Comparing Supportive Properties of Poly Lactic-Co-Glycolic Acid (PLGA), PLGA/Collagen and Human Amniotic Membrane for Human Urothelial and Smooth Muscle Cells Engineering

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Purpose: To compare human urothelial and smooth muscle cells attachment and proliferation using three different matrices; poly lactic-co-glycolic acid (PLGA), PLGA/collagen and human amniotic membrane (hAM).

Materials and Methods: Human urothelial and smooth muscle cells were cultured and examined for expression of urothelium (pancytokeratin and uroplakin III) and smooth muscle cells [desmin and alpha smooth muscle actin (α-SMA)] markers. Cells were cultured on three scaffolds; PLGA, PLGA/collagen and hAM. Thereafter, they were analyzed for cell growth on days 1, 3, 7, 14 and 21 after seeding by 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Scaffolds were fixed and processed for hematoxylin and eosin (H&E) staining and immunohistochemistry against their cell specific markers after 7 and 14 days of culture.

Results: MTT assay results revealed that collagen has improved cell attachment features of PLGA and led to significant increase of MTT signal in PLGA/collagen compared to PLGA ($P < .001$) and hAM ($P < .001$). hAM was a weaker matrix for both cell types as demonstrated in MTT assay and scanning electron microscope (SEM) images. SEM micrographs showed normal phenotype and distribution on PLGA and PLGA/collagen. In the same line, cells formed a well-developed layer either on PLGA or PLGA/collagen, which maintained expression of their corresponding markers.

Conclusion: Our findings demonstrated significant improvement of cell attachment and growth achieved by collagen coating (PLGA/collagen) compared to PLGA and hAM. hAM despite of its natural entity was a weaker matrix for bladder engineering purposes.

Keywords: biocompatible materials; cell proliferation; membranes; artificial; polyglycolic acid; urinary bladder; regeneration; tissue engineering; amnion; muscle; smooth; cell culture techniques.

INTRODUCTION

Various disorders, such as infection, trauma, cancer and congenital abnormalities may affect functional and anatomical characteristics of the bladder and may result in organ damage and even organ loss.[1] Consequently, reconstructive surgery may be needed to save the bladder normal functions. Tissue engineering has been proposed as a novel potential curative approach substituting enterocystoplasty as the most frequent practice which is associated with several complications, including, infection, metabolic disorders, urolithiasis, increased mucus production and malignancies.[2,3] To address this challenge, various matrices have been used in vitro and in vivo for genitourinary tissues which could be categorized as naturally derived materials, acellular tissue matrices and synthetic materials.[4,5] Concerning the scaffolds, biocompatibility, minimal immunogenicity and mechanical properties of matrices (permeability, stability, elasticity, flexibility, plasticity and resorbability at harmonious rate with original tissue) are key parameters for tissue substitutes.[6,7] In this regard, naturally derived materials are biocompatible, resorbable and contain cell recognition groups but possess poor mechanical features and modifications in their structural properties are not easily reachable during preparation processes. In contrast, synthetic scaffolds are versatile and various methods like co-polymering, hybrid scaffolding and addition of cell anchorage groups or bioactive molecules can be recruited to modify or improve their elements.[8]

Amniotic membrane (AM) has been greatly considered in tissue engineering due to its natural essence and supportive features such as reduction of scarring and inflammation; improving wound healing and providing proper performance as a scaffold for cell proliferation and differentiation.[9] In addition, different components of extracellular matrix (ECM) such as, collagens, laminin, fibronectin, nidogen and proteoglycans, exist in the layers of AM which all motivated its application in various disciplines of regenerative medicine.[10-12] Poly lactic-co-glycolic acid (PLGA) is a copolymer of two linear aliphatic polyesters, polylactic acid (PLA) and polyglycolic acid (PGA) and is well-recognized for its superior biodegradability and biocompatibility which lead to its frequent use as polymer for scaffold fabrication.[13] In addition, PLGA has been widely studied and accepted as drug-delivery vehicle[14] and suture material.[15] A numerous array of studies have been focused on PLGA employment in different contexts of tissue engineering.[16-18] However, lack of cell recognition (binding) sites has limited and hindered many potential applications of PLGA and several other synthetic polymers, such as polycaprolactone (PCL), poly-DL-lactide) (PDLLA) and PGA.[19] A growing body of studies have been dedicated to improve different aspects of synthetic polymers such as addition of ECM molecules, to improve cell attachment and function, or various treatments and preparations for alternation in fibers alignment and diameter or exposing new chemical groups.[20,21] In the current work, we studied the capacity of PLGA, PLGA/collagen and hAM as culture matrices for urothelial and bladder smooth muscle cells to compare a naturally derived material, hAM, with a synthetic polymer (PLGA) at first and furthermore, to evaluate the effects of collagen addition on supportive features of PLGA.

