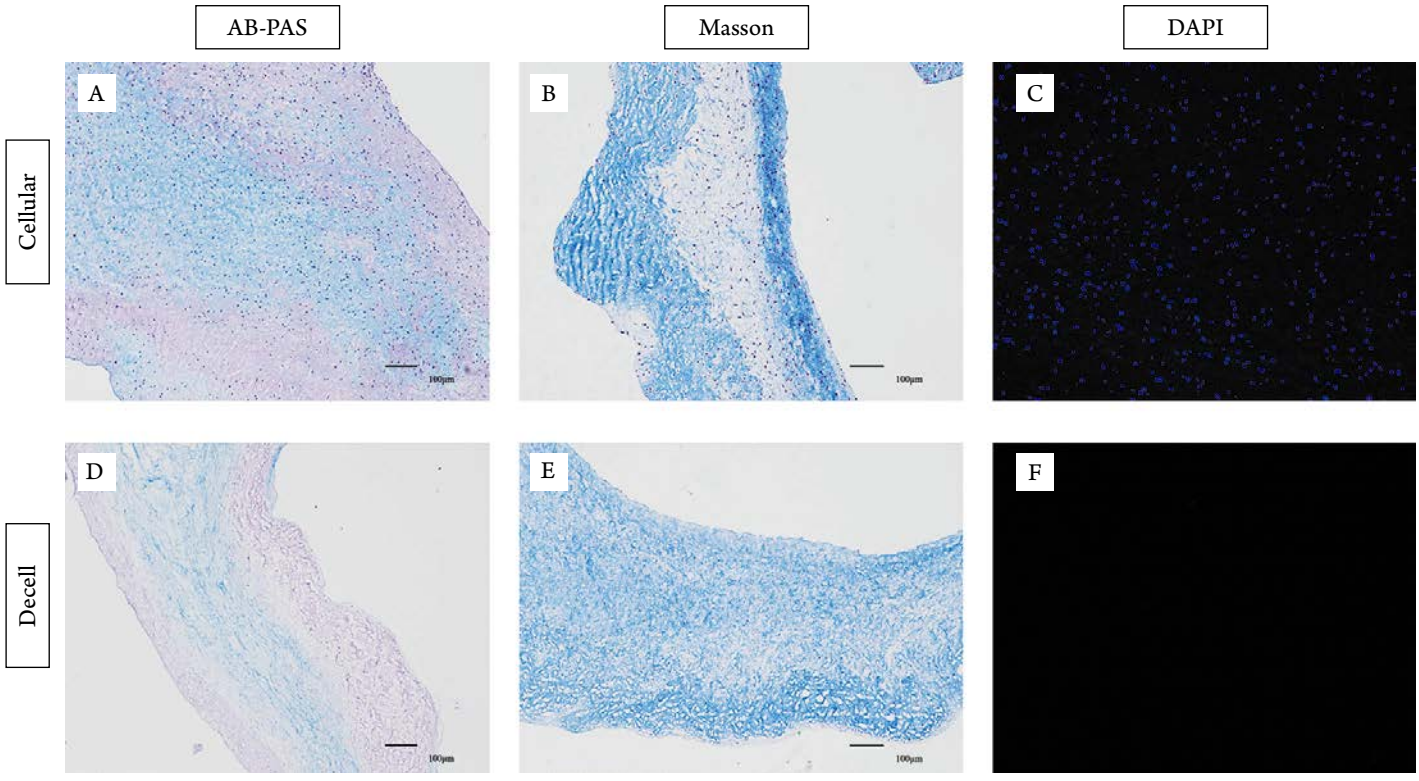
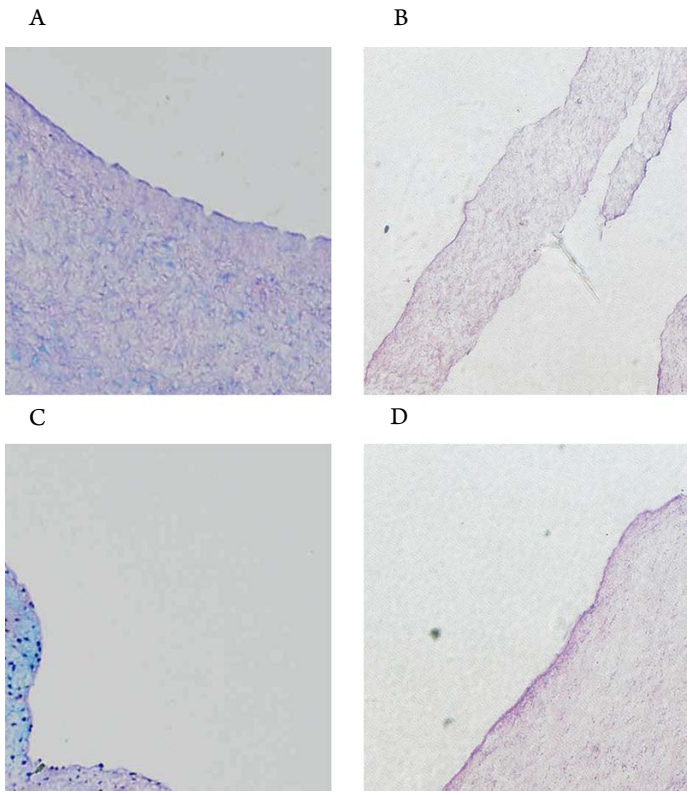


