Simultaneous Effects of High Intensity Interval Training and Human Amniotic Membrane Scaffold on Rat Tibialis Anterior Vascularization and Innervation after Volumetric Muscle Loss Injury

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ABSTRACT

Background: Despite the high regenerative capacity of skeletal muscle, volumetric muscle loss (VML) is an irrecoverable injury. One therapeutic approach is the implantation of engineered biologic scaffolds.

Objective: To investigate the simultaneous effect of high intensity interval training (HIIT) and the use of decellularized human amniotic membrane (dHAM) scaffolds on vascularization, growth factor, and neurotrophic factor gene expression, and muscle force generation in the tibialis anterior (TA) of rats after VML injury.

Methods: VML injury was created in the TA of 24 rats, which were randomly divided into two groups—12 animals with and 12 without the use of a dHAM scaffold. After injury, each group was further divided into two groups of 6 animals each—sedentary and HIIT. Blood vessels were visualized and counted by hematoxylin and eosin staining. The PowerLab converter assay was used to evaluate isometric contraction force. The relative expression of neurotrophic factors and growth factor genes was measured with reverse transcription PCR (RT-PCR).

Results: The number of blood vessels in the whole regenerating areas showed a significant difference in the dHAM-HIIT and dHAM-sedentary groups compared to the sedentary group without dHAM ($p=0.001$ and $p=0.003$, respectively). *BDNF* and *GDNF* mRNA levels in the dHAM-HIIT group were significantly ($p<0.05$) higher than those in other groups; *NGF* mRNA levels did not differ significantly among groups. Isometric contraction force in the dHAM-HIIT group was significantly ($p=0.001$) greater compared to the sedentary group without dHAM.

Conclusion: Combined use of dHAM scaffoldsand HIIT would improve the structure of the injured muscle during regeneration after VML by better vascular perfusion. HIIT leads to greater force generation and innervation by modulating neurotrophic factor synthesis in regenerating muscles.

KEYWORDS: Tissue engineering; Vascularization; Neurotrophic factors; High intensity interval training; Scaffold

INTRODUCTION

Volumetric muscle loss (VML) is defined as the loss of a large portion of skeletal muscle tissue due to trauma during civilian accidents or military actions, surgery, or congenital defects [1]. Despite the self-regenerating ability of skeletal muscle, regeneration after VML injury is inadequate because of the large volume of myofibrils removed [2]. Following injury, long-term dysfunction can cause permanent disability and cosmetic handicap. Routine therapies such as surgical repair with muscle flap transposition or autologous tissue transfer are limited to scar tissue debridement, yet provide some cos-

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metic improvement. However, these clinical methods are ineffective in improving muscle structure and function. Moreover, the innate immune responses are a major challenge in these modes of therapy [2, 3]. Tissue engineering technologies have emerged as promising strategies for regenerative skeletal muscle repair. In this approach, the use of biological scaffolds and extracellular matrices has been proposed [4].

The human amniotic membrane (HAM) is the inner layer of the fetal membranes, and consists of three parts: epithelium, basement membrane, and stroma. The structure of this membrane is characterized by consistency and strength since it is made of type III collagen, proteoglycan, and various structural proteins [5]. HAM, which is used as a biological covering for different types of superficial burns and wounds, has minimal antigenicity, which reduces the risk of infection and increases the speed of wound healing. In addition, HAM is an abundant and readily available option that is not subject to ethical constraints [6].

VML injury not only leads to cosmetic handicaps and structural problems in the skeletal muscle, but also destroys the nerves and blood vessels in the affected area, resulting in a decrease in force generation and partial loss of function in the affected limb [7]. For example, when 20% of the tibialis anterior (TA) mass is defective, the TA exhibits a functional deficit of 29% [8]. Therefore, to regenerate the structure and function of skeletal muscle, the damaged tissue must be vascularized and reinnervated. Decellularized human amniotic membrane (dHAM) provides an appropriate support for cell adhesion and improves blood flow to the underlying tissue by accommodating dense capillary networks at its surface [9]. Due to the decellularization process, cells on the surface of the amniotic membrane are destroyed, which prepares the scaffold for transplantation on the wound [6]. Therefore, a dHAM scaffold was used in this study.

Exercise training is an effective method to improve the function and rehabilitation of injured skeletal muscle [3, 10, 11]. It has been reported that exercise training improves innervation in the injured skeletal muscle and improves force generation by the repaired muscle [3, 11]. It was also reported that exercise training increases capillary network densities in skeletal muscles [12].

The intensity and volume of exercise training are major determinants of the type of exercise. High intensity interval training (HIIT) is a specific training model that includes high intensity exercise frequencies and active rest periods with low intensity [13]. It was reported that long-term HIIT increases the synthesis of muscle protein, and stimulates muscle growth by stimulating growth factors and improving the number and activation of satellite cells in injured muscles [14, 15]. Neurotrophic factors and glial cells are involved in muscle communication with the nervous system, and with the stimulation of synaptic plasticity and neurogenesis. Neurotrophic factor is also released by contracting muscle cells in skeletal muscles during exercise training, and is able to stimulate neurogenesis in damaged muscle tissue. The effects of HIIT on neurotrophic factors and glial cell function have been investigated. However, the available evidence is scarce. Current evidence shows a positive impact of HIIT compared to the traditional training on the expression of neurotrophin secreted from skeletal muscles. Moreover, HIIT is associated with angiogenesis and increased capillary density, and consequently increased blood flow to the injured muscle tissue [12, 16].

