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Cellular energy supply in promoting vascular remodeling of small-diameter vascular grafts: preliminary study of a new strategy for vascular graft development

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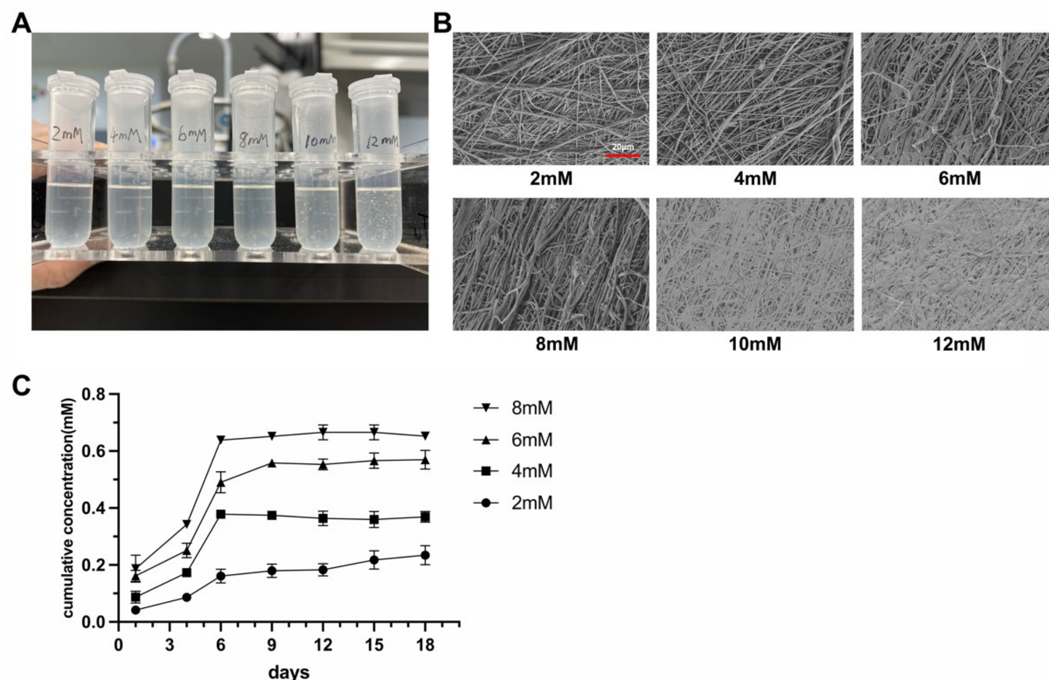


Fig. S1. A-C) The processes of how AICAR concentration used within the vascular graft was determined and the release test of AICAR-modified vascular graft.

we made a series of different concentrations of AICAR electrospinning compositions (AICAR/PLLA/Gelatin) using ultrasonic and warming, including 2, 4, 6, 8, 10, and 12 mM, as shown in Fig. S1A. The results indicated that there were obvious undissolved solids in 10 and 12 mM AICAR electrospinning composition, while no obvious solids were found in the others. AICAR could fully dissolve in the electrospinning composition and form a final concentration less than 8 mM. Furthermore, different concentrations of AICAR electrospinning composition were electro-spun into vascular grafts and the corresponding membranes of grafts were observed using SEM (Fig. S1B). Previous studies have reported that electrospinning is a promising technique for fabricating vascular grafts due to its ease to form grafts with porous and nano- or micro-fibrous topography closely resembling the

architecture of ECM.[1-4] There were obvious nano- or micro-fibrous and proper porous microstructure in the 2, 4, 6, and 8 mM vascular grafts membranes. In the contrast, there was lower porosity and deformed nano-fibrous topography in the 10 and 12 mM vascular graft membranes. To further understand *in vitro* sustained release of AICAR in different concentration vascular grafts, a release experiment was performed within 18 days. It was not surprising that the patterns of AICAR release were similar and different concentrations of AICAR grafts could constantly release AICAR for 6 or 9 days. Especially, the release pattern of the 8 mM AICAR vascular graft reached the highest level of AICAR concentration (0.65 ± 0.0025 mM), which was close to the average working concentration in vitro (1 mM, Fig. S2, supplementary data). Taken together, the 8 mM AICAR concentration electrospinning composition was used to fabricate the vascular graft.

