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Growth and characterization of a tissue-engineered construct from human coronary artery smooth muscle cells

Sulgin A.A.[1], Sidorova T.N.[2], Sidorov V.Y.[3]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Vanderbilt University Medical Center*
1211, Medical Center Dr., Nashville, 37232, USA

[3] *Vanderbilt University*
1221, Stevenson Center Ln., Nashville, 37240, USA

ABSTRACT

Aim. To optimize a bioengineered I-Wire platform to grow tissue-engineered constructs (TCs) derived from coronary artery smooth muscle cells and characterize the mechano-elastic properties of the grown TCs.

Materials and methods. A fibrinogen-based cell mixture was pipetted in a casting mold having two parallel titanium anchoring wires inserted in the grooves on opposite ends of the mold to support the TC. The casting mold was 3 mm in depth, 2 mm in width and 12 mm in length. To measure TC deformation, a flexible probe with a diameter of 365 mcm and a length of 42 mm was utilized. The deflection of the probe tip at various tensile forces applied to the TC was recorded using an inverted microscope optical recording system. The elasticity modulus was calculated based on a stretch-stress diagram reconstructed for each TC. The mechano-elastic properties of control TCs and TCs under the influence of isoproterenol (Iso), acetylcholine (ACh), blebbistatin (Bb), and cytochalasin D (Cyto-D) were evaluated. Immunohistochemical staining of smooth muscle α-actin, desmin and the cell nucleus was implemented for the structural characterization of the TCs.

Results. The TCs formed on day 5–6 of incubation. Subsequent measurements during the following 7 days did not reveal significant changes in elasticity. Values of the elastic modulus were 7.4 ± 1.5 kPa on the first day, 7.9 ± 1.4 kPa on the third day, and 7.8 ± 1.9 kPa on the seventh day of culturing after TC formation. Changes in the mechano-elastic properties of the TCs in response to the subsequent application of Bb and Cyto-D had a two-phase pattern, indicating a possibility of determining active and passive elements of the TC elasticity. The application of 1 μM of Iso led to an increase in the value of the elastic modulus from 7.9 ± 1.5 kPa to 10.2 ± 2.1 kPa ($p < 0.05$, $n = 6$). ACh did not cause a significant change in elasticity.

Conclusions. The system allows quantification of the mechano-elastic properties of TCs in response to pharmacological stimuli and can be useful to model pathological changes in vascular smooth muscle cells.

Key words: tissue engineering, vascular smooth muscle cells, smooth muscle tissue construct.

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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✉ *Sidorov Veniamin Yu.,* e-mail: v.sidorov@vanderbilt.edu.

Выращивание и характеризация тканеинженерной конструкции из гладкомышечных клеток коронарной артерии сердца человека

Сульгин А.А.[1], Сидорова Т.Н.[2], Сидоров В.Ю.[3]

[1] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

[2] *Vanderbilt University Medical Center*
1211, Medical Center Dr., Nashville, 37232, USA

[3] *Vanderbilt University*
1221, Stevenson Center Ln., Nashville, 37240, USA

РЕЗЮМЕ

Цель. Оптимизировать биоинженерную платформу I-Wire для выращивания тканеинженерных конструкций (ТК) из гладкомышечных клеток (ГМК) артериальных сосудов и охарактеризовать механо-эластические свойства полученных ТК.

Материалы и методы. Клеточную смесь на основе фибрина засевали в канал матрицы из полидиметилоксана с вставками из титановой проволоки на противоположных концах канала для горизонтальной поддержки конструкции. Размеры канала: глубина 3 мм, ширина 2 мм и длина 12 мм. Для измерения деформации ТК использовали гибкий зонд диаметром 365 мкм и длиной 42 мм. Отклонение кончика зонда при различной силе растяжения, приложенной к ТК, регистрировали с помощью системы оптической регистрации на основе инвертированного микроскопа. Модуль упругости вычисляли на основе диаграмм растяжения ТК. Были оценены механо-эластические свойства конструкций в контроле и под действием изопротеренола (Изо), ацетилхолина (Ацх), блеббистатина (Бб) и цитохалазина Д (Цито-Д). Для структурной характеризации конструкций использовали метод иммуногистохимического окрашивания конструкций на α-актин гладких мышц, десмин и ядра клеток.

Результаты. Формирование конструкций происходило на 5–6-й день инкубации. Последующие измерения в течении 7 дней не выявили значительных изменений эластичности. Значения величины модуля упругости конструкций составили 7,4 ± 1,5 кПа в первый день после их формирования, 7,9 ± 1,4 кПа – на 3-й и 7,8 ± 1,9 кПа – на 7-й день культивирования. Изменения механо-эластических свойств ТК в ответ на последовательное применение Бб и Цито-Д имели двухфазный характер, что демонстрирует возможность выделения активного и пассивного элементов эластичности гладкомышечных конструкций. Добавление 1 мкМоль Изо приводило к увеличению значения величины модуля упругости с 7,9 ± 1,5 кПа до 10,2 ± 2,1 кПа ($p < 0,05$, $n = 6$). Добавление Ацх не вызывало значимого изменения эластичности.

