This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Directional freezing has been widely employed to prepare highly ordered three-dimensional (3D) porous assemblies. However, in this scenario, there is one concept that has not been extensively explored: by applying directional freezing to a nanoparticle (NP) dispersion supported on a substrate, two-dimensionally (2D) patterned films may be produced. In this study, tunable 2D-patterned of TiO$_2$-NP dispersions on alumina substrates is demonstrated. By imposing different temperature gradients throughout the ceramic dispersion coatings, both homogeneous (non-patterned) and highly aligned patterned topologies (consisting of parallel grooves) were produced. In the case of patterned films, the orientation of the grooves was modulated from those oriented along the freezing direction to those perpendicularly oriented to the temperature gradient. Thermally-induced reduction of the prepared films led to electrically conducting titanium oxide Magnéli phases. The measured resistances were strongly dependent on the orientation of the aligned patterns. To demonstrate the possibility of employing these structured films as electrical stimulation-related supports, a stimulator electronic circuit was developed and connected to the prepared films. Charge-balanced biphasic stimulus pulses with tunable current amplitudes and frequencies were successfully delivered through the conducting 2D-patterned assemblies.

1. Introduction

Nanochemistry aims at extending the traditional length scales of synthetic chemistry to specially exploit the collective properties of organized assemblies. Structurally organized materials (organic, inorganic, nanocomposites) have attracted much attention for many emerging applications such as catalysis, energy storage, nanomedicine, biotechnologies, among others. The idea in all these cases has been mainly based on offering an appropriate combination of an extended internal reactive surface, given for instance by narrow pores (micropores), with facile molecular transport through wide paths leading to and from these micropores. Particularly, materials with aligned micro- and macrostructures are of interest for a wide range of applications such as microfluidics, molecular filtration, electronics, tissue engineering, among others. Likewise, synthetic aligned porous structures may be also used to mimic natural structured materials such as bone and nacre.

Demands for these advanced materials, able of being applied in new and emerging technologies, have triggered the development of different structuring techniques. Compared to dry processing routes, wet shaping processes are able of leading to materials with complex shapes, being effective at the same time to obtain both dense and porous materials. Among the different wet structuring processes, cost-effective freezing techniques (involving solutions, colloidal dispersions) have been developed and explored as unique routes to produce highly organized porous materials, though freeze casting has also been applied to produce dense monoliths. The freezing technique involves three stages: (i) actual freezing, (ii) primary drying, and (iii) secondary drying. The freezing stage is accomplished by contacting a liquid sample with or placing it in a cold bath. This leads to the solidification of the solvent medium which finally determines the resulting microstructure and volume change associated to the phase transformation. In this respect, there is no doubt that water-based systems have been the most investigated ones. During the freezing step, most solutes are segregated from the ice phase, giving rise to a hierarchical assembly characterized by “fences” of matter enclosing ice. The final removal of ice via freeze drying (primary drying stage) allows obtaining macroporous materials, generated from the voids left by the initially formed ice. This step is usually the most time-consuming, which is directly related to the ice sublimation rate and it is determined by factors such as the vacuum level, sample temperature, sample volume and exposed surface area. Secondary drying is related to strongly adsorbed solvent, reason why lower vacuum levels (compared to primary drying) are generally needed. During the freezing stage different conditions, including freezing temperature, solute concentration, solvent type, presence of additives and direction of freezing, can have (and do have) a great impact on the resulting structure. As to the freezing direction, it has been recognized to exert one of the major effects on the final morphology. When the temperature distribution is uniform throughout the sample, anisotropic ice crystal growth is hindered and homogeneous microstructures are obtained. However, by applying a high temperature gradient across the sample (which is termed directional freezing) anisotropic ice crystal growth along the freezing direction is favored. This occurs due to the chemical potential difference among the different ice crystal facets. The final removal of oriented ice generates materials with highly aligned unidirectional macropores.

