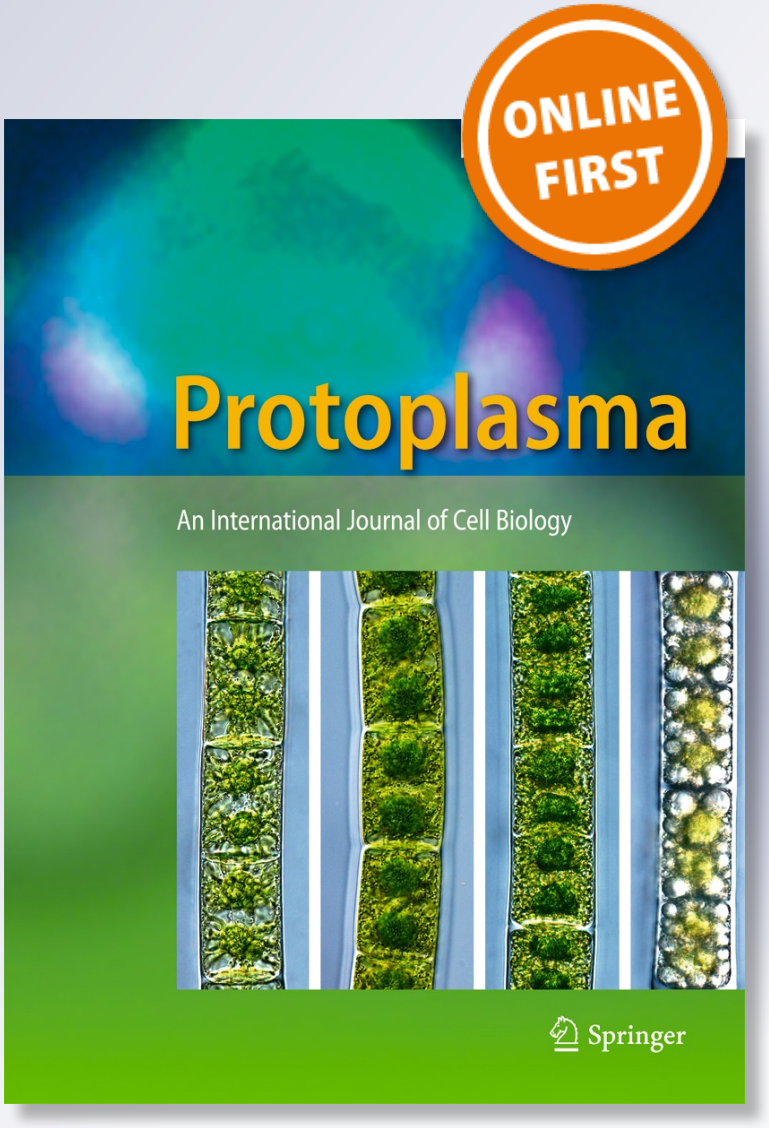
Biocompatibility of different nanostructured TiO_2 scaffolds and their potential for urologic applications

Roghayeh Imani, Meysam Pazoki, Daša Zupančič, Mateja Erdani Kreft, Veronika Kralj-Iglič, Peter Veranič & Aleš Iglič

Protoplasma
An International Journal of Cell Biology

ISSN 0033-183X

Protoplasma
DOI 10.1007/s00709-015-0896-0



Springer

Protoplasma
DOI 10.1007/s00709-015-0896-0

ORIGINAL ARTICLE

Biocompatibility of different nanostructured TiO_2 scaffolds and their potential for urologic applications

Roghayeh Imani[1,2] · Meysam Pazoki[3] · Daša Zupančič[4] · Mateja Erdani Kreft[4] · Veronika Kralj-Iglič[2] · Peter Veranič[4] · Aleš Iglič[1]

Received: 10 June 2015 / Accepted: 6 October 2015

Abstract Despite great efforts in tissue engineering of the ureter, urinary bladder, and urethra, further research is needed in order to improve the patient's quality of life and minimize the economic burden of different lower urinary tract disorders. The nanostructured titanium dioxide (TiO_2) scaffolds have a wide range of clinical applications and are already widely used in orthopedic or dental medicine. The current study was conducted to synthesize TiO_2 nanotubes by the anodization method and TiO_2 nanowires and nanospheres by the chemical vapor deposition method. These scaffolds were characterized with scanning electron microscopy (SEM) and X-ray diffraction (XRD) methods. In order to test the urologic applicability of generated TiO_2 scaffolds, we seeded the normal porcine urothelial (NPU) cells on TiO_2 nanotubes, TiO_2 nanowires, TiO_2 nanospheres, and on the standard porous membrane. The viability and growth of the cells were monitored everyday, and after 3 weeks of culturing, the analysis with scanning electron microscope (SEM) was performed. Our results showed that the NPU cells were attached on all scaffolds; they were viable and formed a multilayered epithelium, i.e., urothelium. The apical plasma membrane of the majority of superficial NPU cells, grown on all three different TiO_2 scaffolds and on the porous membrane, exhibited microvilli; thus, indicating that they were at a similar differentiation stage. The maximal caliper diameter measurements of superficial NPU cells revealed significant alterations, with the largest cells being observed on nanowires and the smallest ones on the porous membrane. Our findings indicate that different nanostructured TiO_2 scaffolds, especially nanowires, have a great potential for tissue engineering and should be further investigated for various urologic applications.

