Multifunctional Nanowire Bioscaffolds on Titanium

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This paper reports a new fabrication of multifunctional nanowire bioscaffolds directly on titanium (Ti) through a simple and scale-up easy hydrothermal reaction of alkali with the Ti metal without using any seeds, templates, TiO_2 powder, or stabilizers. The nanowires root firmly inside the Ti substrate and grow on top to eventually self-assemble into macroporous scaffolds. The effects of the alkali concentration, reaction time, and temperature on the bioscaffold morphologies were investigated. The novel solid-state chemistry for the nanowires' downward/upward co-growth and the accompanied self-assembly were tackled. Thus-formed coating of scaffolds on the metal implant surface, mimicking the natural extracellular matrix in structure, can promote cell adhesion and proliferation on Ti implant and perform controlled on-site drug release and photocatalytic sterilization.

1. Introduction

In biomaterials science it is a longstanding challenge to make a bioscaffold that is both macroporous and mechanically tough.[1−4] A typical natural extracellular matrix may be too fragile to support weight.[5] Smooth coatings on implantable titanium that can support weight, on the other hand, may lack the macropores important for accommodating tissue growths.[6] Hence, fabricating a robust and multifunctional coating of organized ceramic nanostructures on metal surfaces, easily and economically from solution using neither micropatterns nor template, would be an alternative approach to improving the ordinary implantable biomaterials through a tailored nanofabrication.

In literature, three approaches were reported for fabricating coatings of one-dimensional nanostructures directly on substrate. One is the templating synthesis, in which dense, aligned titania nanowires with well-controlled lengths and diameters were fabricated on substrates.[7−11] The second is the syntheses of oriented titanate nanotubes via a nanoseeding method.[12] The third reported a networked TiO_2 nanocoating on Ti from the galvanodynamic anodization.[13] These coatings may either be mechanically not tough enough to be used in bone-replacement or possess pores too small to accommodate the tissue growth.

We here report a detailed study on a tailored metal oxidation (or corrosion) route to large-scale solution fabrications of titanate nanowires at low temperature on the basis of a preliminary work published before.[14] The nanowire was found to root firmly inside Ti substrate of nearly any size and shape via an unusual downward growth. At the same time, the nanowires' tips grew upward on top to eventually self-assemble into scaffolds. The scaffolds, composed of macropores of 2−10 μm in diameter, can promote cell adhesion and proliferation, release drug in a controlled fashion, and perform photocatalytic sterilization. Compared with the typically smooth hydroxyapatite coatings on the Ti,[15] such a nanowire-scaffold coating could make the otherwise ordinary Ti implant multifunctional.

2. Experimental Section

2.1. Preparation of Self-Assembled Titanate Nanowires. Before the synthesis, titanium substrates (foil and mesh from Alfa

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Aesar and Ti TEM grids from Ted Pella) were inserted in 10 mL of acetone at room temperature, sonicated for 10 min, and rinsed with deionized water thereafter. The Ti substrate was then placed in a Teflon-lined vessel containing 10 mL of 1.0 mol/L NaOH solution. Afterward, the vessel was sealed and then hydrothermally heated at 160−250 °C for 2−10 h. Thus-treated Ti substrates, covered by the titanate nanowires scaffolds, were finally collected, rinsed with deionized water, and dried in air.

2.2. Characterizations. The phase purity and crystalline structure of the nanowires were characterized by X-ray diffraction (XRD) on a Philips X'Pert X-ray diffractometer (Cu Kα, $\lambda = 1.5418$ Å) scanning from 4° to 70° (2θ) at a speed of 1°/min. The morphology of the nanowire scaffolds was examined under a scanning electron microscope (SEM, Philips SEM XL30), and a high-resolution transmission electron microscope (HRTEM, JEOL 2010) performed at 200 keV.