MATERIAL AND METHODS

Scaffold Preparation

Frozen amniotic membranes in phosphate buffered saline (PBS) containing 10% dimethylsulfoxide (DMSO) (Royan Institute, Tehran, Iran) were thawed and washed with PBS several times. To remove amniotic epithelial cells, 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) was added and membranes were incubated at 37°C with 5% CO2 for 30 minutes, then scraped using a sterile cell scraper (SPL Life Sciences, Eumhyeon-Ri, Naechon-Myeon, Pocheon-Si, Gyeonggi-Do, South Korea) until full detachment. After being thoroughly washed with PBS, they were cut and loaded with cells.

An electrospinning apparatus (CO881007NYI series, Asian Nanostructure Company, Tehran, Iran) was used for fabrication of nanofibrous mats. This device was equipped with drum collector with 70 mm diameter and 50 mm width. The electrospinning process, which was used in this study, was the conventional process. The proper PLGA solution (12.5%) (Boehringer Ingelheim, Germany,) in 1, 1, 1, 3, 3, 3, hexafluoro-2-propanol (HFIP) has electrospun under the conditions mentioned in Table. Then, nanofibrous mats were coated with collagen via dipping method in 0.1% collagen solution (Sigma-Aldrich Corp., St. Louis, MO, USA). Thereafter, the coated collagen was crosslinked using adjacency to

Table. Electrospinning conditions for poly lactic-co-glycolic acid scaffold preparation.

Drum Linear Speed	Drum Rotational Speed	Feeding Rate	Electrode Distance	Potential Difference
5 mm/sec	2000 round per min	2 mL/h	150 mm	22 kV

glutaraldehyde vapor (Sigma-Aldrich Corp., St. Louis, MO, USA) for 3 hours in order to reduce the collagen solubility in water. Finally, the crosslinked scaffolds were washed by PBS three times to remove residual crosslinker.

Isolation of Smooth Muscle and Urothelial Cells

All necessary cell culture materials were purchased from PAA Laboratories GmbH, PAA Strasse 1,A-4061 Pasching, Austria, except where mentioned otherwise. After obtaining written informed consents, normal human bladder tissues were obtained during open surgery for reasons other than cancer, including open prostatectomy or anti-reflux surgery (n = 11). Tissue biopsies (approximately 0.3-0.5 cm^2) were transferred to laboratory in Dulbecco's modified eagle's medium (DMEM) containing 2% penicillin/streptomycin and 2% fungizone (Invitrogen, Grand Island, NY, USA) on ice. Under sterile condition, each tissue was rinsed with Hank's balanced salt solution (HBSS) and mucosal layer was dissected off the smooth muscle layer. Mucosal and smooth muscle layers were incubated with 2 mg/mL collagenase type XI (Sigma-Aldrich Corp., St. Louis, MO, USA) and 1 mg/mL dispase (Invitrogen, Grand Island, NY, USA) for 1 hour at 37ºC in separate tubes. Cold HBSS was added to digestion solution to dilute out the enzymes followed by passing through 70 µm cell strainer. After 5 minutes centrifugation