The fundamental principles of directional freezing are very well established and documented in excellent reviews. As already mentioned, the formation of porous micro-
macrostructures relies on the rejection of particles (and/or solutes initially dissolved) by the advancing solidification front and the subsequent concentration of them between the ice lamellae. However, this only happens if the breakdown of the solidification front to a non-planar morphology takes place. When the solidification velocity is extremely low, lamellar (and consequently porous) microstructures may disperse. This is because the ice front velocity, dictated by the temperature gradient, is much lower than the critical particle entrapment velocity. This leads to a stable planar advancing ice front, in which case all of the particles and/or solutes are rejected together and directional microstructures along the freezing direction cannot be produced. While all of these aspects have been taken into consideration to prepare materials with complex anisotropic porous microstructures, such as lamellar, dendritic, parallel, columnar and aligned porous scaffolds, these studies have only dealt with three-dimensional (3D) arrangements.

In this scenario, there is one concept that has not been extensively explored yet: by applying directional freezing to a nanoparticle (NP) dispersion supported on a substrate, it would be possible to generate two-dimensionally (2D) patterned films. While it is true that studies on films and membranes prepared by freeze-tape-casting processes have already been published, in these cases only through-thickness porosity has been developed. Only one study on the directional freezing of gold NP dispersions on mica surfaces (obtaining oriented metallic patterns) has been reported. However, systematic evaluation of the possibility of modulating the gold patterns has not been performed. The possibility of controlling both the generation of homogeneous or anisotropic structures and the degree of segregation of NPs by imposing different temperature gradients would allow us to modulate the 2D-pattern obtained.

In this study, titanium dioxide NP (TiO$_2$-NP) aqueous dispersions were induced to self-organize on alumina ($\text{Al}_2\text{O}_3$) substrates during the course of a directional freezing process. Both homogeneous (non-patterned) and highly oriented TiO$_2$ films were successfully prepared by controlling the freezing conditions. In the case of patterned films (consisting of parallel grooves), the orientation of the grooves was modulated from those oriented along the freezing direction to those perpendicularly oriented with respect to the freezing direction. A possible mechanism for the self-organization and tunable orientation was proposed. Finally, structurally-aligned TiO$_2$ films were thermally treated in a reducing atmosphere ($\text{H}_2$/Ar) in order to generate oxygen vacancies in the TiO$_2$ (rutile) structure. This thermally-induced reduction led to mixtures of electrically conducting substoichiometric titanium oxide phases (known as Magnéli phases) which allowed to introduce a new functional property (electrical conductivity) to the structured films. To demonstrate the effect of the orientation of the aligned conductive patterns on the electrical properties, electrical resistances of the films (parallel to and perpendicular to the freezing direction) were determined. The measured resistances were strongly dependent on the orientation of the aligned patterns.

Because of the known electrical, antiinflammatory and biocompatibility properties of the Magnéli phases, in combination with the presence of freezing-induced aligned grooves on the prepared films, these 2D assemblies may find applications on biomedical-related areas. As to this purpose, the possibility of employing the prepared structured films as electrical stimulation-related supports was evaluated. To this end, a stimulator electronic circuit was developed. Charge-balanced biphasic stimulus pulses (with tunable current amplitudes and frequencies) were successfully delivered through the conducting 2D-patterned structures.

To the best of our knowledge, this is the first report on the simultaneous combination of directional freezing and electrically conducting titanium oxide phases to render orientation-dependent electrically active 2D-patterned assemblies, able of being employed as platforms on tunable electrical stimulation applications.

2. Experimental

2.1. Preparation of TiO$_2$ dispersions.

Fig. 1 shows a TEM image of the Degussa P25 TiO$_2$-NPs used for preparing the aqueous dispersions used in this work. The powder was composed of particles of average diameter 20-30 nm. For preparing dispersions, 9.3 g of polyvinylpyrrolidone (PVP, Aldrich, ~1.3MDa) were dissolved in 150 mL of distilled water. Then, 31.5 g of TiO$_2$ were added to the resulting solution and dispersed by means of a T25 Ultra-Turrax instrument at 25000 rpm. TiO$_2$-NP dispersions exhibited long time stability (over 24 h) which represented a suitable time for preparing and processing samples, with no sedimentation nor precipitation occurring during this period.