2.3. Photocatalytic Sterilization. The titanate nanowires were tested under a UV light for inactivating *E. coli* O157:H7. Transparent glass slides and bare titanium foil were selected as the control specimen. *E. coli* O157:H7 (ATCC 43888) were grown in brain heart infusion (BHI) broth (Remel, Lenexa, KS) at 37 °C for 20 h to reach a concentration of 10^8-10^9 cells/mL. Different concentrations of *E. coli* O157:H7 were prepared via sequential dilutions in physiological saline solution. A low-power UV light source (302 nm, 4 W, Ultraviolet Products, San Gabriel, CA) was placed 20 cm above the samples for 5 min.

The bacterial concentration was determined by the standard surface plating-count methods. Briefly, *E. coli* O157:H7 was enumerated by surface plating 0.1 mL dilutions on MacConkey sorbitol agar (Remel) and incubating the plates at 37 °C for 18−24 h. Then the colonies on the agar plate were counted.

A total of 0.5 mL of the inoculum containing 1×10^6 cells of *E. coli* O157:H7 was first dropped in a round area (approximately 10 mm diameter) on a rectangular glass slide (20 mm × 50 mm), a round (20 mm diameter) titanium plate, and a nanowire coated round (20 mm diameter) titanium plate, respectively, under regular indoor lighting (400 W fluorescent lights at ceiling) at 20 °C. After 5 min, each sample was put into a plastic tube pre-filled with 10 mL of phosphate buffer solution and shaken for 2 min to wash the bacteria off the substrate. The sample was washed three times with 0.1 mL of water and then used for microbial tests to determine the number of viable *E. coli* cells on the sample.

2.4. Supported Growths of Tissues from Mesenchymal Stem Cells and Osteoblasts. The scaffolds on titanium were first sterilized in 70% ethanol, rinsed in sterile 0.9% saline, and then put on cell-culture plates. Subsequently, mesenchymal stem cells were prepared and transduced with green fluorescent protein for visulalization,[16] suspended in DMEM-LG media (Gibco, Grand Island, NY) containing 10% fetal bovine serum (Hiclone, Logan, UT), then added onto the culture plates, and allowed to adhere for 24 h. Another medium, containing 10 mM sodium β-glycerophosphate, 100 nM dexamethasone, and 50 nM ascorbate (Sigma, St. Louis, MO), was used to promote the osteoblast differentiation. The in vitro samples were characterized using a fluorescent microscope (Olympus BX 51). For in vivo testing, the scaffold-coated Ti meshes were implanted into severe combined immunodeficient (SCID) mice after the adherence of cells and removed after 4 weeks of the implantation. The in vivo samples were examined under the low vacuum SEM operation mode (Philips SEM XL30). X-ray radiographs were taken with the AXR Minishot 100 beryllium source (Associated X-ray Imaging Corp., Haverhill, MA) with a 20 s exposure at 42 kV. All the studies and procedures were approved by the Institutional IRB and the Animal Care and Use Committee.

3. Results and Discussion

3.1. Effects of Reaction Temperature, Concentration, and Time on Nanowire Growth and Self-Assembly. To study the nanowire structural evolution and self-assembly on Ti (TEM Grid), samples formed from NaOH solutions (ranging from 0.25 to 1.0 mol/L) at 240 °C (rather than under 180 °C)[17] were first characterized under the SEM (Figure 1). Figure 1a,b depicts the SEM images of titanate nanorods, upward grown from a 0.25 mol/L NaOH solution, with the diameter of ~200 nm and length of ~2 μm. When the NaOH concentration was increased to 0.50 mol/L, the nanowires, about 80 nm wide and 5 μm long, vertically standing on the Ti substrate started to "meet with each other" on top (Figure 1c,d). From the 1.0 mol/L NaOH, however, much longer nanowires, nearly 50−100 nm in width and 5−10 μm in length, self-assembled on top into macroporous scaffolds on the Ti (Figure 1e,f). This alkali concentration effect may reflect the nanowire growth kinetics governed likely by the Ti corrosion speed or the concentration[18] of the nanowire structural building blocks in the NaOH solution, which may further enrich our understanding of the chemistry involved in the nanowire-bioscaffold growths.[14] An HRTEM image of the nanowire shows a typical layered structure (Figure 1g) similar to that of the titanate,[19] and the XRD pattern (Figure 1h) of the nanowires resembles that of the layered hydrogen titanates $H_2Ti_nO_{2n+1}\cdot xH_2O$.[17,20] Further, the size and hydration degree of the countercation (e.g. H^+)[20] in the interlayer space may fine-tune the interlayer distance, which could make such nanowire coating robust.[21]