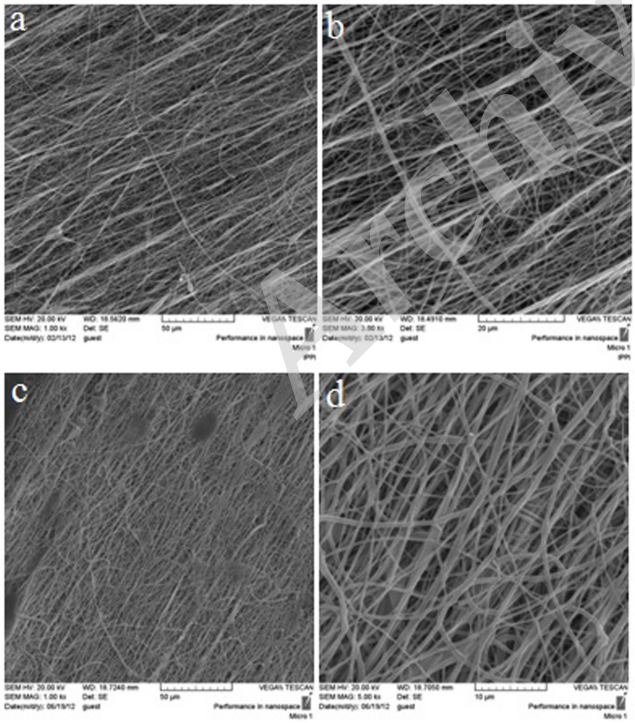

Figure 1. SEM analysis of PLGA and PLGA/collagen; (a and b) electrospun PLGA scaffolds and (c and d) PLGA/collagen.
Keys: PLGA, poly lactic-co-glycolic acid; SEM, scanning electron microscope.

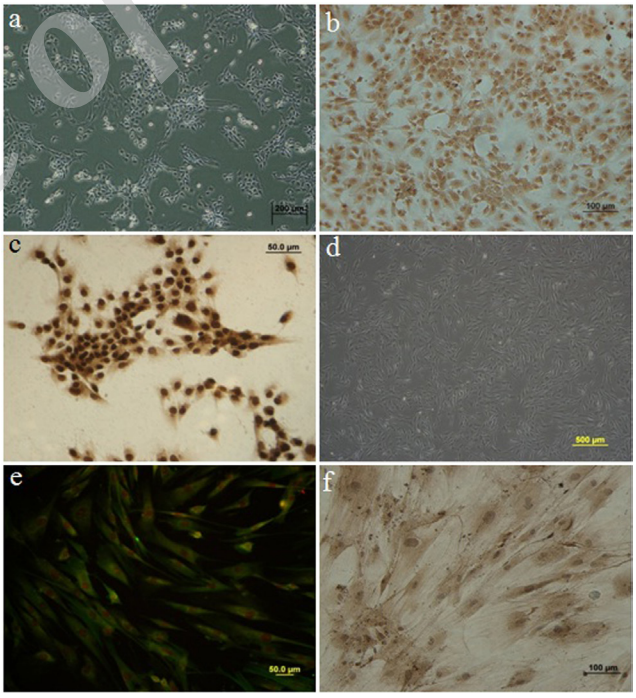

Figure 2. Phenotypic characterization of urothelial and smooth muscle cells; (a) propagated colonies of human urothelial cells in different size 4 days after culture, (b) prominent expression of pancytokeratin in cultured urothelium, (c) strong expression of uroplakin III in colonies, (d) isolated human smooth muscle cells in passage 4, (e) expression of α-SMA and (f) desmin were verified via immunocytochemical assay. Brown color was developed by DAB substrate.
Keys: DAB, 3, 3′-diaminobenzidine; α-SMA, alpha smooth muscle actin.

