Antimicrobial Titania Nanofibers

techsolutions:
Introduction to Manufacturing
Fabrication of Antimicrobial Titania Nanofibers by Electrospinning

ABSTRACT
Titanium dioxide or titania (TiO₂) has well-known photoactive antimicrobial attributes. It also readily forms on titanium implants. Recently conducted in vitro studies have demonstrated increased osteoblast functions of nanostructured TiO₂ necessary to promote the efficacy of orthopedic implants. Titania-based ceramics (bulk material, thin films, nanoscale powders, etc.) are generally synthesized by using either a highly corrosive and toxic titanium tetrachloride (TiCl₄) and titanium oxychloride (TiOCl₂) or rather expensive and moisture-sensitive titanium isopropoxide [Ti(C₃H₇O)₄] as the starting chemicals. In this paper, the technique of electrospinning has been used to fabricate non-woven, breathable titania nanomats† employing titanyl nitrate as a benign and inexpensive precursor.

INTRODUCTION
Titanium dioxide (also known as rutile) in pure or doped form has been extensively used in a number of applications, ranging from food coloring, paints, cosmetics, catalysts, anti-biofouling, photovoltaic solar cells, and sunscreens to gas sensors. In addition, due to its suitable energy band gap (≈3.2 - 3.5 electron volts, eV), titania has also gained interest in photonic band gap crystals for the visible spectrum of light due to its high refractive index (n_rutile ≈ 2.9) and low absorption properties [1]. Recently, self-cleaning wool-polyamide, polyester and cotton textiles coated with TiO₂ have been also reported [2-7]. One of the unique physical properties of titania is its photocatalysis – a photo-activated antimicrobial/disinfecive activity where free radicals generated from TiO₂ oxidize organic matter upon activation by light. This property makes the material a candidate for numerous medical applications where infection control is needed. By interposing an effective procedure based on nanotechnology, the bone healing can be made safer and to take place at an accelerated pace, simultaneously eliminating or mitigating the probability of wound infection. However, the unique photocatalytic property of nanostructured titania as a wound and bone fracture disinfectant has not been exploited hitherto. Constructing non-woven TiO₂ nanothreads and nanomats possessing a three-dimensional scaffold structure and optimal porosity, in conjunction with photo-activated antimicrobial activity, could provide a significant improvement in the management of segmental bone defects particularly in the presence of infection. A photoactive TiO₂ nanomat either in pristine form or impregnated with antibacterial agents can be used as an effective ultralight disinfectant gauze for wound healing upon brief activation by light.

Considering that the natural scaffold (extracellular matrix or ECM) consists of a multilayered fibrous and porous architecture, the possibility of utilizing electrospinning as a novel nanomanufacturing technique applicable to tissue engineering has emerged. Several researchers have explored the feasibility of fabricating bio-threads containing live cells in benign polymeric matrices that could be used for a number of applications including wound healing and tissue growth. Electrospun fibers are found to possess features that bear morphologic similarity to the ECM of natural tissue such as high porosity and effective mechanical properties. They therefore meet the essential design criteria of an ideally engineered scaffold [8-9]. Recently, the authors have successfully carried out preliminary experiments to attach cells to the electrospun polymeric (poly vinyl pyrrolidone or PVP) as well as ceramic (alumina) nanofibers [10].

With the goal of using nanomats to combat wound infection due to its photoactive attributes, this paper describes the fabrication and characterization of non-woven titania nanofibers using a simpler and more benign precursor than that which has usually been used in the case of titania synthesis. In this case, an aqueous titanyl nitrate (TiO(NO₃)₂) was synthesized from water soluble titanium fluoride, as described in the Experimental Procedure section. First, an appropriate ceramic-polymer (ceramer) composite of titania electrospun as a continuous non-woven nanofibrillar mat was fabricated from an optimized mixture of suitable inorganic and polymeric precursor blend. The ceramer composite was then processed carefully and the transformation of ceramer to high purity, crystallized and morphologically optimized titania was followed by a well-conceived heat-
orientation of the syringe pump in this work was horizontal. A stainless steel needle was attached to the syringe, which was mounted on a programmable syringe pump. The preferred volume (v/v) ratios and stirred into homogeneous viscous solutions. Each of these mixtures (1:1, 1:2 and 2:3 v/v) was drawn and to re-form hydrated titania. Therefore, the precursor was synthesized only when needed and was used soon after preparation. A white precipitate of titanium hydroxide (Ti(OH)$_4$) was formed and was allowed to settle for 2 hours. The supernatant liquid was tested by adding a few drops of ammonium hydroxide. Absence of the formation of fresh precipitate indicated that the reaction was complete.