Заключение. Представленная система позволяет количественно оценивать механо-эластические свойства ТК в ответ на фармакологическое воздействие и может быть полезна в моделировании патологических изменений в ГМК сосудов.

Ключевые слова: тканевая инженерия, васкулярные гладкомышечные клетки, гладкомышечная тканеинженерная конструкция.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Vascular smooth muscle cells (VSMCs) are the main type of cells in the vascular system that determine vascular tone, as well as peripheral and arterial blood pressure. A feature of VSMCs is their incomplete differentiation and, as a consequence, high plasticity associated with changes between contractile and secretory phenotypes in response to biochemical or mechanical stimulation [1]. It has been shown that a change in the VSMC phenotype is involved in such pathologies as atherosclerosis, aortic aneurysm, and arterial hypertension [2–4].

At present, in most experimental studies of the mechanisms of vascular diseases *in vitro*, traditional two-dimensional cultures of VSMCs are utilized. Such experimental models represent a non-physiological environment, which makes it impossible to maintain the original phenotype of cells and complicates interpretation of data. The modern development of tissue engineering methods allows us to reproduce quite well the basic physiological properties of various types of tissues [5–7]. The advantages of engineered three-dimensional tissue constructs (TCs) are the appropriate mechanical environment, ensuring the stability of the response to external stimulation over a long period of time, the relative ease of genetic manipulations, and the unique potential for studying the biophysical characteristics of tissues *in vitro* [8].

Thus, the development of an adequate *in vitro* model both for studying the mechanisms of vascular diseases and for testing drugs is of paramount importance. This work is aimed at optimizing the novel I-Wire bioengineering platform developed at Vanderbilt University [9, 10], growing TCs using arterial VSMCs and characterizing the mechano-elastic properties of the artificial tissue constructs.

MATERIALS AND METHODS

Preparation of PDMS mold and cell mixture. In this work, a polydimethylsiloxane (PDMS) mold with a horizontal support (titanium wire with a diameter of 0.25 mm and a length of 12 mm, Sigma-Aldrich, USA) for the TC was utilized. To prepare the mold, a template with six cavities was made from monolithic acrylic plastic using a drill with a diameter of 0.79 mm and a numerically controlled milling machine (MicroProtoSystems, USA). Two thin edges of the same material were inserted into each cavity to form channels for the supporting titanium wire.

The cavities were filled with liquid PDMS (SYLGARD ™ 184 kit, DowCorning, USA) mixed with a hardener in a ratio of 10:1 and degassed. Then, the assembled structure was placed in an oven for 6 hours at 65 °C for polymerization. The final PDMS matrix had a channel with a depth of 3 mm, a width of 2 mm and a length of 12 mm, as well as two grooves to accommodate the anchor wire. Next, each PDMS mold was transferred to the well of a 6-well plate and glued to the bottom using liquid PDMS. To increase the hydrophobicity, the channels of the PDMS devices were treated with 0.2% Pluronic® F-127 solution (Sigma-Aldrich, USA) and then washed with deionized water. Then the 6-well plate with molds was sterilized by UV radiation for 30 minutes.

To grow TCs using commercial VSMCs (HCASMC, ThermoFisher, USA), a fibrin-based cell matrix was prepared. It has been shown that fibrin degradation products, which are produced during the maturation of the construct, promote the proliferation of VSMCs and stimulate the formation of the extracellular matrix [11] . In preliminary experiments for optimizing the conditions for the formation of the construct, a cell mixture of fibroblasts and VSMCs in a proportion of 1:10 was used. When only VSMCs were utilized, the final concentration of cells and other components of the medium was the same.