Then, a time study was conducted for understanding the self-assembly of the nanowires. After a hydrothermal reaction for 30 min at 240 °C in a 1.0 mol/L NaOH solution, dense nanorods (about 50−100 nm wide and 200 nm long, see Figure 2a) have grown on the Ti. After 60 min, the nanorods became 400 nm long and bent on top (Figure 2b). A longer time (e.g. 2 h) for the reaction would result in even longer nanowires, 50−100 nm in width and 2−5 μm in length, that can bend on top to start to self-assemble into one another (Figure 2c) rather than keep growing vertically and randomly. After a 4-h reaction, the nanowires eventually self-assembled on top into bundles that in turn formed "ridges" and "valleys" about 2−10 μm wide and 2−5 μm deep on the Ti (Figure 2d). Probably, the process was governed by the surface tension effects,[22] static charge,[23] H-bonding, or even dehydration between surface hydroxyl groups on the adjacent nanowires.

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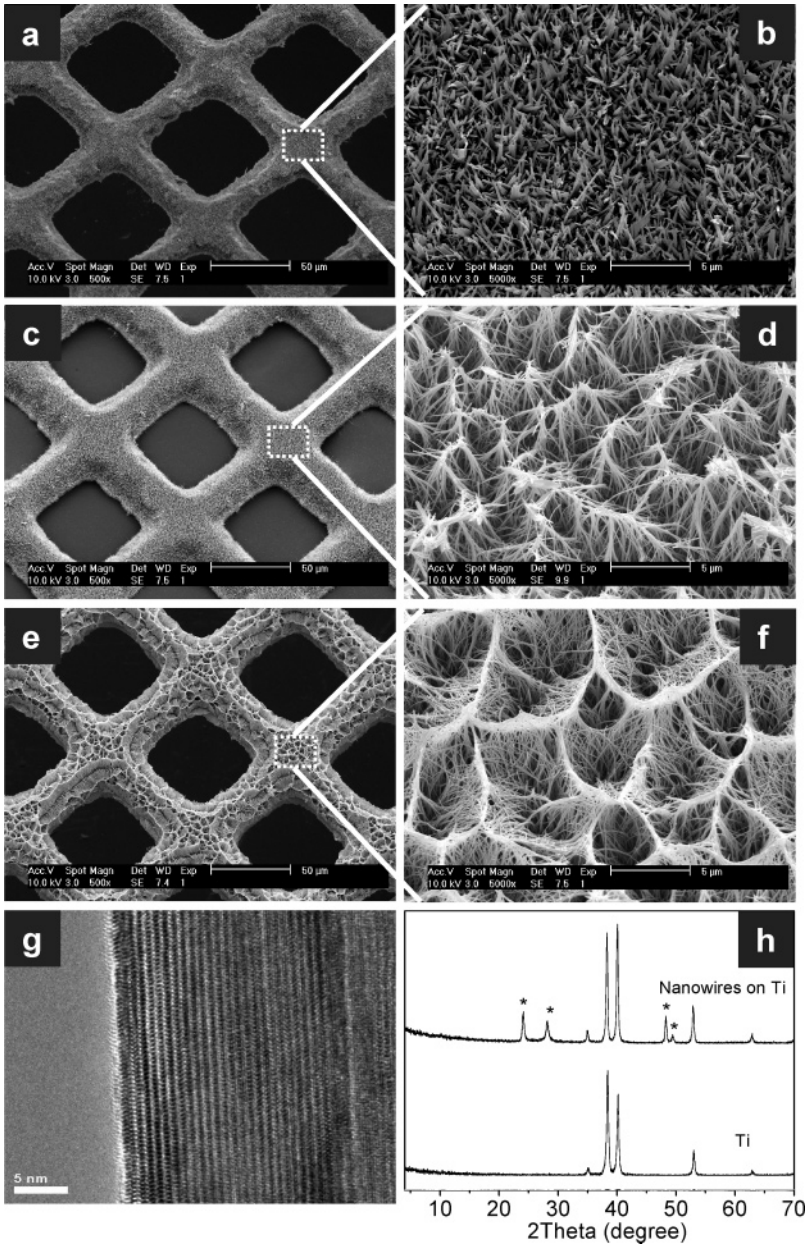