Figure 4. AB-PAS staining of heart valve of EPCs in the control group (**A**), HE staining of heart valve of EPCs in the control group (**B**); AB-PAS staining of heart valve of EPCs in the experimental group (**C**), HE staining of heart valve of EPCs in the experimental group (**D**). Original magnification: AB-PAS ×100, HE ×40



Discussion

Novel therapeutic interventions to halt heart valve deterioration must focus on strategies that target the cellular events controlling tissue degeneration and rejection. A common problem with implantation is the calcification of the valve over time, and thus, leading to structural valve deterioration [15, 16]. Accelerating the formation of functional endodermis on the valve surface can effectively reduce the incidence of these events. Tissue engineering aims to address this issue through in vitro fabrication of living autologous grafts with growth, repair, and remodeling capacity. Previous studies have confirmed the possibility of using EPCs from peripheral blood or bone marrow as an alternative cell source to create engineered tissue constructs [17–19]. However, EPCs are a fairly rare cell population, and, when administered intravenously, only a very small fraction of EPCs reach the target region and participate in re-endothelialization. How to increase this cell number is an urgent problem.

HGF is an angiogenic factor that stimulates EC proliferation and migration [20, 21]. HGF binds its receptor, c-Met, and stimulates c-Met kinase activation, which in turn triggers transphosphorylation of c-Met and downstream signaling events [22]. Unlike most RTKs, which recruit Gab1 indirectly via Grb2, Gab1 can be recruited to activated c-Met through a direct mechanism based on interaction with tyrosine-phosphorylated c-Met via the Met-binding domain (amino acids 450–532) [7, 23]. Our previous study has shown that Gab1 mediates HGF/c-Met proliferation and migration signaling in human EPCs in vitro [13].

The focus of this study was to investigate the re-endothelialization of decellularized heart valves by HGF-induced EPCs over-expressing Gab1. Decellularized matrix is an ideal scaffold that has been frequently used in animal models for fabricating engineered heart valve grafts [24]. However, there is currently no scientific evidence that decellularized heart valves will experience a partial or complete autologous cell repopulation or endothelialization following clinical implantation, as occurs in animal models [25]. The endothelial layer acts as a natural barrier that prevents platelet aggregation and fibrin deposition on the valve surface [26].

The absence of an endothelial layer is a main reason for the calcification and degeneration of artificial or natural grafts, which are currently used in clinical settings. Indeed, clinical trials suggest that constructed TEHVs form a decellularized matrix covered with EPCs that can overcome calcification and thrombogenicity without losing their excellent hemodynamic properties [27]. In contrast, four clinical valve replacements with decellularized valves without cell seeding failed within one year owing to degeneration and rupture of the valves [28]. Therefore, establishing a confluent and functional endothelial cell layer on decellularized valve scaffolds before implantation is essential [26–28].

Our results showed that after seeding onto the decellularized valve matrix, the HGF-induced Gab1 overexpressing EPCs were viable and formed an integrated neo-endothelium on the surface of the decellularized matrix in vitro. Their continued proliferation implied some capacity of the neo-endothelium for growth and self-repair. Importantly, this endothelial cell monolayer stained positive for vWF and CD31.

