Both sides nanopatterned tubular collagen scaffolds as tissue-engineered vascular grafts

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Abstract

Two major requirements for a tissue-engineered vessel are the establishment of a continuous endothelium and adequate mechanical properties. In this study, a novel tubular collagen scaffold possessing nanopatterns in the form of channels (with a 650 nm periodicity) on both sides was designed and examined after seeding and co-culturing with vascular cells. Initially, the exterior of the tube was seeded with human vascular smooth muscle cells (VSMCs), cultured for 14 days, and then human internal thoracic artery endothelial cells (HITAECs) were seeded on the inside of the tube and cultured for a further week. Microscopy revealed that nano-scale patterns could be reproduced on collagen with high fidelity and preserved during incubation in vitro. The VSMCs were circumferentially orientated with the help of these nanopatterns and formed multilayers on the exterior, while HITAECs formed a continuous layer on the interior, as is the case in natural vessels. Both cell types were observed to proliferate and retain their phenotypes in the co-culture.

1. Introduction

According to the Department of Health and Human Services of the United States Center for Chronic Disease Prevention, cardiovascular diseases are the predominant cause of death in the USA, killing more people than cancer every year, with 685,000 deaths in 2003 (National Center for Chronic Disease Prevention and Health Promotion of USA, 2009). The ideal vascular graft has not yet been found; autogenous grafts are currently the best conduits but are not always available or suitable (Ratliffe, 2000). There is therefore, a continuous search for a graft that is fully biocompatible, and able to take over the function of diseased or injured medium-sized vessels.

A tissue-engineered vascular graft should fulfil a number of requirements. One important one is that its mechanical properties approach those of native vessels. Another is that it should consist of a continuous layer of endothelium to prevent surface thrombosis and subsequent vascular blockage. A tissue-engineered vascular graft must also have a highly organized internal structure that combines collagen for strength and elastin to provide compliance and recoil. Hence, organized layers of interlinked collagen and elastin are a necessity for strong, elastic vessels (Mitchell and Niklason, 2003).

The choice of a scaffold material is critical, since stiff materials cause compliance mismatch, which leads to neointimal hyperplasia and atherosclerosis. On the other hand, materials with low ultimate tensile strength (UTS) rupture under normal blood pressure (Salacinski et al., 2001). As a result, many different materials have been studied for their suitability for vascular tissue engineering in terms of cell spreading, viability and construct mechanical properties, both as films and tubes (Shum-Tim et al., 1999; Berglund et al., 2003; Chong et al., 2007; Kurane et al., 2007; Thomas et al., 2007; Grenier et al., 2009).
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Synthetic polymers have the advantage of higher mechanical strength than biological ones but surface treatment is generally required for enhanced cell–material interaction. Poly(ε-caprolactone) (PCL) films have been tested after seeding with VSMCs, and to increase the cell adhesion the films were modified by either collagen immobilization or surface hydrolysis (with sodium hydroxide), the latter causing a UTS drop (Chong et al., 2007). A collagen-immobilized surface gave the best result in terms of VSMC adhesion and proliferation. Polyglyconate-based electrospun tubular scaffolds with a random fibre distribution and 200–400 nm fibre diameters have been mechanically tested and found to have enough mechanical strength for vascular tissue-engineering applications (Thomas et al., 2007). However, the polyglyconate had to be blended with biopolymers, such as gelatin and elastin, to mimic the natural artery, and a layered structure was obtained by stepwise electrospinning. The UTS dropped to 2.5 MPa from 14.5 MPa after blending, a value still comparable to those of natural arteries. In one study, tissue-engineered vascular grafts that were prepared by using autogenous cells and biodegradable polyglycolic acid and polyhydroxyalkanoate polymers were tested as ovine pulmonary arteries; this resulted in adequate mechanical strength, with collagen and DNA content approaching that of the native artery over time upon implantation in the systemic circulation without any surface modification (Shum-Tim et al., 1999). Grenier et al. tested three-dimensional (3D) porous polyurethane scaffolds with VSMCs and showed that these scaffolds promoted cell attachment and proliferation; however, the attachment was uneven and the problem was solved by coating the scaffold with Matrigel (Grenier et al., 2009). An extreme case of a vascular grafts utilizes cell sheets rolled around mandrels (without a scaffold material), and these were found to possess good mechanical strength (burst strength of 2000 mmHg) and suturability but limited patency after 3–4 weeks of transplantation (L’Heureux et al., 1998). Use of natural polymers as scaffold materials gives better results than with synthetic polymers in terms of cell–material interaction, but these have lower mechanical properties. Smooth muscle and endothelial cells seeded on electrospun silk fibroin scaffolds, respectively, proliferated, preserved their phenotype and deposited ECM (Zhang et al., 2008). In another study, tubular electrospun silk fibroin scaffolds were sequentially seeded with VSMCs and endothelial cells and cultivated under physiological pulsatile flow (Zhang et al., 2009). Dynamic flow conditions gave better results when compared to static culture controls in terms of cell proliferation, alignment, phenotype and ECM production, while the tube was coated with Matrigel to enhance endothelial cell retention under physiological shear conditions. A tubular scaffold made from collagen supported with an acellular crosslinked collagen sleeve to enhance its mechanical properties demonstrated viable cells and appropriate histology (Berglund et al., 2003). Tubes made from collagen seeded with endothelial and smooth muscle cells were investigated under shear conditions (4 dynes/cm²), which resulted in appropriate changes in the morphology of the cells that faced the luminal surface (Takei et al., 2007). Elastin was also studied as a scaffold material for vascular tissue engineering (Kurane et al., 2007). Acellular elastin tubes prepared from porcine carotid arteries were implanted subdermally in adult rats and cell infiltration was studied for 28 days. Cell infiltration was greater when the elastin tubes were filled with agarose gel releasing basic fibroblast growth factor, and most of the infiltrated cells were fibroblasts.

