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Coating of Polyurethane Scaffold With Arabinogalactan Leads to Increase of Adhesion to Fibroblast Cells by Integrin Molecules Pathway

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ABSTRACT

The purpose of this study was to determine the effect of polyurethane blood vein scaffold (PUBVS) and polyurethane blood vein scaffold impregnated with arabinogalactan (PUBVSIAG) on the cell attachment by integrin signaling molecules. After exposure of HFF cells to PUBVS and PUBVSIAG, both cell attachment and cell viability were determined. Also, the expression of *integrin* signaling genes was evaluated by both Real-time PCR and western blot. This study showed that both PUBVSIAG and arabinogalactan 5% had the same toxicity in all incubation times. Significant differences were shown between the viability of HFF cells when exposed to PUBVS and PUBVSIAG ($P < 0.001$). At all incubation times, PUBVSIAG had higher attached cells compared with PUBVS ($P < 0.001$). In case of PUBVSIAG, the expression of *alpha integrin, beta integrin, fibronectin*, and *laminin* was higher than PUBVS ($P < 0.001$). Western blot analysis confirmed the high expression of *integrin* molecules. In vivo test showed the reconstruction of new micro-vessels.

Artificial scaffold is a three-dimensional structure in which the cells are homed [1]. Nowadays, there are different scaffolds that can be used for transplantation [2]. Remarkably, these scaffolds are commonly biocompatible. Polymers are basic materials to build scaffolds in various engineering applications [3]. Synthetic polymers have predictable physical and chemical properties that can be modified or optimized for specific application [4]. In addition, they can be made in different shapes. Nowadays, Dacron and polyurethane are used to build artificial blood scaffold [5]. Laboratory research has shown that polyurethane elastomers have high mechanical and chemical properties in comparison with other polymers [6]. In tissue engineering, the main important issue is attachment of cells. This means that cells must be homed in the pores of scaffold, and then proliferated. Surface molecules, such as *integrins*, have basic role [7]. In mammals, there are at least 24 types of *intrigins*, consisting of α and β subunits. They are commonly located at the cell surface. Most *integrins* bind to extracellular matrix molecules, and some of them are involved in cell-cell responses. Also, *integrins* act as a receptor that can send messages to the cell for migration, cell structure, and cell cycle. Great researches have been done to identify new molecules involved in *integrin* signaling pathway, including *Talin*, *vinicullin*, *FAK*, *Src*, *Paxillin*, *Cavolin*, *fibronectin,* and *laminin*[8].

Arabinogalactan is a hydrocolloid that increases the viscosity of different materials, and widely used in many medical application. As known, different plants can secrete gum. This secreted substance has antimicrobial properties. *Amygdalas lycioides* is an almond species that is specific in Iran. This plant is resistant to drought and dehydration and has a high adaptation to environmental stresses [9]. Since this plant can secrete Arabinogalactan, it may be used for medical application [10].

The purpose of this study was to determine the effect of polyurethane blood vein scaffold (PUBVS) and polyurethane blood vein scaffold impregnated with arabinogalactan (PUBVSIAG) on the cell attachment and the expression of *alpha integrin, beta integrin, fibronectin,* and *laminin.* Moreover, the efficacy of PUBVSIAG was checked invivo.

Roswell Park Memorial Institute 1640 (RPMI1640), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropanol, chloroform, PU, and all antibodies were purchased from Sigma-Aldrich Chemical Co, (St Louis, MO, USA). All primers were sourced from Takapoo Zist Company, Iran. RNA extraction buffer (RiboX) and cDNA synthesis kits were purchased from Sinaclon Company, Iran. The real-time Mastermix was provided by Applied Biosystems Company, USA. First, a cubic piece of polyurethane was cut with height of 10 cm and width of 2.5 cm. Then, a wooden rod with a length of 15 cm and diameter of 0.5 cm was passed through the middle of the piece. In the next step, a plastic yarn completely wrapped around it, and then it was placed in boiling water for 15 min. Next, yarn was removed and it was restored. Then, the wooden rod was removed and the two ends of the piece were cut. Fig. 1 from a to d shows preparation of polyurethane blood vein scaffold, step by step. In order to sterilize the scaffold, the blood vein scaffolds were placed in 70% alcohol for 15 min, and it was squeezed out. It was also placed at 37 °C for one day

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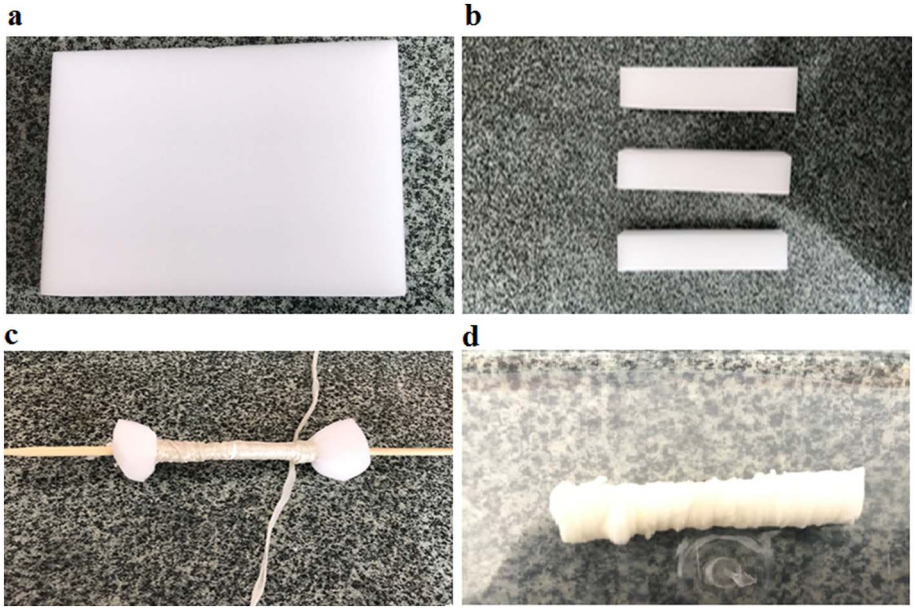

