Low-intensity pulsed ultrasound enhances angiogenesis in rabbit capsule tissue that acts as a novel vascular bed in vivo

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Abstract

Background. In vivo prevascularization followed by pedicled transfer has emerged as a promising strategy for tissue engineering in recent years. We recently demonstrated that capsule tissue could serve as a novel axial in vivo vascular bed, although its high-density microvessels could only be maintained for about a week.

Objectives. In this present study, we aimed to demonstrate whether low-intensity pulsed ultrasound (LIPUS) promotes angiogenesis in capsule tissue.

Materials and methods. After successful induction of capsule tissue using a skin expander, 24 rabbits were randomly divided into the LIPUS group and the control group. The LIPUS group received LIPUS treatment 3 times per week. After 2 and 4 weeks of treatment, angiogenesis of the capsule tissue was assessed using in vivo and in vitro methods, including contrast-enhanced ultrasound (CEUS), photoacoustic imaging (PAI), photoacoustic microscope (PAM), and CD31 immunohistochemistry.

Results. In vivo assessments (CEUS, PAI and PAM) showed that tissue perfusion, hemoglobin content and vascular density were all significantly higher in the LIPUS group, which was consistent with CD31 immunohistochemistry. The LIPUS also promoted protein and mRNA expression of vascular endothelial growth factor α (VEGFα) and basic fibroblast growth factor (bFGF) in capsule tissue. Furthermore, cell experiments showed that LIPUS enhanced tube formation of human microvascular endothelial cells (HMECs) and promoted secretion of VEGFα and bFGF.

Conclusions. The LIPUS treatment promoted angiogenesis of the capsule tissue by stimulating release of angiogenic factors such as VEGFα and bFGF from endothelial cells, making the capsule tissue more potent and sustained when acting as in vivo vascular bed.

Key words: low-intensity pulsed ultrasound, capsule tissue, angiogenesis, vascular bed, tissue engineering

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Background

Timely vascularization to establish blood supply after implantation is essential for successful reconstruction and is one of the major challenges currently faced by tissue engineering. Classical approaches, including optimization of the material properties of the scaffolds, addition of angiogenic factors and inclusion of microvascular networks in the constructs, are barely suitable for large grafts.[1] Prevascularization on in vivo or in vitro vascular beds and fabrication of vascularized constructs with vascular pedicles have been proven to be promising solutions to this problem.[2,3]

Capsule tissue induced by an expander can serve as an in vivo vascular bed due to its unique blood supply. Bengtson et al. demonstrated that an isolated flap of capsule tissue can survive as a local pedicle flap to provide sufficient inherent vascularity to support a split-thickness skin graft in pigs.[4] This study was the first to use the capsule tissue as an in vivo vascular bed. Schoeller et al. utilized pouch-like capsule tissue to cultivate urothelial cells suspended in fibrin glue and fabricate a pre-laminated flap for bladder reconstruction in a rat model.[5] In addition, capsule tissue can be used as a tissue-engineered blood vessel for arteriovenous grafting by changing its shape to a tubular shape.[6] Moreover, capsule tissue can be directly used to rebuild tissue defects or redefine the tissue border in clinical practice.[7,8]

In our previous study, the capsule tissue induced by an expander served as a novel in vivo vascular bed for cultivating smooth muscle cell sheets or buccal mucosa and fabricating vascularized and pedicled constructs that can be successfully used for bladder or urethra reconstruction.[9–12] However, according to our findings, the vascular density within the capsule tissue was highest 1 week after the full expansion, rapidly declined in the next week, and then tended to remain stable at a relatively low level.[9] This phenomenon may limit the application of the method in multilayered engineered tissues, which usually need a few weeks to stack together and form integrated tissues.

Low-intensity pulsed ultrasound (LIPUS) has been proven to be effective in promoting angiogenesis under many conditions, such as cardiac dysfunction, tissue injury and tissue engineering.[13–15] However, LIPUS has rarely been applied to promote angiogenesis in the vascular bed for tissue engineering.

Thus, in the present study, we aimed to determine whether LIPUS ameliorates vascularization of the capsule tissue through therapeutic angiogenesis and enhances the potency and durability of this in vivo vascular bed for tissue engineering.

Objectives

The purpose of the study was to promote angiogenesis in the capsule tissue by LIPUS, which may enhance the efficacy and sustainability of this in vivo vascular bed for tissue engineering, and to preliminarily investigate the mechanism of this proangiogenic effect.

Materials and methods

Animals and study design

The animal protocol was approved by the Ethics Committee of Shanghai Sixth People's Hospital affiliated with Jiao Tong University, China. Twenty-four male New Zealand white rabbits, purchased from Shanghai Jiao Tong University and raised in the same feeding room, were randomly divided into 2 groups (n = 12 per group) using a random number table. Only the authors in charge of research design and conduct of the treatment were aware of the group allocation. The LIPUS group was treated with LIPUS 3 times per week (every Monday, Wednesday and Friday) after a successful induction of the capsule tissue (2 weeks after implantation of an expander), and the control group received the same treatment with the LIPUS device turned off. Angiogenesis in the capsule tissues of rabbits in the 2 groups was evaluated 2 and 4 weeks after LIPUS treatment (n = 6 per time point for each group). Obvious complications such as infection, wound disruption or poor healing at any point throughout the whole process were criteria for exclusion.

Expander capsule induction

Following general anesthesia, inguinal incisions were made at an average length of 2 cm, and the superficial circumflex iliac vessels (SCIs) were carefully isolated from the surrounding tissue. An empty spherical skin expander (10 mL) was placed around separated SCIs to induce the capsule tissue (Fig. 1A). Starting from day 8 after the implantation, 3 mL of saline solution was injected into the expanders every other day until the expanders were fully expanded (Fig. 1B). Full expansion took 2 weeks after skin expander implantation, and the capsule tissue was fully formed at this time point.