As for the average working concentration of AICAR *in vivo*, We could hardly evaluate the AICAR release of the implanted vascular in a rabbit, but the release of AICAR in PBS, which was viewed as a similar behavior of drug release in the animal body,[5, 6] could be assessed. The result of the release experiment showed that the AICAR-modified vascular grafts used in transplantation surgery could sustainably release AICAR for nearly 9 days (Fig.S1C, line of 8 mM), with an average working concentration of 0.65 ± 0.0025 mM on the 9th day. According to the AICAR-release test, the loading amount of AICAR in the electrospun vascular grafts was calculated by the following equation:

Actual loading amount (mg/mg)= m_0/m (1)

m_0 (mg) = Concentration (mol/L) × Volume (ml) × Molecular Weight (g/mol)

(2)

Where m_0 is the mass of AICAR in the electrospun vascular graft and m refers to the total mass of the electrospun vascular graft (13 mg). The volume is 2 ml, and the Concentration of AICAR on the 9th day was 0.65 ± 0.0025 mM. The Molecular Weight of AICAR is 258.23 (g/mol). We could get the actual loading amount of AICAR in the vascular graft was 0.025823 (mg/mg).

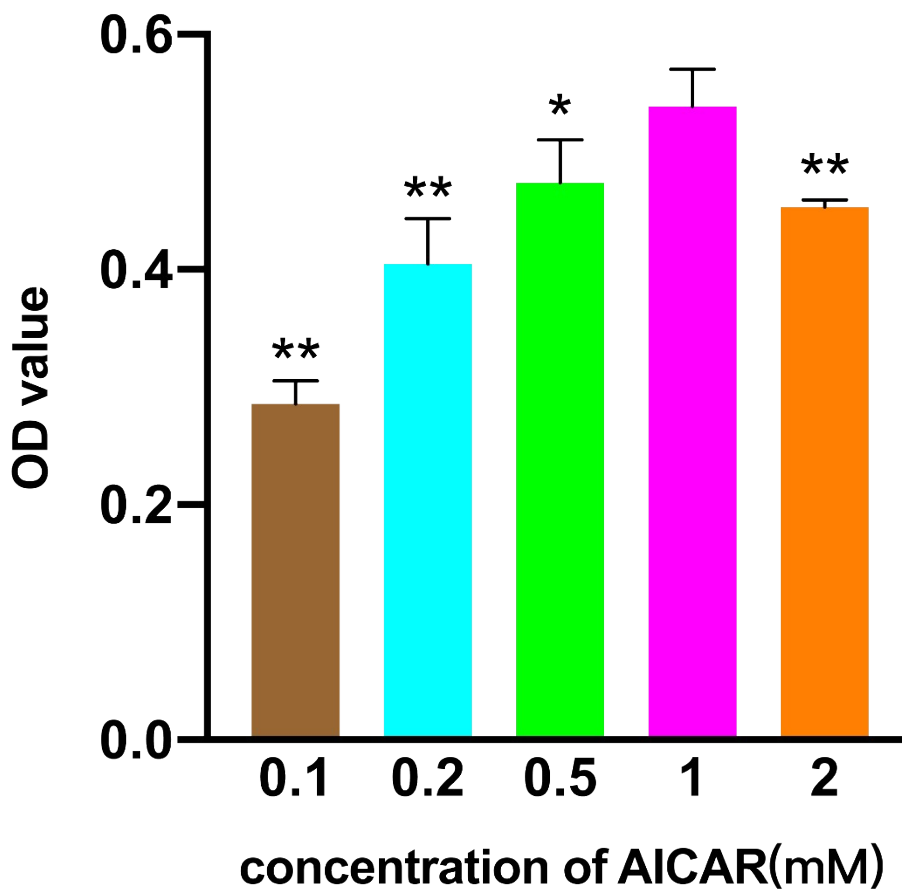


Fig. S2. CCK-8 assay was performed to assess the effectiveness of several diverse concentrations (0.1, 0.2, 0.5, 1, 2 mM) of AICAR on the viability of HUVECs in the inflammation condition. *p < 0.01 and **p < 0.01versus 1 mM group. Results are mean ± SD, n = 3.