![Fig. 1 TEM image of TiO$_2$ nanoparticles used. Bar: 200 nm.](image)

2.2. Preparation of alumina-supported TiO$_2$ films.

Before film deposition, alumina substrates (2.5 cm x 1 cm) were washed with aqueous detergent solution followed by ethyl alcohol and acetone, and finally heated for drying. The prepared TiO$_2$-NP dispersions were used for the deposition of films on clean substrates by a squeegee technique, using adhesive Scotch-tape to control the thickness of the coatings. Dispersions thus deposited did not drain out from the substrates during vertical dipping due mainly to the high viscosity of the prepared slurries (tests performed previously to evaluate this condition demonstrated that hours are in fact needed for the deposited dispersions to flow down with gravity). Films were subsequently stored in a 100% relative humidity incubator at room temperature in order to avoid solvent evaporation, and kept under this condition until they were directionally frozen.

This journal is © The Royal Society of Chemistry [year]
2.3. Directional freezing of alumina-supported TiO$_2$ films.

The prepared TiO$_2$-NP films were vertically dipped at variable advancing ice front rates into a liquid nitrogen bath maintained at 77 K and under room pressure (Fig. 2).

![Fig. 2 Schematic representation of the directional freezing process.](Image)

Surface 2D-patterning was modulated by the temperature gradient imposed to the samples. This was carefully controlled with the immersion rate at which samples were induced to freeze. Four conditions were evaluated: (1) films submitted to high freezing rates (30 mm/min), (2) films submitted to intermediate freezing rates (15 mm/min), (3) films immersed at low rates (5 mm/min), and (4) films exposed to cold nitrogen vapours (samples did not contact the liquid phase, and were frozen at the lowest rate: ~ 1 mm/min). Once the films start freezing, the imposed temperature gradient keeps the growing ice into a steady state at a self-controlled and reproducible rate which finally governs the whole directional freezing process.

Finally, the unidirectionally frozen samples were freeze-dried for 48 h (100 mtorr and -45 °C) using a VirTis Benchtop SLC (2KBTES-SS) freeze-drier.

2.4. Thermal treatments on freeze-dried films.

The lyophilized films were heat-treated at 1000 °C for 1.5 h, with heating and cooling rates of 5 °C/min, for sintering in an air atmosphere. For this purpose, an electric high-temperature furnace (Carbolite RHF 17/6S) was used.

Electrically conducting films were obtained after a high-temperature reducing process. Sintered films were treated at 1000 °C for 3 h under H$_2$/Ar (5% H$_2$) atmosphere in order to obtain Ti sub-oxide phases. Both sintered and reduced films were finally characterized.

2.5. Experimental design for biphasic electrical stimulation.

The stimulator electronic circuit used as an excitation source was composed of two sub-systems: (1) an embedded control board (ECB) responsible for handling the user interface and generating the two pulse-width modulation (PWM) signals, and (2) an analog circuit assembly (ACA) responsible for signal adaptation and control (Scheme 1).

Stimulating PWM outputs were easily configured by means of a keyboard and an LCD display. PWM signals were adjusted in frequency, amplitude and duty cycle. The programmed signals then fed the output stage of the analog circuit for final biphasic current pulse adaptation by means of four operational amplifiers (OA 1, 2, 3 and 4 in Scheme 1). The output frequency was set within the 1 Hz to 1 kHz range, and current amplitudes were modulated from 5 to 50 µA. Films under test were placed on two copper paths as electrical contacts (indicated by a black arrow in Scheme 1). In order to measure the circulating current along the conducting films, an analog operational circuit was employed as a current to voltage converter. Finally, the output voltage was tested with a Tektronix TDS2024C oscilloscope. All stimulations were performed at room temperature.

2.6. Characterization techniques.

The morphology and size distribution of commercial TiO$_2$ NPs were assessed by means of transmission electron microscopy (TEM) in a Philips CM200 microscope.

Film surface morphologies were investigated by scanning electron microscopy (SEM) on metalized samples by means of a Jeol JXA-8600 instrument.

Grazing incident X-ray diffraction (GI-XRD) patterns of the prepared films were recorded with an X’Pert Pro PANalytical diffractometer, employing the Cu K$_\alpha$ line (wavelength 1.5406 Å). The electrical voltage and current were 40 kV and 30 mA, respectively. Data were collected for 20 angles ranging between 10° and 80° with a step size of 0.02° 2θ and 0.5s acquisition time per step.