The Ti(OH)$_4$ suspension was allowed to settle overnight and tested again for additional precipitation the next day. The supernatant liquid was decanted, and the precipitate was washed with DI water and centrifuged several times until the decanted liquid acquired a near neutral pH (~8). Fifteen milliliters of concentrated nitric acid (HNO$_3$) was added to the hydrated solid Ti(OH)$_4$[TiO$_2$·3H$_2$O] under constant stirring and gentle heating until it dissolved completely giving a clear transparent solution of 0.214 M strength. The resulting solution was diluted with 230 ml of DI water, to which 20 ml of 7.4 M ammonium hydroxide (NH$_4$OH) was added slowly under constant stirring. A white precipitate of titanium hydroxide (Ti(OH)$_4$) was formed and was allowed to settle for 2 hours. The supernatant liquid was tested by adding a few drops of ammonium hydroxide. Absence of the formation of fresh precipitate indicated that the reaction was complete.

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In order to optimize the electrospinning conditions, the precursor solutions (TN and PVP) were mixed in different volume/volume (v/v) ratios and stirred into homogeneous viscous solutions. Each of these mixtures (1:1, 1:2 and 2:3 v/v) was drawn into a 10 ml capacity clinical syringe. A precision-tip 25-gauge stainless steel needle was attached to the syringe, which was mounted on a programmable syringe pump. The preferred orientation of the syringe pump in this work was horizontal. A custom-made direct current power supply with a high voltage system (30 kV maximum) using a modified version of a circuit design developed at NASA Glenn Research Center [11] was used for electrospinning (e-spinning). One terminal of the power supply was connected to the needle, while the other was connected to a grounded stainless steel collector plate.

However, for the ease of sample handling and subsequent thermal processing, ceramic plates instead of metal were used as a collector. In order to enhance the fiber collection area, a modified collection set-up was devised. Two (4.5 in. x 4.5 in.) dense aluminum plates (0.0625 in. thick) were employed. The plates were kept a half inch apart and connected together to a common junction by attaching short lengths of electrical wires to the center of each plate through blocks of one square inch of aluminum foil stuck to the back of the plates with double-sided tape. This allowed the fibers to spread and collect across the plates and including the empty space between them. Other details of this set-up are described elsewhere [12-13].

Using the high-voltage power supply, an electrical impulse was applied between the needle and the collectors in order to initiate the e-spinning. After the voltage was turned on, the syringe pump was started. The voltage was tweaked precisely until the fibers began to form steadily and collect on the plate, placed three inches away from the tip of the needle; the optimized voltage in this case was found to be 16 kV. A flow rate of 0.02 ml/h was chosen, and the ceramic fibers were spun continuously with short intermittent interruptions of the run for periodic cleaning of the clogged needle tip.

After spinning was complete, small amounts of the as-spun composite fibers were used for characterization by scanning electron microscopy. This exercise allowed the determination of which mixture of the two precursors was the more optimal in terms of the quality of the fibers (free from intertwining, twisting, branching, liquid globule entrapment, etc.). The remaining fibers collected on the ceramic plates were fired at 700°C for one hour in static air as per the following heating rate-temperature-soak time profile: 22°C (room temperature) to 500°C at a rate of ½ °C/min. with a hold at 500°C for one hour; 500°C to 700°C at a rate of ½ °C/min. with a hold at 700°C for one hour, followed by cooling from 700°C to the room temperature at a rate of 1 °C/min. The rather small heating and cooling rates were chosen so as to ensure the removal of organic components without destroying the nanofibrillar morphological features in the end product and also to avoid the disintegration of the titania nanofibers into powdery grains. Subsequent to cooling, the samples were collected for characterization by a host of analytical techniques, such as X-ray diffraction, scanning and transmission electron microscopy, energy dispersive spectroscopy and selected area electron diffraction. The results of photoactivity evaluation and biocidal efficacy of the electrospun titania nanofibers will be reported subsequently.

