Regulation of Matrix Metalloproteinase in Vascularization of Endothelial Cells On Extracellular Matrix Secreted By Mesenchymal Stem Cell Sheet

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Abstract

Background: We have previously developed a prevascularized platform by seeding endothelial cells on the mesenchymal stem cell sheet and the technology provides a promising vascularization potential for wide applications in tissue engineering. However, the mechanism behind the interactions between stem cell sheet-derived matrix and endothelial cells (ECs) are still unknown. The purpose of this study is to investigate the role of MMPs in angiogenesis process *in vitro*.

Methods: Human bone marrow-derived mesenchymal stem cells (hBMSCs) were cultured continually to form a thin cell sheet, and then green fluorescent protein (GFP)-labeled human umbilical vein endothelial cells (HUVECs) were seeded on the cell sheet to form a prevascularized system. Fibronectin, collagen I and MMPs immunofluorescence staining were used to observe their interaction changes. After that, HUVECs and hBMSCs were sorted by flow cytometry and RT-PCR was performed to quantify related gene expression of ECs and stem cells. In order to further explore the possible mechanism, the broad-spectrum MMPs inhibitor GM6001 was added into the medium to observe the effects of vascular network formation.

Results: The immunofluorescence staining results showed that HUVECs secreted MMP-2 and membrane-type matrix metalloproteinase-1 (MT1-MMP) in the early stage of angiogenesis. MMPs degraded and remodeled the ECM to create space for vascular formation. HUVECs also promoted hBMSCs to secrete tissue inhibitors of metalloproteinase-2 (TIMP-2), which can stabilize blood vessels by avoiding excessive vascularization or vascular degradation in the later stage. Thus, HUVECs/hBMSCs co-culture group were beneficial for angiogenesis than the other two control groups. With the addition of the broad-spectrum matrix metalloproteinase inhibitor GM6001, RT-PCR results showed that the formation of EC lumen was blocked, while the mean fluorescence density of fibronectin and collagen I were increased.

Conclusions: our study suggests that the MMP-2, MT1-MMP and TIMP-2 can synergistically regulate the angiogenesis. In addition, HUVECs and hBMSCs interact with each other in the process of vascular formation, and jointly regulate and promote the construction of lumen. These results also indicate a promising potential using the hBMSCs sheet as a platform for broad applications in engineering vascularized tissues.

Background

Although tissue engineering has been rapidly developed in the field of regenerative medicine research in the past few decades, it has not been widely used in clinical practice. The inability to supply blood in time after complex tissue substitutes transplanted into the host is the biggest obstacle that limits the clinical application of tissue engineering technology. Our previous studies have shown that seeding endothelial cells on the mesenchymal stem cell sheet to form a prevascularized cell sheet is more conducive to the integration with the host vasculature and the establishment of functionalized blood vessels[1], such method has been widely used in skin, bone tissue and adipose tissue. This

prevascularized cell sheet technology may be a promising prevascularized platform for the revitalization of scaffolds in bone tissue engineering. It is well known that angiogenesis is a highly regulated event that involves complex interactions between endothelial cells and extracellular matrix (ECM). Large amounts of studies have shown that in the process of angiogenesis, ECM provides a suitable microenvironment for the migration of endothelial cells (ECs) and the formation of lumens [2]. This process involves the proteolysis of ECM molecules, in which matrix metalloproteinases (MMPs) degrade ECM, especially fibronectin (FN) and type I collagen (Col I), to form tunnel space for ECs migration and tube formation [3-5]. ECM also contains various secreted growth factors and cytokines, which play essential roles in cell migration, adhesion, proliferation and differentiation [6].