Keywords Nanostructured TiO_2 scaffolds · Anodization · Chemical vapor deposition · Normal porcine urothelial cells · Urologic application

Handling Editor: Christos D. Katsetos

✉ Aleš Iglič
ales.iglic@fe.uni-lj.si

[1] Laboratory of Biophysics, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, Ljubljana, Slovenia

[2] Laboratory of Clinical Biophysics, Faculty of Health Sciences, University of Ljubljana, Zdravstvena 5, Ljubljana, Slovenia

[3] Department of Chemistry, Ångström Laboratory, Physical Chemistry, Uppsala University, Box 523, SE 75120 Uppsala, Sweden

[4] Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, Ljubljana, Slovenia

Introduction

Titanium dioxide (TiO_2) is one of the most significant biocompatible materials for medical applications due to its non-toxicity, great tensile strength, flexibility, high corrosion, and resistance to body fluid effects; therefore, it is not rejected by the body (Williams 2008). Meanwhile, the importance of nanoscale surface topography and roughness of biomaterials is, along with chemical surface modifications, gradually becoming acknowledged as a crucial factor for cell attachment, survival, proliferation, and differentiation as well as for tissue acceptance (Wagner, Dullaart et al. 2006; Gongadze, Kabaso et al. 2011). Indeed, it was demonstrated that nanostructured titanium oxide represents an even better biomimetic material than flat TiO_2 (Gongadze, Kabaso et al. 2011). This was confirmed by various experiments conducted with different cell types, e.g., osteoblasts (Gongadze, Kabaso et al.

Published online: 24 October 2015

2011), mesenchymal cells (Park, Bauer et al. 2007), fibroblasts (Carbone, Marangi et al. 2006), endothelial cells (Park, Bauer et al. 2009), osteosarcoma cells (Carbone, Marangi et al. 2006), and recently also with urothelial cells, which showed improved cell adhesion and growth (Alpaslan, Ercan et al. 2011) on nanostructured titanium oxide even without coating with extracellular matrix proteins.

Recently, an increasing number of in vitro studies have exploited an inexpensive and highly reproducible electrochemical anodization method for obtaining a self-assembled layer of vertically oriented TiO_2 nanotubes with a diameter between 15 and 100 nm (Alpaslan, Ercan et al. 2011; Imani, Kabaso et al. 2012; Lee, Mazare et al. 2014). Moreover, other methods are emerging, e.g., chemical vapor deposition (CVD), which yield different nanostructured TiO_2 scaffolds (Liu, Zhang et al. 2012; Selvaraj, Jursich et al. 2013) and urge to be tested for medical applicability. Many diverse pathologies affect the lower urinary tract covered by urothelial cells from the ureter to bladder and urethra, including compromised ureter due to kidney stones or tumor (Mardis and Kroeger 1988; Romih, Korosec et al. 2005), injured ureters in abdominopelvic surgeries (Al-Awadi, Kehinde et al. 2005), bladder and urethral dysfunction, congenital anomalies, trauma and malignancies (Atala 1999; Southgate, Cross et al. 2003; McAninch 2005). These conditions have a high incidence and a long-term impact on the patient's quality of life as well as on the economy of health systems all over the world. Until now, some successes were documented regarding reconstructive urologic medicine (Jerman, Kreft et al. 2015), namely promising research in the field of the ureter reconstruction (Fu, Xu et al. 2012), attempts towards the implantation of the tissue-engineered bladder (Fraser, Thomas et al. 2004; Atala, Bauer et al. 2006; Subramaniam, Turner et al. 2012), and also the urethra (Raya-Rivera, Esquiliano et al. 2011). Nevertheless, many contradictory results in basic research and in preclinical and clinical trials demand further searching for improved technologies and constructs, which would have a potential to benefit patients. Besides, it has not yet been determined what kind of scaffold might best support the urothelial growth without prolonged regeneration and even extensive fibrous tissue overgrowth (Vance, Miller et al. 2004).

Titanium-based materials are widely used in a broad range of clinical applications, especially for bone implants in orthopedic or dental medicine. Many in vitro studies were performed with osteoblasts and osteoblast-like cells, indicating that even small variations of titanium surface topography and roughness affect different cell reactions, e.g., cell attachment, proliferation, and differentiation (Zhu, Chen et al. 2004; Luthen, Lange et al. 2005; Gongadze, Kabaso et al. 2011; Pazoki et al. 2012). In 2011, the first paper was published proposing anodized TiO_2 nanotubes as a novel scaffold for the ureter stents, but only a short-term culturing was tested (Alpaslan, Ercan et al. 2011). The establishment of a normal urothelium with differentiated superficial urothelial cells, named umbrella cells, is a process that takes in in vitro conditions 1 to 3 weeks (Kreft, Sterle et al. 2005; Kreft, Sterle et al. 2006; Visnjar and Kreft 2013; Visnjar and Kreft 2015). Our goal in the present study was to investigate the long-term culturing of normal urothelial cells on nanostructured TiO_2 scaffolds with different topography. TiO_2 scaffolds were synthesized with the anodization method and chemical vapor deposition (CVD). With these different methods, we prepared not only the nanotubes but also the nanowires and the nanospheres, which could have a wide range of urologic applications, including a novel scaffold design for the ureter stent as well as proximal urethra tissue engineering. The NPU cells were attached, viable and proliferative, and formed a urothelium with tightly bound superficial cells after a 3-week culturing on all three nanostructured TiO_2 scaffolds tested here. Moreover, our results showed some differences in the sizes of the superficial urothelial cells, while the majority of them exhibited microvilli on their apical surface in all the experiments. We believe that different TiO_2 scaffolds are very promising and should be further explored as stents for urologic applications.

Materials and methods

Synthesis of TiO_2 nanowires and nanospheres

The synthesis of TiO_2 nanowires and nanospheres was performed according to the chemical vapor deposition (CVD) method reported previously by the in-house built CVD reactor (Pazoki et al. 2012). The CVD reactor consisted of a horizontal glass tube reactor with a 5 cm diameter and 30 cm long reactor. $TiCl_4$ was used as the titanium precursor, while oxygen gas and water were used as oxygen sources. Glass substrates were cleaned by sonication in dilute nitric acid, acetone, and deionized water, and placed in the reactor in two zones: zone A (for the synthesis of nanowires) and zone B (for the synthesis of nanospheres). The standard working temperature in the center of the reactor was 250 °C. The measured temperatures in zone A and in zone B were 230 and 210 °C, respectively. Water vapor and $TiCl_4$ were introduced into the reactor by bubbling Ar and N_2 as carrier gasses, kept in respective bubblers at 20 °C, as well as an independent flow of O_2. The standard flow rates for Ar (H_2O), N_2 ($TiCl_4$), and O_2 were 30, 560 and 560 sccm, respectively.