Cardiac fibroblasts (NHCF-V, Lonza, USA) and VSMCs were collected from T-175 flasks (ThermoFisher Scientific, USA) using TrypLE™ Express Enzyme (Thermo Fisher Scientific, USA). The total cell density was adjusted to 10^6, 2×10^6, or 4×10^6 cells/ml. Thereafter, the cells were mixed with fibrinogen (100 µl, 20 mg/ml, Sigma-Aldrich, USA), aprotinin (33 µg/ml, Sigma-Aldrich, USA), thrombin (10 µl, 100 U/ml, Sigma-Aldrich, USA), and penicillin/streptomycin (1%, Gibco, USA). In addition, Matrigel™ (100 µl, BD Biosciences, USA) was applied depending on experimental conditions. After dilution, the final cell density was 0.5×10^6, 10^6, or 2×10^6 cells/ml. Next, the cell mixture was pipetted into the channel of each PDMS device and cells were incubated at 37 °C and 5% of CO_2 for one hour for fibrin polymerization. Later, 3 ml of Gibco™ Medium 231 (ThermoFisher Scientific, USA) was added to each well of the plate. This medium contained aprotinin (33 µg/ml, Sigma-Aldrich, USA), tranexamic acid (400 µmol, Sigma-Aldrich, USA), antibiotics (1%, Gibco, USA) and either growth supplement (Gibco™ Smooth Muscle Growth Supplement, SMGS) (Thermo Fisher Scientific, USA) or differentiation supplement (Gibco ™ Smooth Muscle Differentiation Supplement, SMDS) (ThermoFisher Scientific, USA) as needed.

The medium was replaced every second day. After the formation of TCs, their mechano-elastic properties were measured using an optical recording system based on an inverted microscope (Fig. 1). The matured smooth muscle fibers were 350–450 μm in diameter and 7 mm long (Fig. 2, *a*).

Setup to measure mechano-elastic properties in engineered TCs. To measure elasticity, a flexible sensor made of polyetheretherketone tubing (Putnam Plastics, USA) with an outer diameter of 365 μm, an inner diameter of 120 μm, and a length of 42 mm was utilized. The sensor was glued to a console made of organic glass, which, in turn, was attached to a holder plate with two magnets to control the sensor position. The console, holder plate and flexible sensor were mounted by friction mounting to the condenser of an inverted fluorescence microscope (Eclipse Ti, Nikon, USA) equipped with a microcontroller (MS-2000 Flat-Top XYZ Automated Stage, ASI, USA), which allows for precise movement of the platform in two dimensions (Fig. 1). The elasticity of the flexible sensor was calibrated; it demonstrated a linear dependence of the strain on the applied force (Fig. 2, *b*). The optical registration of displacement of the flexible sensor tip was carried out with a digital camera (Zyla sCMOS Camera, Andor Technology, Northern Ireland) at equal intervals of the displacement of the platform with a multiwell plate, so that each displacement corresponded to a certain applied force, the value of which was calculated using a calibration graph.

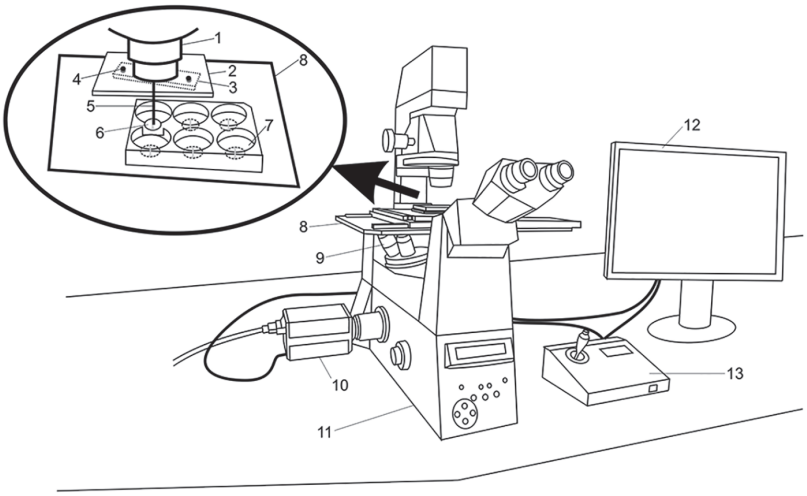


Fig. 1. Schematic diagram of the setup for measuring the mechano-elastic properties of tissue-engineered constructs: *1* – condenser, *2* – plate holder, *3* – console, *4* – magnet, *5* – flexible sensor, *6* – PDMS device with a construct, *7* – well of the multiwell plate, 8 – motorized platform, *9* – lens, *10* – CMOS camera, *11* – inverted microscope, *12* – computer, *13* – microcontroller