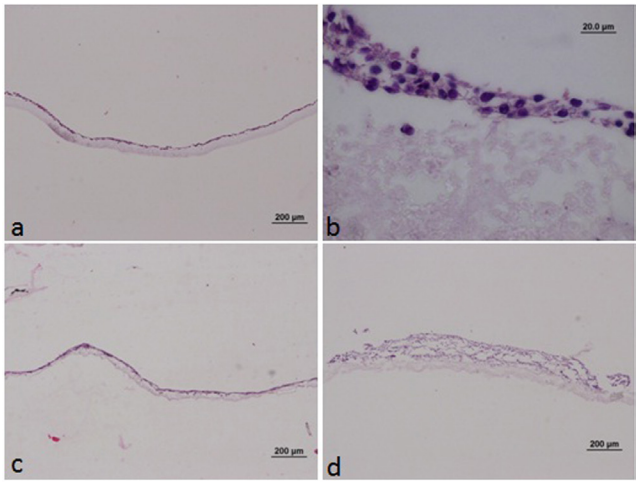

Figure 3. Histological analysis of scaffolds after cell seeding with H&E staining; (a and b) human urothelial cells on the surface of PLGA/collagen formed a continuous sheet after 7 days of culture containing 2 to 3 layer of cells and (c and d) human smooth muscle cells 7 days after loading on PLGA/collagen made multiple layers.
Keys: PLGA, poly lactic-co-glycolic acid; H&E, hematoxylin and eosin.

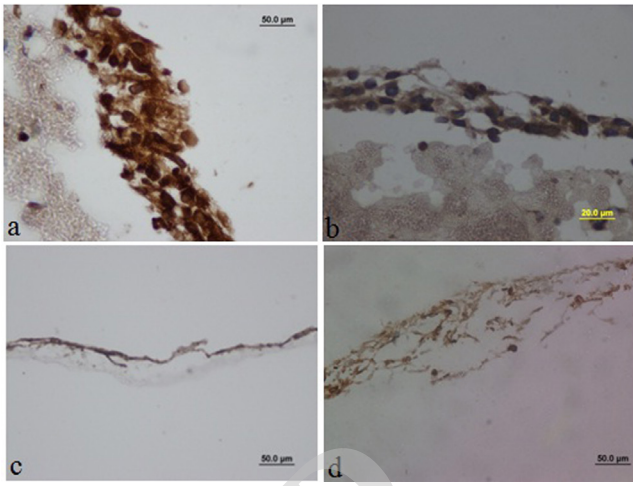

Figure 4. Immunohistochemical expression of human urothelial and smooth cell markers after proliferation on scaffolds; (a) apparent expression of pancytokeratin and (b) uroplakin III in urothelial cells after 7 days on PLGA/collagen, (c) expression of α-SMA and (d) desmin were also continued after 7 days of culture. No distinctive difference in the expression level of markers was observed between PLGA/collagen and PLGA.
Keys: PLGA, poly lactic-co-glycolic acid; α-SMA, alpha smooth muscle actin.

at 1500 at 4ºC, pellets of urothelial and muscle cells were resuspended in keratinocyte serum-free medium supplemented with 50 µg/mL bovine pituitary extract, 5 ng/mL human epidermal growth factor (all from Invitrogen, Grand Island, NY, USA), 30 ng/mL cholera toxin subunit B (Sigma-Aldrich Corp., St. Louis, MO, USA) and 2% and high glucose DMEM supplemented with 10% fetal bovine serum (FBS), respectively. Cell suspensions were plated in T-25 tissue culture flasks (SPL Life Sciences, Eumhyeon-Ri, Naechon-Myeon, Pocheon-Si, Gyeonggi-Do, South Korea) and incubated at 37°C with 5% CO2.

Immunocytochemistry

To characterize cultured cells, immunocytochemical expression of four markers was studied. Cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100 (Sigma-Aldrich Corp., St. Louis, MO, USA) in PBS, and blocked with 1% bovine serum albumin (Sigma-Aldrich Corp., St. Louis, MO, USA) for 1 hour. Rabbit polyclonal anti-uroplakin III, anti-pancytokeratin, anti-desmin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and mouse monoclonal anti-human α-SMA (Abcam Ltd. 31 Cambridge Science Park, Milton Road. Cambridge CB4 0FX, UK) antibodies were diluted 1:50 in blocking buffer and incubated overnight at 4°C. Primary antibodies binding sites were probed with secondary antibody including in VECTASTAIN Universal Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) or fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody (Sigma-Aldrich Corp., St. Louis, MO, USA) for 30 minutes at room temperature (RT). For chromogenic system (uroplakin III, pancytokeratin and desmin), staining was followed by biotinylated horseradish peroxidase incubation at RT for 30 minutes. 3, 3′-diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA, USA) substrate was used to reveal expression of markers and allowed to develop for 3 minutes. Counterstaining was performed using hematoxylin or propidium iodide (Sigma-Aldrich Corp., St. Louis, MO, USA) for chromogenic and fluorescent systems, respectively. Finally, cells were examined using an inverted microscope (CKX41, Olympus, Shinjuku, Japan) and photographed by DP71 camera (Olympus, Shinjuku, Japan)