Synthesis of TiO_2 nanotubes

TiO_2 nanotubes were synthesized according to the electrochemical anodization method (Imani and others 2012), described previously (Imani, Kabaso et al. 2012). In short, titanium foils were degreased and TiO_2 nanotubes were grown by the electrochemical anodization of these foils (thickness 0.25 mm; purity 99.5 %; Sigma-Aldrich, St. Louis, MO, USA) in a two-electrode electrochemical cell at room temperature. A DC power supply was used as the voltage source, and the titanium foil served as anode and platinum foil as cathode. The titanium foils were biased at 60 V for 1 h to grow a nanotubular TiO_2 surfaces (TiO_2 nanotubes), which were thereafter rinsed with deionized water and dried in air. Before they were used as scaffolds for NPU cells, they were sterilized in 70 % ethanol for 5 min and dried in air.

Characterization of different nanostructured TiO_2 scaffolds

The surface topography of synthesized nanostructured TiO_2 scaffolds was observed with a Hitachi S4700 scanning electron microscope (SEM, Hitachi-S4160). The crystal structure properties of the TiO_2 scaffolds (after annealing at 450 °C for 1 h) were obtained from hard X-ray low-angle 1 reflectivity measurements, using a Philips PW1710 powder diffractometer with a copper anode source (Cu-K alpha, lambda=1.54 Å), operating at 0.8 kW and with an accuracy of 0.015° 2-theta.

Urothelial cell cultures

The experiments were approved by the Veterinary Administration of the Slovenian Ministry of Agriculture and Forestry (permit no. 34401-1/2010/6) in compliance with the Animal Health Protection Act and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes. The porcine urinary bladders were obtained from a local abattoir. Primary urothelial cell cultures were prepared as described previously in the UroM medium (Kreft, Sterle et al. 2005; Kreft, Di Giandomenico et al. 2010; Visnjar, Kocbek et al. 2012; Visnjar and Kreft 2015). The UroM medium consisted of equal parts of MCDB153 medium (Sigma-Aldrich, Taufkirchen, Germany) and advanced Dulbecco's modified essential medium (Invitrogen, Life technologies, Austria), supplemented with 0.1 mM phosphoethanolamine (Sigma-Aldrich), 15 μg/ml adenine (Sigma-Aldrich), 0.5 μg/ml hydrocortisone (Sigma-Aldrich), 5 μg/ml insulin (Sigma-Aldrich), 4 mM glutamax (Gibco), 100 μg/ml streptomycin, and 100 U/ml penicillin. Normal porcine urothelial cells (NPU cells) were propagated and sub-cultured in the UroM medium with 0.9 mM extracellular Ca^{2+} concentration and 2.5 % final concentration of fetal bovine serum (UroM ($-Ca^{2+}$ + S_{FBS})). When the NPU cells of the VI passage reached the confluence, they were incubated in TrypLE Select (Gibco) at 37 °C for 5 min, resuspended in the UroM ($-Ca^{2+}$+ S_{FBS}) medium, centrifuged at 200*g* for 5 min, and plated at seeding density of 1×10^5 cells/cm^2 on the three different nanostructured TiO_2 scaffolds and on the 0.4-mm porous membrane (synthetic polyethylene terephthalate (PET) membranes, BD Falcon, Bedford, USA). All four urothelial models were maintained for 3 weeks at 37 °C in a humidified atmosphere of 5 % CO_2 in the air. The growth of NPU cells was monitored by inverted phase-contrast microscope (Leica) everyday, and the UroM ($-Ca^{2+}$ + S_{FBS}) medium was replaced every other day.

Scanning electron microscopy

Three-week NPU cell cultures were prepared for scanning electron microscopy (SEM) as previously described (Visnjar, Kocbek et al. 2012; Visnjar and Kreft 2013). Briefly, the NPU cells together with different scaffolds were fixed in 4 % formaldehyde and 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at 4 °C for 3 h, and rinsed in 0.1 M cacodylate buffer, pH 7.4, at 4 °C overnight. After dehydration, the samples were dried at the critical point, spattered with gold, and examined with a Jeol JSM 840A scanning electron microscope.

Statistical analysis

We measured the maximal caliper diameter of randomly sampled NPU cells imaged by SEM and grown on TiO_2 nanowires (n=20), TiO_2 nanotubes (n=20), TiO_2 nanospheres (n=20), and PM (n=20). The average and the standard errors were calculated for each scaffold and PM. All data were analyzed using the standard analysis of variance (ANOVA), followed by a two-tailed Student's t test; p values <0.05 were considered statistically significant.

Results

The surface topography of the nanostructured TiO_2 scaffolds synthesized in the zone A (230 °C) of the CVD reactor as observed by SEM (Fig. 1a) was homogenous throughout the sample and resembled the wires. Therefore, we named these scaffolds nanowires. The diameter of each nanowire was approximately 200 nm. In the zone B (210 °C) of the CVD reactor, spherical structures were synthesized (Fig. 1e), and we named these structures nanospheres. Similarly as nanowires, the average diameter of nanospheres was around 200 nm. The TiO_2 nanotubes