Conclusion

This study confirms and extends previous findings that autologous, immunologically neutral EPCs are a convenient cell source for construction of TEHVs. Thus, HGF-induced EPCs overexpressing Gab1 can endothelialize the decellularized matrix and create functional TEHVs that may then be preconditioned in a bioreactor before clinical implantation.

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Human ethics

The Medical Ethics Committee of The Affiliated Hospital of Qingdao University granted ethical approval to carry out the study within its facilities.

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REFERENCES

1. Dohmen PM, da Costa F, Yoshi S, Lopes SV, da Souza FP, Vilani R et al. Histological evaluation of tissue-engineered heart valves implanted in the juvenile sheep model: is there a need for in-vitro seeding? The Journal of Heart Valve Disease. 2006;15(6):823–9. PMID: 17152791
2. Cebotari S, Lichtenberg A, Tudorache I, Hilfiker A, Mertsching H, Leyh R et al. Clinical Application of Tissue Engineered Human Heart Valves Using Autologous Progenitor Cells. Circulation. 2006;114(1 Suppl): I132–7. DOI: 10.1161/CIRCULATIONAHA.105.001065
3. Sales VL, Mettler BA, Engelmayr GC, Aikawa E, Bischoff J, Martin DP et al. Endothelial Progenitor Cells as a Sole Source for Ex Vivo Seeding of Tissue-Engineered Heart Valves. Tissue Engineering Part A. 2010;16(1):257–67. DOI: 10.1089/ten.tea.2009.0424
4. Jordan JE, Williams JK, Lee S-J, Raghavan D, Atala A, Yoo JJ. Bioengineered self-seeding heart valves. The Journal of Thoracic and Cardiovascular Surgery. 2012;143(1):201–8. DOI: 10.1016/j.jtcvs.2011.10.005
5. Rippel RA, Ghanbari H, Seifalian AM. Tissue-Engineered Heart Valve: Future of Cardiac Surgery. World Journal of Surgery. 2012;36(7):1581–91. DOI: 10.1007/s00268-012-1535-y
6. Bianconi V, Sahebkar A, Kovanen P, Bagaglia F, Ricciuti B, Calabrò P et al. Endothelial and cardiac progenitor cells for cardiovascular repair: A controversial paradigm in cell therapy. Pharmacology & Therapeutics. 2018; 181:156–68. DOI: 10.1016/j.pharmthera.2017.08.004
7. Nakaoka Y, Komuro I. Gab Docking Proteins in Cardiovascular Disease, Cancer, and Inflammation. International Journal of Inflammation. 2013; 2013:141068. DOI: 10.1155/2013/141068
8. Nakaoka Y, Nishida K, Fujio Y, Izumi M, Terai K, Oshima Y et al. Activation of gp130 Transduces Hypertrophic Signal Through Interaction of Scaffolding/Docking Protein Gab1 with Tyrosine Phosphatase SHP2 in Cardiomyocytes. Circulation Research. 2003;93(3):221–9. DOI: 10.1161/01.RES.0000085562.48906.4A
9. Shioyama W, Nakaoka Y, Higuchi K, Minami T, Taniyama Y, Nishida K et al. Docking Protein Gab1 Is an Essential Component of Postnatal Angiogenesis After Ischemia via HGF/c-Met Signaling. Circulation Research. 2011;108(6):664–75. DOI: 10.1161/CIRCRESAHA.110.232223
10. Aasrum M, Ødegård J, Sandnes D, Christoffersen T. The involvement of the docking protein Gab1 in mitogenic signalling induced by EGF and HGF in rat hepatocytes. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2013;1833(12):3286–94. DOI: 10.1016/j.bbamcr.2013.10.004
11. Sang H, Li T, Li H, Liu J. Down-Regulation of Gab1 Inhibits Cell Proliferation and Migration in Hilar Cholangiocarcinoma. PLoS ONE. 2013;8(11): e81347. DOI: 10.1371/journal.pone.0081347
12. Zhao J, Wang W, Ha CH, Kim JY, Wong C, Redmond EM et al. Endothelial Grb2-Associated Binder 1 Is Crucial for Postnatal Angiogenesis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31(5):1016–23. DOI: 10.1161/ATVBAHA.111.224493