The aim of the present study was to produce a tissue-engineered vascular graft with appropriate mechanical properties and an adequate cell population, by coupling the inherent biocompatibility and good cell–material interaction of collagen with enhanced mechanical properties provided with the help of nanopatterns on the surface of a collagen scaffold. We have previously studied the effects of 300–650 nm patterned collagen surfaces on VSMCs and endothelial cells separately, and showed that, besides the expected good biocompatibility, the presence of the nanopatterns aligned the VSMCs, resulting in improved tensile properties, probably through oriented organization of the secreted extracellular matrix (Zorlutuna et al., 2009a) and enhanced endothelial cell retention under physiological shear flow conditions, possibly because the nanopatterned surfaces resemble their native substrate more than a smooth surface and also provide an increased surface area for cell–material interactions (Zorlutuna et al., 2009b). These previous studies, therefore, confirmed the benefits of surface patterning and cell guidance.

In the present study, in the light of our previous individual outcomes, a novel double-sided nanopatterned tubular collagen scaffold was designed and examined with vascular smooth muscle cells and endothelial cells in co-culture. These collagen scaffolds were patterned along the flow axis on the inside and normal to the flow on the outside. Seeding was sequential: first, VSMCs were seeded on the exterior and then ECs were seeded on the interior of the tubes. Cell proliferation, phenotype and histology of the tissue-engineered construct were investigated to determine the suitability of this double-sided nanopatterned tubular collagen construct for tissue engineering a small–medium calibre vascular graft.

2. Materials and methods

2.1. Template preparation

X-ray interference lithography facilities at Bilkent University Physics Department (Ankara, Turkey) were used to create the templates with nano-channels. Briefly, a photoresist (AZ 5214) of 500 nm thickness was coated on a silicon wafer, exposed to a laser (λ = 325 nm) for 10 min and developed. Later, patterns were transferred to silicon by using an epoxy replica. The templates prepared consisted of parallel channels of equal
CO2 concentration of the incubator was increased to (Sigma) and 10 µM, pH 7.4, 10 mM), cut longitudinally and the endothelial cells more than 3 h. The vein was washed with PBS (pH Sigma, USA] and stored at room temperature for not µM with 10% FCS (Hyclone, USA) and 10% FCS, 75 µM, with 1 M NaCl and then with 2 M NaCl and finally with distilled water. The double-sided nanopatterned tubular scaffolds were examined with SEM for pattern fidelity.