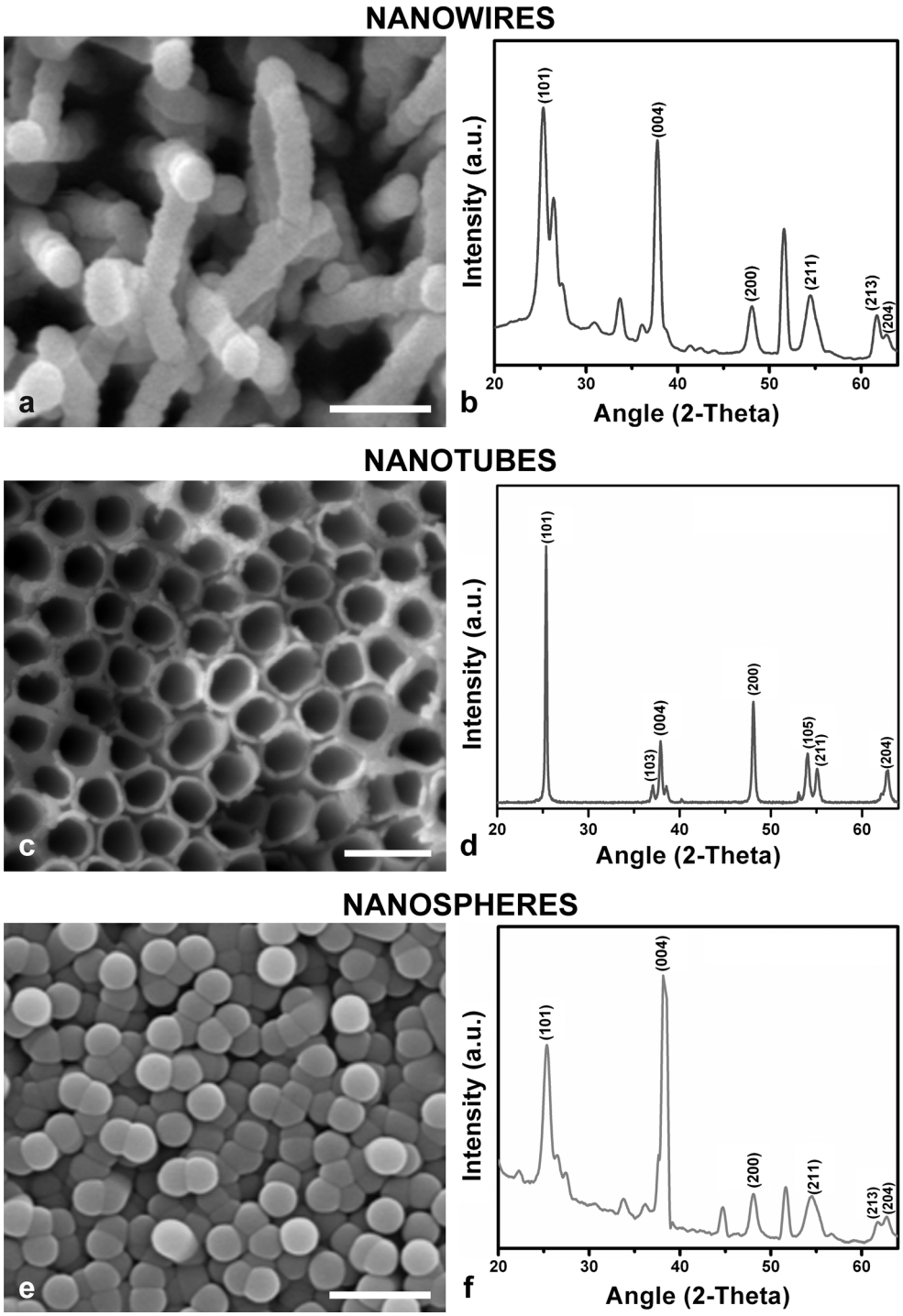


Fig. 1 Characterization of TiO_2 nanowires (**a**, **b**), TiO_2 nanotubes (**c**, **d**) and TiO_2 nanospheres (**e**, **f**). SEM micrographs show typical surface topography of different TiO_2 scaffolds (**a**, **c**, **e**) and XRD patterns showing their crystal structure properties are represented in graphs (**b**, **d**, **e**). *Scale bars*: **a** and **e** 500 nm, **c** 200 nm

(Fig. 1c) synthesized by the anodization method were similar as reported previously (Imani, Kabaso et al. 2012). The internal nanotube diameter was around 50–100 nm, while the length was up to 10 μm. The walls of nanotubes were smooth and around 20-nm thick.

The XRD patterns of all the three different nanostructured TiO_2 scaffolds confirm that after annealing at 450 °C, all of them are in the anatase phase. All the peaks observed at $2\theta = 25.3$, 36.9, 38.5, 48, 53.8, 55, 61.5, and 62.7 in the XRD patterns are consistent with anatase (101), (103), (112), (200), (105), (211), (213), (204) planes (JCPDS number: 21–1272) (Fig. 1b, d, f).

The normal porcine urothelial (NPU) cells grown for 3 weeks on PM were viable, proliferative, polygonally

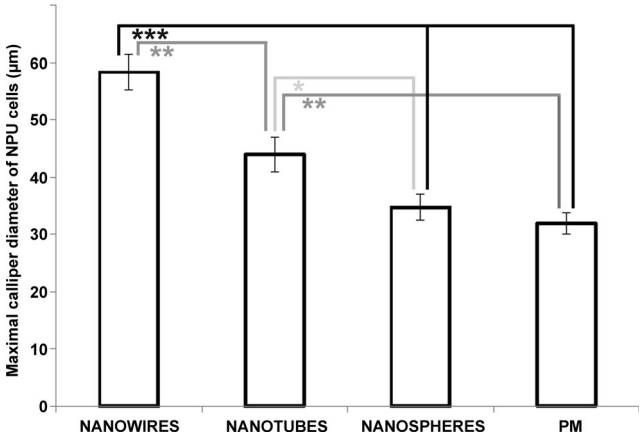


Fig. 2 The mean maximal caliper diameters (±SE) of superficial NPU cells cultured for 3 weeks on different nanostructured TiO_2 scaffolds (nanowires, nanotubes, nanospheres) and on the porous membrane (PM). There are statistically significant differences between the pairwise comparisons of the grouped data, i.e., nanowires vs nanotubes (**$p<0.005$), nanospheres (***$p<0.001$) and porous membrane (***$p<0.001$), and nanotubes vs nanospheres (*$p<0.05$) and porous membrane (**$p<0.005$)

shaped, tightly attached to each other, and reached 100 % confluence as previously described in details (Visnjar and Kreft 2013). Similar characteristics were observed also for the NPU cells grown for 3 weeks on all the three different nanostructured TiO_2 scaffolds. Nevertheless, the maximal caliper diameter of superficial NPU cells differed significantly (Fig. 2). The largest caliper diameter being 58.1±3.1 μm was observed on nanowires, while the smallest was found to be on the porous membrane, with maximal caliper diameter being 31.9±1.8 μm. The cells grown on nanotubes had a significantly larger diameter than the cells grown on nanospheres and on the porous membrane, whereas, there was no statistically significant difference between the cells grown on nanotubes and the cells grown on nanospheres. Furthermore, the cells grown on nanospheres were similar to the cells grown on the porous membrane regarding the maximal caliper diameter.