RESULTS AND DISCUSSION

Hollow titania fibers have been prepared by soaking the electrospun poly (lactic acid) in a 1:19 (v/v) solution of titanium (IV) isopropoxide and isopropanol, followed by hydrolysis, vacuum drying and calcination at 450°C for ten hours at a ramp rate of 15 °C/min., three hours of which were in a nitrogen atmosphere and seven hours in an oxygen atmosphere. [14] The amorphous
titania fibers were shown to be porous and 500 nm across. As is evident, the technique involves several time-consuming processes: electrospinning of the polymeric skeleton first; preparing of inorganic sol, followed by gel formation and its hydrolysis onto the electrospun polymeric fibers before the organics are removed to yield the desired ceramic nanofibers. Moreover, the authors of reference 14 claim that the surface of the fiber is not flat but contains small oval indentations, which are due to rapid phase separation during the electrospinning process, where the solvent-rich regions result in pore formation. This statement, equivalent to the postulation of the existence of a ‘concentration gradient’ region in the dynamic process of fiber formation, is rather speculative and unsubstantiated; this is particularly so, in the light of the absence of any visible ‘thinning’ along the fiber length. If this were true, some collapsing and narrowing of the uniform tubes to form ‘sheets’ should certainly be observed in the high magnification micrographs included in the reference.

A similar procedure has also been adopted to synthesize metal (tin and titanium) oxide coated polymer nanofibers with a diameter on the order of 100 nm and a coating thickness ranging from 20 to 80 nm. [15] The preparatory technique used is also quite elaborate and involved. It uses complex salts, such as ammonium hexafluorotitanate and ammonium hexafluorostannate, as the precursors and requires a halide scavenger in the form of boric acid. Furthermore, as seen from the SEM images, the metal oxide coating is uneven and non-uniform, and the TEM images confirm this. Other researchers have used a sol of titanium and silicon to electrospin silica-doped titania nanofibers onto a rotating drum. [16] The ceramic fibers were obtained by firing them for two hours in the temperature range of 500-1000°C. However, while the SEM images of the as-spun fibers and those dried (incorrectly referred to as ‘calcined’ in the reference) at 100°C for two hours alone are shown, no micrographs of the fired samples were included. Hence, the morphological features of the samples calcined in the range 500-1000°C is unknown.

The fabrication of anatase titania porous nanofibers of controlled diameter using an ethanol solution containing both PVP and titanium (IV) isopropoxide via electrospinning, followed by calcination in air at 500°C, has been reported. [17] This research shows that the average fiber diameter ranged between 20 and 200 nm depending upon a number of parameters, such as the strength and ratio of PVP and titanium (IV) isopropoxide solutions, the strength of the applied electric field, and the flow rate of the precursor solution.

As can clearly be seen from above, the synthesis of pure and/or doped titania nanofibers via electrospinning: (a) involves elaborate routes, or (b) uses either a complex compound (ammonium fluorotitanate, for example) in combination with a halide scavenger, or a relatively expensive and moisture-sensitive titanium (IV) isopropoxide, as a precursor. Moreover, a technique of coating an electrospun polymeric skeleton with titania precursor followed by calcination does not produce the as-desired uniform and homogeneous ceramic fibers. In contrast, the present work describes the procedure of synthesizing a simple titanium precursor (viz., titanyl nitrate) from a less reactive and benign source (TiF₄) and employs it in the fabrication of high-quality titania nanofibers.

As stated above, in order to ascertain an optimized composition that is conducive to yield uniform ceramer nanofibers, the aqueous solution containing titanyl nitrate and the PVP solution in ethanol were mixed in three different v/v ratios: 1:1, 1:2 and 2:3, keeping other experimental variables (applied electric field strength, flow rate, the distance between the needle and the collector plates, etc.) constant. The SEM images of the as-spun nanofibers from each batch are shown in Figure 1.

As seen from Figure 1 (a-b), the fiber spun from a 1:1 (v/v) mixture of the inorganic and organic components are characterized by the presence of a large fraction of nearly spherical liquid globules connecting various segments of the fibers. Thus, it appears that this mixture is subject to the combined phenomena of electrospaying (spherilization of the charged droplet upon discharging) and electrospinning. A large population of globules, rather than linear fibers, suggests that the mixture might not have attained the optimal viscosity in order to satisfy the conditions of forming perfect non-woven fibers. For this purpose, the polymeric content of the solution was increased in the ratio TN:PVP = 1:2. The SEM images displayed in Figure 1 (c-d) indicate that this strategy helped, as the density of liquid blobs greatly diminished,
though was not eliminated totally. Some evidence of fiber bending can also be seen, which indicates that the ratio of the two components in the spinning mixture still needs to be optimized. The 2:3 (v/v) mixture of TN:PVP appeared to form the most desired microstructure when electrospun, as could be seen from the nonwoven fibers of uniform thickness in Figure 1 (e-f). The subsequent discussion pertains to the fibers which were electrospun from a homogeneous solution containing the inorganic and organic precursors in the volumetric ratio of 2:3.