Film topographical features (grooves depth) were determined with a KLA Tencor Alpha Step D100 profilometer, at a scan rate of 0.03 mm/s.

The resistance of the conducting films was calculated from the slope of current-voltage plots. Measurements were carried out at room temperature, both along and perpendicularly to the freezing direction, by means of a Xantrex XFR 300-4 DC power supply unit and a HP 34401A multimeter, with a distance of 0.8 cm between test leads.

3. Results and discussion

Fig. 3 shows a photograph and SEM images of a sintered TiO$_2$ film prepared from the highest freezing rate (30 mm/min). From now on, this sample will be referenced as 30-TiO$_2$ film.
Homogeneous ~ 2-3 µm thick films (Fig. 3 inset) were obtained on alumina substrates, which retained their structural stability after sintering. According to the high freezing rate employed, non-patterned surfaces were obtained (see also Fig. S1, Supplementary Information). This is expected when high ice formation velocities are induced. A high rate of immersion into liquid nitrogen prevents generating a high temperature gradient throughout the sample, which leads to isotropic ice growth and consequently to homogeneous microstructures. Structures like the one shown in Fig. 3b (and Fig. S1 in Supplementary Information) are consistent with high particle expulsion hindrance and randomly oriented ice crystal growth. Similar results were obtained for the 30-TiO$_2$ film before sintering. This corroborated that the thermal treatment did not disrupt the initial non-patterned homogeneous structure.

Fig. 4 shows the GI-XRD pattern corresponding to the 30-TiO$_2$ film heat-treated at 1000 °C. The diffraction pattern corresponds to a single phase which was indexed to the rutile phase of TiO$_2$, in agreement with the JCPDS 76-0649 file. The same pattern was registered for every film treated at 1000 °C, regardless of the immersion rate applied during the directional freezing stage.

In order to assess the possibility of modulating the film surface topology, different immersion rates were then tried. This would in principle allow entering the velocity gap where disruption of the planar advancing ice front takes place, leading to Mullins-Sekerka instability and consequently to anisotropic ice growth. The evaluation of the modulated 2D-patterning according to the freezing conditions will be analyzed in what follows.

Fig. 5 shows SEM images, captured under different magnifications, of a TiO$_2$ film immersed into liquid nitrogen at a lower rate (15 mm/min) compared to that of the 30-TiO$_2$ sample. This sample will be referred to as 15-TiO$_2$ film.

Experimental evidence showed that it is effectively possible to induce the 2D breakdown of the solidification front to a non-planar advancing ice morphology, by simply modifying the directional immersion rate. This effect has been already reported for 3D porous structures, however, to the best of our knowledge, this is the first time that this morphological transition is evidenced on patterned films. Region 1 depicted on Fig. 5a corresponds to the furthest end of the TiO$_2$ film with respect to the cooling liquid surface. This region is composed of highly aligned grooves along the freezing direction and it is preceded by a transitional region (region 2, neither ordered nor disordered) which links to a homogeneous non-oriented topology (region 3) (see details in Fig. S2, Supplementary Information).

By further lowering the immersion rate into liquid nitrogen (which is, by increasing the temperature gradient throughout the sample), the degree of order was extended to the whole film surface. This was accomplished on a film immersed at 5 mm/min. This sample will be referred to as 5-TiO$_2$ film. Fig. 6 shows SEM micrographs corresponding to a sintered 5-TiO$_2$ film (see microstructure details in Fig. S3, Supplementary Information).

Sample 5-TiO$_2$ exhibited highly oriented parallel patterns extending throughout the whole surface, with grooves ~ 5 µm wide and ~ 1.5 µm depth, as measured by profilometry. A detail of groove morphology is presented in Fig. 7.
partitions (perpendicularly oriented to the freezing direction). This finally leads to the bridging of primary TiO$_2$ paths (aligned along the freezing direction) by shorter ceramic NP partitions (perpendicularly oriented to the freezing gradient). The surface seen between ridges is composed of TiO$_2$ nanoparticles (although a ‘naked’ alumina surface may be also identified) but less interconnected compared to the joining bridges. A closer inspection of the surface is shown Fig. S4 in the Supplementary Information.