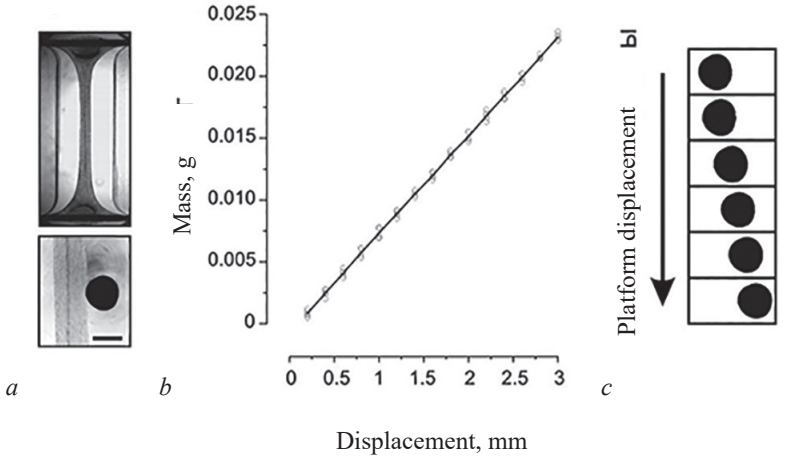


Fig. 2. Calibration graph of elasticity and displacement frames of the flexible sensor tip: *a* – general view of the construct and its central part with the end of the flexible sensor; scale bar – 300 μm, *b* – linear dependence of the change in mass on the offset position of the console with a flexible sensor ($N = 5$, $R = 0.99997$**), *c* – processed binary images of the sensor position during a gradual shift of the motorized platform with the multiwell plate

Measurement protocols and immunohistochemical staining. Two protocols were utilized in this study. The first protocol consisted of a series of sequential measurements of the position of the flexible sensor end when the platform was displaced for every 200 μm before the introduction of substances and after incubation with substances for two hours. According to the second protocol, the registration was carried out at a constant applied force F_t = 0.062 mN for 95 min. During the first 15 min, control registrations were performed. Next, 30 μm of blebbistatin (Bb) and 50 μm of cytochalasin D (Cyto-D) were added after 20 min and 55 min from the start of the protocol, respectively. To assess the ability of the cells to respond to β-adrenergic and cholinergic stimulation, the TC was incubated in standard media containing 1 μm of isoproterenol (Iso) (Sigma-Aldrich, USA) and 5 μM of acetylcholine (ACh) (Sigma-Aldrich, USA) for 60 min. In these experiments, the first protocol was applied.

Bb and Cyto-D were utilized to assess the effect of pharmacological stimuli on the contractile function and elasticity of the extracellular matrix of the TC. Blebbistatin is a reversible inhibitor of ATPase activity of myosin II [12], while cytochalasin D blocks actin polymerization, thereby disrupting the organization of the cytoskeleton filament network, but does not affect the extracellular matrix [13].

For immunohistochemical staining, the TCs were fixed in a paraformaldehyde solution (4%) for 15 min, washed three times with sodium phosphate buffer, then embedded in paraffin blocks and sliced into 5-μm sections. The antigen availability was ensured by incubation of sections at 94 °C in 10 mM sodium citrate solution (pH 6.0) for 10 min. The following antibodies were used in this study: primary murine monoclonal antibodies against smooth muscle cell α-actin (α-SMA) (Sigma-Aldrich, USA), rabbit monoclonal antibodies against desmin (Abcam, USA), secondary antibodies against mice (ThermoFisher, USA) and rabbit (ThermoFisher, USA) conjugated to Alexa (568 and 488 nm), and DAPI for nuclei staining (Fluoromount-G 368/451, SouthernBiotech, USA). Color images were acquired with a Zeiss LSM780 confocal microscope (Zeiss, Germany).

Data analysis and statistics. Image analysis was performed with MATLAB software (MathWorks, USA). The contrast of the image was adjusted to observe only the end of the flexible sensor. Then a binary image was created and the coordinates of the centroid were calculated in each frame (Fig. 2, *c*). Based on the obtained data, the change in the TC length, the value of the force acting along the structure (F_c), and the value of the elastic modulus were calculated. A detailed description of the calculation of the elasticity modulus and a diagram of the geometry of the structure is presented in the supplemental material of a previously published work [9]. Therefore, we provide only a brief description of the calculation procedure.

The elastic modulus was calculated as the ratio of the dependence of strain on stress according to the following equation [14]:

$$E = \frac{L_0 F_c}{A_0 \Delta L},$$

where *F*c is the force acting along the TC, *L*o is the initial length of the TC, *A*o is the cross-sectional area, and Δ*L* is the change in the length of the TC.

Given the position of the TC in space, the formula for calculating the modulus is modified according to [9]:

$$E = \frac{2L_0 F_t \sqrt{(d_s - d_t)^2 + a^2}}{\pi D^2 \Delta L (d_s - d_t)},$$

where F_t – the force exerted by the flexible sensor, d_c – the distance between the initial and current position of the platform, d_t – the distance between the initial and current position of the end of the flexible probe, *a* – half of the length of the TC in a relaxed state, and *D* – the diameter of the TC. The diameter of the TC was calculated based on the average value of three measurements: at the midpoint and at two points at a distance of 1 mm to the right and left of the midpoint.

Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, USA) and OriginLab R2018 (OriginLab Corporation, USA). Statistical comparisons between the control and experimental groups were made with Student's t-test for dependent samples, one-dimensional analysis of variance for repeated measurements (ANOVA) in the case of three or more groups, and non-parametric paired Wilcoxon test. The change in length in response to the applied force, measured as a percentage of the initial length of the preparation, is presented as the mean and standard error of the mean. Differences in the results were considered statistically significant at a significance level of $p < 0.05$. In the process of calibrating a flexible sensor, a correlation analysis was used to assess the linearity of its elastic properties depending on the load.

RESULTS AND DISCUSSION

The main results of preliminary experiments to optimize the protocol revealed that (*a*) the use of a medium with SMDS did not cause the formation of tissue fibers; (*b*) the addition of Matrigel™ accelerated gel condensation and the formation of TCs; (*c*) the VSMC density of 10^6 cells/ml was optimal to enable the rapid growth of the TCs; and (*d*) the addition of 10% fibroblasts did not affect the rate of TC formation.

We utilized fluorescence microscopy to characterize the structure of the TCs. Figure 3 illustrates TC immunohistochemical staining against smooth muscle α-actin, desmin and nuclear DNA with DAPI, as well as hematoxylin and eosin staining. α-Actin constitutes the largest protein fraction in the VSMCs, plays a major role in the contraction via interaction with myosin, and also participates in the formation of the cytoskeleton through its polymerization [15]. Desmin is the main protein that makes up the intermediate filament of the contractile apparatus and is located in both the Z-line of the striated muscle and in the dense bodies of smooth muscle tissue [16]. The immunohistochemical staining data demonstrate the structural homogeneity, as well as uniform and dense distribution of smooth muscle cells in the TCs.

To verify the stability of the mechano-elastic properties of the TCs, control measurements were performed within 7 days after construct formation. The tension diagrams are shown in Fig. 4, A. The linear part of the diagram was utilized to calculate the elastic modulus (Fig. 4, *b*). Substantial TC elongation is observed when a transverse tensile force (F_t) is applied greater than 0.07 mN. Analysis of tensile diagrams did not reveal statistically significant changes between measurements on the 1st, 3rd and 7th days (F (2.10) = 0.225, p = 0.8, n = 6). The value of the elastic modulus was 7.4 ± 1.5 kPa at the beginning of the experiment, 7.9 ± 1.4 kPa on the third day and 7.8 ± 1.9 kPa on the seventh day.

