Expert Opinion

Self-ordered nanopore and nanotube platforms for drug delivery applications

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The application of nanotechnology to medicine termed as 'nanomedicine' is recognised as an emerging field with enormous potential for developing new therapeutic concepts. A range of nanoscale materials have been explored in the last few years for drug delivery to address the problems associated with conventional drug therapies such as limited drug solubility, poor biodistribution, lack of selectivity and unfavourable pharmacokinetics. Among them, nanoporous materials with ordered and controlled pore structures, high surface area and pore volume, attracted great attention, particularly for implantable drug delivery systems. This review presents the recent progress in this field focused on electrochemically engineered nanopores/nanotube materials such as nanoporous alumina and nanotubular titania. The basic concept of fabrication of these unique materials using a self-ordering process, description of their structural properties, biocompatibility and recent applications for therapeutic implants is presented.

Keywords: drug delivery, electrochemical anodisation, nanoporous alumina, nanotube titania

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1. Introduction

To avoid problems associated with conventional drug therapies related to limited drug solubility, poor biodistribution, lack of selectivity and unfavourable pharmacokinetics, considerable research has been directed in the last two decades towards the development of new and more efficient drug delivery systems [1-3]. Hydrophobic drugs do not dissolve in the blood and do not reach their target, which reduces their pharmacological efficiency [3]. Bioactive agents such as proteins, nucleic acids, enzymes and genes administered through oral or intravenous routes can be degraded prematurely by metabolism or by enzymatic conditions existing in gastrointestinal tracts [3,4]. These challenges have contributed to the development of new controlled drug delivery systems to achieve various goals, such as delivery of therapeutic agents to the desired site, enhancing bioavailability and drug protection.

The application of nanotechnology to medicine, referred to as 'nanomedicine', is recognised as an emerging field with enormous potential for the development of new therapeutic concepts [5-7]. Knowledge of materials at the nanoscale may accelerate the improvement of drug delivery systems, especially in treating life-threatening conditions such as cancer and heart disease [8,9]. A range of new nanoscale materials have been explored in recent years for drug delivery applications, including nanoparticles, nanofibres, dendrimers, liposomes, polymer micelles, carbon nanotubes, fullerenes, nanogels, nanocrystals, viral vectors and virus-like particles (VLP) [5,7-11]. Among them nanoporous and nanotube carriers, owing to their unique features such as low cost fabrication, controllable pore/nanotube structure, tailored surface chemistry, high surface area, high loading capability, chemical resistivity and mechanical rigidity, have engaged a special niche in drug delivery technology [12-14]. Drug

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delivery systems based on mesoporous silica prepared by organic synthesis and porous silicon fabricated by electrochemical process have been widely explored in the last few years [12-17]. However, owing to their exceptional properties, increased research interest has recently been focused on electrochemical synthesis of self-organised nanopores and nanotube materials from transition and valve metal oxides [11,14,18-20]. Nanoporous anodic aluminium oxide (AAO) and nanotubular titania (TiO_2) are popular examples, and typical images showing their highly ordered pore and nanotube structures are presented in **Figure 1**.

This review presents recent developments on applications of self-ordered nanoporous and nanotubular platforms for the delivery of therapeutics, focused primarily on nanoporous alumina and nanotubular titania. First, the phenomenon of the electrochemical formation of nanopores and nanotubes by a self-ordering process is explained. Then fabrication, properties and biocompatibility of these two remarkable materials are addressed briefly, including the current stage of knowledge and recent studies on their implantable drug delivery applications. Finally, examples of nanopore and nanotube materials fabricated from other biocompatible metals and their alloys are discussed.