2.2. Scaffold preparation
Collagen type I was isolated from Sprague–Dawley rat tails and its purity was determined by SDS–PAGE. Films were prepared by a modified solvent-casting method, using an acetic acid solution of collagen type I, and then these films were converted into tubes. In order to prepare double-sided patterned collagen films, collagen solution (150 µl, 20 mg/ml, in 0.5 M acetic acid) was placed onto one of the templates and then the second template was placed on top, with its patterns perpendicular to the one at the bottom. It was allowed to dry and was peeled off the surface. Then, these films were rolled around Teflon mandrels (diameter 3.5 mm) and chemically crosslinked in this position with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC; Pierce, USA) and N-hydroxysulphosuccinimide (NHS; Sigma, USA) (Zorlutuna et al., 2009a; Staros et al., 1986). After incubating for 2 h at room temperature (RT), the tubes were washed with phosphate buffer (0.1 M, pH 9.1) for 1 h, with 1 M NaCl and then with 2 M NaCl and finally with distilled water. The double-sided nanopatterned tubular scaffolds were examined with SEM for pattern fidelity.

2.3. Cell culture
Human VSMCs were isolated from human saphenous vein sections (~5 cm in length) obtained from bypass surgery at Bayındır Hospital (Ankara, Turkey), in accordance with the rules of their Ethical Committee. The sections were put into transport medium (DMEM [Gibco, USA]:HamF12 [Gibco, USA], 1:3, supplemented with 10% FCS [Hyclone, USA] and 10 µl/ml antibiotics [Sigma, USA]) and stored at room temperature for not more than 3 h. The vein was washed with PBS (pH 7.4, 10 mM), cut longitudinally and the endothelial cells were scraped off with a No. 15 scalpel blade. The vein was then further cleaned to remove remaining endothelial cells. The de-endothelialized vein was cut into smaller pieces (~4 mm²) and put into 35 mm Petri dishes pre-coated with gelatin (Sigma), with the luminal surface facing down. In order to improve the adhesion of explants onto the gelatin-coated Petri dish surface, a small volume (500–700 µl) of DMEM:Ham F12 (3:1), supplemented with 30% FCS, 75 µl/ml ECGS (Sigma) and 10 µl/ml antibiotics were added and the CO2 concentration of the incubator was increased to 8% until cells migrated out of the biopsy samples. Within 2 weeks, cells started to migrate out of the biopsy samples. When the explant cultures reached subconfluency (90%), they were trypsinized with 0.25% trypsin–EDTA solution (Sigma) by incubating for 1 min and counted using a NucleoCounter (ChemoMetec A/S, Denmark). 5000 cells/cm² were seeded on poly(L-lysine)-coated coverslips for immunostaining for phenotype confirmation, and the rest of the cells were transfected into T75 tissue culture flasks and incubated in the standard medium (3:1 DMEM:Ham F12, supplemented with 10% FCS and antibiotics) until they reached confluency. At subconfluency, the cells were either passaged until passage 6 or they were frozen at lower passage numbers for future use.

Human internal thoracic artery endothelial cells (HITAECs) were obtained from the European Collection of Cell Cultures (UK) and cultured in HITAEC growth medium at 37 °C in a carbon dioxide incubator. The cells were passaged by incubation in 0.25% trypsin–EDTA for 1 min at room temperature solution at subconfluency.

2.4. Co-culture of VSMCs and HITAECs on tubular scaffolds
Double-side nanopatterned tubular collagen scaffolds still wrapped around the mandrels were placed into 24-well plates. Teflon sheets were used to cover the bottom of the wells. They were sterilized by incubation in EtOH (70%) for 3 h and washed four times with PBS (10 mM, pH 7.4). VSMCs (passage 4) were detached from the tissue culture flasks by using 0.25% trypsin–EDTA for 1 min at 37 °C, centrifuged for 5 min at 3000 rpm and resuspended in their standard medium (3:1 DMEM:Ham F12, supplemented with 10% FCS and 1% penicillin–streptomycin). Cell number was determined using a NucleoCounter. 2 × 10⁵ cells in 2 ml were seeded on each tubular scaffold. They were incubated at 37 °C in a carbon dioxide incubator for 14 days. The medium was changed every other day. After 14 days, the mandrel was removed and the inside of the scaffold was seeded with HITAECs (passage 3) at a density of 5 × 10⁴ cells/tube and incubated for another week. After HITAEC seeding, the medium was changed to HITAEC medium and was refreshed every day. As a control, the tubes were seeded on the outside with VSMCs and cultured for 21 days without endothelial cell seeding on the inside.