After employing the lowest dipping rate attainable with the immersion device (5 mm/min), an attempt to further lower the freezing rate was made. This would allow, a priori, to take control over the relative dominance of ice nucleation rate and crystal growth. For this purpose, the cold vapours of the freezing liquid were used. This was accomplished by submitting a TiO$_2$ film to nitrogen vapour, not placing the sample in contact with the liquid nitrogen. The film was kept at a distance of 3 cm from the liquid surface and it remained at that distance until it was completely frozen.

It has been widely reported that very slow freezing rates on aqueous dispersions lead to the rejection of particles by the planar advancing ice front. Thermodynamically, the interfacial free energy between the particles and the solidifying front ($\sigma_{pl}$) should be larger than the sum of interfacial free energies associated with solid-liquid ($\sigma_{sl}$) and particle-liquid ($\sigma_{pl}$) interactions for the particles to be effectively rejected forward as the planar ice advances ($\sigma_{sp} > \sigma_{sl} + \sigma_{pl}$). This effect has been successfully employed to collect particles on one side of the studied system once solidification was achieved, representing a useful method for removing pollutants (water purification). In addition to the thermodynamic criterion, the rejection of NPs will depend on extra forces exerted on them. For particles close to the solidification front, the most important forces are repulsive and drag ones. The relationship between these forces can in fact reinforce the thermodynamic contribution. Taking these concepts into consideration, only dragging of the TiO$_2$ NPs would be expected from a very low freezing rate imposed to the films, and non-patterned morphologies would have to be predicted. However, it has been already demonstrated that NPs can only be concentrated by the advancing ice front up to a certain breakthrough point. The TiO$_2$ NPs may be pushed along the solid-liquid interface until the capillary drag force is counterbalanced by the force resulting from the osmotic pressure of the suspension. If this happens, patterned films (but in this case, perpendicularly oriented to the freezing direction) might be produced. This is, in fact, demonstrated herein.

Fig. 8 depicts SEM images of the film produced after the freezing process into nitrogen vapour. The freezing rate (~1 mm/min) was measured from the advancing solidification front seen on the substrate being frozen (the dispersion/ice interface was clearly visualized as a horizontal opaque layer advancing upwards). This film will be referenced as 1-TiO$_2$ sample.

Fig. 8 SEM images of the surface developed in sintered 1-TiO$_2$ film. Arrows indicate the freezing direction. Bars: 100 µm (a) and 200 µm (b).

Highly-aligned TiO$_2$ patterns perpendicularly oriented to the freezing direction were obtained. The morphologies seemed to be similar to those obtained from the so-called “ice lenses” which have been already reported for other ice templated colloidal dispersions. A 90-degree tilting change (compared to 5-TiO$_2$ sample) was produced by simply exposing the film to liquid nitrogen vapour (Fig. 8a) (See details in Fig. S5a, Supplementary Information). This behaviour may be explained by a continuous sequence of dragging and ‘engulfing’ events, both temporally and spatially interspersed. The growing planar ice front would reject the NPs as it advances on the alumina substrate, engulfing them when the osmotic pressure of the deposited suspension equals the dragging force. However, tilted grooves (at about 45º with respect to the freezing direction) were also observed in the same sample (Fig. 8b, indicated by ellipses) (See also details in Fig. S5b, Supplementary Information). This effect may be associated with a change of temperature gradient orientation during the freezing process, as a consequence of the freezing conditions (the nitrogen atmosphere has to be precisely controlled). The tilted features closely depend on the relationship between the crystal growth rate along the preferred crystal growth direction and the rate along the temperature gradient direction. Changes in direction of the temperature gradient due to vapour fluctuations could have induced, in this case, orientational changes during TiO$_2$ NP rejection. Although this structured film was generated from a phenomenon which is mechanistically different from that previously discussed for the other films, highly ordered TiO$_2$ paths separated by grooves ~ 20-30 µm wide were also obtained.

The presence (in 5-TiO$_2$ and 1-TiO$_2$ films) or absence (in 30-TiO$_2$ film) of 2D patterns could produce a significant influence on transport properties associated with the film anisotropic features, and this is precisely one of the most interesting aspects that can be exploited from these 2D structures.
Directionally frozen 3D porous scaffolds developed from TiO$_2$ NP dispersions have been demonstrated to be excellent supporting materials. The presence of aligned ‘highways’ throughout the whole scaffolds has been shown to be useful not only to the transport of analytes through the as-developed macroporosity, but of incident radiation as well (orientation-dependent light harvesting devices). This might also be present, in good agreement with the review article by Szot et al. Analogous results were obtained for all of the treated films, however, significant differences in electrical resistances were evidenced depending on both structuring features and pattern orientation.