MMP-2, a member of the MMPs family, belongs to gelatinase and can specifically degrade FN and Col I. However, MMP-2 is secreted in the process of zymogen formation and needs membrane-type matrix metalloproteinase-1 (MT1-MMP) to trigger before it can work. MT1-MMP can not only activate MMP-2, but also directly degrade ECM and regulate blood vessel formation [7]. On the other hand, tissue inhibitors of matrix metalloproteinases (TIMPs) can bind to inactivated MMPs or activated MMPs to specifically inhibit the activity of MMPs. Under physiological conditions, MMPs and their inhibitors form MMP-TIMP dimer to maintain the dynamic balance of tissue development [8]. Once the balance is broken, it could lead to excessive pathological vascularization or vascular degeneration [9]. These signaling events stimulate intracellular vacuole formation in ECs and activate vessel regression both *in vitro* and *in vivo* [10-13]. To understand the complex mechanisms that govern the angiogenesis process, many studies have been performed about the invasion and migration of ECs on three-dimensional (3D) hydrogel matrix with ECs seeded or ECs and other cell types co-cultured [14-16]. Although these studies demonstrated that collagen-based hydrogel has the advantage of acting as ECM proteins to investigate the interaction mechanisms between ECM and cells, it is difficult to recapitulate the complex angiogenesis process *in vivo* and the related mechanism in the interplay of cell-derived native ECM and cells. The mechanism behind these results is still unknown and inconsistent. Therefore, it is necessary to investigate how the components of the ECM of stem cell-derived cell sheets regulate the migration of ECs to form the lumen and how ECs remodel ECM to create a favorable environment for the formation of the lumen.

In this study, we first seeded GFP-labeled HUVECs on the cell sheet to form a prevascularized cell sheet, which is a typical tissue engineering technique. It contains a complete ECM and some important cell surface proteins, which contribute to the establishment of mature vasculature in bone tissue engineering. We hypothesized that MMPs degraded ECM of stem cell sheets to create relative the space for cell migration, thus leading to the tube formation. At the same time, HUVECs stimulated stem cells to differentiate and secreted TIMP to stabilize the vessel. By studying the secretion of MMP-2, MT1-MMP and TIMP under different conditions, we aimed to explore the mechanism of hBMSC-derived sheet ECM affecting tube formation and how HUVECs rearranges the matrix structure.

Methods

2.1. Cell culture. The GFP-labeled HUVECs were gifted by Yunqing Kang's lab at Florida Atlantic University and cultured in EC medium (EGM-2 medium; Lonza). The hBMSCs (Lonza) were cultured in low glucose DMEM. Both of them were supplemented with 10% fetal bovine serum (FBS, HyClone), 1% guluronicacid-penicillin-streptomycin (GPS), and 1% glutamine at 37°C and 5% CO₂. The cell medium was changed every 3 days. The passage-two cells were used in the experiments below.

2.2. Preparation and Characterization of Prevascularized HUVEC/hBMSC Cell Sheet. The hBMSCs were cultured in 6 well plates at a cell density of $1 \times 10^5/\text{cm}^2$ in low glucose DMEM (L-DMEM medium) only the first day. After 1 day, the L-DMEM medium was changed to high glucose DMEM (H-DMEM) cocktail containing 50 $\mu\text{g}/\text{mL}$ ascorbic acid and 30 mM glucose to promote the production of ECM [17]. After a thin hBMSC sheet is formed, HUVECs were then seeded on the sheet surface at a cell density of $5 \times 10^4/\text{cm}^2$ for seven days. The mixed medium of EC and H-DMEM (1:1) was used for co-cultured of hBMSCs sheet and HUVECs. At the same time, HUVECs were seeded on a tissue culture plate as a control. A fluorescent microscope was used to observe the HUVEC migration on the hBMSC sheet at designated time points of 1, 3 and 7 days. The media were changed every 3 days.

2.3. MMPs inhibitor assays. The co-cultured group with an inhibitor was carried out in 6-well plates pre-formed with hBMSC cell sheet. When the HUVECs were seeded onto the surface of the hBMSC sheet at a cell density of $5 \times 10^4/\text{cm}^2$, the broad-spectrum MMPs inhibitors GM6001 (Santa Cruz) was added to the culture media at 10 μM to observe the network formation on the 7th day. The media with GM6001 were changed every 3 days.