Figure 1. SEM study on the effect of NaOH concentration on the nanowire formation on Ti meshes in 10 mL of solution at 240 °C for 4 h. Parts a and b are low- and high-magnification SEM pictures for the sample from 0.25 mol/L NaOH. Parts c and d are low- and high-magnification SEM pictures for the sample from 0.5 mol/L NaOH. Parts e and f are low- and high-magnification SEM pictures for the sample from 1.0 mol/L NaOH. g, An HRTEM image showing the layered titanate lattice of a nanowire. h, XRD patterns of the Ti and the nanowire-on-Ti (all asterisk-denoted peaks are for titanate, and other peaks for Ti).

On this basis, the temperature effect on the scaffold morphology evolution in this nanosynthesis was likewise studied in a 1.0 mol/L NaOH solution for 4 h at different temperatures. At 210 °C, about 50−100 nm wide and 200 nm long nanorods have grown on the Ti (Figure 3a). By increasing the temperature to 220 °C, the average height of the nanowires extended to 1−2 μm (Figure 3b). An even higher reaction temperature (230 °C) would give rise to even longer nanowires (about 50−100 nm thick and 2−5 μm long) that likewise bent on top to start to self-assemble into bundles (Figure 3c). After a reaction at 240 °C, the nanowires hierarchically self-assembled into three-dimensional macroporous scaffolds, with typical pores around 2−10 μm in diameter and 2−5 μm in depth (Figure 3d). The shape and size of the "valleys" in Figure 1f are different from those in Figures 2d and 3d, showing that the scaffold pore size can be fine-tuned over a wide range by controlling the reaction temperature, concentration, and time, which could help us optimize the scaffold structure that can meet various application needs.

3.2. Upward and Downward Co-Growth of the Organized Nanowires. To understand the solid-state chemistry involved in the nanowire growth, we have conducted an SEM study on 45°-cross-section samples and found three interesting sections (Figure 4a) on each sample. As depicted in Figure 4a, the top region is full of titanate nanowire scaffolds, and the bottom region is the metallic Ti. The middle portion, however, is a corrosion region composed of mostly titanate nanoparticles and some nanowires vertically rooted on the nanoparticles (Figure 4b,c),[24,25] suggesting a continuous

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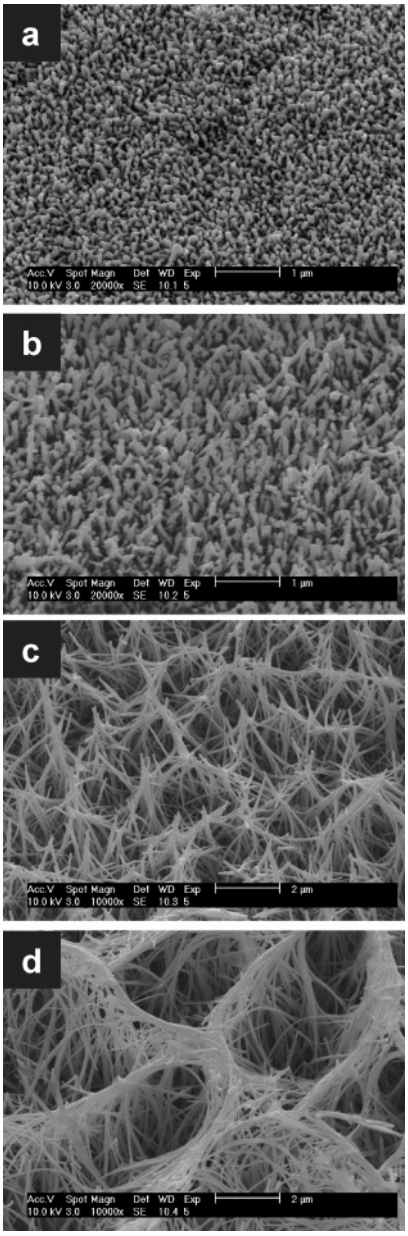

Figure 2. SEM analysis of the time effect on nanowire self-assembly on the Ti in 1.0 mol/L NaOH at 240 °C. a, 0.5 h. b, 1 h. c, 2 h. d, 4 h.