13. Fan Q, Zhang L, Zhu W, Xue S, Song Y, Chang Q. Up-regulation of Grb2-associated binder 1 promotes hepatocyte growth factor-induced endothelial progenitor cell proliferation and migration. PeerJ. 2019;7: e6675. DOI: 10.7717/peerj.6675
14. Ravishankar P, Zeballos MA, Balachandran K. Isolation of Endothelial Progenitor Cells from Human Umbilical Cord Blood. Journal of Visualized Experiments. 2017; 127:56021. DOI: 10.3791/56021
15. Schoen FJ, Levy RJ. Calcification of Tissue Heart Valve Substitutes: Progress Toward Understanding and Prevention. The Annals of Thoracic Surgery. 2005;79(3):1072–80. DOI: 10.1016/j.athoracsur.2004.06.033
16. Siddiqui RF, Abraham JR, Butany J. Bioprosthetic heart valves: modes of failure. Histopathology. 2009;55(2):135–44. DOI: 10.1111/j.1365-2559.2008.03190.x
17. Schmidt D, Breymann C, Weber A, Guenter CI, Neuenschwander S, Zund G et al. Umbilical Cord Blood Derived Endothelial Progenitor Cells for Tissue Engineering of Vascular Grafts. The Annals of Thoracic Surgery. 2004;78(6):2094–8. DOI: 10.1016/j.athoracsur.2004.06.052
18. Schmidt D, Mol A, Neuenschwander S, Breymann C, Gössi M, Zund G et al. Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. European Journal of Cardio-Thoracic Surgery. 2005;27(5):795–800. DOI: 10.1016/j.ejcts.2005.01.064
19. Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW et al. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nature Medicine. 2001;7(9):1035–40. DOI: 10.1038/nm0901-1035
20. Ackah E, Yu J, Zoellner S, Iwakiri Y, Skurk C, Shibata R et al. Akt1/protein kinase B is critical for ischemic and VEGF-mediated angiogenesis. Journal of Clinical Investigation. 2005;115(8):2119–27. DOI: 10.1172/JCI24726
21. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A et al. MicroRNA-92a Controls Angiogenesis and Functional Recovery of Ischemic Tissues in Mice. Science. 2009;324(5935):1710–3. DOI: 10.1126/science.1174381
22. Ha CH, Wang W, Jhun BS, Wong C, Hausser A, Pfizenmaier K et al. Protein Kinase D-dependent Phosphorylation and Nuclear Export of Histone Deacetylase 5 Mediates Vascular Endothelial Growth Factor-induced Gene Expression and Angiogenesis. Journal of Biological Chemistry. 2008;283(21):14590–9. DOI: 10.1074/jbc.M800264200
23. Barrow-McGee R, Kermorgant S. Met endosomal signalling: In the right place, at the right time. The International Journal of Biochemistry & Cell Biology. 2014; 49:69–74. DOI: 10.1016/j.biocel.2014.01.009
24. Dohmen PM, Ozaki S, Nitsch R, Yperman J, Flameng W, Konertz W. A tissue engineered heart valve implanted in a juvenile sheep model. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2003;9(4):BR97–104. PMID: 12709659
25. Stock UA, Schenke-Layland K. Performance of decellularized xenogeneic tissue in heart valve replacement. Biomaterials. 2006;27(1):1–2. DOI: 10.1016/j.biomaterials.2005.05.100
26. Kasimir M-T, Weigel G, Sharma J, Rieder E, Seebacher G, Wolner E et al. The decellularized porcine heart valve matrix in tissue engineering: Platelet adhesion and activation. Thrombosis and Haemostasis. 2005;94(09):562–7. DOI: 10.1160/TH05-01-0025
27. Dohmen PM, Lembcke A, Hotz H, Kivelitz D, Konertz WF. Ross operation with a tissue-engineered heart valve. The Annals of Thoracic Surgery. 2002;74(5):1438–42. DOI: 10.1016/S0003-4975(02)03881-X
28. Breuer CK, Mettler BA, Anthony T, Sales VL, Schoen FJ, Mayer JE. Application of Tissue-Engineering Principles toward the Development of a Semilunar Heart Valve Substitute. Tissue Engineering. 2004;10(11–12):1725–36. DOI: 10.1089/ten.2004.10.1725