Cell Seeding on Scaffolds

Scaffolds were sterilized in 70% ethanol for one hour followed by three washes of PBS to remove ethanol. Scaffolds were incubated with culture medium for 30 minutes. After trypsinization and determination of cell concentration, 250 µL of cell suspension containing 100 000 viable cells was

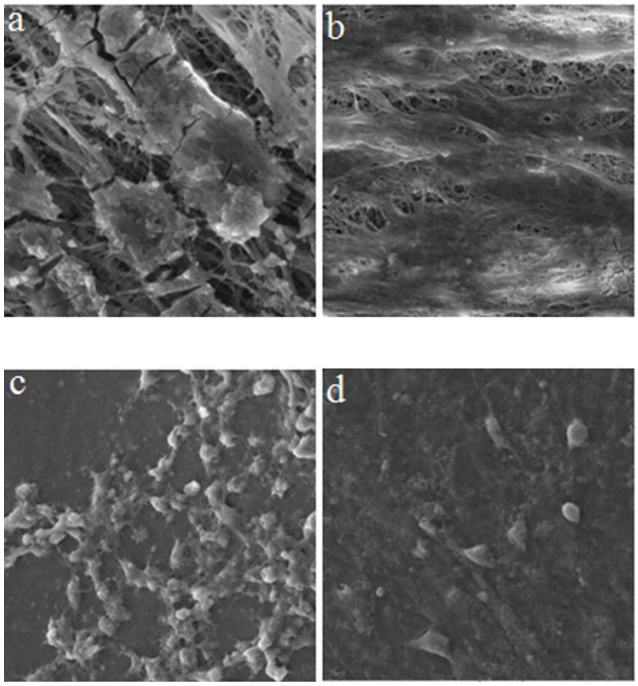

Figure 5. Representative images of SEM of PLGA, PLGA/collagen and hAM after cell seeding; (a) human urothelial cells colonized normally on the PLGA/collagen after 5 days (3000 ×), (b) smooth muscle cells proliferated on PLGA after 5 days while scaffolds fibres could be observed between cells (1500 ×), (c) colonies of human urothelial cells on hAM is loose and dispersing after 3 days (1500 ×) and (d) human smooth muscle cells could be seen infrequently on hAM on day 3 post seeding (1500 ×). No difference in distribution and growth pattern of cells was detected between PLGA and PLGA/collagen.

Keys: PLGA, poly lactic-co-glycolic acid; AM, amniotic membrane; hAM, human amniotic membrane; SEM, scanning electron microscopy.

transferred onto scaffolds or hAM. Cells were allowed to attach and propagate at 37°C and 5% CO2. Owing to high density of cells seeded on scaffolds, the medium was refreshed every other day.

Histological Analysis of Scaffolds

To evaluate cell layering and expression of above-mentioned markers, Hematoxylin and Eosin staining and immunohistochemistry were performed on 5 µm sections of formalin-fixed paraffin-embedded scaffolds on days 7 and 14 after cell seeding. Briefly, sections were dewaxed in xylene (Merck KGaA, Darmstadt, Germany), rehydrated in descending grades of ethanol, and permeabilized with 0.2% Triton X-100 (Sigma-Aldrich Corp., St. Louis, MO, USA). To block non-specific binding sites, 1% normal horse serum was added to sections for 30 minutes. Primary antibodies against urothelial and muscular markers were diluted in blocking buffer (1:100) and incubated at 4°C for overnight. Biotinylated universal secondary antibody (VECTASTAIN Universal Elite ABC Kits; Vector Laboratories, Burlingame, CA, USA) was applied for 30 minutes. Biotinylated horseradish peroxidase was added afterward and the expression sites were revealed by DAB chromogene (Vector Laboratories, Burlingame, CA, USA). Finally, slides were counterstained in Hematoxylin, mounted with Entellan (Merck KGaA, Darmstadt, Germany), and observed using a light microscope (BX41, Olympus, Shinjuku, Japan).