LIPUS application

After full expansion, LIPUS treatment was applied 3 times per week for 2 or 4 weeks using a portable ultrasound device (LIFU-DT100; Institute of Ultrasound Imaging of Chongqing Medical University, Chongqing, China). Comprehensive comparison of previous studies suggested the following conditions for LIPUS treatment: a pulse frequency of 1.0 MHz, an output intensity of 200 mW/cm^2, a duty cycle of 20%, and a repetition rate of 100 Hz.[14–16] Rabbits were anesthetized, and the skin around the expander was cleaned. Then, LIPUS was administered to the skin around the expander for 20 min for each rabbit (Fig. 1C). The probe was slowly moved over the skin to ensure that the capsule tissue was treated evenly

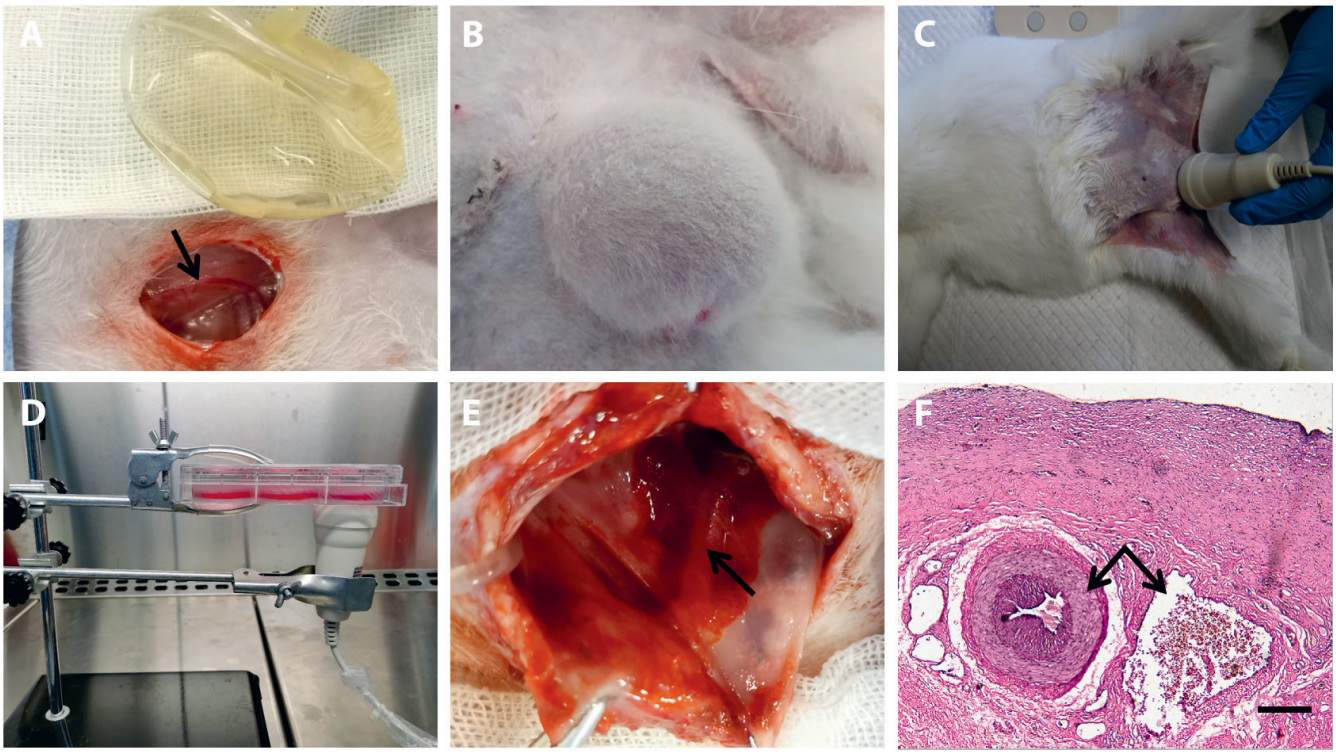

Fig. 1. Induction of the capsule tissue using a skin expander. A. A skin expander was placed to surround the separated superficial circumflex iliac vessels (SCIs) in the groin; B. The skin expander was fully injected 2 weeks after the implantation; C. The application of LIPUS on rabbit capsule tissue; D. The application of LIPUS on HMECs in vitro; E. Gross appearance of the induced capsule tissue; F. H&E staining showed that SCIs were located in the central layer of the capsule tissue. Black arrow – SCIs; scale bars = 200 μm

and to prevent local overheating. The rabbits in the control group received the same anesthesia and treatment procedures with the device turned off.

Contrast-enhanced ultrasound

Contrast-enhanced ultrasound (CEUS) was performed with a MyladTwice eHD instrument (Esaote, Genoa, Italy) using a CA541 probe (1–8 MHz bandwidth). The rabbits were anesthetized and placed in the prone position. The best image plane was confirmed under greyscale ultrasonography scanning. The examination data were acquired immediately after a 0.1 mL bolus injection of SonoVue (Bracco Suisse SA, Geneva, Switzerland) followed by a saline flush into the ear vein of each rabbit. The images were obtained before and during microbubble passage through the capsule tissue. The region of interest (ROI) was manually selected in each image based on the border of the capsule tissue, and the time-intensity curves were plotted using Sonomath software (developed by the Department of Ultrasound in Medicine, Shanghai Sixth People's Hospital affiliated with Jiao Tong University, Shanghai, China).