The objective of this study was to investigate the simultaneous effect of HIIT and the use of dHAM scaffolds on vascularization, neurotrophic factor and growth factor gene expression, muscle function and force generation in the TA of rats after VML injury.

MATERIALS AND METHODS

Animals

Twenty-four adult Wistar rats were obtained from the animal laboratory of Shahid Chamran University of Ahvaz, and maintained at a temperature of 22±1 °C and humidity of

65±5% with a 12-h dark/12-h light cycle. The animals were fed standard pellets and had unrestricted access to water and food. All animal care and handling procedures were in accordance with the guidelines of the National Research Committee (USA) on the Care and Use of Laboratory Animals, 8th edition. All stages of the study were approved by the Research Ethics Committee of Shahid Chamran University of Ahvaz (EE/97.24.370024/scu.ac.ir).

Experimental Design

VML injury was induced in the TA muscle of all 24 rats. The animals were randomly divided into two groups of 12 animals—12 with and 12 without the membrane scaffold. The animals recovered for two weeks after VML injury; then each group was subdivided into two groups of six animals—sedentary group and HIIT group. The HIIT group animals were trained on the treadmill for 8 weeks, then anesthetized and killed with CO_2 to measure isometric contractility in their injured muscles. Tissues were removed and stored in liquid nitrogen at -80 °C for molecular assays.

In Vitro Studies

Preparation of biological scaffolds

Human amniotic membrane was dissected according to a protocol designed by Shi, *et al* [17]. Amniotic membrane specimens were obtained from mothers who underwent cesarean delivery, and who provided their informed consents in writing to donate this material for the study. At the time of tissue donation, serological tests were performed to screen mothers for diseases such as syphilis, HIV, and viruses such as hepatitis B and C. The amniotic membrane was mechanically separated from the placenta and then washed three times with PBS. The membrane was sterilized in 75% alcohol/water for 10 sec and then washed with shaking in PBS containing penicillin and streptomycin (200 U/mL) for 1 day. For the decellularization process, HAM was treated in sequence with 1% Triton X-100 for 14 h, 2000 U/L lipase PBS for 10 h at 37 °C, and 2000 U/L DNAase PBS for 3 h at 37 °C. Finally, the dHAM was cut into slices about 2.0×2.0 cm and stored at 4 °C.

Characterization of dHAM

Decellularization of HAM was confirmed by hematoxylin and eosin (H&E) staining. Samples were fixed in 10% neutral-buffered formalin, then dehydrated with a graded series of ethanol and embedded in paraffin wax. After sectioning, they were examined with a Nikon E-200 microscope (Nikon, Tokyo, Japan).

DNA quantification assay

To assess the total DNA contents within each of the test articles, the dHAM scaffold materials were cut into thin strips and digested with Proteinase K at 37 °C for up to 144 hrs or until no visible scaffold material remained. Digested scaffolds were then centrifuged at 2980 g for 30 min to precipitate any remaining proteins. Supernatants were purified with phenol-chloroform-isoamyl alcohol (25:24:1) followed by centrifugation at 9000 g for 30 min. Aqueous layers were removed and ethanol precipitated at -20 °C for at least 8 hours to isolate any DNA present. Samples were dehydrated in a vacuum manifold and rehydrated in 1X TE buffer. DNA content was quantified using the Picogreen DNA assay (Invitrogen) following manufacturer's instructions.

Vessel and capillary formation

New vessels and capillaries in intact and regenerating tissues were identified in H&E-stained slides (Thermo Fisher Scientific, Massachusetts, USA). In brief, the tissues were sectioned, embedded in paraffin and cut into 5-μm-thick sections. The sections were examined with a Nikon E-200 microscope and digital camera; the areas of interest were quantified with Image J software (NIH, Bethesda, MD, USA) using a color deconvolution plugin. New vessels and capillaries were quantified as the percentage of the area they occupied within 500 μm of the scaffold periphery [11].

Quantitative reverse transcription PCR

Total RNA was extracted from frozen TA muscle tissue specimens that contained approximately 50–100 mg of the regenerating area, using Trizol according to the manufacturer's protocol for reverse transcription to obtain cDNA. Then 2 μL cDNA was combined with SYBR Green Ex Taq II (Tli

Table 1: Primer sequences used for gene expression analysis

Target gene		Primer sequences (5'–3')	Amplicon length (bp)
BDNF	Forward	*TTGAGCACGTGATCGAAGAGC*	238
	Reverse	*GTTCGGCATTGCGAGTTCCAG*	
GDNF	Forward	*GACTCCAATATGCCCGAAGA*	178
	Reverse	*TAGCCCAAACCCAAGTCAGT*	
NGF	Forward	*ACCTCTTCGGACACTCTGGA*	168
	Reverse	*GTCCGTGGCTGTGGTCTTAT*	
VEGF	Forward	*ACTCCAGGGCTTCATCATTG*	224
	Reverse	*AATTGAGACCCTGGTGGACA*	
GAPDH	Forward	*TCAACAGCAACTCCCACTCTTCC*	242
	Reverse	*ACCCTGTTGCTGTAGCCGTATTC*	