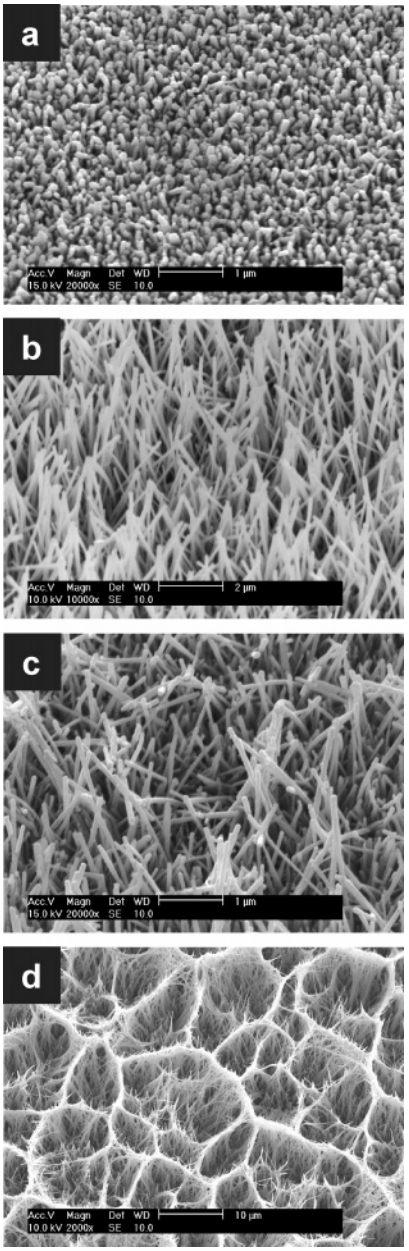

Figure 3. SEM examination of the temperature effect on the nanowire self-assembly on the Ti in 1.0 mol/L NaOH for 4 h. a, 210 °C. b, 220 °C. c, 230 °C. d, 240 °C.

downward in situ growth of both the nanoparticles and the nanowire. In the time-study samples, the corroded part, about 4 μm thick after 30 min, 10 μm thick after 2 h, and 20 μm thick after 4 h of the reaction, form a dense base for the nanowires to root deeply inside (Figure 4b). As the corrosion region grows downward, the nanowire may grow downward. Only with the downward growth could the nanowire keep a nearly vertical orientation all the time, as revealed by Figure 4b. Without the downward growth, the scaffold might quickly become free-standing due to the continuous dissolution of the nanoparticles by the alkali in solution, which is the case in a previous work.[12] This cross-section SEM study implies that much detailed work needs to be done on the complex co-growth and self-assembly mechanisms to help us understand why the nanowire-scaffold coating on Ti is robust and suggests that the nanosynthesis could make the otherwise ordinary metal corrosion more exciting and fruitful than before.

Further, a second growth for 4 h at 140 °C was conducted on the nanowire, because the multiwalled nanotubes preferably formed below 150 °C[17,26−29] could be easily identified. After the second growth, typical open-ended nanotubes can be seen on top of the nanowire (Figure 4d), with an outer diameter of about 80 nm and inner diameter of 50 nm, confirming an upward growth at the nanowire tip in solution during the corrosion. On this basis, the upward and downward co-growth has been revealed and is outlined in Figure 4e. These findings have suggested a new and probably