Photoacoustic imaging

Photoacoustic imaging (PAI) was performed using a PrexionLED AcousticX instrument (PreXion Co. Ltd., Tokyo, Japan) with a 10 MHz transducer (L100AAN) immediately after CEUS. The lower part of the bodies of the rabbits were placed in a basin with warm water, and the sensor was pressed against the local skin to achieve better acoustic coupling between the sensor and the capsule tissues. The imaging parameters were set as follows: display mode: PA+B; frequency: 9 MHz; gain: 45 dB and 60 dB; and depth: 4 cm. Images were acquired at a wavelength of 850 nm corresponding to oxygenated hemoglobin. The results were recorded as images. The ROI was manually selected in each image based on the border of the capsule tissue, and the signal intensity was calculated using ColorQuantification software (developed by the Department of Ultrasound in Medicine, Shanghai Sixth People's Hospital affiliated with Jiao Tong University).

Photoacoustic microscope scanning

A photoacoustic microscope (PAM) (Hadatomo™ Z WEL5200; Advantest, Tokyo, Japan) was used for three-dimensional (3D) imaging of the vasculature within the capsule tissue under the following settings: frequency: 500 MHz, wavelength: 532 nm, measurement range: 9 × 9 × 6 mm. The results were recorded, and the 3D distribution of the vessels was reconstructed using the software for enhanced calculation of the vascular density.

Histological analysis

After general anesthesia, the capsule tissue of each rabbit was fully exposed. Then, the capsule tissues were harvested and placed in liquid nitrogen or 4% paraformaldehyde. The samples were embedded in paraffin and sectioned into 4-μm thick sections, and hematoxylin and eosin (H&E) staining was performed according to the standard protocols. In addition, the sections were deparaffinized and blocked with 3% bovine serum albumin (BSA) for 30 min followed by incubation with a mouse anti-CD31 monoclonal antibody (1 : 500; Abcam, Cambridge, UK). The images were acquired using fluorescence microscopy. Each slide was carefully examined at ×40 magnification to identify the area with the highest density of vessels. Then, 3 fields of view were selected at ×200 magnification. The number of CD31-positive vessels and the area ratios were quantified using ImageJ software (National Institutes of Health, Bethesda, USA).

ELISA

The tissue samples were stored at 2–8°C after thawing and manually ground to homogeneity in phosphate-buffered saline (PBS) followed by centrifugation at 10,000 × g for 20 min at 4°C. The supernatant was collected, and the protein levels of vascular endothelial growth factor α (VEGFα) and basic fibroblast growth factor (bFGF) in the supernatant were measured using enzyme-linked immunosorbent assay (ELISA) kits (Keshun, Shenzen, China) according to the manufacturer's instructions.

Real-time PCR

RNA was extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, USA). Total RNA was converted to cDNA using an RT reagent kit (TaKaRa, Tokyo, Japan). The primer sequences were as follows: (forward) 5'-TGGCAGAAGAAGGAGACAATAA-3' and (reverse) 5'-GCACTCCAGGCTTTCATCATT-3' for VEGFα; (forward) 5'-AGACTGCTGGCTTCTAAATGTGTT-3' and (reverse) 5'-TTCGTTTCAGTGCCACATACCA-3' for bFGF; and (forward) 5'-CCGCCCAGAACATCATCCCT-3' and (reverse) 5'-GCACTGTTGAAGTCGCAGGAGA-3' for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Reverse transcription was performed at 37°C for 15 min; then, quantitative real-time polymerase chain reaction (qPCR) was performed using a SYBR5 Premix Ex Taq kit (TaKaRa). The qPCR was performed for 5 min at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 65°C. The results are presented as the copy number of the target gene relative to that of *GAPDH*, which was used as a housekeeping gene.

Cell culture and LIPUS treatment

Human microvascular endothelial cells (HMECs) were purchased from the American Type Culture Collection (ATCC, Manassas, USA) and used for in vitro experiments. The HMECs were reseeded into six-well cell culture plates overnight in complete MCDB 131 medium (Gibco, Waltham, USA) and then stimulated with LIPUS (Fig. 1D). The in vitro-cultured cells were in a fragile external environment different from the ones in vivo, and a previous study showed that an intensity over 200 mW/cm^2 might be harmful to cultured endothelial cells.[17] Thus, the parameters for cell treatment were set as follows: frequency of 1 MHz, intensity of 100 mW/cm^2 and duration of 10 min per day for 3 days. Control cells were subjected to the same treatment with the machine turned off. After the last stimulation, the cells were cultured in complete medium for another 30 min and harvested for tube formation assays and protein extraction.

Endothelial cell tube formation assay and ELISA

Matrigel matrix (Becton Dickinson Biosciences, Franklin Lakes, USA) was added to prechilled 96-well plates at a dose of 50 μL/well and then incubated at 37°C for 3 min. The HMECs suspended in complete medium at a density of 2 × 10^4 cells/well were seeded in Matrigel-coated wells. After incubation for 8 h, tube formation was detected using an inverted microscope. The tube formation indicators (total length and total nodes) were measured using ImageJ software.

The HMECs were lysed using whole cell lysis buffer (Keshun) containing 1% phenylmethylsulfonyl fluoride (PMSF) and placed on ice for 30 min to extract total protein. Then, the protein levels of VEGFα and bFGF in the lysates were measured using ELISA kits (Keshun) according to the manufacturer's instructions.