2.4. Immunofluorescent staining. Immunofluorescence studies were performed on each group on day 7. After washing briefly with PBS twice, the sheets were fixed in 3.0% paraformaldehyde for 15 min. Cell sheet samples were blocked in a 5% goat serum-PBS buffer solution for 30 min at room temperature. Afterwards, a primary antibody mouse anti-human FN (ab18265, Abcam, dilution 1: 300) in 1% BSA-PBS was added into the sample and incubated overnight at 4 °C. Then a secondary antibody goat anti-mouse (Alexa Fluor 594, 2 $\mu\text{g}/\text{mL}$, Invitrogen) in 1% BSA-PBS buffer was added and incubated in the dark for 1 hour at room temperature. A DAPI (5 g/mL) solution was added to counterstain the cell nuclei for 10 minutes and then the samples were washed using PBS. Primary antibodies Col I (ab90395), MT1-MMP (sc-30074) and TIMP-2 (sc-21735, Santa Cruz, dilution 1: 300) experienced the same immunofluorescent staining, respectively. The fluorescent staining was recorded by confocal microscopy (Olympus). For each group, we chose five pictures at least to analyze the difference using the software image J.

2.5 Flow cytometry. Flow cytometry was adopted to sort hBMSCs and ECs in order to detect each gene more accurately. In co-culture samples, we abandon the media, wash the cell sheet, add trypsin 1 ml every hole, and shake the 6-well plates to make the enzyme infiltrate the whole sheet, suck out and transfer the suspension cell to the sample tube with serum when some surface cells were observed slip in a microscope. The desired amount of cells was collected in the same way in the original hole. In other group, non-co-cultured cells were gotten in conventional ways.

2.6 Reverse transcription PCR (RT-PCR). RT-PCR was conducted to analyze the expression of several genes of hBMSCs and ECs. Table 1 lists the genes analyzed. RNA assay kit (Molecular Probes) was used to extract RNA according to the manufacturer's instructions. An equal amount of total RNA from each sample was used to synthesize cDNA templates, according to the manufacturer's protocol. The cDNA for each sample (2 µl) was amplified via PCR for 35 cycles in a total reaction volume of 25 µl. The reaction mixture was comprised of 0.4 uM of the appropriate forward and reverse primers, and 12.5 ul of 2x SYBR Premix Ex Taq[™] II DNA-Polymerase. Forward and reverse primer sequences and amplicon sizes are listed in Table 1. RT-PCR determined the expression level of CI, FN, TIMP-2, MT1-MMP, MMP-2 and CD31. The relative expression level was calculated using the $2^{-\Delta\Delta C_t}$ method, with the CT values normalized using GAPDH as an internal control.

Table 1 Description of primers used for RT-PCR

Gene	Primers (F =forward; R=reverse)	Amplicon size (bp)
Col I	F 5' -TCC GAC CTC TCT CCT CTG AA- 3' R 5' -AAA CTG CTT TGT GCT TTG GG -3'	125
FN	F – AAG GTT CGG GAA GAG GTT GT-3' R: – CGA GTC ATC CGT AGG TTG GT-3'	72
TIMP-2	F 5'-TCT GGA TGG ACT GGG TCA CA-3' R 5'-CTT GAT GCA GGC GAA GAA CTT-3'	
MT1-MMP	F : 5'-GCTTGCAAGTAACAGGCAAA-3' R: 5'-AAATTCTCCGTGTCCATCCA-3'	589
MMP-2	F: 5'-CAAGTGGTCCGTGTGAAGTA-3' R: 5'-CTTGCGGTCATCATCGTAGT-3'	504
CD31	F 5'-AACAGTGTTGACATGAAGAGCC-3' R 5'-TGTA AACAGCACGTCATCCTT-3'	
GAPDH	F: 5'-CCCTGTTGCTGTAGCCGTA-3' R: 5'-CCGGTGCTGAGTATGTCTG-3'	442

2.7 Statistical analysis. The data were analyzed using using SPSS Version 20.0 software package (SPSS Inc., Chicago, IL, USA). (SPSS). A Student's t-test was used to detect the difference between the two groups with $P < 0.05$ accepted as showing a significant statistical difference.