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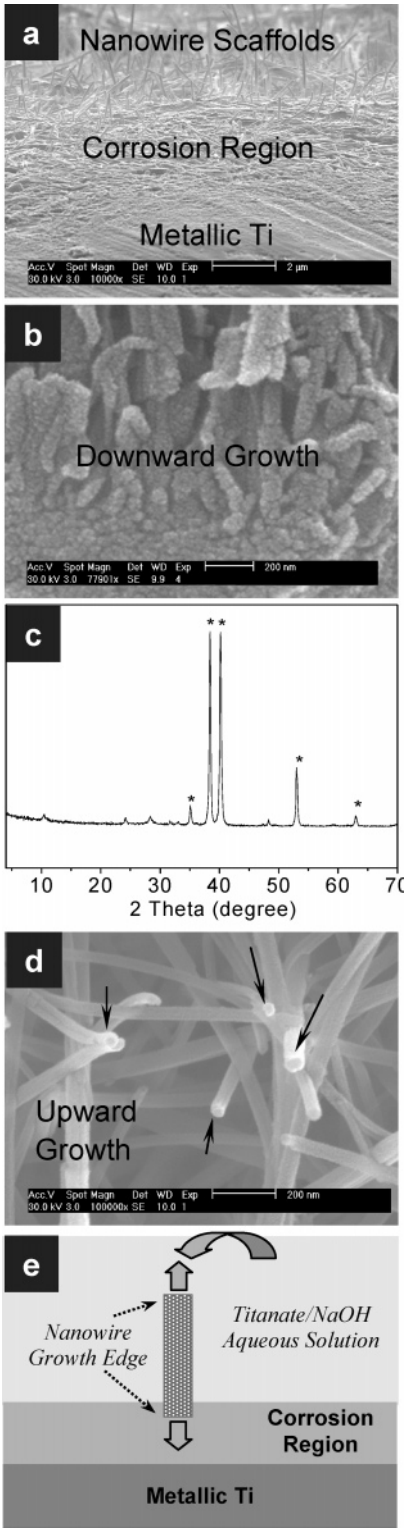

Figure 4. SEM investigation on the upward and downward co-growth. a, An SEM image from a 45° cross-section sample showing top, middle, and bottom regions. b, The high-resolution SEM picture from middle region depicts nanowires vertically rooting on nanoparticles. c, The XRD pattern of the titanate in the corrosion region on Ti (star-denoted peaks correspond to the Ti and others attribute to titanate NWs). d, The nanotube structures grew upward at tip of the nanowire from solution. e, A new co-growth mechanism suggested by parts a−d.

generalized corrosion−nanofabrication involving three simultaneous processes that are the downward growth, the upward growth, and the self-assembly of the nanowires, which was not tackled previously[14] in solid-state nanosynthesis.