Statistical analyses

All statistical analyses were performed using GraphPad Prism v. 8.0 software (GraphPad Prism, San Diego, USA). The results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using Student's t-test for two-group comparisons. A value of $p < 0.05$ were considered statistically significant.

Results

Characteristics of the capsule

Capsule tissues were successfully induced in all of the rabbits, and no rabbit was excluded due to obvious complications during the whole process. The morphology of the capsule tissue was hollow with a smooth surface. Pulsatile SCIs were located inside the capsule tissue, and numerous small vessels originated from axial SCIs and extended to the periphery of the capsule (Fig. 1E).

The H&E staining showed 3 layers of the capsule tissue: the cellular layer (closest to the expander), central layer and fibrous layer. The SCIs were located in the central layer surrounded by numerous microvessels (Fig. 1F).

Effects of LIPUS on angiogenesis in the capsule tissue

Angiogenesis in the capsule tissue was assessed in vivo with CEUS (Fig. 2A), PAI (Fig. 2B) and PAM (Fig 2C), which detected tissue perfusion, hemoglobin content and vessel density, respectively. The recordings were processed using the corresponding software as described in the Materials and methods section to quantify the signal intensity (Fig. 2D–F). The results showed that the signal intensities of CEUS, PAI and PAM were significantly higher in the LIPUS group ($p < 0.01$) 2 and 4 weeks after LIPUS treatment (Fig. 2G–I).

The results of CD31 immunohistochemistry showed a significantly higher number of vessels and greater CD31-positive areas in the LIPUS group (Fig 3A,B). Although there was a decline in the LIPUS group at 4 weeks ($p < 0.01$), the vascular density remained relatively high. In addition, there was no significant difference between the 2 time points in the control group.

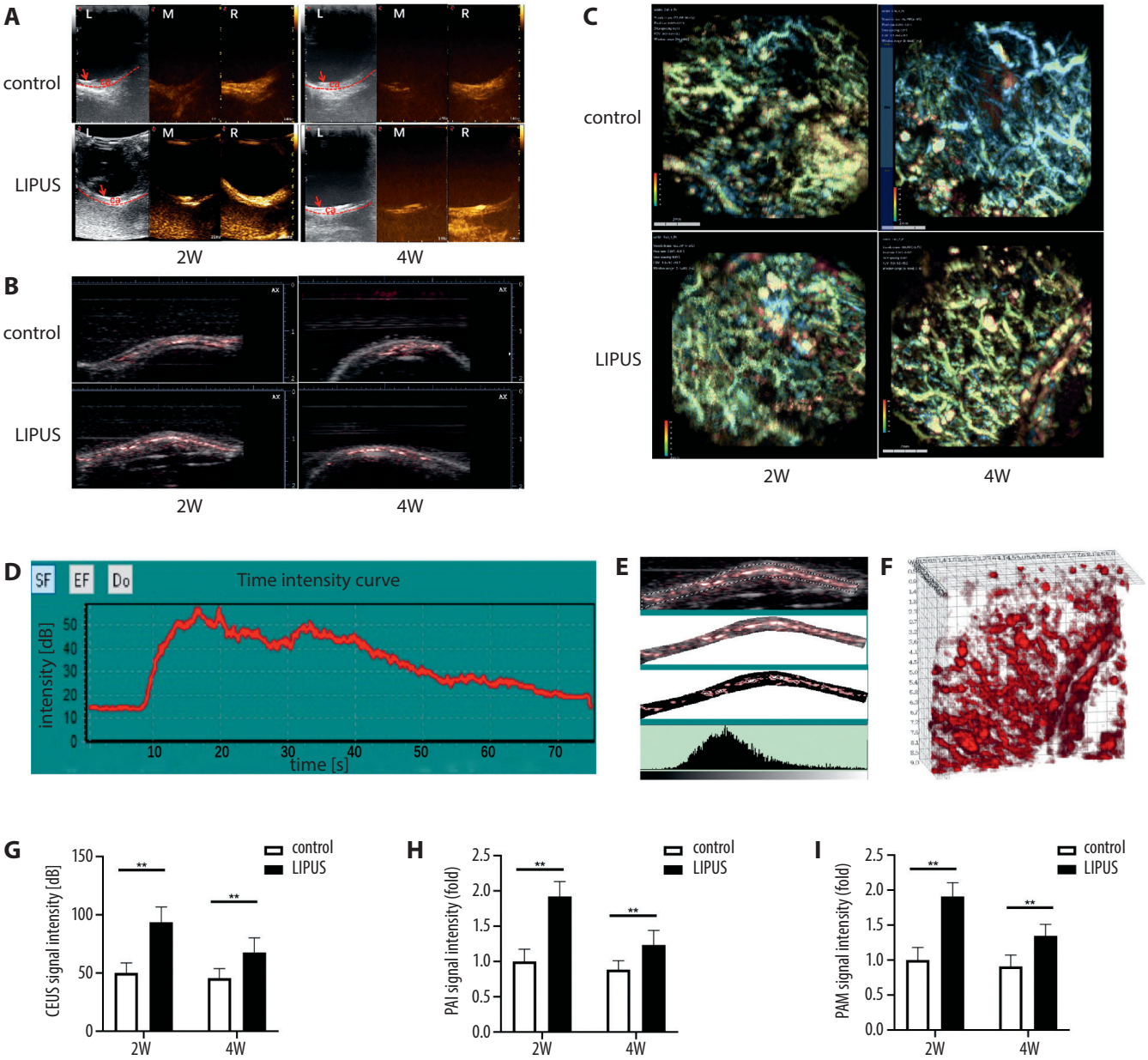

Fig. 2. LIPUS promoted tissue perfusion, increases in hemoglobin content and vascular density in the capsule tissue detected using CEUS, PAI and PAM, respectively. A. CEUS images of each group. Red arrow – the wall of the expander; L – greyscale ultrasonography scanning of the capsule tissue; M – before the injection of microbubbles; R – microbubbles passing through the capsule tissue; ca – capsule tissue; B. PAI of each group; C. Images obtained with PAM of each group (color scale: percentage of oxygen saturation); D. Time-intensity curve of CEUS; E. Quantitative analysis of the PAI signal intensity; F. Reconstructed 3D distribution of the vessels used for quantification; G–I. Comparison of CEUS, PAI and PAM signal intensities in each group and at each time point. The data are shown as mean ±SD; **$p < 0.01$