Results

1. Network Formation on the hBMSCs Sheet.

We constructed a cell sheet successfully at the bottom of six-well plates for 14 days, which presented a slight edge contraction and could be lifted. Figure. 1 (a, b) shows the dense morphology of the hBMSCs sheet, which contained the cells and rich ECM. After obtaining a dense hBMSCs sheet, we seeded the GFP-HUVECs on the hBMSCs sheet and cultured them in an endothelial medium. Once seeded, HUVECs adhered and migrated spotty distribution on the hBMSCs sheet after 3 hours. At this point, we promptly added the 10 uM inhibitor GM6001. After 1 day, the HUVECs of the co-cultured group without GM6001 elongated spindle-shaped morphology and aligned into cords (Fig. 2a). In addition to collectively undergoing morphogenesis, HUVECs proliferated, migrated and rearranged to form new blood vessels. With time increasing, these cords matured to form tubes with hollow lumens through the formation and coalescence of intracellular vacuoles and migrated to connect with each other and formed networks on day 3 (Fig. 2b). These networks continued to strengthen and formed typical lumen-like structures. At the same time, we found the number of ECs reduced (Fig. 2c). However, after the addition of the inhibitor, these phenomena disappeared, the HUVECs were arranged in disorder and almost no tube was formed (Fig. 2d-f). As a control, we seeded HUVECs on a tissue culture plate at the same time. Results showed that HUVECs adhered to the well plate (Fig. 2g-i) and proliferated to reach confluence, but they did not form any network during the culture time.

2. Dynamic remodeling of Col I and FN

Our immunofluorescence staining for Col I and FN showed a significant reconstruction of matrix structures. In the hBMSCs sheet alone group, Col I protein structure arranged randomly (Fig. 3a), but FN showed more ordered reticularly morphology (Fig. 3d). After HUVECs seeded, the morphology of Col I showed a more orderly arrangement around cells (Fig. 3b) and FN became scattered (Fig. 3e). With the addition of GM6001, Col I became disordered and arranged without directional compared to FN (Fig. 3c, f), which seems to be consistent with the results of hBMSCs sheet alone group.

To determine the changed amounts of Col I and FN, we analyzed the fluorescence intensity by the software image J (Fig. 4). The results showed that no matter whether or not HUVECs were seeded, the mean fluorescence density (MFD) of Col I was higher compared with FN in the ECM of hBMSCs. Although there was no statistical difference, it was consistent with the experimental results of Liu et al [18]. Along with the addition of HUVECs, both the MFD of matrix decreased, especially Col I, so we proposed the addition of HUVECs rearranged Col I and FN, and even secreted some kind of enzyme to dissolve them. When the inhibitor GM6001 was added into the hBMSCs group, there was no difference in MFD between FN and Col I compared with hBMSCs without GM6001.. This result suggests that GM6001 did not change the microenvironment of hBMSCs sheets. However, when the GM6001 was added into HUVECs/hBMSCs groups, the MFDs of Col I and FN markedly increased compared to the HUVECs/hBMSCs groups without the inhibitor (Fig. 4), which indicated that the MMP secreted by HUVECs was inhibited. This further confirms our above conjecture.

3. Immunofluorescence staining for MT1-MMP and TIMP-2.

To study the interaction change of MMP-TIMP dimer in the ECM of cell sheet, immunofluorescence staining of MT1-MMP and endogenous MMP inhibitor TIMP-2 were performed. Staining results showed that MT1-MMP and TIMP-2 expression did not exist in the hBMSCs alone group (Fig. 5c, f). In the HUVECs alone group (Fig 5e), MT1-MMP was slightly expressed and no expression of TIMP-2 was observed (Fig 5b, e). However, in the co-cultured group of HUVECs/hBMSCs, the expression of MT1-MMP was significantly augmented (Fig. 5a) and the expression of TIMP-2 was observed (Fig. 5d). These results suggested that the co-cultured group remarkably stimulates the secretion of MT1-MMP and TIMP-2. The secretion of MT1-MMP promotes the degradation of ECM and facilitates the ECs migration and tube formation, while the secretion of endogenous inhibitor TIMP-2 inhibits this process, so that the formed capillary vessels can be stabilized. The co-culture of the hBMSCs sheet and HUVEC was beneficial to this balance.