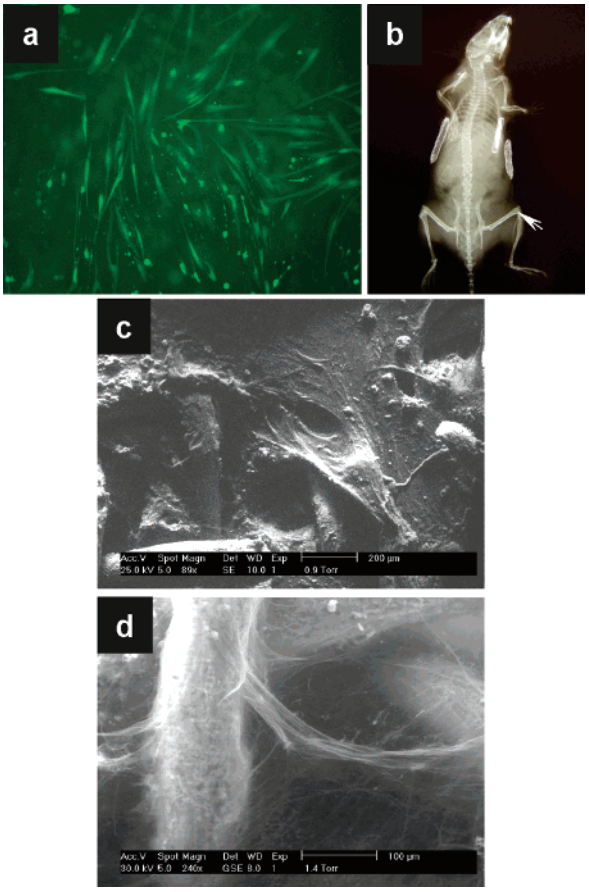

Figure 5. Tissue growth on the nanowire scaffold-coated Ti. a, A fluorescent photograph of the tissues formed after 42 days from an in vitro growth. b, An X-ray radiograph of the bioscaffolds after 4 weeks of implantation inside an SCID mouse. Parts c and d are low- and high-magnification SEM images of tissues grown in vivo for 4 weeks.

Table 1. Inactivation of *E. coli* O157:H7 (0.5 mL of the Inoculum Containing 1 × 10^6 Cells of *E. coli* O157:H7) on Nanowires under UV Irradiation (302 nm, 4 W, 20 cm) for 5 min

substrate	glass	titanium	nanowire-coated titanium
number of *E. coli* O157:H7 (no UV for 5 min)	$10^{6.5}$	$10^{6.3}$	$10^{5.4}$
number of *E. coli* O157:H7 (302 nm UV for 5 min)	$10^{5.6}$	$10^{6.1}$	$10^{2.1}$
reduction	90%	37%	99.99%

3.3. Simple, Quick, Low Cost Efficient Photocatalytic Sterilization. The titanate nanowires are known to have novel photocatalytic properties in water,[30,31] suggesting an unusual potential to develop a new photocatalytic sterilization protocol for cleaning such scaffold coating on Ti implants. The data outlined in Table 1 indicate that after a low-power UV irradiation for 5 min, *E. coli* O157:H7 on the glass slide and bare titanium plate were reduced by one log (90%) and 0.4 log, respectively, while that on the nanowires were reduced by four logs (99.99%). The bacteria reduction on the scaffold is several orders of magnitude more efficient than that on non-coated surfaces, suggesting that this is truly a quick, simple, economic, easy scale-up, and effective photocatalytic sterilization strategy for nearly any sized or

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shaped Ti implants pre-coated with the nanowire scaffolds. After the sterilization, the *E. coli* cells on the nanowire scaffold show the typical deformed morphology. The nanowires on Ti are intact after autoclaving at elevated temperatures and pressures, ethanol dipping, water rinsing, and swabbing, implying that the nanowire-coated new Ti implant can support weight and quickly sterilize bacteria.

3.4. Effective Growths of Tissues from Mesenchymal Stem Cells. Naturally, the large and open "valleys" on the coating can facilitate cellular activities.[32−35] The mesenchymal stem cells after a 1-day in vitro growth on the scaffold pre-coated with fibronectin suggests a good compatibility between the cell and the scaffold, same as what was reported previously.[14] After 42 days, tissues formed (Figure 5a) on the bioscaffold. On this basis, a Ti mesh tube about 5 mm wide and 10 mm long, pre-coated similarly, was implanted into SCID mice (Figure 5b). After 4 weeks, the scaffold became fully covered by tissues (Figure 5c,d). The bioscaffold's nanoscale integrity has remained intact after the in vivo and in vitro tests. The drug release property has been observed on such nanowire-bioscaffold (see Supporting Information), which is similar to what has been reported previously.[14]

4. Summary

The above results suggest that we have developed a simple method for fabricating robust coatings of ceramic nanowire-based bioscaffolds on titanium surface in large scale at low cost. The nanowires can grow via a newly revealed upward-downward co-growth route, and the scaffold was formed via a self-assembly of the nanowires. Thus-formed scaffolds may mimic the nature's extracellular matrix and exhibit a good cellular compatibility, mechanical toughness, on-site drug release function, and structural robustness. Such multifunctional bioscaffolds could be potentially useful in bone replacement, drug release, stenting, photocatalysis, high-temperature oil cracking, photocatalytic sterilization of surgical and food processing environments, solar energy conversion, and water cleaning, to name a few.

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Supporting Information Available: Controlled drug release on the nanowire-coated Ti foil (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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