2.5. Cell proliferation
On days 1, 7, 14 and 21, Alamar Blue assay was performed in order to assess the cell number on the scaffolds (n = 3). Briefly, cells were incubated in 10% Alamar Blue (BD Biosciences) in colourless DMEM (Gibco) for 1 h and the OD was measured at 570 and 595 nm, using a kinetic microplate reader (Maxline Vmax®, Molecular Devices). From these OD values, percentage reduction was calculated and converted to cell numbers using a calibration curve.
2.6. Microscopy (SEM, fluorescence)

On days 1, 7, 14 and 21 the cells on the scaffolds were fixed with either formaldehyde (4%) or glutaraldehyde (2.5% in cacodylate buffer 0.1 m, pH 7.4), for immunostaining or SEM, respectively. Immunostaining was done by using anti-α-smooth muscle actin (Sigma) as the primary antibody for VSMCs and by using anti-PECAM-1 (CD31) (Sigma) as the primary antibody for HITAECs and using Alexa 488 (Molecular Probes) as the secondary antibody for both. After fixing the cells with paraformaldehyde (4%; Sigma) for 30 min at RT, the cells were permeabilized with Triton X-100 (1%; Sigma) for 5 min and washed three times with PBS. They were then incubated in the blocking solution (0.5% BSA, 0.1% Tween 20, 0.1% FCS, 0.1% sodium azide in PBS) for 30 min at RT and in the primary antibody solution for 2 h at 37 °C. After three washes with PBS, the cells were incubated for 1 h in the secondary antibody solution at 37 °C. After washing with PBS the cells were examined with a fluorescence microscope (IX70, Olympus, Japan).

2.7. Histology

Cell-seeded scaffolds were examined histologically on day 21. The cells were fixed with formaldehyde (4%) for 24 h

Figure 1. SEM images of tubular collagen scaffolds: (a) scheme of the tubular scaffold; (b) interior of the double-side patterned tubular scaffold; (c) exterior of the double-side patterned tubular scaffold; (d) inside of the unpatterned tubular scaffold; (e) outside of the unpatterned tubular scaffold
and incubated in EtOH (96%) for 5 h and then transferred into xylol and incubated for another 2 h, and finally embedded in paraffin for 4 h at 62°C. 5 μm thick sections were obtained with a microtome (RM 2125RT, Leica, Germany) and deparaffinized by incubating at 70°C for 25 min and in xylol for 30 min. After washing with EtOH (96%) and with distilled water, the sections were stained with haematoxylin and eosin (H&E; Biooptica, Italy). The sections were examined under a light microscope (IX70, Olympus, Japan).

3. Results and discussion

3.1. Pattern transfer fidelity

Pattern transfer fidelity on both sides of the tubular scaffold was examined with SEM. Figure 1a shows a scheme of the tubular construct, showing the direction of the patterns and organization. SEM shows properly reproduced patterns on both the inside (Figure 1b) and the outside (Figure 1c) of the tubular construct. When compared to an unpatterned control (Figure 1d, e), the presence of the nanopattern is strikingly evident.

3.2. Cell proliferation

Proliferation of the cells on collagen tubes nanopatterned on both sides was determined by Alamar Blue assay. During the first 2 weeks only the VSMCs were on the tubes. The percentage Alamar Blue reduction was converted into cell numbers using a calibration curve constructed for VSMCs. Figure 2 shows that VSMCs proliferated steadily on the tubes prior to HITAEC seeding on day 14. Proliferation was also studied with the Alamar Blue test after seeding the HITAECs on the insides of the tubes. This time, the proliferation was represented as percentage reduction rather than as cell number, because the tube contained a co-culture of two cell types with no way of differentiating the respective effects of individual cell types on reduction. The steady increase in the reduction of the dye, however, shows that cells continued to proliferate after the co-culture was initiated on day 14 (Figure 2). On day 21, the reduction of the Alamar Blue dye for the co-culture was 32%, whereas it was 28% for the VSMCs-only seeded tubes (Figure 2). The 10% increase in the dye reduction values at day 24 is consistent with the extra 10% cell loading with endothelial cell seeding (500 000 VSMCs and 50 000 HITAECs). Here we assume that the cells did not affect each other. This was further supported by microscopy.