Abbreviations: BDNF, Brain-derived neurotrophic factor; GDNF, Brain-derived neurotrophic factor; NGF, Nerve growth factor; VEGF, Vascular endothelial growth factor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase

RNaseH Plus, Bio-Rad, Hercules, CA, USA) and 200 nM forward/reverse primers using a Rotor-Gene Q 5plex system (Qiagen, Hilden, Germany). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression (housekeeping gene) was used as an internal control to determine the ΔCT value. Expression levels for mRNA were determined with the $2^{-\Delta\Delta Ct}$ method by normalizing each group to the untreated sedentary group. Primer pairs were synthesized with the DNA Oligos design tool (Sigma-Aldrich, Missouri, USA) (Table 1).

In Vivo Studies

VML injury model and recovery

One hour before the surgery, the animals were given 20 mg/kg ibuprofen by oral gavage. They were then anesthetized by an intraperitoneal injection of 5 mg/kg ketamine and 2 mg/kg xylazine. The surgery and creation of the VML injury were carried out according to the protocol of Wu, *et al* [1]. Briefly, a long incision was made along the lateral aspect of the shin through the skin and muscle fascia. Then, in the bulk of the TA muscle, two parallel longitudinal incisions 10-mm long, and two horizontal incisions 7-mm wide and 3-mm deep, were made with a sterile size no. 11 scalpel. Approximately 20% of the total muscle mass was removed.

In the dHAM groups, bio-scaffolds were immediately transplanted into the site of the muscle defect. Each piece of dHAM scaffold was carefully inserted in the wound; the excess portions were cut away. The fascia and skin were then closed with a prolene suture (0-6). The animals were kept at body temperature for recovery with a manual heating plate. In the group without dHAM, the defect site was closed without dHAM.

The animals recovered for 2 weeks after surgery. In the first 24 hrs, 20 mg ibuprofen was given to the animals every 6 hrs as an analgesic by oral gavage. All rats received systemic antibiotics—10–20 mg/kg enrofloxacin every 24 hr via drinking water for 10 days.

Exercise training program

Animals in the experimental groups were familiarized with the treadmill during 2 weeks of recovery. The animals in the exercise group were placed on the treadmill for 3 days a week and walked at a speed of 5 m/min for 10 min. At the end of recovery, each group with and without the dHAM scaffold was randomly divided into two subgroups—HIIT or sedentary. Exercise training was used 5 days per week for 8 weeks, and each session consisted of three sets of HIIT which included: 1) Warm up: running for 5 min at 30–40% VO_{2max}; 2) Main training: 32 min interval running (8 intervals). Each interval consisted of 3 min of