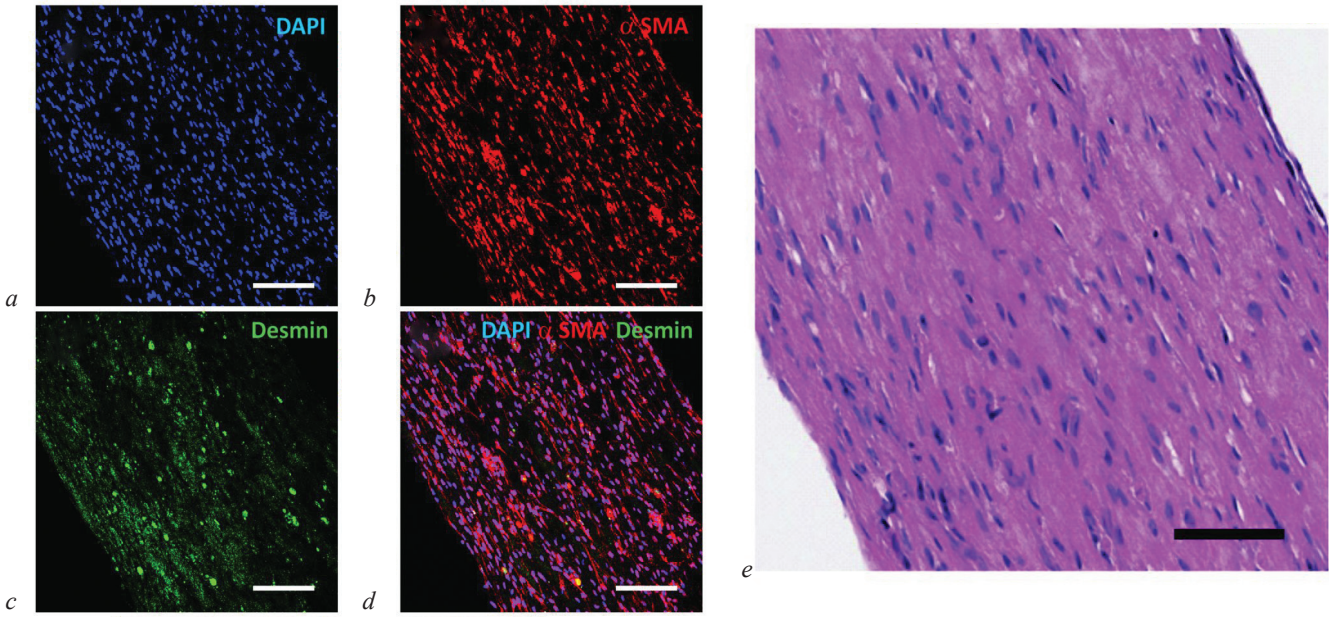


Fig. 3. Immunohistochemical analysis of longitudinal sections of the construct: *a* – DNA staining with DAPI, *b*, *c* – staining for the main markers of the contractile phenotype α-actin (red) and desmin (green), *d* – combined image, *e* – hematoxylin and eosin stain. The scale bar is 100 μm

In the literature, the elasticity data of smooth muscle TCs vary depending on the method of engineering. In particular, elasticity depends on load: static or variable; the composition of the culture medium; the geometry of constructions: ring-shaped [5, 6] or fiber-shaped [7]; the type of matrix: collagen-based, fibrin-based; as well as the origin of the VSMCs and their final density in the cell's mixture [5, 17–20]. The data obtained in individual VSMCs are less variable and are mainly determined by the phenotype of cells [21–23]. Specifically, when measuring elasticity by atomic force microscopy in constructs grown using VSMCs isolated from the thoracic aorta of a monkey (*Macaca fascicularis*) and collagen matrix, elastic modulus

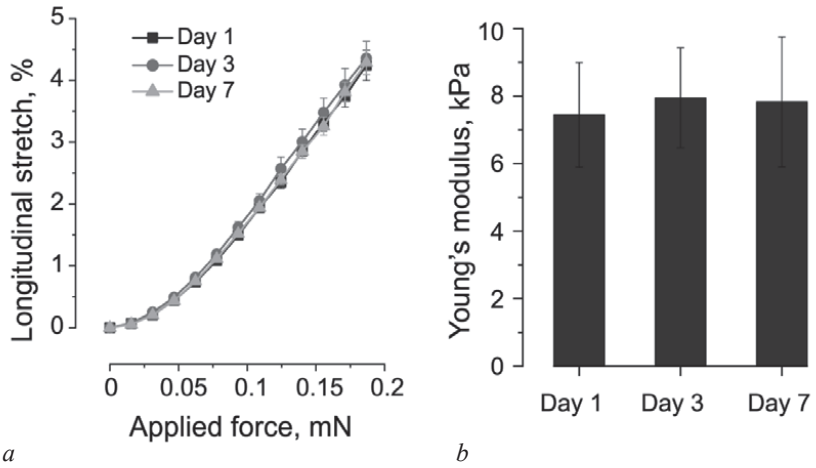


Fig. 4. The stability of the mechanical properties of tissue-engineered constructs: *a* – stress-stretch diagram of the constructs. Repeated measurements at 1st, 4th and 7th days, *b* – calculated elastic modulus (Young's modulus). The data are presented as Mean ± *SEM, N* = 6. There are no statistical differences between the three groups

values were within 13.7 ± 2.4 kPa and 23.3 ± 3.0 kPa [24]. The authors attributed the observed variability to age-related changes in the expression of actin and β1 subunit of integrin [25]. The elasticity assessed with the same method, but in individual VSMCs isolated from the thoracic aorta of healthy young rats (*Wistar-Kyoto*), varies between 5 and 14 kPa [21, 23]. Similar values of the elastic modulus (~13.7 kPa) were demonstrated in individual VSMCs isolated from rat (Sprague – Dawley) skeletal muscle arterioles and cultured for 3–7 days after the first passage [22].

D. Seliktar et al. performed isometric measurements of elasticity using a force sensor in ring-shaped three-dimensional TCs grown in a collagen matrix (2 mg/ml) using rat thoracic aorta VSMCs (10^6 cells/ml) after 8 days of culture, which showed a value of 68 kPa [18]. In another work, wherein the authors compared the elastic properties of the ring-shaped constructs grown with fibrin-based (2 mg/ml) or collagen-based (2 mg/ml) matrixes but with the same type of VSMCs (rat aorta, 10^6 cells/ml), it was shown that after 5 days of culture, collagen-based TCs had significantly greater rigidity (191 kPa) as compared with fibrin-based constructs (19 kPa) [20]. An increase in the concentration of collagen and fibrin in the cell mixture up to 4 mg/ml led to a rise in rigidity to 242 kPa and 28 kPa, respectively.