Scanning Electron Microscopy (SEM) of Scaffolds

Scaffolds and hAM were fixed in 2.5% glutaraldehyde (Merck KGaA, Darmstadt, Germany) for 24 hours followed by two consecutive washes in PBS for 20 minutes. Samples were dehydrated in ascending concentrations of ethanol, and then, air-dried for 24 hours. They were mounted on stubs, coated with gold-palladium, and examined by SEM (Tescan Vega-II, Las Vegas, Nevada, 89118, USA). Bare scaffolds were analyzed without any processing.

MTT Assay

At each time point (1, 3, 7, 14 and 21 days after cell seeding), 1 mL of 0.5 mg/mL MTT solution (Sigma-Aldrich Corp., St. Louis, MO, USA) was added to each scaffold for 3 hours at 37°C and 5% CO2 in the dark. After formation of formazan during reduction process, isopropanol containing 0.01 N HCl (Merck KGaA, Darmstadt, Germany) was added for 30 minutes at 37°C in the dark with moderate agitation to disrupt the cells and solubilize formazan. Absorbance at 560 nm (BioPhotometer, Eppendorf AG, Hamburg, Germany) was measured as an indicator of live cells, which were contributed to the reduction of MTT.

Statistical Analysis

Data were exhibited as the mean ± standard error. ANOVAs statistical analysis was used to evaluate cell viability during 14 days of cultivation on matrices using SPSS software (the Statistical Package for the Social Sciences, Version 18.0, SPSS Inc, Chicago, Illinois, USA). *P* values less than .05 were considered statistically significant.

RESULTS

Structure and Chemical Features of Scaffolds

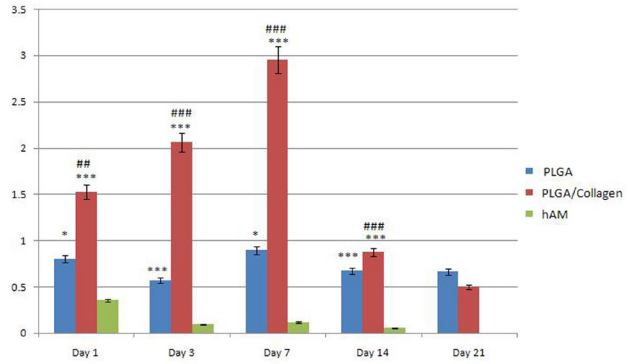

Figure 6. MTT assay for urothelial cell viability after culture on scaffolds and hAM; (a) human urothelial cells showed more significant increase for MTT signal on PLGA/collagen opposed to PLGA and hAM. Asterisks represent comparisons between PLGA or PLGA/collagen with hAM, while # stands for PLGA vs. PLGA/collagen comparisons.
Keys: PLGA, poly lactic-co-glycolic acid; hAm, human amniotic membrane; MTT, 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide.
* $P < .05$, ** $P < .01$, *** $P < .001$.

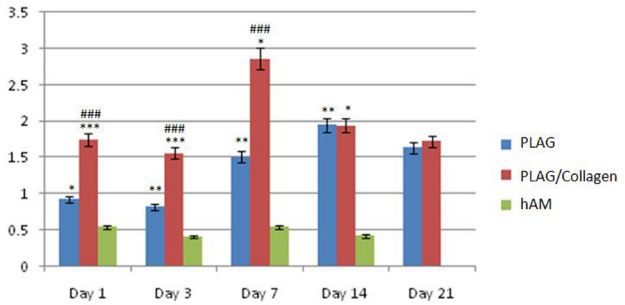

Figure 7. MTT assay for bladder smooth muscle cell viability after culture on scaffolds and hAM; (a) human bladder smooth muscle cells showed more significant increase for MTT signal on PLGA/collagen opposed to PLGA and hAM. Asterisks represent comparisons between PLGA or PLGA/collagen with hAM, while # stands for PLGA vs. PLGA/collagen comparisons.
Keys: PLGA, poly lactic-co-glycolic acid; hAm, human amniotic membrane; MTT, 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide.
* $P < .05$, ** $P < .01$, *** $P < .001$.