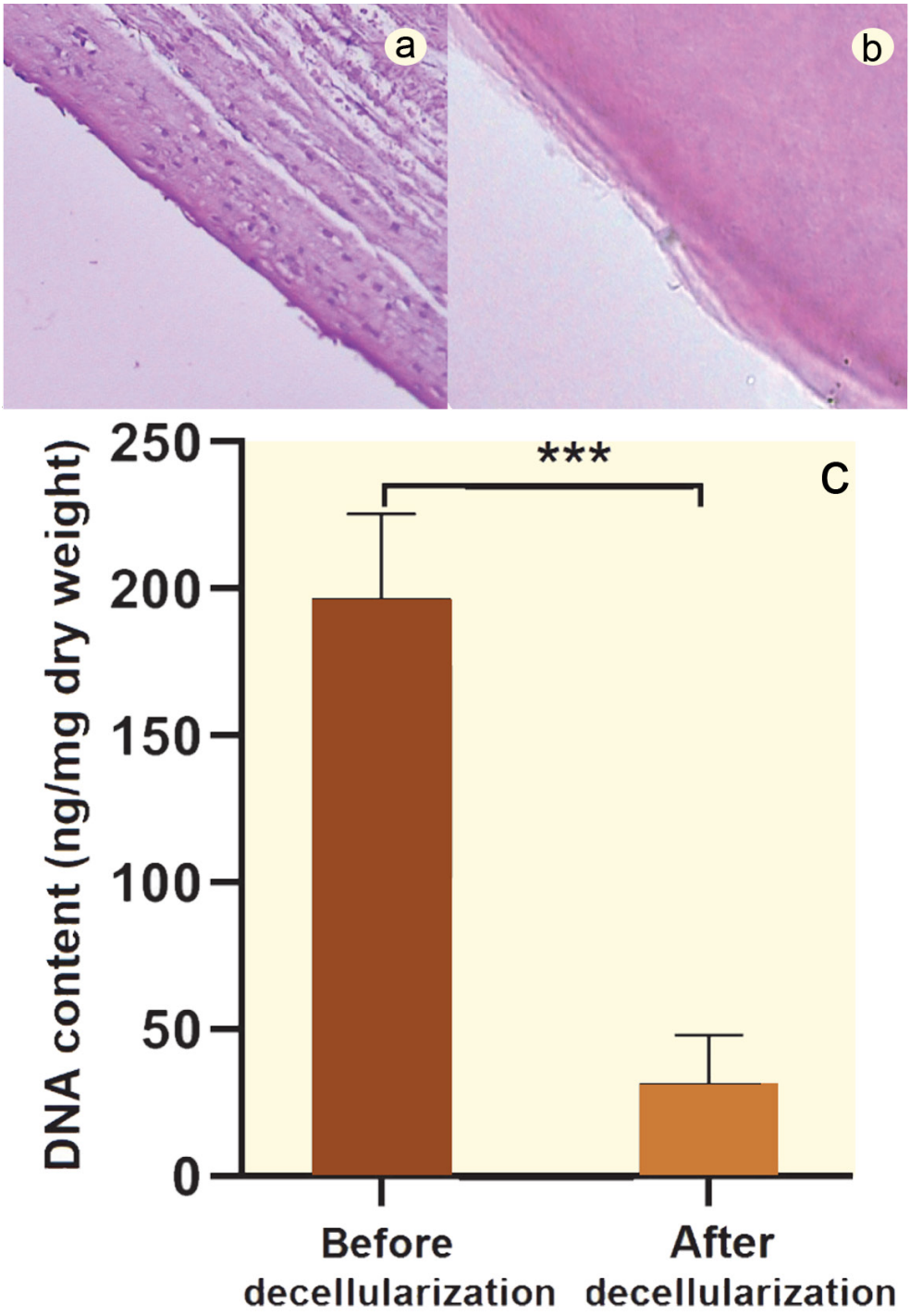

Figure 1: (a) H&E staining of fresh HAM, and (b) successful decellularization of HAM tissue. The photos were taken at 1500× magnification with a Nikon Plan Fluor 40× oil immersion objective

running at 85–90% VO_{2max} and 2 min at 30–35% VO_{2max} for active recovery; and 3) Cool down: 5 min at 30–40% VO_{2max}.

Exercise intensity estimates for each week were adjusted based on previous studies indicating a strong relationship between speed and VO_{2max} in rats. Therefore, the intensity of training was increased by 0.02 m/s each week [18].