Previous studies have examined the effect of co-culture on vascular cells. When endothelial cells are co-cultured directly on smooth muscle cells, their proliferation rate is unaffected (Lavender et al., 2005). Human saphenous vein smooth muscle cells and endothelial cells co-cultured on 2D PGA scaffolds showed a uniform VSMC distribution and a continuous endothelial layer, with significantly higher endothelin and 6-keto-prostaglandin F1α level compared to controls (only ECs or only VSMCs), suggesting no adverse cell interaction in co-culture (Wen et al., 2005). Powell et al. (1996) showed that when co-cultured with endothelial cells, smooth muscle cells could lose their synthetic phenotype, causing mechanical strength decrease, but this also enhanced smooth muscle cell proliferation (Powell et al., 1996). However, there are other studies which contradict this, and where co-culture led to a reduced cell proliferation (Imegwu et al., 2001). In another study, myofibroblasts were co-cultured with endothelial cells on poly(glycolic acid) (PGA) and poly(4-hydroxybutyrate) substrates to investigate the effect of co-culture on ECM composition and mechanical properties (Pullens et al., 2009). It was observed that 1 week co-culture did not cause mechanical weakness and only a slight decrease in the secreted collagen, but when the co-culture period was extended to 2 weeks there was mechanical weakening. VSMCs and endothelial cells have also been co-cultured on PGA tubular scaffolds, in a perfusion bioreactor under pulsatile shear stress, for short (2 day) and extended (15 day) co-culture periods after an initial VSMC culture of 23 or 10 days, respectively (Williams and Wick, 2005). Co-culture of 15 days resulted in a significantly increased cell proliferation and decreased ECM secretion, with more uniform cell distribution and a more contractile VSMC phenotype when compared with 2 days of co-culture. These studies suggest that co-culturing for 1 week might be the optimum duration for proper proliferation rate and ECM secretion.
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3.3. SEM and immunostaining

Tubes seeded only with VSMCs were examined with SEM on day 21. Figure 3a shows the inside of the tubular scaffold. There are no cells to see, since the Teflon mandrel was not removed and therefore no cell seeding was done on the inside. The nanopatterns in the inside of the tube are distinct even after 21 days of incubation in the cell culture, indicating that they would help to retain the endothelial cells under flow shear conditions in the co-culture experiments, as expected from our earlier results. SEM images of the outside of the tubular scaffold, seeded with only VSMCs on day 21, shows VSMC cell orientation along the pattern and an aligned cell sheet (Figure 3b, c); the edge of the sheet (Figure 3d) confirms the retained nanopattern.

With co-cultured samples, on days 1, 7 and 14, the tubes were stained only with anti-α-smooth muscle actin because endothelial cells had not yet been seeded, but on day 21, 1 week after HITAEC seeding, staining was done with both anti-α-smooth muscle actin (for the VSMCs) and anti-CD31 (PECAM; for HITAECs). Figure 4a–d shows the cells stained with anti-α-smooth muscle actin, confirming the phenotype of the VSMCs on the outside of the tube. Since anti-α-smooth muscle actin stains the cytoskeletal elements of the VSMCs, the alignment of the cytoskeleton indicates the organization of the cells along the patterns. As expected, the inside of the tube was stained positive for the endothelial cell marker CD31, confirming the presence of the endothelial cells (Figure 4e, f). The endothelial cells formed a continuous layer, since CD31, as a surface receptor, marks the cell membranes and demonstrates a covered surface.

VSMCs seeded on the tubes were examined with SEM at the same time points (Figure 5). An increase in cell number and cell alignment was observed as the duration of the incubation period increased. In Figure 5b, the patterns on the surface of the tubes could be seen to be in the direction of cell alignment, although a clear pattern could not be observed because the entire surface was covered with the cells. On day 21, a multilayer of VSMCs could be seen and this was also confirmed by microscopic examination (Figure 5g, h). When Figures 3b and 5g are compared, it can be seen that the morphologies of the cells are quite similar, suggesting that co-culturing with HITAECs did not alter VSMC morphology. Figures 6a and b show that HITAECs cover the inner surface of the tubes as a near-continuous layer.