According to the SEM micrographs, it was observed that the mats had a partial alignment, and no significant changes were detected in their structures after being coated with collagen. But fiber diameters showed a little enhancement (569.91 ± 228.78 nm vs. 484.85 ± 270.48 nm, $P = .43$), which may be as a result of fiber swelling or addition of collagen coating (Figure 1).

Human Bladder Smooth and Urothelial Cells

Small colonies of urothelial cells appeared 24 hours after initial plating and proliferated to form large colonies, which contained cubical cells with epithelial features after 4 days of culture (Figure 2a). Immunocytochemical studies showed the expression of uroplakin III and pancytokeratin in cultured urothelium (Figures 2b and 2c). Urothelial cells were passaged after 70% confluency. Cultured smooth muscle cells were distinguished with their elongated morphology and strong expression of α-SMA as well as moderate expression of Desmin (Figures 2d to 2f).

Cell Layering and Histological Characterizations of Scaffold

Cell-scaffold interaction is much looser than the native tissue; thus, the invasive process of sectioning and staining leads to cell shedding. Despite cell detachment, a well-developed and continuous layer of cells was observed in most microscopic fields in PLGA and PLGA/collagen (Figure 3), but no cell remained on hAM surface (data not shown). No significant difference was observed between days 7 and 14. Because of cell detachment, a meaningful comparison of PLGA and PLGA/collagen regarding layering was not possible. We examined the cells after seeding on scaffolds for expression of markers. Expression of pancytokeratin and uroplakin III was continued in urothelial cells (Figures 4a and 4b) while smooth muscle cells maintained the expression of desmin and α-SMA on scaffolds surface (Figures 4c and 4d).

Distribution and Morphology of the Cells on Scaffolds

Scanning electron microscopy is a valuable method for monitoring cell morphology and distribution on electrospun scaffolds in comparison with light microscopy. Five days after seeding, urothelial cells showed expansion on PLGA and PLGA/Collagen beside regular appearance for colonies (Figure 5a). Smooth muscle cells also proliferated efficiently and spread all over the surface (Figure 5b). No obvious difference was recognized between PLGA and PLGA/Collagen in each cell type. On the other hand, cells on hAM, as expected from immunohistochemistry results, showed very low density, and dispersing colonies of urothelial cells were noticed after 3 days of culture, confirming unfavorable attributes of hAM for prolonged cell culture (Figure 5c). Smooth muscle cells were also rarely observed on hAM (Figure 5d).

Viability of Cells on Scaffolds

MTT assay is a convincing way to compare the capabilities of scaffolds related to initial cell attachment and proliferation. MTT assay provides indirect information regarding cell proliferation. In supportive scaffolds, before contact inhibition due to limited surface of scaffolds, cells proliferate to a summit. In the present study, cells on supportive scaffolds (PLGA and PLGA/collagen) showed a similar pattern. Collagen was used to include cell recognition site on PLGA and promote cell adhesion and proliferation. Initial cell attachment (the first day post seeding), cell viability and proliferation increased significantly in PLGA/collagen scaffolds compared to PLGA for urothelial ($P < .01$ for day 1 and $P < .001$ for days 3, 7 and 14) and smooth muscle cells ($P < .001$ for days 1, 3 and 7) (Figures 6 and 7). hAM was not as supportive as PLGA and PLGA/collagen; hence, MTT absorbance of both cells significantly increased on synthetic scaffolds versus hAM (Figures 6 and 7).