4. Genes expression.

To determine the specific role of each cell in this process, we decided to quantify the genes expression levels of MMP-2, MT1-MMP, CD31, TIMP-2, Col I and FN by RT-PCR. The genes expression of MMP-2, MT1-MMP and endothelial-specific marker CD31 (Fig. 6a) were remarkably up-regulated in the co-cultured HUVECs/hBMSCs group compared to those in HUVECs alone group. This result indicates that the hBMSCs sheets promoted the expression of these genes. After adding inhibitor GM6001, genes expression of MT1-MMP, MMP-2, and CD31 in both HUVECs alone group and HUVECs/hBMSCs coculture group are significantly down-regulated. Notably, the degree of down-regulation of these genes in co-cultured groups is much higher than that in HUVECs alone group, which suggests that the ECM microenvironment of hBMSCs plays a crucial role in triggering the expression of the genes.

We further investigated the gene expressions of HUVECs-derived MMP-2, MT1-MMP, CD31 and hBMSCs sheet-derived TIMP-2, FN, and Col I. Results show that in the HUVECs/hBMSCs group, the MMP-2, MT1-MMP, CD31 were highly expressed and with the addition of inhibitor GM6001 significantly reduced the expression of MMP-2 in the co-cultured group and CD31 in the HUVECs group. However, the inhibitor GM6001 did not affect the gene expression of TIMP-2 in hBMSCs and HUVECs/hBMSCs. It is not surprising since GM6001 is an inhibitor of MMPs. Notably TIMP-2 expressed a higher level in the co-cultured group compared to the hBMSCs group, which implies that the addition of HUVECs promoted the expression of TIMP-2. The expression of FN and Col I, two primary genes in the ECM of hBMSCs sheet, increased in the hBMSCs alone group after the addition of the inhibitor GM6001, while the gene expression in HUVECs/hBMSCs group was remarkably lower than in hBMSCs alone group.

Discussion

The most important feature of hBMSCs cell sheet is that it retains the best microenvironment, ECM, for cell growth [19]. VEGF was usually considered as a key molecule in the formation of new blood vessels in earlier time, but now there are more and more evidences showing the dynamics of ECM changes are equally important, especially in regulating the morphogenesis of blood vessels and the stabilization of

new blood vessels. Proteases are closely involved in every stage of the angiogenesis process, including from the initial remodeling of the basement membrane of the blood vessel to the final trimming of the vasculature. Moreover, ECM affects the formation of blood vessels in physical and chemical aspects, including providing protease cleavage sites, allowing stem cells and endothelial cells to remodel ECM. In the whole process of blood vessel formation, only by strictly controlling the expression, distribution and activation of protease can the ECM be degraded and reconstructed at the blood vessel formation site [20]. Thus, the aim of this study was to investigate the effect of the interaction between hBMSCs sheet and HUVECs on the formation of vascular networks and the related mechanisms behind the phenomena involving possible MMPs and their inhibitors. Our study showed that the hBMSCs sheet facilitated the HUVEC migration and further network formation. After seeded onto the sheet, the original flat polygonal shape of HUVECs was observed to turn into a tendency for the elongate interconnected branch structure to form tubes. Similar phenomena was not found on the tissue culture plate. This finding implies that hBMSC-derived ECM provided critical signaling support for ECs survival and migration. Similar results was found in the Soucy's study [21], in which HUVECs became a slender shape and the area occupied by cells reduced after HUVECs seeded on fibroblast-derived matrix. Richard et al. [22] also found that three-dimensional interstitial Col I stimulated ECs *in vitro* to form a spindle-shaped morphology and to align into cords similarly to those observed during angiogenesis *in vivo*. These studies strongly implies that the migration and interconnection of ECs during the process of lumen formation are related to ECM components [23]. More importantly, it was also showed that the ECM structure of hBMSCs sheet re-arranged to facilitate a proper space for the migration of ECs, which lead to the lumen formed. Our previous studies found [24] that the network started to form on the surface of a hBMSCs sheet and gradually migrated into the bottom of the hBMSCs sheet. This result confirmed the re-arrangement of the cell sheet ECM during the tube formation of seeded HUVECs.