The homogeneous non-patterned 30-TiO$_2$ film exhibited the lowest resistance values (3-4 kΩ) compared to its freeze-patterned counterparts (5-TiO$_2$ and 1-TiO$_2$ samples) (see Table 1).

### Table 1 Characteristics and electrical resistances of the prepared films

<table>
<thead>
<tr>
<th>Sample</th>
<th>Freezing Rate (mm/min)</th>
<th>Patterning</th>
<th>Resistance Dependent on Surface Orientation</th>
<th>Electrical Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-TiO$_2$</td>
<td>30</td>
<td>absent</td>
<td>no</td>
<td>3-4 kΩ</td>
</tr>
<tr>
<td>5-TiO$_2$</td>
<td>5</td>
<td>along the f. d.</td>
<td>yes</td>
<td>3-4 kΩ</td>
</tr>
<tr>
<td>1-TiO$_2$</td>
<td>1</td>
<td>perpendicular to the f. d.</td>
<td>yes</td>
<td>open circuit</td>
</tr>
</tbody>
</table>

**f. d.: freezing direction**

The resistance of 30-TiO$_2$ sample did not show any dependence on surface orientation, as expected from non-patterned conducting surfaces due to the isotropic and continuous network of interconnected grains. As to the freezing-patterned films, they interestingly evidenced strong orientation-dependent electrical resistances. Higher resistance values, compared to 30-TiO$_2$ sample, were explained on the presence of grooves taking part of the film topology. For the 5-TiO$_2$ film, measurements determined along the freezing direction gave values of 3-4 kΩ (the same as that for the non-patterned sample), while it displayed a resistance of ~ 10 kΩ perpendicular to the structured patterns. On the other hand, for the 1-TiO$_2$ film, measurements determined along the freezing direction (perpendicularly to the ordered fringes) gave an open circuit response, which was attributed to the poor film continuity as revealed by SEM (Fig. 8, and Fig. S4 in Supplementary Information). Measurements performed perpendicular to the freezing direction (along the aligned conductive paths) gave resistance values of 30-40 MΩ. Such high values were assigned to the partial contact among titanium oxide particles along conducting strips (Fig. 8, and Fig. S4 in Supplementary Information). The experimental results demonstrated that, in fact, the anisotropic features effectively exert a strong influence on electrical transport properties of the 2D-ice templated films. As a consequence, the possibility of employing these conducting assemblies as electrical stimulation-related supports was evaluated.

Electrical stimulation (ES) has been shown to be a promising technique for a variety of stimulus-assisted medical treatments. Electric pulses have been employed to induce responses in nerve, skin and skeletal tissues. In particular, it is known that ES affects neuronal activity by triggering a change of voltage gradient through neuronal membranes, reason why this approach has recently raised much interest as a potential therapeutic tool for the treatment of injuries related to the peripheral nervous system (PNS). As to this application,
whether monophasic or biphasic current pulses is better for peripheral nerve regeneration is still an issue of debate. While it is true that monophasic stimuli can lead to the orientation of cell membrane proteins creating in this way an “electrical cue” for regeneration,\textsuperscript{51,55} it is widely accepted that biphasic stimulation has several advantages over the monophasic approach. Among these advantages, biocompatibility, charge balance and maintenance of a constant pH are the most remarkable ones. In addition to all of these electrical stimulation-related aspects, it has been also demonstrated that “guidance cues” (given by topographyally-structured surfaces made up of parallel grooves) provide considerable in vitro neurite guidance information.\textsuperscript{53} In the same line of research, Rajnicek and McCaig investigated the mechanism by which contact guidance, given by grooved surfaces, affected the pattern of growth cones of both embryonic Xenopus spinal neurites and rat hippocampal neurites.\textsuperscript{54} In summary, not only electrical stimulation but topographical features as well have been demonstrated to exert strong morphogenetic stimuli for developing neurons, with all of these characteristics being very well stated in a recent review.\textsuperscript{55} All this knowledge could in principle be thought to have a strong impact on in vitro stimulating approaches towards a better understanding of the mechanisms involved on neuronal evolution.