Effects of LIPUS on the expression of protein and mRNA of VEGFα and bFGF in the capsule tissue

We then determined the effect of LIPUS treatment on the secretion of VEGFα and bFGF in the capsule tissue. The expression of protein and mRNA of VEGFα and bFGF in the capsule tissue was detected using ELISA and real-time PCR (RT-PCR), respectively. The results demonstrate that LIPUS enhanced the expression of VEGFα and bFGF at the protein and mRNA levels (Fig. 3C,D).

Effects of LIPUS on HMECs

An in vitro tube formation assay was performed to assess the potential proangiogenic effects of LIPUS treatment on HMECs. The results indicated that LIPUS treatment apparently promoted tube formation in treated HMECs compared with that in the control cells ($p < 0.01$), including total length and total nodes (Fig. 4A,B). Furthermore, the results of ELISA of HMEC lysates showed that LIPUS treatment also enhanced the secretion of VEGFα and bFGF in HMECs ($p < 0.01$, Fig. 4C). These results indicate that LIPUS stimulated VEGFα and bFGF expression in HMECs and enhanced tube formation.

Discussion

The lack of timely and functional vascularization has become a common shortcoming in tissue engineering.[18] For example, the clinical results of bladder reconstruction showed that engineered bladder tissues usually develop into fibrotic tissue due to insufficient blood supply.[19] Therefore, in recent years, the field of tissue engineering has turned towards angiogenesis, which is being actively pursued by a variety of physical and chemical methods, such as co-culture with endothelial cells and application of angiogenic growth factors using topographical engineering or 3D bioprinting.[1] However, these traditional strategies cannot produce a marked effect on large or thick tissue since the average growth rate of newly formed vasculature in transplanted tissue is only approx. 5 μm/h.[20] Pre-vascularization on a vascular bed with a pedicle, which can be finally transplanted together with the construct, has been recently proven to be an effective strategy because the vascular pedicle can provide sufficient and continuous blood supply for the graft.

Capsule tissue is a valuable in vivo vascular bed for tissue engineering[4] due to its unique blood supply and the flexibility to generate it in locations without sacrificing any normal tissue. In our previous studies, a tissue expander