The interaction process between the re-arrangement of the cell sheet ECM and migration of seeded ECs should be dynamic and complex. Similar to fibroblasts and adipocytes need to be guided by urokinase-type plasminogen activator in the process of vascularization, the infiltration of hBMSCs sheet blood vessels requires protease anchoring at the front of neovascularization and degradation of ECM in a tightly specific guided manner, which provides space for the formation of blood vessels. Many studies have demonstrated that MMPs and their endogenous inhibitors balance the process to prevent excessive blood vessel formation or regression [9-13]. In this study, we also examined the interaction between the MMPs and their inhibitors involving in our hBMSCs sheet/HUVECs system. GM6001, a specific inhibitor of MMPs was added to block the roles of MMPs in the degradation of ECM. We found that HUVECs group could secrete a small amount of MT1-MMP (Fig. 5b), but increased secretion level in the HUVECs/hBMSCs group (Fig. 5a). The result indicated that the hBMSC sheets promoted HUVECs to secrete MT1-MMP. Considerable evidence indicated that the culture of ECs in a three-dimensional Col I matrix increased the total amount of MMP-2 and MT1-MMP mRNA and proteins [25], our results also implied that ECM matrix proteins, especially Col I contained in the BMSC sheet stimulated the secretion of MMP-2 and MT1-MMP. At the same time, we found that the gene expression of CD31 increased when HUVECs were seeded on the cell sheet, and that the addition of inhibitor lowered the gene expression in

both HUVECs group and co-cultured group (Fig. 6a). Our data indirectly support the conclusion that the ECM of hBMSCs sheet was in favor of the interaction with ECs for their adhesion, migration, and formation of blood vessels, while the matrix-metalloproteinase inhibitor blocked this interaction process. Moreover, this result also demonstrated that MT1-MMP mediated the interaction process. In addition, as another important ingredient of ECM, FN play a key role in promoting cell adhesion, proliferation and signal transduction. Ardila et al believed that the FN is an active ingredient in stimulating the formation of ECs lumen, while other ECM molecules will only provide the necessary ingredients for the environment [26]. Therefore, in this study we used stem cell-derived cell sheets that provided the native ECM components to study the interaction between ECM microenvironment and ECs.

Many studies have demonstrated that ECs facilitate the growth of blood vessel via secreted MMPs and cytokines [8], including MT1-MMP, degrading ECM and creating space for ECs migration and the formation of a tube. On the other hand, hBMSCs which have been exposed to endothelial growth supplements differentiate into perivascular cells in the stimulation of ECs. These mesenchymal stem cell (MSC)-derived pericytes could keep the vessels stability by secreting inhibitors of MMPs and protect the perivascular niche from high levels of MMPs. In our study, the hBMSCs sheet without HUVECs did not secrete TIMP (Fig. 5f). However, when co-cultured HUVECs and hBMSCs sheet for 7 days, the gene for TIMP-2 was increased (Fig. 5d), which means HUVECs stimulate the MSCs to secrete TIMP to balance the action of MMP-2 [27]. Additionally, according to Lee et al [28] the stem cells-derived matrix contains large amounts of Col I and FN, which was more conducive to the secretion of MT1-MMP. We detected both of the essential ingredients by RT-PCR and immunofluorescence staining. Col I and FN have a high content in hBMSCs sheet, especially Col I is higher than FN (Fig. 4). Furthermore, ECs secreted more MT1-MMP after co-cultured with hBMSCs (Fig. 5a and Fig. 6a). Previous studies have shown that ECM controls all stages of angiogenesis through three aspects: structural support [28], direct signal transduction [29] and dynamic remodeling, and this process is regulated through MMPs and TIMPs. Similar to these previous reports, we detected the gene expression of MT1-MMP and TIMP-2 increased in the co-cultured group and found that they interacted with each other to stimulate the secretion of MT1-MMP and TIMP-2, thus promoted the lumen formation and stabilization synergistically [16, 30].