The elasticity values of smooth muscle TCs attained in our work are close to those measured in individual VSMCs. This could be explained by the use of fibrin in the cell mixture. Fibrin-based matrix is widely used in tissue engineering [26]. Fibrin gel has vasoactive and high adhesive properties and promotes rapid cell adaptation and proliferation in constructs [11], but due to its structural features it has considerable elasticity [27]. It should be noted that because of high plasticity of VSMCs [28, 29], the elasticity of constructs can substantially change during culturing [18, 19]. This can complicate the interpretation of the data, especially in long-term experiments, such as using long-term mechanical stimulation [18]. The application of fibrinolysis inhibitors (aprotinin, tranexamic acid) and low serum supplement (SMDS) in our work contributed to the stability of the elastic properties of the TCs after their maturation (Fig. 4).

Figure 5 depicts the results of a separate experiment to test the mechano-elastic properties of TCs under the influence of Bb and Cyto-D. It can be seen that the addition of 30 μm of Bb causes stretch of the TC from 1.2% to 1.45% (Fig. 5, *a*). Subsequent treatment with Cyto-D at a concentration of 50 μm promotes further relaxation of the TC and an increase in elongation to 1.8%. This correlates with changes in elasticity corresponding to the applied force of 0.062 mN in the tensile diagram of the TC shown in Fig. 5, *b*. It is known that Bb is a selective inhibitor of the ATPase activity of various isoforms of myosin II in striated muscle cells [12] and smooth muscle cells [30], while Cyto-D alters the mechanical properties by depolymerization of the cytoskeleton actin filaments [13]. Accordingly, the addition of Bb inhibits myosin-related contraction, whereas subsequent incubation with Cyto-D destroys the actin-mediated tonic contraction and increases the relaxation of the TC.

Adrenergic and cholinergic stimulations are one of the most important systems involved in the regulation

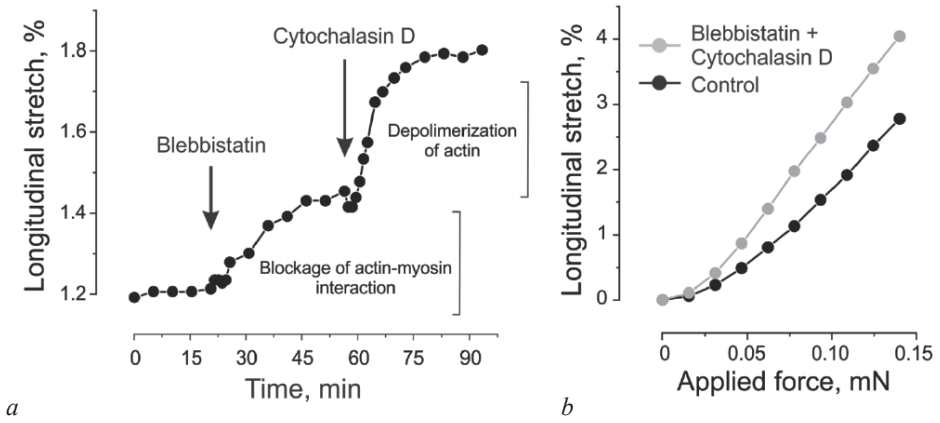


Fig. 5. Effect of blebbistatin and cytochalasin D on the mechano-elastic properties of TCs: *a* – changes in the elasticity of the construct in response to successive treatment by blebbistatin and cytochalasin D at a constant force of 0.062 mN, *b* – stress-stretch diagrams of the constructs at control and after application of 30 μm blebbistatin and 50 μm cytochalasin D

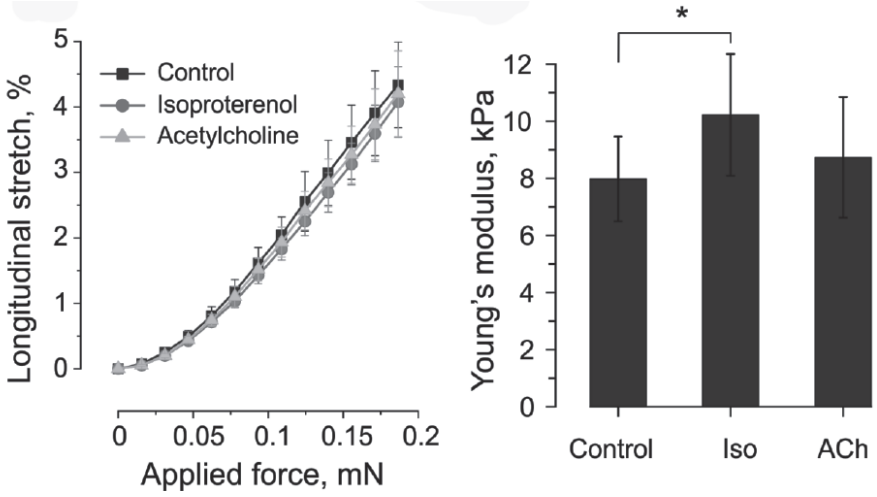
of cardiovascular tone. It is known that β-adrenergic stimulation through activation of adenylate cyclase and an increase in cAMP affects the level of intracellular Ca^{2+} [31, 32]. Several mechanisms causing relaxation were proposed, including hyperpolarization through Ca^{2+} activated potassium channels, reduction of the sensitivity of contractile elements to Ca^{2+} due to phosphorylation of the kinase of myosin light chains, a decrease in the content of cytosolic Ca^{2+} through regulation of Ca^{2+} transporting systems of the sarcoplasmic reticulum and plasmalemma, etc. [33, 34]. On the other hand, VSMCs are in close structural and functional interaction with endothelial cells, which can regulate vascular tone through paracrine interaction or through intercellular channels connecting two types of cells [35]. Therefore, adrenergic regulation of VSMCs contractility mediated by endothelial cells is also possible [35–37].