DISCUSSION

Tissue engineering has emerged due to incorporation of material science, engineering and cell biology and achieved increasing importance in regenerative strategies over the last decade. Scaffolds and cell sources are two essential elements of tissue engineering. Supporting matrices, scaffolds, in tissue engineering should simply incorporate with host tissue and provide favorable signals for cells to adhere, proliferate, differentiate and resume their normal function. Fabrication of scaffolds by electrospinning method has recently received growing attention in tissue engineering. Fibrous nature of electrospun scaffolds can imitate ECM ultra structure and their non-woven format in addition to high surface/volume ratio and porosity, play significant role in distribution of nutrients, cell migration, attachment and proliferation.[22,23] Proper capacity of electrospun scaffolds has received support from the results of numerous tissue engineering studies of bone, cartilage, blood vessels.[24,25] Our findings showed significant effect of collagen for improvement of cell attachment, followed by increased number of cells after cultivation. Cultured urothelial cells revealed their typical appearance and were positive for expression of uroplakin III and pancytokeratin and remained proliferative. Similar to our results, some studies have revealed the expression of Uroplakin III in the primary culture of urothelium.[26,27] Conversely, Southgate and colleagues reported that uroplakin III could not be detected in the primary culture of urothelial cells.[28] Differences in culture conditions, species, method of detection and its sensitivity may lead to the conflicting results. Preserving normal phenotype of cells is a key prerequisite of scaffolds in tissue engineering. In the current study, both cell types on PLGA and PLGA/collagen preserved the expression of their pertinent markers after 7 and 14 days of culture. Consistent with the expression of markers, SEM observations demonstrated normal appearance for colonies of urothelium and monolayer of smooth muscle cells on PLGA and PLGA/collagen which could be continued up to confluency ending to a continuous layer (data not shown) which is critical for urothelium function.[29] MTT assay has been frequently used for assessment of cytotoxic effects of scaffolds.[30] Herein, it was demonstrated that coating with collagen can enhance initial cell plating on PLGA which lead to significant increase in cell number during 14 days of culture as revealed by MTT assay. On the other hand, cell attachment on hAM after 1 day was significantly lower than PLGA/collagen and PLGA. Cells did not grow on hAM over the time and MTT signal gradually declined. Despite of formation of a continuous cell layer on PLGA and PLGA/collagen, invasive process of sectioning detached the superficial cells from scaffold surface and limited more detailed analysis of layering. PLGA with or without coating by collagen not only elicited any cytotoxic effect on cells but also supported their proliferation, layering and colonization.

Current knowledge of nanotechnology proposed new methods to fabricate scaffolds which imitate ECM structure with their nano-size dimensions. Diameter of fibers in our work was about 500 nm which could be efficient for cell deposition and slow diffusion of nutrients, and growth factors.[31] Nano-scaffolds could provide more suitable environment for cells owing to superior adsorption of ECM proteins, such as fibronectin.[32] Additionally, it was reported that nanometer surface features could hamper calcium oxalate stone formation.[33] Regarding bladder engineering technologies, nanoscale PLGA, PCL and polyether urethane (PU) were more supportive matrices compared to micron ones.[34,35]

Implantation of cell-seeded scaffolds is an important issue of tissue engineering which incorporate cell based therapy and scaffolding; so investigation to find appropriate scaffolds for

cell cultivation is an essential issue. In this regard, superior regenerative properties of scaffolds seeded with cells have been reported.[36] PLGA was selected owing to its previously reported exploit for reconstruction of different organs especially bladder;[37,38] nevertheless, preparation methods and properties were primarily different form current study. On the other hand, hAM is intrinsically a collagenous membrane with promising reports for tissue engineering.[10-12] We previously showed that hAM could be a superior supportive matrix compared to peritoneum and omentum membranes.[39] So, it was assumed that hAM can be a proper matrix for cell adhesion and cultivation. Moreover, it was showed that denuded hAM was a better matrix than intact hAM.[40] In our study, initial adherence of cells on hAM was satisfactory but cells did not proliferate further and their morphology was quite different from PLGA and culture plates. This phenomenon could be addressed to low porosity of hAM and its mechanical features. On the contrary, PLGA showed encouraging results for cells growth and collagen, ,as it was predicted, significantly improved cell adhesion.

CONCLUSION

PLGA is a suitable scaffold for human bladder regeneration regarding cell attachment, growth and preserving normal phenotype of cells. In addition, collagen can improve supportive nature of PLGA. hAM despite the natural origin and favorable composition may not be useful in this framework without more modification.

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CONFLICT OF INTEREST

None declared.

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