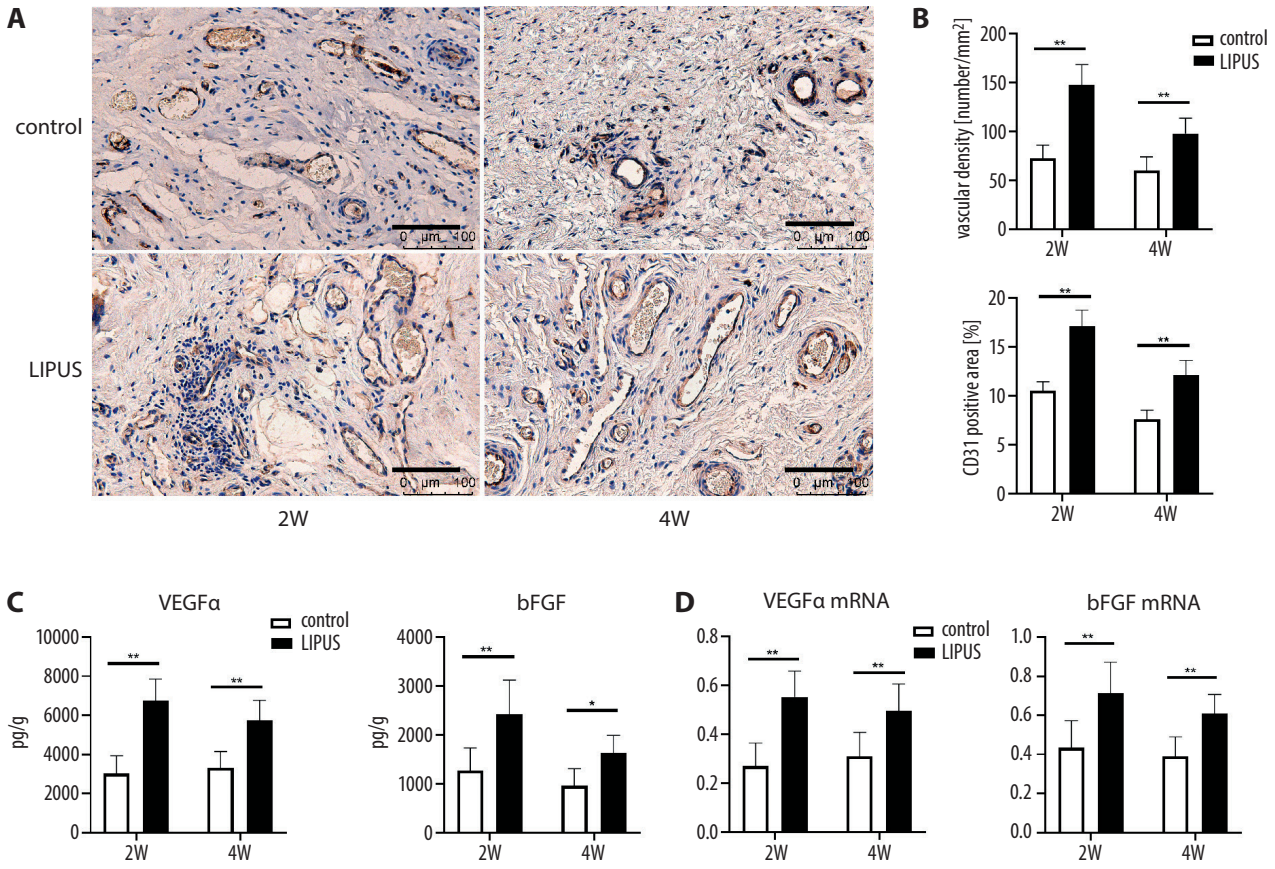

Fig. 3. LIPUS increased the number and area of blood vessels and upregulated the expression of VEGFα and bFGF at the protein and mRNA levels in the capsule tissue. A. CD31 immunohistochemical staining of each group; B. The number of CD31-positive vessels and CD31-positive areas in each group; C. Expression of VEGFα and bFGF detected using ELISA; D. Expression of VEGFα mRNA and bFGF mRNA detected using real-time PCR. The data are shown as mean ±SD; **$p < 0.01$; *$p < 0.05$; scale bars = 100 μm

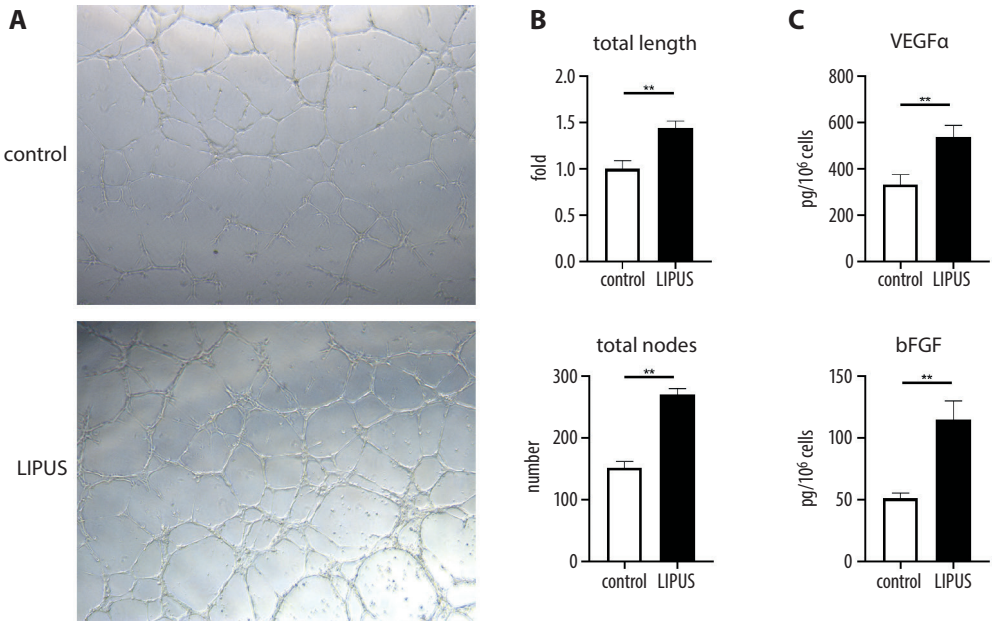

Fig. 4. LIPUS enhanced tube formation and upregulated the expression of VEGFα and bFGF in HMECs. A. Tube formation assay of HMECs; B. Quantified results of tube formation assay; C. Expression of VEGFα and bFGF in HMECs detected using ELISA. The results are expressed as mean ±SD; **p < 0.01

was placed to surround separated SCIs in the groin region to generate capsule tissue that contained an axial vascular pedicle influenced by SCIs. The effectiveness of the capsular vascular bed was verified by comparison with the subcutaneous vascular bed.[9] Subsequently, we used the capsule tissue as a vascular bed to cultivate free buccal mucosa grafts or multilayered smooth muscle cell sheets and fabricated pedicled vascularized constructs that can be successfully used for anterior and posterior urethra or bladder reconstruction, respectively.[10–12] The results were encouraging since the vascular pedicle continuously provided independent blood supply for the construct. However, we still encountered certain problems in the previous experiments. We demonstrated that the vascular density of the capsule tissue continued to increase after full expansion and reached its peak approx. 1 week later. Then, the vascular density rapidly decreased in the next week and remained stable. Thus, high efficiency of the capsular vascular bed can be maintained only for several days, which may limit the application of this method in the fabrication of multilayered constructs, such as full-thickness bladder tissue, that usually need multistage implantations and a long build time. Therefore, maintaining a sufficient blood supply of the capsule tissue for a sufficiently long time represents one of the major challenges.

The change in vascular density in the capsule tissue is related to its formation. Capsule tissue is the product of foreign body reaction against the implanted expander, which can be generally divided into 5 phases: 1) protein adsorption; 2) acute inflammation; 3) chronic inflammation; 4) foreign body giant cell formation; and 5) fibrosis or fibrous capsule formation.[21,22] Macrophages are predominant in the chronic inflammation phase and secrete many proangiogenic growth factors, such as VEGF, FGF and platelet-derived growth factor (PDGF), which play important role in angiogenesis. Therefore, a subsequent decrease in inflammation results in gradual maturation of the initial capsule tissue into less cellular and more collagenous and thicker tissue. The concentration of angiogenic growth factors is thus reduced, leading to a decrease in vascular density.