Conclusion

In summary, this study shows the interaction between ECs and hBMSC sheets in the process of the formation of blood vessels. We successfully observed the vessel lumen formation and matrix remodeling, and proved that MMP-2, MT1-MMP and TIMP-2 play a key pair role in ECM remodeling and the formation of blood vessels .

Abbreviations

ECM: extracellular matrix; MMPs: matrix metalloproteinases; ECs: endothelial cells; hBMSCs: human bone marrow-derived mesenchymal stem cells; GFP: green fluorescent protein; MT1-MMP: membrane-type matrix metalloproteinase-1; TIMP-2: metalloproteinases-2; COL I: collagen I; FN: fibronectin; HUVECs:

human umbilical vein endothelial cells; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; GPS: guluronicacid-penicillin-streptomycin; MFD: mean fluorescence density; 3D: Three-dimensional; MSC: Mesenchymal stem cell;

Declarations

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Authors' contributions

Lina Ren contributed to data analysis and interpretation, drafting and editing of the manuscript. Jinda Li and Dan Yang contributed to the experimental design and the experimental process. Xuan Zhang contributed to data collection and organization. Yuan Shen contributed analysis and interpretation. Rui Chen contributed to data analysis and interpretation and drafting. Dongyang Ma and Liling Ren provided fund support for the experiment, supervised the whole process of the experiment, and made the final proofreading of the manuscript. The final manuscript read and approved by all authors.

Availability of data and materials

All data generated and/or analyzed during this study are included in this manuscript and its supplementary information files.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Morphology of hBMSCs sheet. a Morphological characteristics of HUVECs on hBMSCs sheet after 21 days culture. Scale bar, 100 μm . b hBMSCs grew into a layer of milky white film-like substance attached at the bottom of the six-well plates, which presented a slight edge contraction and could be peeled off.

Figure 2

Migration analysis of HUVECs on hBMSCs sheet. After seeding HUVECs on hBMSCs sheet a 1 day, b 3 days and c 7 days, a continuous process of HUVECs networks on a hBMSCs sheet. d-f Morphology of HUVECs seeded on hBMSCs sheet within GM6001. g-i Morphology of HUVECs seeded on tissue culture plates. d-i HUVEC did not form networks. The red arrows show the lumen formation. All scale bars, 100 μ m.

Figure 3

Dynamic remodeling of Col I and FN. Arrangement of Col I in the a hBMSCs worksheet group, b co-culture normal group and c co-culture inhibitor group. Distribution of FN in the d hBMSCs worksheet group, e co-culture normal group and f co-culture inhibitor group. (red: Col I/FN; blue: DAPI). All scale bars, 100 μ m.

Figure 4

The mean fluorescence density (MFD) of Col I and FN in each group. (*P< 0.05, n= 5)

Figure 5

Expression of MT1-MMP and TIMP-2 in each group. Fluorescent images show the result of MT1-MMP in the a co-cultured normal group, b HUVECs group and c MSCs group; d-f The result of TIMP-2 in the co-cultured normal group, HUVECs group and MSCs group (red: MT1-MMP/TIMP-2; blue: DAPI). All scale bars, 50 μm ..

Figure 6

mRNA expression of MT1-MMP, MMP-2 and the surface markers CD31 from a endothelial cells; TIMP-2, Col I, FN from b hBMSCs detected by RT-PCR (*P< 0.05, n= 3).