The SEM analysis of the NPU cell apical membrane surface topography revealed that all the cells were interconnected by prominent cell borders (Fig. 3). Besides that, the majority of the superficially positioned cells grown on all the three different nanostructured TiO_2 scaffolds and on the porous membrane exhibited microvilli on their apical surface (Fig. 3), indicating that they were at a similar differentiation stage. Some of the cells grown on nanowires (Fig. 3a) and the porous membrane (Fig. 3d) also showed ropy ridges, which are typical markers of successive differentiation following microvilli (Kreft, Romih et al. 2002; Veranic, Romih et al. 2004; Visnjar, Kocbek et al. 2012). However, some cells grown on nanotubes and nanospheres exhibited unusual ridges, which we named pleomorphic ridges (Fig. 3b, d). A detailed observation of the shape of both types of ridges indicated that they seem to be composed of interconnected microvilli, but these interconnections differed between ropy and polymorphic ridges. In ropy ridges, microvilli seemed to be aligned in a thick row and the interconnections between them were of the same thickness as microvilli (Fig. 4a, d), which is in agreement with the previously published data (Kreft, Romih et al. 2002; Kreft, Sterle et al. 2005; Visnjar, Kocbek et al. 2012). However, in the pleomorphic ridges, the interconnections between microvilli were thinner than the microvilli and therefore no row-like but rather network-like pattern was observed (Fig. 4b, c). Moreover, within a network of thin pleomorphic ridges individual microvilli were observed.

Discussion

Despite numerous studies conducted over the last 3 decades in the field of tissue engineering of the ureter, bladder, and urethra (Southgate, Cross et al. 2003; Atala 2006; Raya-Rivera, Esquiliano et al. 2011; Yoo, Olson et al. 2011; Fu, Xu et al. 2012), the nanostructured TiO_2 scaffolds have only recently been proposed as promising scaffolds for urothelial tissue (Alpaslan, Ercan et al. 2011). Since a different topography of titanium surface is of fundamental importance for the cell's survival, attachment, proliferation, and differentiation (Wagner, Dullaart et al. 2006; Gongadze, Kabaso et al. 2011), we synthesized the three different nanostructured TiO_2 scaffolds (e.g., nanotubes, nanowires, and nanospheres) and tested their biocompatibility with normal urothelial cells. The normal porcine urothelial cells plated on these TiO_2 scaffolds adhered and grew with similar characteristics as the cells grown on the porous membrane. Nevertheless, some variations regarding their diameter and apical surface morphology were observed. Our results suggest that the nanostructured TiO_2 scaffolds should be further explored for urologic applicability.

Numerous in vitro studies exploited the TiO_2 nanotubes with diameters ranking between 15 and 100 nm. The majority of those studies indicated that small diameter (15 nm) nanotubes provided stronger adhesion (Imani, Kabaso et al. 2012), growth, and differentiation of different cell types (Park, Bauer et al. 2007; Park, Bauer et al. 2009; Alpaslan, Ercan et al. 2011; Gongadze, Kabaso et al. 2011). Nevertheless, our results showed that normal urothelial cells attached and grew on 50–100 nm TiO_2 nanotubes as efficiently as on the synthetic PET porous membrane. Moreover, superficial urothelial cells reached a significantly larger diameter when grown on the TiO_2 nanotubes rather than on the porous membrane. Since superficial urothelial cells with their specific apical