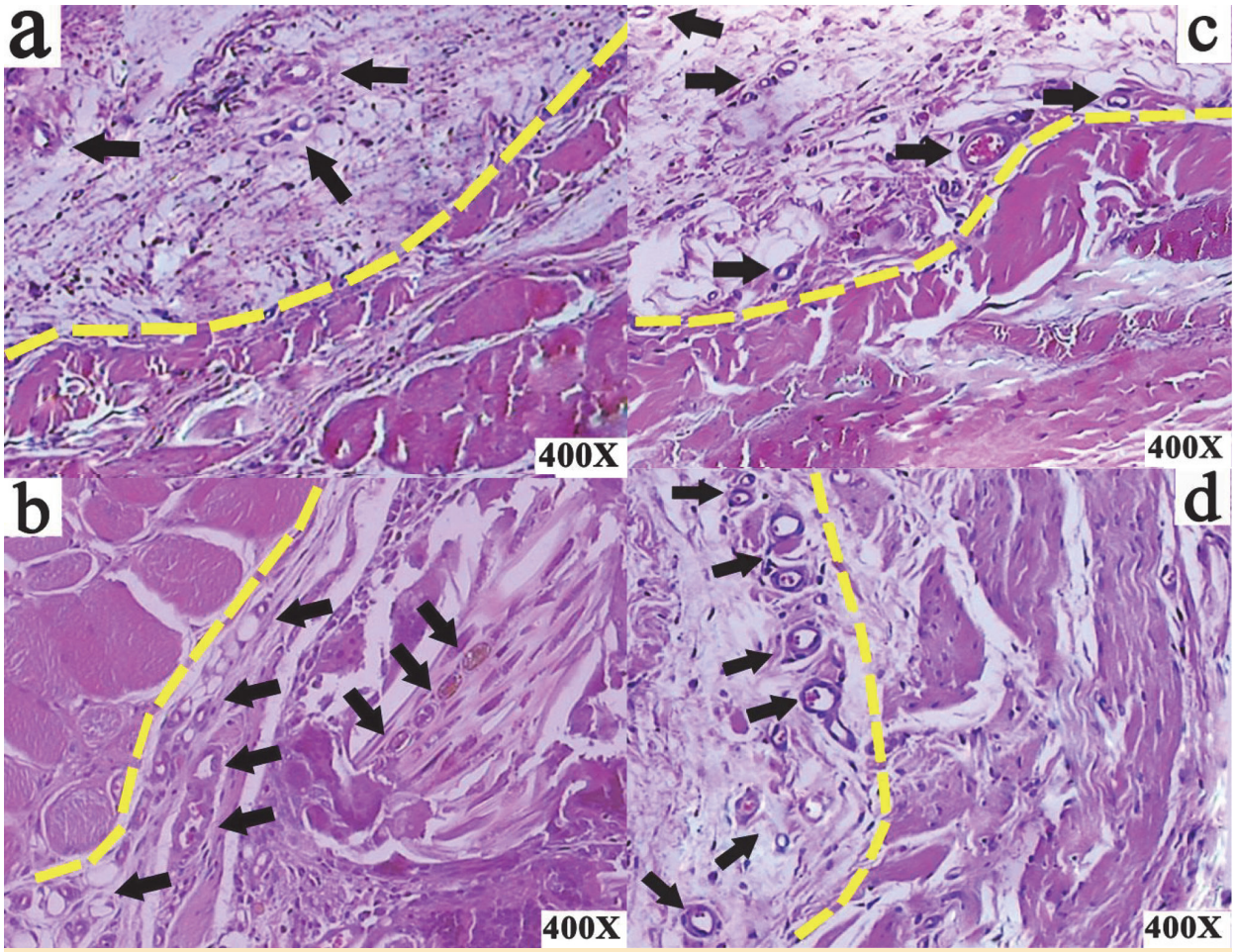

Figure 2: Magnified view of the engineered scaffold. The dotted yellow line marks the interface between native skeletal muscle tissue and regenerating tissue repaired with the dHAM bio-scaffold. Black arrows indicate new surface vessels and capillary networks. All images were taken at 400× magnification. (a) Vascularization without the scaffold in the sedentary group with few small vessels. (b) Vascularization without the scaffold in the HIIT group with significant number of vessels and capillary networks. (c) dHAM in the sedentary group. Despite the lack of training, the appearance of blood vessels due to the use of scaffolds is evident. (d) dHAM bio-scaffold in the HIIT group with the greatest growth of large number of blood vessels. The vascular network appeared to be denser than in all other groups.

Functional tests

Isometric contraction force of the TA muscle was measured in vivo in anesthetized rats. The body temperature of the rats was maintained at 36–37 °C. The lower limbs were fixed with pins and bars at right angles to the ankle and knee joint. For in vivo measurements, the TA was isolated by tenotomizing the agonist dorsiflexor muscles. The maximum isometric contraction strength was determined by stimulating the peroneal nerve and calculating the average value of three contractions. Muscle strength signals were recorded at approximately 150 Hz with a pulse width of 0.1 mg at a range of voltages (2–8 V) with a PowerLab converter (ADInstrumentes, Nagoya, Japan).

Statistical Analysis

The target gene expression was quantified with the $2^{-\Delta\Delta Ct}$ formula. Data were analyzed with one-way ANOVA and Tukey post-hoc tests. All data were reported as the mean±SD. A p value <0.05 was considered statistically significant. All statistical analyses and graphs were plotted with GraphPad Prism ver 8 software.