In our experiments, the incubation of TCs with Iso at a concentration of 1 μm caused a small but significant decrease in elasticity with an increase in the applied tensile strength above 0.05 mN ($p < 0.05$, $n = 6$), as well as a significant increase in the elastic modulus ($p < 0.05$, $n = 6$) from 7.9 ± 1.5 kPa to 10.2 ± 2.1 kPa (Fig. 6). In early studies on the effects of β-adrenergic stimulation on vascular tone and contractility of VSMCs, the experiments were mainly carried out *in vivo* or *in vitro* on isolated preparations of animal vessels, where the relaxing effect is typical [32, 33, 35]. In isolated VSMCs, a double mechanism of regulation of calcium channels was demonstrated. At nanomolar concentrations, Iso increased the L-type calcium channel current, while at micromolar concentrations it had the opposite effect [38]. Since we used only one type of cells (VSMCs) to engineer constructs, the dependence of the response mechanism on the concentration of Iso may underlie the observed decrease in elasticity.

The effect of ACh on VSMCs is mediated by M_3 muscarinic receptors [39]. According to the concept of participation of endothelial cells in the humoral regulation of vascular tone, endothelial cells play a primary role in response to cholinergic stimulation, whereas the adjoining VSMCs are passive recipients of nitric oxide [40]. However, from early works it is known that in the case of an immediate effect of ACh on VSMCs, ACh binds directly to the M_3 receptor, activates phospholipase C and can induce a contractile effect through the inositol triphosphate pathway [41]. In our experiments, the application of ACh caused only a slight decrease in the TC elasticity (Fig. 6, B). The small effect is most likely explained by a homogeneous cell population, i.e., the absence of endothelial cells in the cell mixture during the engineering of the constructs. Future experiments with the addition of a fraction of endothelial cells will allow us to evaluate the role of interactions between various types of cells in both cholinergic and adrenergic regulation of contractility of smooth muscle TCs.

CONCLUSION

In the current work, we for the first time utilized the I-Wire experimental platform to engineer and characterize smooth muscle TCs using VSMCs isolated from the human coronary artery.



a *b*

Fig. 6. Changes in the elasticity of tissue-engineered constructs in response to adrenergic and cholinergic stimulation: *a* – stress-stretch diagrams in the control, under isoproterenol and acetylcholine *b* – change of the modulus of elasticity (Young's modulus) after application of isoproterenol and acetylcholine. The data are presented as *Mean* ± *SEM*. **p* < 0.05, *N* = 6

We showed that a cell density of 10^6 cells/ml is optimal for tissue fiber formation with uniform and dense distribution of cells. The addition of Matrigel™ to the cell mixture accelerates formation of the construct. The combined application of Bb and Cyto-D makes it possible to isolate and evaluate the active and passive elements of elasticity of the TC. Addition of Iso caused an increase in the rigidity of the structures, while incubation with ACh did not have a significant effect on the TC elasticity. Since endothelial cells play an important role in the regulation of vascular smooth muscle tone, in order to reproduce a more complete regulation system in engineered TCs, further experiments should be conducted with co-culture of both VSMCs and endothelial cells, as well as with the use of elastin and collagen to form a stiffer extracellular matrix.

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Authors information

Sulgin Aleksandr A., Student, Medical Biological Department, Siberian State Medical University, Tomsk, Russian Federation.

Sidorova Tatiana N., Cand. Sci. (Biol.), Research Scientist, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, 37232, USA.

Sidorov Veniamin Yu., Cand. Sci. (Biol.), Research Assistant Professor, Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, 37240, USA.

(✉) **Sidorov Veniamin Yu.,** e-mail: v.sidorov@vanderbilt.edu.

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