The LIPUS is a noninvasive and straightforward technology that has been proven to be effective in promoting angiogenesis under many conditions. In the present study, we aimed to verify the angiogenic effect of LIPUS on the capsular vascular bed. The LIPUS treatment was applied 3 times per week after the expander was fully filled with saline solution, and the results show that tissue perfusion and vascular density of the capsule tissue were significantly enhanced by LIPUS treatment. After 2 weeks of LIPUS treatment, the vascular density of the capsule tissue almost reached the peak level of the untreated tissue registered 1 week after full expansion. Although the vascular density in the LIPUS group was decreased after 2 weeks, it remained relatively high, indicating that LIPUS treatment of the in vivo vascular bed enhanced the efficacy and sustainability of the capsule tissue. In addition, LIPUS resulted in prolonged upregulation of VEGFα and bFGF, 2 fundamental growth factors involved in angiogenesis, at the protein and mRNA levels.

The potential mechanism for the angiogenic effect of LIPUS has been explored before. Previous studies demonstrated that endothelial cells are sensitive to ultrasound waves, and LIPUS can alter their morphology, proliferative activity, gene expression, and protein secretion.[23] The acoustic streaming of LIPUS affects caveolae in endothelial cells, which play a key role in mechanotransduction via embedded mechanosensors, such as integrin and caveolin. Mechanical stimuli are then transmitted to several intracellular signaling pathways, such as the PI3K-Akt, Hippo and HIF-1α signaling pathways, to upregulate the expression of angiogenic growth factors and thus

enhance angiogenesis.[23–25] In addition, ultrasound can induce sonoporation on the endothelial cell membrane that results in an influx of calcium ions, which is correlated with this bioeffect.[26] Moreover, ultrasound can be used for the promotion of angiogenesis and monitoring of angiogenesis in vivo. The CEUS is a common method for the detection of angiogenesis, and targeted ultrasound contrast agents can produce stronger signals associated with angiogenesis.[27] The PAI technique is based on a combination of laser and ultrasound, which is particularly suitable for visualization of microvasculature due to high optical absorption of hemoglobin; it has proven valuable for monitoring neovascularization in tissue engineering.[28] The PAM is a new method for in vivo imaging of the microvasculature using high contrast and deep penetration, and has great potential for biomedical research.[29] Real-time in vivo monitoring of the vasculature enhances visualization of angiogenesis to provide an intuitive understanding of these processes for investigators.

Limitations

The results of the present study are encouraging; however, some limitations remain. First, we selected a single set of parameters of LIPUS treatment based on previous studies. The best stimulation condition for capsule tissue requires additional exploration. Second, the effect of LIPUS on inflammation of the capsule tissue was not investigated, and inflammation plays an important role in capsule formation and angiogenesis. Finally, we only examined the proangiogenic effect on endothelial cells, while several types of cells in the capsule tissue, such as fibroblasts and smooth muscle cells, might be stimulated using LIPUS to some extent.

Conclusions

The LIPUS treatment of vascular beds in vivo can enhance angiogenesis of the capsule tissue by stimulating microvascular endothelial cells to secrete proangiogenic growth factors. This approach can enhance the potency and sustainability of the capsule tissue and is thus extremely important for thick or multilayered constructs.