RESULTS

Cell and Tissue Morphology, and Characterization of the VML Model

Fresh dHAM scaffolds stained with H&E are

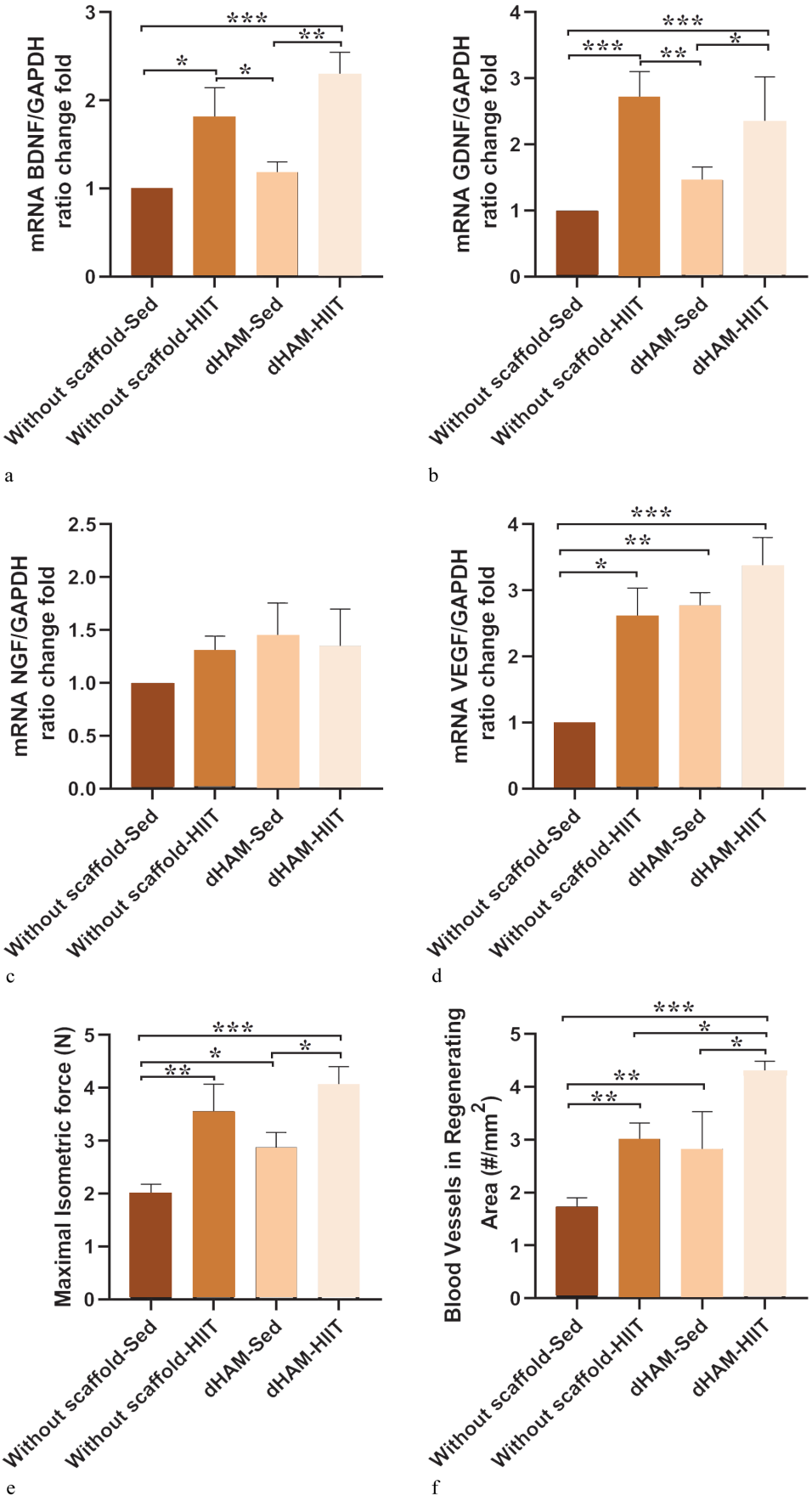

Figure 3: Results of one-way ANOVA and Tukey *post-hoc* tests. Expression of neurotrophic factor genes in the regenerating TA muscle defect (a, b, c). Specimens from the defect area in sedentary and HIIT groups were compared for (a) BDNF, (b) GDNF, and (d) NGF expression. Maximal isometric force *in vivo* (N) (e) and number of blood vessels in the regenerating area (per mm^2) (f). Values are means±SD. Statistically significant comparisons: *p<0.05, **p<0.01, ***p<0.001.

shown in Figures 1a and 1b. Cells were successfully removed from the HAM tissue. To characterize the muscle defect, we compared the ablated TA muscle with the same uninjured muscle from the opposite hind limb. Our surgical ablation model was compatible with the standard method reported by Wu, *et al.* In each surgically created defect, approximately 30% of the TA muscle mass was lost [1].

Quantification of Residual DNA Following Decellularization

The mean±SD DNA content of human amniotic membrane before decellularization was 191±25 ng/mg; after decellularization, it dropped significantly (p=0.001) to 33±5.2 ng/mg (Fig. 1c).

Histological Characterization

The number of blood vessels in the regenerating area of the scaffold periphery was significantly (p=0.001) different between the two HIIT groups and the sedentary group without the scaffold. In both the HIIT and sedentary groups that received the bio-scaffold, the number of blood vessels in the regenerating area was significantly (p=0.003) greater than in the sedentary group without the bio-scaffold (Fig 2).

Neurotrophic Factor and VEGF Gene Expression

Levels of neurotropic factor gene expression were compared in the sedentary and HIIT group. One-way ANOVA for the GAPDH mRNA expression ratio showed that the dHAM group that received HIIT had significantly higher BDNF mRNA levels than the sedentary group without the scaffold (p=0.001) and the sedentary group with the scaffold (p=0.004). GDNF gene expression was significantly higher in the dHAM-HIIT group than the no-dHAM-sedentary group (p=0.001), and was also significantly higher in the no-dHAM-HIIT group than the dHAM-sedentary group (p=0.002). The levels of nerve growth factor (NGF) mRNA did not differ significantly in any of the groups. The levels of VEGF gene expression were significantly higher in the dHAM-HIIT group (p=0.0001), the dHAM-sedentary group (p=0.003) and the no-dHAM -HIIT group (p=0.01) compared to the no-dHAM-sedentary group (Fig 3).

Maximal Isometric Force

In vivo force production measurements showed that the HIIT protocol in the dHAM group resulted in larger increases in the force production and transmission in regenerating muscle tissue after VML compared with the sedentary group without the bio-scaffold (p=0.001). The force production was also significantly (p=0.005) greater in the HIIT group without the scaffold compared to sedentary group without the scaffold (Fig 3).

DISCUSSION

The present study investigated the effect of combined exercise training and the use of biological scaffolds on skeletal muscle structure and function after VML injury. In this study, for the first time, decellularized HAM scaffolds were used to repair a VML injury. Other innovations in the present study include the use of HIIT for the first time for rehabilitation and repair after a VML injury. The advantages of using the dHAM scaffold compared to other scaffolds are its natural origin, availability and abundance.