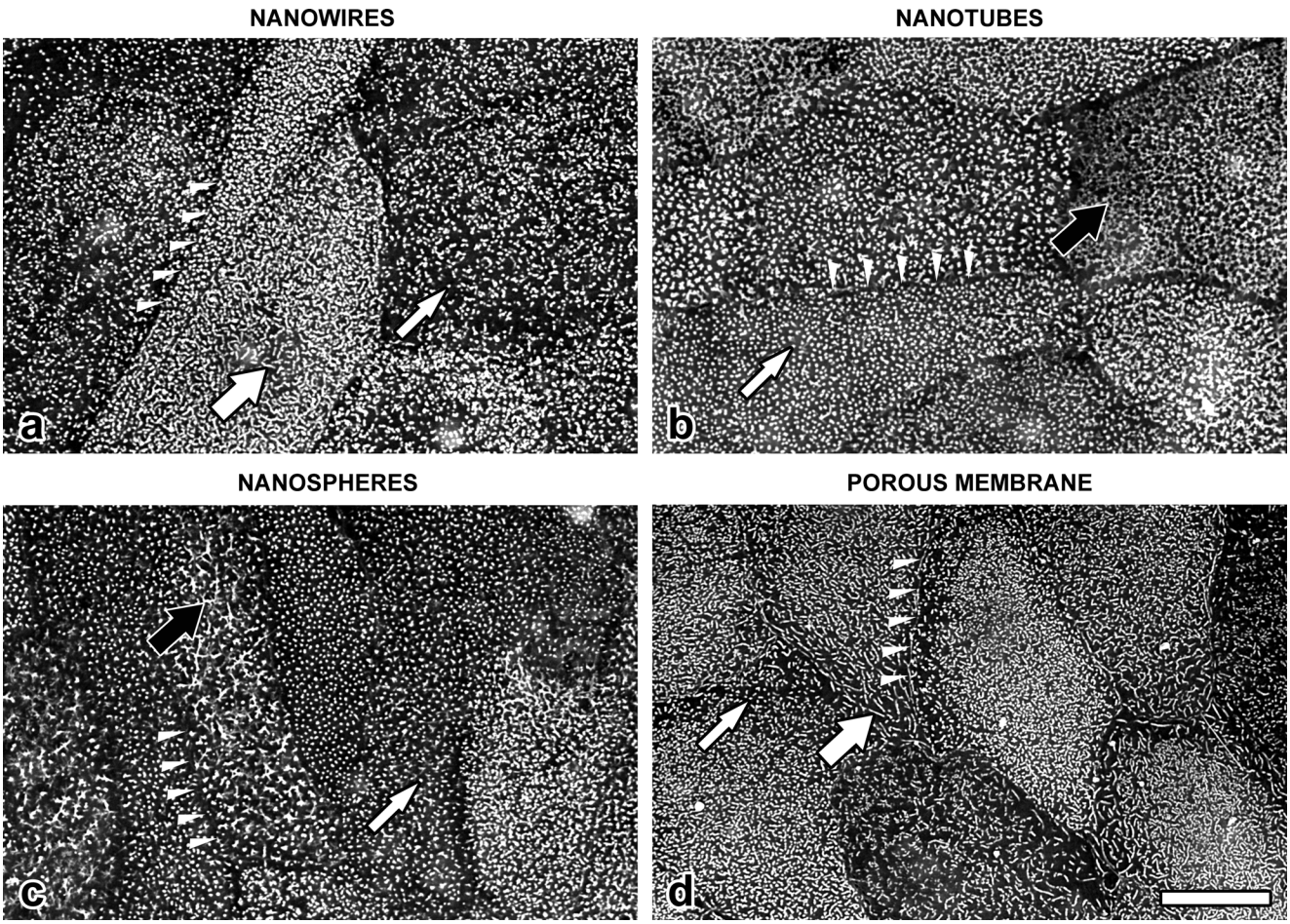


Fig. 3 Normal porcine urothelial (NPU) cells after 3 weeks culturing on the TiO_2 nanowires (**a**), TiO_2 nanotubes (**b**), TiO_2 nanospheres (**c**) and on the porous membrane (**d**) imaged by SEM. The overview of the apical plasma membrane showing the cell borders between superficial NPU cells (*arrowheads*). SEM additionally shows that superficial NPU cells have an apical surface mostly shaped in microvilli (*thin white arrows*). The apical surface of NPU cells grown on nanowires and porous membrane also exhibits ropy ridges with thick interconnections between microvilli (*thick white arrows* in a and d), while the apical surface of NPU cells grown on nanotubes and nanospheres displays pleomorphic ridges (*thick black arrows* in b and c). *Scale bar*: 10 μm

plasma membrane structure called urothelial plaques (Kreft and Robenek 2012; Zupancic, Romih et al. 2014) contribute to the blood-urine barrier formation and maintenance, their larger surface could contribute to better functionality of the urothelium. As we have shown previously, the stretching of superficial urothelial cells is also one of the earliest cellular events during the urothelial superficial wound-healing process (Kreft, Sterle et al. 2005). Additionally, it was recently reported, that proteins bind more effectively to the large diameter (100 nm) than to the small diameter (15 or 50 nm) nanotubes (Kulkarni, Flasker et al. 2015). This should represent an advantage for future coating of the nanotubes with extracellular matrix proteins (e.g., collagen or fibronectin), which might have an even higher potential in regenerative medicine.

Although it seems that TiO_2 nanowires and nanospheres are cytotoxic to cells when they are introduced to cells as suspension and are therefore internalized (Hamilton, Wu et al. 2009; Park, Shim et al. 2013), our results showed that urothelial cells were viable and grew on the TiO_2 nanowires and nanospheres, prepared as scaffolds, as effectively as on the porous membrane, which is an optimal carrier of the urothelial cells cultured in vitro. Moreover, the structuration of the apical surface of the superficial urothelial cells grown on nanowires was similar to the differentiation stage of the cells grown on the porous membrane. Ropy ridges are known to be a characteristic of the superficial urothelial cells in the middle of the differentiation process in vivo (Romih and Jezernik 1996; Romih, Koprivec et al. 2001) and in vitro (Kreft, Romih et al. 2002; Kreft, Sterle et al. 2005; Visnjar, Kocbek et al. 2012; Zupančič, Romih et al. 2014). Therefore, we assume that the cells grown on nanowires have the highest potential to become terminally differentiated and form urothelial plaques. Surprisingly, the superficial urothelial cells