Fig. 1. Preparation of polyurethane blood vein scaffold, step by step (from a to d).

to complete evaporation of alcohol. The sterilized scaffold was kept in the refrigerator at 4 °C in sterile falcon. To detect the presence of sufficient pores in the scaffold, it was investigated under an optical microscope. In a sterile condition, 10 mL of arabinogalactan 5% *w/v* was poured into a falocn. Then, a piece of PUBVS was placed inside it, and incubated at 37 °C for 24 h. Next, it was washed in distilled water for one minute to remove excess arabinogalactan. After washing, it was cut with height and width of 1 cm. All pieces were kept in a sterile flask at 4 °C.

In the next step, HFF cells were cultured in presence of RPMI1640, 100 units of penicillin-streptomycin, and FBS 10%. After one week, the medium was completely discharged and 2 mL of trypsin EDTA 0.25% was added and incubated for 2 min at 37 °C. The isolated cells were transferred to a sterile Falcon and centrifuged for 5 min at 1500 rpm. After centrifugation, fresh medium was added to it, and the density of cell was measured using Neubebear slide. Then, 4 mL of HFF cell suspension was separately added to PUBVSIAG and PUBVS. Next, they were incubated at 37 °C for 1, 2, 4, and 8 days. After incubation, 100 μL of 5 mg/mL MTT solution was added. After 3 h, the optical density of each one was read at 450 nm. Then, the percentage of live cells was determined using Eq. (1). In negative control, there were no PUBVSIAG and PUBVS.

$$\text{Percentage of live cells} = (\text{Optical density of negative control} / \text{Optical density test tube}) \times 100 \quad (1)$$

To find the quantity of adhered cells, after incubation times, PUBVSIAG and PUBVS was placed in a 10% formalin, and then stained by hematoxylin and eosin (H&E). Of each group, 30 slides were randomly investigated, and the quantity of adhered cells was counted by a light microscope. For western blot test, HHF cells were lyzed, in presence of protease inhibitor. Proteins were separated by electrophoresis and then transferred onto cellulose membranes. The membranes were blocked with 5% milk in Tris buffered saline containing 0.1% Tween 20 and then incubated with primary antibodies, including *alfa integrin* and *beta integrin*. HRP-conjugated secondary antibodies were used to detect the primary antibodies. For gene expression, total RNA was first extracted by RiboX buffer. In the next step, 1 μg of total RNA and 1 μL of random hexamer were mixed and the final volume was reached to 10 μL with DEPC water. Next, 10 μL of RT Premix 2 × was added to the mixture, and incubated at 50 °C for 60 min. In the final step, 2 μL of cDNA, 2 μL of forward primer, and 2 μL of reverse primer of the interested gene were mixed to 10 μL of SYBR® Green Real-Time PCR Master Mix. Then, all strip tubes were entered into Real-time PCR machine (ABI 1 plus, USA), and run. *Algha integrin, Beta integrin, fibronectin, laminin* and *GAPDH* were analyzed in this study. The expression of these genes was calculated by ΔΔCT formula.

For in vivo evaluation, newzland rabbits were anesthetized with injection of intramuscular ketamine (30 mg/kg) and intravenous pentobarbital (30 mg/kg). Through a longitudinal incision, brachial artery was exposed. Then, a piece of PUBVSIAG was placed as an end-to-end anastomosis to the common brachial artery using a 6–0 Prolene suture. All care and handling of the animals were provided according to quideline of Laboratory Animals of Islamic Azad University. After 3 months, midportion segments of the implanted blood vessel were fixed with 10% (vol/vol) formaldehyde solution, and then stained with H&E.

All results were reported as mean ± standard deviation (SD). To detect significance differences between groups, one-way ANOVA method was used. For this purpose, SPSS software (SPSS 20.0 Inc., Chicago, IL) was used, and *P*-values < 0.05 were considered as statistically significant.

Fig. 2a shows the viability of HFF cells when exposed to PUBVSIAG, PUBVS, and arabinogalactan 5% at different incubation times, from 1 to 8 days. As seen, the viability of all exposure groups was increased by the increase of incubation time. Moreover, both PUBVSIAG and arabinogalactan 5% had the same toxicity in all incubation times. Significant differences were shown between viability of HFF cells when exposed to PUBVS and PUBVSIAG or arabinogalactan5% ($P < 0.001$). Fig. 2b demonstrates the number of adherent cells when exposed to PUBVSIAG and PUBVS at different incubation times, from 1 to 8 days. Importantly, at all incubation times, PUBVSIAG had higher attached cells compared with PUBVS ($P < 0.001$). Table 1 shows the relative expression of *algha integrin, beta integrin, fibronectin,* and *laminin* when exposed to PUBVSIAG and PUBVS at different incubation times, from 1 to 8 days. In case of PUBVSIAG, the relative expression of all genes was higher than PUBVS ($P < 0.001$). Fig. 3 illustrates western blot of *alpha integrin* and *beta integrin* when exposed to PUBVSIAG and PUBVS at different incubation times, from 1 to 8 days. Same as gene expression data, western blot experiment also shows the higher expression of *alpha* and *beta integrin* when exposed to PUBVSIAG, compared with PUBVS. The H&*E*-stained sections showed reconstruction of the artery, with structures very similar to those of the native artery. We can observe novel micro vessel with endothelial cell after 3 months (Fig. 4).

In tissue engineering, there are three important things, including