The mean number of H&E-stained blood vessels was determined in sample areas of the regenerating tissue. The number of blood vessels in the dHAM-HIIT group was significantly higher than that in the other three groups. In addition, vessel diameter was thicker in the dHAM-HIIT group than the other groups. Although earlier studies suggested that scaffolds can regenerate injured skeletal muscle when co-cultured with differentiated stem cells [2, 4, 19], some researchers such as Badylak and his group believe that decellularized scaffolds alone can improve regeneration in injured skeletal muscles [20]. Previous studies show that transplantation of a dHAM scaffold in superficial wounds alone increases blood vessel numbers and is associated with the progression of angiogenesis [9, 17]. Similarly, the present results show that regardless of exer-

cise training, the number of vessels in the two dHAM groups was significantly higher than in the non-scaffold groups. This suggests that the dHAM scaffold alone improved angiogenesis and vascularization.

Expression of vascular endothelial growth factor (VEGF) gene, which is an indicator of increased angiogenesis, was increased in the HIIT groups with or without the scaffold, and also in the dHAM-sedentary group. Exercise training generally increases capillary density by increasing VEGF and endothelial progenitor cells [12, 21]. A possible mechanism by which HIIT enhances angiogenesis is increased blood flow to the capillaries, amplification of shear stress, and the consequent positive regulation of endothelial nitric oxide synthase (eNOS) and NADPH oxidase 2 (NOX2) levels. It was reported that HIIT—more than other types of training—increases the expression of proteins involved in angiogenesis and stimulates the secretion of VEGF and endothelial progenitor cells [12, 16, 22]. Therefore, the combination of HIIT and the dHAM scaffold may act synergistically to improve skeletal muscle vascularization after VML injury.

The present study also investigated the expression of neurotrophic factor genes. The results showed that regardless of whether the dHAM scaffold was used or not, BDNF and GDNF gene expression was significantly greater only in the HIIT group. The expression of NGF did not differ significantly in any groups. Another notable finding is that the dHAM scaffold was not associated with differences in the expression of BDNF and GDNF genes in the two HIIT groups. Previous studies suggested that neurotrophic factors directly and indirectly influence skeletal muscle regeneration [23, 24]. A possible mechanism underlying this effect is the ability of these factors to favor survival, growth and differentiation of new neurons in regenerating muscle tissue [25].

The mechanism by which neurotrophic factors respond to exercise training and especially HIIT remains to be elucidated. But it is thought that muscle contraction during exercise training stimulates a biochemical signaling cascade, which increases BDNF levels in the brain or leads to increased BDNF secretion in skeletal muscle tissue [26]. However, BDNF and GDNF are more active in traumatic conditions than NGF, whereas NGF is more active in pathological conditions. The lack of statistical significance for the differences between groups in NGF expression in the present study may be related to this situation [24].

Another outcome investigated in the present study was the change in isometric contraction strength and force generation in the injured muscle after an 8-week HIIT intervention. Because of the direct relationship between innervation and muscle strength during rehabilitation of the injured muscle, force generation was greater in the HIIT groups than the sedentary groups. Although few studies have investigated the effect of HIIT on neural aspects and functional adaptations, it has been reported that HIIT can increase motor unit power by stimulating neuromuscular adaptations [27].

The main limitation of the present study was the ethics problem to use other scaffolds or other treatment. A further potential limitation was the use of exogenous scaffolds that were not derived from skeletal muscle. The limited number of animals and the lack of immunohistochemical staining such as myosin heavy chain (MHC), Neurofilament and PECAM1 were other limitations of this study. In future studies it would be informative to test the use of differentiated mesenchymal stem cells, which are one of the main contributors to muscle regeneration, in co-cultures with the scaffold. The use of multilayer amniotic membrane to repair VML injury is another approach that should be investigated. In addition, nanofiber coatings or electrospun fibers could be tested for their ability to provide greater strength and favor cell adhesion in bio-scaffolds. Although tissue engineering technology is being investigated for the treatment of VML injuries in large muscles, it can also be potentially used to treat congenital problems such as cleft lip or small-muscle defects.

Our study showed that when human amniotic membrane scaffolds were combined with HIIT, muscle structure and function were likely to show more improvement after VML injury in rats than when either HIIT or the dHAM scaffold were used alone. Vascularization and innervation are two basic mechanisms in skeletal muscle repair and regeneration. High intensity interval training may amplify some physiological processes in injured muscles by increasing the expression of angiogenesis-related and neurotrophic factors. The present findings are potentially useful for the development of tissue engineering technologies and rehabilitation programs after traumatic muscle loss.