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References

1. Kant RJ, Coulombe KLK. Integrated approaches to spatiotemporally directing angiogenesis in host and engineered tissues. *Acta Biomater.* 2018;69:42–62. doi:10.1016/j.actbio.2018.01.017
2. Epple C, Haumer A, Ismail T, et al. Prefabrication of a large pedicled bone graft by engineering the germ for de novo vascularization and osteoinduction. *Biomaterials*. 2019;192:118–127. doi:10.1016/j.biomaterials.2018.11.008
3. Rnjak-Kovacina J, Gerrand YW, Wray LS, et al. Vascular pedicle and microchannels: Simple methods toward effective in vivo vascularization of 3D scaffolds. *Adv Healthc Mater.* 2019;8(24):e1901106. doi:10.1002/adhm.201901106
4. Bengtson BP, Ringler SL, George ER, DeHaan MR, Mills KA. Capsular tissue: A new local flap. *Plast Reconstr Surg*. 1993;91(6):1073–1079. doi:10.1097/00006534-199305000-00016
5. Schoeller T, Lille S, Stenzl A, et al. Bladder reconstruction using a prevascularized capsular tissue seeded with urothelial cells. *J Urol*. 2001;165(3):980–985. PMID:11176526
6. Geelhoed WJ, van der Bogt KEA, Rothuizen TC, et al. A novel method for engineering autologous non-thrombogenic in situ tissue-engineered blood vessels for arteriovenous grafting. *Biomaterials*. 2020;229:119577. doi:10.1016/j.biomaterials.2019.119577
7. DeGeorge Br Jr, Campbell CA. Techniques to refine the upper outer breast aesthetic subunit in alloplastic breast reconstruction: The lateral capsular flap. *Plast Surg (Oakv)*. 2016;24(2):83–88. doi:10.4172/plastic-surgery.1000966
8. Josiassen M, Kudibal MT, Gramkow C, Trojahn Kølle SF. Closure of thoracic wall defect using breast implant capsule tissue as a rotation flap: A case report. *Case Reports Plast Surg Hand Surg*. 2018;5(1):39–40. doi:10.1080/23320885.2018.1476150
9. Jia Z, Guo H, Xie H, et al. Construction of pedicled smooth muscle tissues by combining the capsule tissue and cell sheet engineering. *Cell Transplant*. 2019;28(3):328–342. doi:10.1177/0963689718821682
10. Guo HL, Peng XF, Bao XQ, et al. Bladder reconstruction using autologous smooth muscle cell sheets grafted on a pre-vascularized capsule. *Theranostics*. 2020;10(23):10378–10393. doi:10.7150/thno.47006
11. Guo HL, Wang L, Jia ZM, et al. Tissue expander capsule as an induced vascular bed to prefabricate an axial vascularized buccal mucosa-lined flap for tubularized posterior urethral reconstruction: Preliminary results in an animal model. *Asian J Androl*. 2020;22(5):459–464. doi:10.4103/aja.aja_133_19
12. Guo HL, Jia ZM, Wang L, et al. Tubularized urethral reconstruction using a prevascularized capsular tissue prelaminated with buccal mucosa graft in a rabbit model. *Asian J Androl*. 2019;21(4):381–386. doi:10.4103/aja.aja_43_19
13. Kang PL, Huang HH, Chen T, Ju KC, Kuo SM. Angiogenesis-promoting effect of LIPUS on hADSCs and HUVECs cultured on collagen/hyaluronan scaffolds. *Mater Sci Eng C Mater Biol Appl*. 2019;102:22–33. doi:10.1016/j.msec.2019.04.045
14. Ogata T, Ito K, Shindo T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates contractile dysfunction of pressure-overloaded heart in mice. *PLoS One*. 2017;12(9):e0185555. doi:10.1371/journal.pone.0185555
15. Kamatsuki Y, Aoyama E, Furumatsu T, et al. Possible reparative effect of low-intensity pulsed ultrasound (LIPUS) on injured meniscus. *J Cell Commun Signal*. 2019;13(2):193–207. doi:10.1007/s12079-018-0496-9
16. Hanawa K, Ito K, Aizawa K, et al. Low-intensity pulsed ultrasound induces angiogenesis and ameliorates left ventricular dysfunction in a porcine model of chronic myocardial ischemia. *PLoS One*. 2014;9(8):e104863. doi:10.1371/journal.pone.0104863
17. Su Z, Xu T, Wang Y, et al. Low-intensity pulsed ultrasound promotes apoptosis and inhibits angiogenesis via p38 signaling-mediated endoplasmic reticulum stress in human endothelial cells. *Mol Med Rep*. 2019;19(6):4645–4654. doi:10.3892/mmr.2019.10136
18. Gholobova D, Terrie L, Mackova K, et al. Functional evaluation of prevascularization in one-stage versus two-stage tissue engineering approach of human bio-artificial muscle. *Biofabrication*. 2020;12(3):035021. doi:10.1088/1758-5090/ab8f36
19. Adamowicz J, Pokrywczynska M, Van Breda SV, Kloskowski T, Drewa T. Concise review: Tissue engineering of urinary bladder. We still have a long way to go? *Stem Cells Transl Med*. 2017;6(11):2033–2043. doi:10.1002/sctm.17-0101
20. Utzinger U, Baggett B, Weiss JA, Hoying JB, Edgar LT. Large-scale time series microscopy of neovessel growth during angiogenesis. *Angiogenesis*. 2015;18(3):219–232. doi:10.1007/s10456-015-9461-x
21. Kastellorizios M, Tipnis N, Burgess DJ. Foreign body reaction to subcutaneous implants. *Adv Exp Med Biol*. 2015;865:93–108. doi:10.1007/978-3-319-18603-0_6
22. Klopfleisch R, Jung F. The pathology of the foreign body reaction against biomaterials. *J Biomed Mater Res A*. 2017;105(3):927–940. doi:10.1002/jbm.a.35958

23. Huang JJ, Shi YQ, Li RL, et al. Angiogenesis effect of therapeutic ultrasound on HUVECs through activation of the PI3K-Akt-eNOS signal pathway. *Am J Transl Res*. 2015;7(6):1106–1115. PMID:26279754
24. Xu XM, Xu TM, Wei YB, et al. Low-intensity pulsed ultrasound treatment accelerates angiogenesis by activating YAP/TAZ in human umbilical vein endothelial cells. *Ultrasound Med Biol*. 2018;44(12):2655–2661. doi:10.1016/j.ultrasmedbio.2018.07.007
25. Costa V, Carina V, Conigliaro A, et al. miR-31-5p Is a LIPUS-mechanosensitive microRNA that targets HIF-1α signaling and cytoskeletal proteins. *Int J Mol Sci*. 2019;20(7):1569. doi:10.3390/ijms20071569
26. Hassan MA, Campbell P, Kondo T. The role of Ca(2+) in ultrasound-elicited bioeffects: Progress, perspectives and prospects. *Drug Discov Today*. 2010;15(21–22):892–906. doi:10.1016/j.drudis.2010.08.005
27. Lau C, Rivas M, Dinalo J, King K, Duddalwar V. Scoping review of targeted ultrasound contrast agents in the detection of angiogenesis. *J Ultrasound Med*. 2020;39(1):19–28. doi:10.1002/jum.15072
28. Ogunlade O, Ho JOY, Kalber TL, et al. Monitoring neovascularization and integration of decellularized human scaffolds using photoacoustic imaging. *Photoacoustics*. 2019;13:76–84. doi:10.1016/j.pacs.2019.01.001
29. Yang F, Wang Z, Zhang W, et al. Wide-field monitoring and real-time local recording of microvascular networks on small animals with a dual-raster-scanned photoacoustic microscope. *J Biophotonics*. 2020;13(6):e202000022. doi:10.1002/jbio.202000022