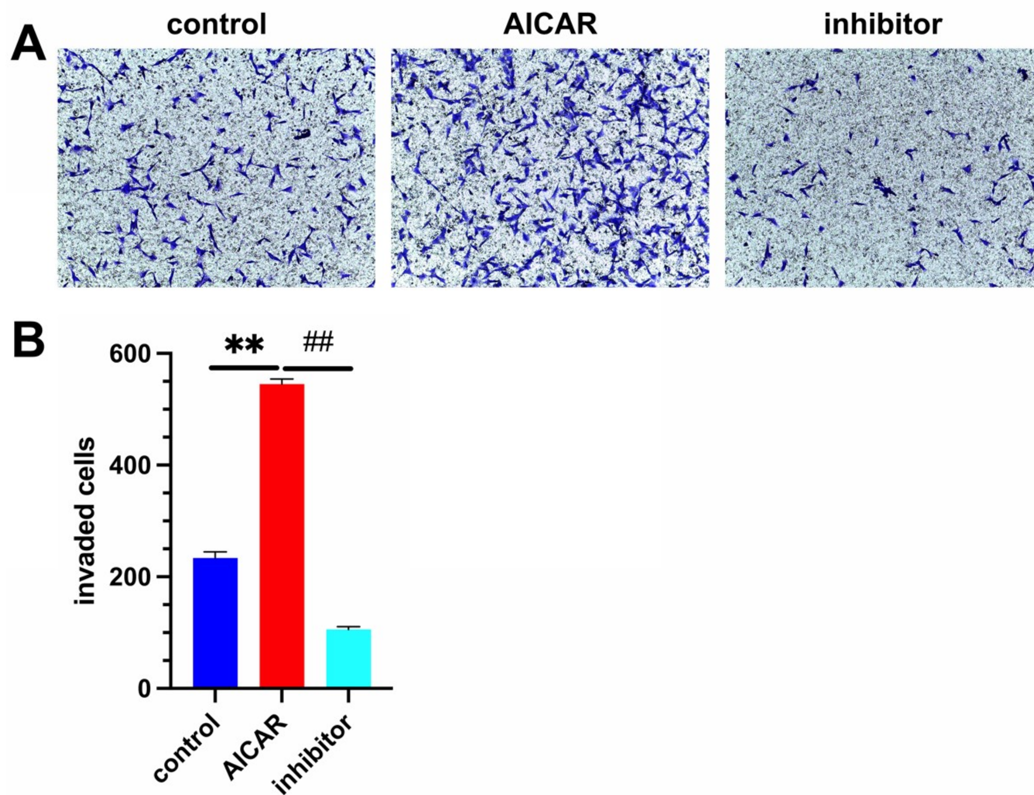


Fig. S3. A, B) Representative images and quantitative estimation of the *in vitro* transwell assay (n=6). Briefly, 8 mm long vascular grafts with or without AICAR were placed on the Transwell (Corning, 3422) lower chamber and the lower chamber contained basic medium. A total of 1×10^4 HUVECs were seeded into the upper chamber after 10 μg/mL LPS pre-treatment. After 24 h, the cells that migrated through the basolateral membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Pictures of invaded HUVECs were observed and collected by an optical microscope (Mshot, China).