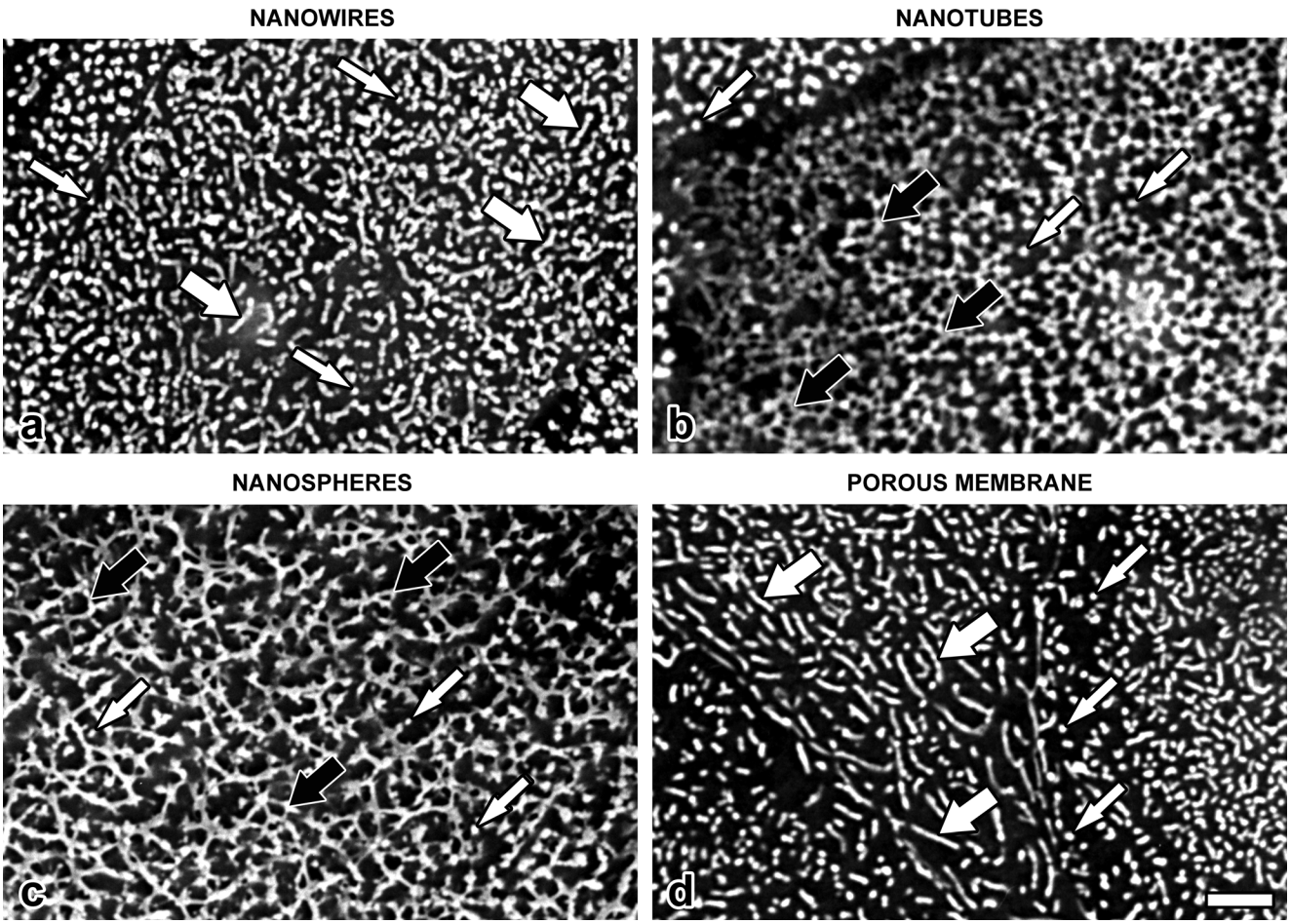


Fig. 4 Normal porcine urothelial (NPU) cells after 3 weeks culturing on the TiO_2 nanowires (**a**), TiO_2 nanotubes (**b**), TiO_2 nanospheres (**c**) and on the porous membrane (**d**) imaged by SEM. Individual microvilli (*thin white arrows*) are present on the apical surface of NPU cells grown on all three TiO_2 scaffolds and on porous membrane. NPU cells grown on nanowires and porous membrane also exhibit ropy ridges (*thick white arrows*), where microvilli seems to be aligned in a row. Nevertheless, the apical surface of NPU cells grown on nanotubes and nanospheres reveals a network of pleomorphic ridges (*thick black arrows*). *Scale bar*: 2 μm

grown on nanowires had the largest mean maximal caliper diameter, which was 1.7 times greater than the mean maximal caliper diameter of the cells grown on the porous membrane. This notion should be taken into consideration and further exploited in future studies.

The apical surface morphology of superficial urothelial cells was similar when cells were grown on all the three different nanostructured TiO_2 scaffolds and on the standard porous membrane. Moreover, the SEM analysis revealed undisturbed cell borders suggesting that urothelial cells formed functional epithelia sheets. The apical plasma membrane of the majority of the cells was shaped into microvilli, thus, confirming a similar differentiation stage of superficial urothelial cells grown on all the three different nanostructured TiO_2 scaffolds and on the standard porous membrane. Nevertheless, some cells grown on nanotubes and nanospheres also exhibited unusual ridges, which we named pleomorphic ridges. These structures could be a result of the aberrant differentiation pathways, but the underlying mechanisms are unknown.

In conclusion, we must point out that the nanostructured TiO_2 nanotubes, nanowires, and nanospheres represent promising scaffolds for normal urothelial cells and should be further studied in a greater detail. It is intriguing to wonder, why the largest superficial urothelial cells were grown on nanowires in the present study. Since promising results were already obtained with nanotubes for the ureter stent applications (Alpaslan, Ercan et al. 2011), nanowires should also be further investigated in this regard. Moreover, besides the nanoscale patterns of topography, additional chemical functionalization of the nanostructured titanium scaffolds should be exploited. Until now, collagen and fibrinogen coatings resulted in an improved urothelial regeneration (Eberli, Susaeta et al. 2007; McManus, Boland et al. 2007) and therefore seem to be the first choice for titanium scaffold optimization.

Acknowledgments We express gratitude to Sanja Čabraja, Nada Pavlica Dubarič, Linda Štrus, and Sabina Železnik for their technical assistance. This study was supported by a grant from the Slovenian Research Agency ARRS P2-0232 and P3-0108.

Compliance with ethical standards The experiments were approved by the Veterinary Administration of the Slovenian Ministry of Agriculture and Forestry (permit no. 34401-1/2010/6) in compliance with the Animal Health Protection Act and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Al-Awadi K, Kehinde EO et al (2005) Iatrogenic ureteric injuries: incidence, aetiological factors and the effect of early management on subsequent outcome. Int Urol Nephrol 37(2):235–241

Alpaslan E, Ercan B et al (2011) Anodized 20 nm diameter nanotubular titanium for improved bladder stent applications. Int J Nanomedicine 6:219–225

Atala A (1999) Future perspectives in reconstructive surgery using tissue engineering. Urol Clin N Am 26(1):157–165, **ix-x**

Atala A (2006) Recent applications of regenerative medicine to urologic structures and related tissues. Curr Opin Urol 16(4):305–309

Atala A, Bauer SB et al (2006) Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 367(9518):1241–1246

Carbone R, Marangi I et al (2006) Biocompatibility of cluster-assembled nanostructured TiO2 with primary and cancer cells. Biomaterials 27(17):3221–3229

Eberli D, Susaeta R et al (2007) Tunica repair with acellular bladder matrix maintains corporal tissue function. Int J Impot Res 19(6):602–609

Fraser M, Thomas DF et al (2004) A surgical model of composite cystoplasty with cultured urothelial cells: a controlled study of gross outcome and urothelial phenotype. BJU Int 93(4):609–616

Fu WJ, Xu YD et al (2012) New ureteral scaffold constructed with composite poly(L-lactic acid)-collagen and urothelial cells by new centrifugal seeding system. J Biomed Mater Res A 100(7):1725–1733

Gongadze E, Kabaso D et al (2011) Adhesion of osteoblasts to a nanorough titanium implant surface. Int J Nanomedicine 6:1801–1816

Hamilton RF, Wu N et al (2009) Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity. Part Fibre Toxicol 6:35