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REFERENCES

1. Wu X, Corona BT, Chen X, Walters TJ. A standardized rat model of volumetric muscle loss injury for the development of tissue engineering therapies. *Biores Open Access* 2012;**1**:280-90.
2. Kesireddy V. Evaluation of adipose-derived stem cells for tissue-engineered muscle repair construct-mediated repair of a murine model of volumetric muscle loss injury. *Int J Nanomedicine.* 2016;**11**:1461.
3. Quarta M, Cromie M, Chacon R, Blonigan J, Garcia V, Akimenko I, Hamer M, Paine P, Stok M, Shrager JB. Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss. *Nat Commun* 2017;**8**:15613.
4. Pantelic MN, Larkin LM. Stem Cells for Skeletal Muscle Tissue Engineering. *Tissue Eng Part B Rev* 2018;**24**:373-91.
5. Fouad H, Sabry D, Elsetohy K, Fathy N. Therapeutic efficacy of amniotic membrane stem cells and adipose tissue stem cells in rats with chemically induced ovarian failure. *J Adv Res* 2016;**7**:233-41.
6. Gholipourmalekabadi M, Sameni M, Radenkovic D, *et al*. Decellularized human amniotic membrane: how viable is it as a delivery system for human adipose tissue-derived stromal cells? *Cell Prolif* 2016;**49**:115-21.
7. VanDusen KW, Syverud BC, Williams ML, *et al*. Engineered skeletal muscle units for repair of volumetric muscle loss in the tibialis anterior muscle of a rat. *Tissue Eng Part A* 2014;**20**:2920-30.
8. Corona BT, Machingal MA, Criswell T, *et al*. Further development of a tissue engineered muscle repair construct in vitro for enhanced functional recovery following implantation in vivo in a murine model of volumetric muscle loss injury. *Tissue Eng Part A* 2012;**18**:1213-28.
9. Song M, Wang W, Ye Q, *et al*. The repairing of full-thickness skin deficiency and its biological mechanism using decellularized human amniotic membrane as the wound dressing. *Mater Sci Eng C Mater Biol Appl* 2017;**77**:739-47.
10. Aurora A, Garg K, Corona BT, Walters TJ. Physical rehabilitation improves muscle function following volumetric muscle loss injury. *BMC Sports Sci Med Rehabil* 2014;**6**:41.
11. Nakayama KH, Alcazar C, Yang G, *et al*. Rehabilitative exercise and spatially patterned nanofibrillar scaffolds enhance vascularization and innervation following volumetric muscle loss. *NPJ Regen Med* 2018;**3**:16.
12. Hoier B, Passos M, Bangsbo J, Hellsten Y. Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Exp Physiol* 2013;**98**:585-97.
13. Biglari S, Gaeini AA, Kordi MR, GhardashiAfousi A. [The Effect of 8 Weeks High-intensity Interval Training on Myostatin and Follistatin Gene Expression in Gastrocnemius Muscle of the Rats]. *Journal of Arak University of Medical Sciences* 2018;**21**:1-10. [in persian]
14. Bazgir B, Fathi R, Valojerdi MR, *et al*. [Satellite cells contribution to exercise mediated muscle hypertrophy and repair]. *Yakhteh* 2017;**18**:473. [in persian]
15. Nederveen J, Joanisse S, Séguin C, *et al*. The effect of exercise mode on the acute response of satellite cells in old men. *Acta Physiol* 2015;**215**:177-90.
16. Cocks M, Wagenmakers AJ. The effect of different training modes on skeletal muscle microvascular density and endothelial enzymes controlling NO availability. *J Physiol* 2016;**594**:2245-57.

17. Shi P, Gao M, Shen Q, *et al*. Biocompatible surgical meshes based on decellularized human amniotic membrane. *Mater Sci Eng C Mater Biol Appl* 2015;**54**:112-19.

18. Kraljevic J, Marinovic J, Pravdic D, *et al*. Aerobic interval training attenuates remodelling and mitochondrial dysfunction in the post-infarction failing rat heart. *Cardiovasc Res* 2013;**99**:55-64.

19. Mizuno H. The potential for treatment of skeletal muscle disorders with adipose-derived stem cells. *Curr Stem Cell Res Ther* 2010;**5**:133-36.

20. Turner NJ, Badylak SF. Regeneration of skeletal muscle. *Cell Tissue Res* 2012;**347**:759-74.

21. Amaral SL, Papanek PE, Greene AS. Angiotensin II and VEGF are involved in angiogenesis induced by short-term exercise training. *Am J Physiol Heart Circ Physiol* 2001;**281**:H1163-H9.

22. Cocks M, Shaw CS, Shepherd SO, *et al*. Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. *J Physiol* 2013;**591**:641-56.

23. Lavasani M, Lu A, Peng H, *et al*. Nerve growth factor improves the muscle regeneration capacity of muscle stem cells in dystrophic muscle. *Hum Gene Ther* 2006;**17**:180-92.

24. Sakuma K, Yamaguchi A. The recent understanding of the neurotrophin's role in skeletal muscle adaptation. *BMC Biotechnol* 2011;**2011**:201696 [PMID: 21960735 PMCID: Pmc3179880 DOI: 10.1155/2011/201696].

25. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 2001;**24**:677-736.

26. Domínguez-Sanchéz MA, Bustos-Cruz RH, Velasco-Orjuela GP, *et al*. Acute effects of high intensity, resistance, or combined protocol on the increase of level of neurotrophic factors in physically inactive overweight adults: the brainfit study. *Front Physiol* 2018;**9**:741. doi: 10.3389/fphys.2018.00741.

27. Burgomaster KA, Hughes SC, Heigenhauser GJ, *et al*. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol* 2005;**98**:1985-90.