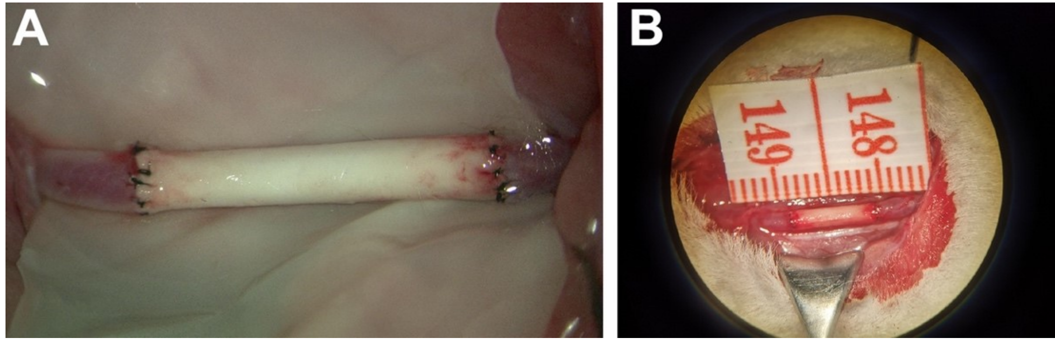
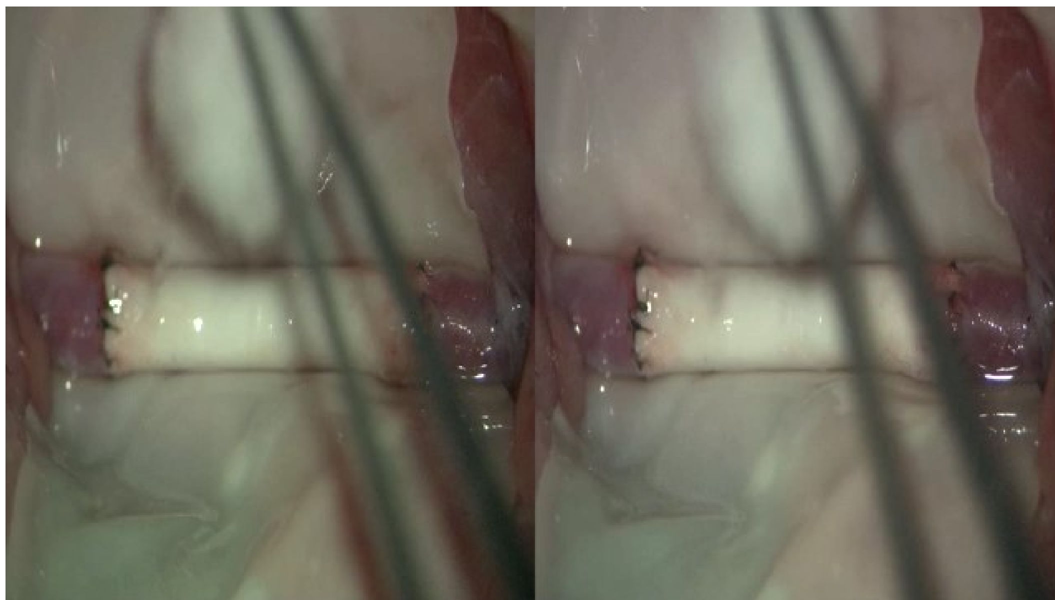


Fig. S4. A, B) The fabricated vascular grafts were successfully transplanted in the right common carotid artery of New Zealand white rabbits.



Video. S1. The video showed the success of vascular grafting.

Table 1. Primers used for RT-qPCR.

Gene	Primer Sequence (5′ → 3′)	PCR Product (bp)

IL-6	F: CCAGAGCTGTGCAGATGAGT R: CCAGAGCTGTGCAGATGAGT	122
IL-8	F: AGTGCATAAAGACATACTCC R: AGTGCATAAAGACATACTCC	105
18srRNA	F: CCTGGATACCGCAGCTAGGA R: CCTGGATACCGCAGCTAGGA	112

F, forward; R, reverse;

Table 2. Primers used for RT-qPCR.

Gene	Primer Sequence (5′ → 3′)	PCR Product (bp)
iNOS	F: TTCAGCTACGCCTTCAACACC R: ATTCCCAAATGTGCTTGTCACC	105
CD163	F: AATCCCAGACACTATTGCCAT R: CCTCCACCTACCAAGCGAAG	121
GAPDH	F: ATCATCTCCGCCCCTTCTGC R: GAGCCCTTCCACAATGCCAA	165

F, forward; R, reverse;

Table 3. Patency evaluation using Doppler Ultrasound.

	AICAR group		Control group		P-value*
	unobstructed	blocked	unobstructed	blocked	
6 weeks	13	1	1	8	＜0.01
12 weeks	6	1	0	4	0.015

* Based on Fisher's exact test, which was performed to evaluate significant differences between pairs in patency rate. A p-value of less than 0.05 was considered statistically significant.

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