Imani R, Kabaso D et al (2012) Morphological alterations of T24 cells on flat and nanotubular TiO2 surfaces. Croat Med J 53(6):577–585

Jerman UD, ME Kreft et al. (2015) "Epithelial-Mesenchymal Interactions in Urinary Bladder and Small Intestine and How to Apply Them in Tissue Engineering." Tissue Eng Part B Rev

Kreft ME, Di Giandomenico D et al (2010) Golgi apparatus fragmentation as a mechanism responsible for uniform delivery of uroplakins to the apical plasma membrane of uroepithelial cells. Biol Cell 102(11):593–607

Kreft ME, Robenek H (2012) Freeze-fracture replica immunolabelling reveals urothelial plaques in cultured urothelial cells. PLoS ONE 7(6):e38509

Kreft ME, Romih R et al (2002) Antigenic and ultrastructural markers associated with urothelial cytodifferentiation in primary explant outgrowths of mouse bladder. Cell Biol Int 26(1):63–74

Kreft ME, Sterle M et al (2006) Distribution of junction- and differentiation-related proteins in urothelial cells at the leading edge of primary explant outgrowths. Histochem Cell Biol 125(5):475–485

Kreft ME, Sterle M et al (2005) Urothelial injuries and the early wound healing response: tight junctions and urothelial cytodifferentiation. Histochem Cell Biol 123(4–5):529–539

Kulkarni M, Flasker A et al (2015) Binding of plasma proteins to titanium dioxide nanotubes with different diameters. Int J Nanomedicine 10:1359–1373

Lee K, Mazare A et al (2014) One-dimensional titanium dioxide nanomaterials: nanotubes. Chem Rev 114(19):9385–9454

Liu H, Zhang Y et al (2012) Synthesis and characterization of TiO2@C core-shell nanowires and nanowalls via chemical vapor deposition for potential large-scale production. J Colloid Interface Sci 367(1):115–119

Luthen F, Lange R et al (2005) The influence of surface roughness of titanium on beta1- and beta3-integrin adhesion and the organization of fibronectin in human osteoblastic cells. Biomaterials 26(15):2423–2440

Mardis HK, Kroeger RM (1988) Ureteral stents. Materials. Urol Clin N Am 15(3):471–479

McAninch JW (2005) Urethral reconstruction: a continuing challenge. J Urol 173(1):7–7

McManus M, Boland E et al (2007) Electrospun nanofibre fibrinogen for urinary tract tissue reconstruction. Biomed Mater 2(4):257–262

Park EJ, Shim HW et al (2013) Comparison of toxicity between the different-type TiO(2) nanowires in vivo and in vitro. Arch Toxicol 87(7):1219–1230

Park J, Bauer S et al (2009) Narrow window in nanoscale dependent activation of endothelial cell growth and differentiation on TiO2 nanotube surfaces. Nano Lett 9(9):3157–3164

Park J, Bauer S et al (2007) Nanosize and vitality: TiO2 nanotube diameter directs cell fate. Nano Lett 7(6):1686–1691

Pazoki M, Taghavinia N et al (2012) CVD-grown TiO2 particles as light scattering structures in dye sensitized solar cells. Rsc Adv 2:12278–12285

Raya-Rivera A, Esquiliano DR et al (2011) Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. Lancet 377(9772):1175–1182

Romih R, Jezernik K (1996) Reorganisation of the urothelial luminal plasma membrane in the cyclophosphamide treated rats. Pflugers Arch 431(6 Suppl 2):R241–R242

Romih R, Koprivec D et al (2001) Restoration of the rat urothelium after cyclophosphamide treatment. Cell Biol Int 25(6):531–537

Romih R, Korosec P et al (2005) Differentiation of epithelial cells in the urinary tract. Cell Tissue Res 320(2):259–268

Selvaraj SK, Jursich G et al (2013) Design and implementation of a novel portable atomic layer deposition/chemical vapor deposition hybrid reactor. Rev Sci Instrum 84(9):095109

Southgate J, Cross W et al (2003) Bladder reconstruction—from cells to materials. Proc Inst Mech Eng H 217(4):311–316

Subramaniam R, Turner AM et al (2012) Seromuscular grafts for bladder reconstruction: extra-luminal demucosalisation of the bowel. Urology 80(5):1147–1150

Vance RJ, Miller DC et al (2004) Decreased fibroblast cell density on chemically degraded poly-lactic-co-glycolic acid, polyurethane, and polycaprolactone. Biomaterials 25(11):2095–2103

Veranic P, Romih R et al (2004) What determines differentiation of urothelial umbrella cells? Eur J Cell Biol 83(1):27–34

Visnjar T, Kocbek P et al (2012) Hyperplasia as a mechanism for rapid resealing urothelial injuries and maintaining high transepithelial resistance. Histochem Cell Biol 137(2):177–186

Visnjar T, Kreft ME (2013) Air-liquid and liquid-liquid interfaces influence the formation of the urothelial permeability barrier in vitro. Vitro Cell Dev Biol Anim 49(3):196–204

Visnjar T, Kreft ME (2015) The complete functional recovery of chitosan-treated biomimetic hyperplastic and normoplastic urothelial models. Histochem Cell Biol 143(1):95–107

Wagner V, Dullaart A et al (2006) The emerging nanomedicine landscape. Nat Biotechnol 24(10):1211–1217

Williams DF (2008) On the mechanisms of biocompatibility. Biomaterials 29(20):2941–2953

Yoo JJ, Olson J et al (2011) Regenerative medicine strategies for treating neurogenic bladder. Int Neurourol J 15(3):109–119

Zhu X, Chen J et al (2004) Cellular reactions of osteoblasts to micron- and submicron-scale porous structures of titanium surfaces. Cells Tissues Organs 178(1):13–22

Zupancic D, Romih R et al (2014) Molecular ultrastructure of the urothelial surface: insights from a combination of various microscopic techniques. Microsc Res Tech 77(11):896–901

Zupančič, D., R. Romih, et al. (2014). "Molecular ultrastructure of the urothelial surface: Insights from a combination of various microscopic techniques." Microsc Res Tech