For all of this, and envisioning a potential use of the prepared films as in vitro electrical-test supports, a stimulator electronic circuit was developed and connected to the patterned films. Fig. 10 shows photographs of the two modules (ECB and ACA, see Scheme 1) that take part of the stimulating electronic circuit, and a capture of the biphasic signal obtained by testing the 5-TiO\textsubscript{2} film.

The film was connected to the analog circuit via gold electrodes deposited (by sputtering) at both film ends (Fig. 10b). The applied stimulation allowed delivering electrical currents of different amplitudes and charge-balanced biphasic pulses of different frequencies. Current values from 5 to 50 µA and stimulus signals from 1 Hz to 1 kHz were successfully delivered through the film.

The 30-TiO\textsubscript{2} film was employed as control sample, which gave the same electrical response as that of 5-TiO\textsubscript{2} specimen. The only difference between these samples is the topographical features. Sample 5-TiO\textsubscript{2} combines the two specific ideas of this work, namely the electrical stimulation and the oriented patterned topology.

The results shown here (taking specially into account the current and frequency ranges that can be scanned, and the biocompatibility properties recognized to Magnéli phases) give support to the potential use of these electrically conducting structured platforms on in vitro cell-related stimulation therapies.

4. Conclusions

Two-dimensionally patterned TiO\textsubscript{2} films were successfully prepared by directional freezing of TiO\textsubscript{2} NP aqueous dispersions deposited on alumina substrates. By carefully selecting the adequate freezing rate, it was possible to modulate the surface morphological features. On going from higher (30 mm/min) to lower (1 mm/min) freezing rates, the following sequence of 2D-assembled films was produced: non-patterned (homogeneous) films → patterned films with grooves highly aligned along the freezing direction → patterned films with grooves perpendicularly oriented to the freezing direction.

Electrically conducting films were obtained by treating sintered samples at high temperature in a reducing atmosphere. The morphological features (presence or absence of oriented 2D patterns) produced a strong influence on the electrical properties. While homogeneous films exhibited the lowest resistances and showed no dependence on measurement orientation, highly-oriented grooved films evidenced significant orientation-dependent electrical resistance values. In order to evaluate the possibility of using these patterned systems as in vitro electrical stimulation-related supports, a stimulator electronic circuit was developed and connected to the prepared films. Charge-balanced biphasic stimulus pulses, with tunable current amplitudes and frequencies, were successfully delivered through the 2D assemblies. This gave support to the potential use of the combined approach (stimulating circuit + patterned conducting films) on in vitro-assisted medical studies. Work is in progress in this direction.

The proposed method towards tunable 2D-patterned films exhibits the following interesting features: (i) cost-effective facilities are required, (ii) NPs of different nature (metallic, semiconducting, polymeric) could be a priori employed, provided their dispersions are stable, (iii) the procedure can be scaled up on substrates of several centimeters, and (iv) the proposed idea could be implemented on different water-soluble/dispersable polymeric-NP combinations, leading to novel multifunctional composite films. All these features could be used to prepare new materials displaying a wide range of properties made to order.

Acknowledgements

This work was supported by the National Research Council (CONICET, Argentina), the National Agency for the Promotion of Science and Technology (ANPCyT, Argentina), the University of Mar del Plata and the University of Buenos Aires (Argentina).
Authors specially thank Victor N. Romeo for the construction of the controlled-immersion device. Thanks are due to Humar Avila Vanegas for Fig. 2.

Electronic Supplementary Information

Surface topographical details by SEM characterization are available.

Notes and references

Graphical Abstract for

2D-ice templated titanium oxide films as advanced conducting platforms for electrical stimulation

Hernán E. Romeo, Fernando Trabadelo, Matías Jobbágy and Rodrigo Parra

Controlling film surface properties (topography and chemistry) while executing electrical stimulation is one of the most addressed research topics in materials chemistry. In this work, titanium oxide conducting films were 2D-structured by directional freezing and tested as advanced platforms for electrical stimulation.