Vascular smooth muscle cell alignment was previously observed using bovine pulmonary artery smooth muscle cells on poly(methyl methacrylate) and PDMS surfaces possessing nanochannels of 350 nm width, 700 nm pitch and 350 nm depth (Yim et al., 2005). Results showed that more than 90% of the cells were orientated, while cell
proliferation was decreased. In our previous studies, on the other hand, no effect of alignment of VSMCs could be observed on cell attachment or proliferation (Zorlutuna et al., 2009a). The observed difference could be due to the differences in the cell sources and the scaffold material. Especially, the hydrophobic surface used by Yim et al. (2005) probably had less interaction with the cells than did collagen. The alignment of human coronary artery smooth muscle cells on nano-scale topography was also reported for synthetic polymeric fibres with 500 nm diameter (Xu et al., 2004). A recent study showed double-sided patterned tubular PCL scaffolds for vascular tissue-engineering applications, with one side with micro-channels and the other with nanopits (Seunarine et al., 2008), but this did not progress to seeded cells on the scaffold. To the best of our knowledge, the present study is the first that shows alignment of vascular smooth muscle cells on nano-scale patterned tubular collagen scaffolds with a successful co-culture of VSMCs and endothelial cells on tubular nanopatterned scaffolds.

3.4. Histology

Histological examination on day 21 with 5 μm thick sections, with unseeded tube serving as control (Figure 7a and b), showed the HITAECS to be a monolayer on the inside of the tube and the VSMCs on the exterior as a multilayer, as occurs in natural vessels (Figure 7c–f). It was reported that a co-culture of mouse aortic smooth muscle cells and bovine carotid artery endothelial cells seeded on tubular collagen gels (Takei et al., 2007) had similar histological results and cell distribution, but in that study the vascular smooth muscle cells were not confined just to the outside of the tube, but also penetrated into the scaffold wall, forming multilayers in the wall. This penetration was probably because the gel form of the collagen used was more permeable to cells. In the current study, such invasion is likely only after collagen degradation. A crosslinked collagen structure would seem to be preferable because of its durability and mechanical properties, important for clinical handling and direct blood vessel replacement.
Figure 5. SEM images of the outside of the nanopatterned tubular scaffold, seeded with VSMCs: (a) day 1 (x500); (b) nanopatterns on the surface near the VSMCs on day 1 (x1000); (c) day 7 (x500); (d) day 7 (x1000); (e) day 14 (x500); (f) day 14 (x1000); (g) day 21 (x500); (h) day 21 (x1000)
Figure 6. SEM images of the inside of the nanopatterned tubular scaffolds seeded with HITAEcs on day 21: (a) ×500; (b) ×1000

Figure 7. Bright-field microscopy images of haematoxylin and eosin-stained scaffolds. Unseeded tubular scaffolds on day 21: (a) ×100; and (b) ×400. VSMC- and HITAEC-seeded tubular scaffolds on day 21: (c) ×100; (d, e) ×200; (f) ×400

4. Conclusion

A functional tissue-engineered vessel is the optimum solution for vascular reconstruction, and with a patterned scaffold natural vessel morphology can be mimicked. A tubular collagen scaffold with nanopatterns on both sides was seeded with vascular smooth muscle cells and endothelial cells. Nanopatterns were successfully transferred and maintained through crosslinking of the collagen films. Nanopatterns on the outside successfully orientated the smooth muscle cells circumferentially, as in the natural vessel; this is expected to enhance the ultimate tensile strength and the nanopatterns on the inside is
expected to increase cell retention under blood flow conditions. Both effects have been individually confirmed in previous studies. When seeded and co-cultured in double-sided nanopatterned tubes, both cell types retained their phenotypes and proliferative capacity. In conclusion, a both-sides nanopatterned scaffold appears to be able to satisfy the major requirements of vascular tissue engineering and thus could serve as an artificial blood vessel.

Acknowledgements

We would like to acknowledge Professor Atilla Aydinli and Askin Kokbas of Bilkent University (Ankara, Turkey) for the patterned templates. We also gratefully acknowledge grants from the State Planning Organization of Turkey (DPT), Project No. BAP 01.08.DPT.2003K120920-20, and the Scientific and Technical Research Council of Turkey (TUBITAK), Grant No. TBAG108T576.

References


National Center for Chronic Disease Prevention and Health Promotion, Chronic Disease Overview, United States Government.

Online, 2009; URL: http://www.cdc.gov/nccdphp/overview_text.


Zhang X, Baughman CB, Kaplan DL. 2008; In vitro evaluation of electrospun silk fibroin scaffolds for vascular cell growth. Biomaterials 29: 2217–2227.