The morphological features of the fired nanofibers of electrospun titania are shown in Figure 2a. The intact nature of the fibers in the layered mats of titania that were present in the as-spun material can be discerned in the calcined sample as well. This is by virtue of the judicious firing scheme adopted in this work; even a slightly higher ramp rate has been found to cause severe fiber rupturing, rendering them into a powdery mass, due to faster combustion of the polymer with a concomitant and sudden release of a copious amount of gaseous products. The energy dispersive spectrum (EDS) of the same is shown in Figure 2b, where the signals due to oxygen and titanium alone are seen; no peak belonging to carbon is present, meaning that the heating profile selected in this work was able to eliminate polymeric components quantitatively.

The TEM images of the nanofibers fired at 700°C as per the schedule described in the previous section are shown in Figure 3. It is evident that the heat treatment used in the present work has preserved the fibrous artifact in the processed material. Moreover, the titania fibers are porous and less than 100 nm across; they are comprised of interconnected monosized grains, making the structure quite breathable and therefore amenable for the intended medical application. Using the wavelength of the electron beam (0.0335Å) and the length of the SAED pattern on the film, the interplanar distances (d-spacings) for successive diffractions were calculated. They match the d-spacings reported in literature for the anatase phase of titania. This is corroborated by the X-ray diffraction pattern of the powdered nanofibers, as shown in Figure 4; the selected area electron diffraction signature of the calcined fibers is also shown as an inset.

**CONCLUSION**

Titanium dioxide nanofibers were successfully electrospun as a polymeric composite from a benign and inexpensive titanium precursor, viz., titanyl nitrate and polyvinyl pyrrolidone. The as-spun ceramer fibers possessing uniform thickness upon firing were formed into breathable titanium dioxide nanomats of anatase modification upon a single-stage firing in air at 700°C for one hour.

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Zainab Al-Firdaus was part of this research during summer 2007, when she was an 8th grade student at Perrysburg Junior High School. She is currently a sophomore at Perrysburg High School.

**NOTES & REFERENCES**

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1 Nanomats are 3-dimensional carpet-like, supportless, independent, modular structures with nearly identical porosity in their bulk. These specialized nanostructured materials can be used either as such or in combination with other components, such as dyes or drugs or both, to enhance their anticipated functional attributes.


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AMMTIAC Success Story:
Commercial Manufacturer Benefits from DoD Technology Transfer

Beyond the primary charge of providing technical solutions to the warfighter, it is also a mission of the Information Analysis Centers (IACs) to transfer, or “spin-off,” innovative technologies to the US industrial base, as AMMTIAC has recently done. Through a commercial inquiry, AMMTIAC provided a manufacturer of wire and cable machinery with a machining solution that employed technology originally developed for the US Army to solve a production issue and improve product quality.

The manufacturer identified the root cause of the production issue as a poor surface finish on machine components called forming rolls. These rolls are grooved pieces of high strength steel used to shape individual strands of wire so that they can be wrapped together to form a larger cable. Due to the weight of the components (60 lbs each) and the complexity associated with machining the wire grooves, the proper surface finish needed to shape the wire strands is not readily achieved by traditional machining methods (e.g., turning, grinding, etc). Engineers from AMMTIAC collaborated with the component manufacturer to evaluate possible solutions and decided the best approach is to apply a superfinish to the complex surface geometry of the forming rolls to achieve the desired surface finish.

Chemically Accelerated Vibratory Surface Finishing (CAVSF), more frequently referred to as Superfinishing, is a process that was originally developed by the US Army to improve the surface finish and fatigue life of components in helicopter transmissions. Superfinishing uses a chemically-formulated conversion coating to oxidize the peaks on a surface after which they are placed in a vibratory media container, where the oxidized peaks are removed, leaving a microscopically-smooth surface finish. Metallic components that have undergone a superfinishing operation have demonstrated a 300% improvement in the fatigue life of the surface. Transition of super-finishing technologies has netted significant results in the automobile racing community, as it has significantly improved the overall performance and service life of car transmissions and ring and pinion gears.

Application of superfinishing technology to the forming rolls produced a drastic improvement in the quality of the wire being produced. The improved surface finish (less than four micro-inches) has allowed the machinery manufacturer to produce individual strands of wire that are free of defects, eliminating a long-standing production issue. The success achieved by superfinishing the forming rolls has lead the machinery manufacturer to consider permanently using the superfinishing process in the production of forming rolls. In addition, AMMTIAC is working with the machinery manufacturer to identify other machine components that would benefit from the use of superfinishing technology.