2. Self-organised electrochemical formation of nanopore and nanotube structures

Self-assembly, or the spontaneous organisation of small units into large-scale ordered and stable structures, is ubiquitous in nature and spans a wide range of scales, from the molecular, nano, micro and macro to the planetary scale. Self-assembly and self-organised (or self-ordered) processes are recognised as cost-effective, and an elegant route in nanotechnology for the spontaneous generation, ordering and hierarchical organisation of complex and functional nanomaterials at the molecular and nano scale [21,22]. Several self-assembly synthetic approaches based on chemical, electrochemical, sol-gel and hydrothermal methods have been introduced [22,23]. The recent progress in electrochemical approaches for self-organised formation of nanostructures, such as highly ordered nanoporous alumina and nanotubular titania, placed this method as one of the most popular nanofabrication strategies, with more than 1000 publications in the last 5 years [11,18-20,24-27].

The basic set-up and schematic of the self-ordering process based on electrochemical anodisation is outlined in **Figure 2**. Anodic oxidation (or anodisation) is an electrochemical process by which an electrochemically active species is oxidised by the passage of current or an applied voltage in an appropriate electrolyte. During this process in an electrochemical cell, the metal serves as the anode connected to the positive terminal of a direct current (DC) power supply, while a chemically stable metal such as platinum and carbon serves as the cathode (**Figure 2A**). When the circuit is closed, electrons are withdrawn from the metal at the positive terminal, allowing ions at the metal surface to react with water to form an oxide layer on the metal. The overall electrochemical reactions that occur during electrochemical anodisation of metal are presented in **Figure 2A**. At the beginning of anodisation, the metal surface is covered with compact and uniform oxide film (**Figure 2B**, stage I). However, the distribution of the electric field (E) in the oxide is strongly influenced by surface morphological fluctuations generating more locally focused electric field (**Figure 2B**, stage II). As a result of field-enhanced dissolution in oxide films, the pores start to form (**Figure 2B**, stage III), and finally uniformly distributed pores are formed when the pore growth process reaches a steady-state (**Figure 2B**, stage IV). The typical current density curve during this process showing these stages is presented in **Figure 2C**. The final porous oxide layer is formed as a result of the continuous process, which involves competition between dissolution of oxide at the oxide/electrolyte interface and oxidation of metal at the oxide/metal interface [19]. However, these pores are not ordered at the beginning, but during the anodisation process as the pores grow into the bulk metal and improve their arrangement after a long anodisation time. The driving force for self-assembly has been attributed to mechanical stress caused by the repulsive forces between neighbouring pores that lead to ideal self-organisation of AAO with perfect hexagonal arrangement of pores (**Figure 1A, B**) [26]. Depending on the type of metal, electrolyte and voltage, two typical oxide growth morphologies can be obtained by the self-organising anodisation process, including porous and tubular structures (**Figure 3**) [19]. Metals such as Al, Nb, Ta and Hf were found to form pore structures, and several other metals (Ti, Ta, Hf, W, Zr, Nb, TiNb, TiZr, TiAl, TiAlNb, etc.) form tubular structures (**Figure 3**). The formation mechanism for the oxides with nanotubular morphology is governed essentially by the same self-ordering principle and growth mechanism for porous oxide such as highly ordered AAO. Tuning the pore to nanotube morphology has been demonstrated for some metals (Ti, Zr, Ta, Hf) by changing the anodisation parameters such as temperature, applied voltage, electrolyte, pH viscosity and solvent [19].

3. Nanoporous anodic aluminium oxide

3.1 Fabrication and properties

Nanoporous AAO fabricated by electrochemical anodisation of aluminium is now one of the most popular nanomaterials because of its simple and low-cost fabrication, and remarkable properties such as chemical, thermal stability, hardness, high surface and highly ordered pore structures [18,26-28]. Its applications are diverse and include molecular separation, template synthesis, catalysis, solar cell, energy storage, biosensing, cell growth and drug delivery [26-35]. The structure of AAO can be described as a close-packed hexagonal and perpendicular orientated array of columnar cells, each containing a central pore, of which the size and interval can be controlled by changing the anodisation conditions (**Figure 4A**) [26,28].

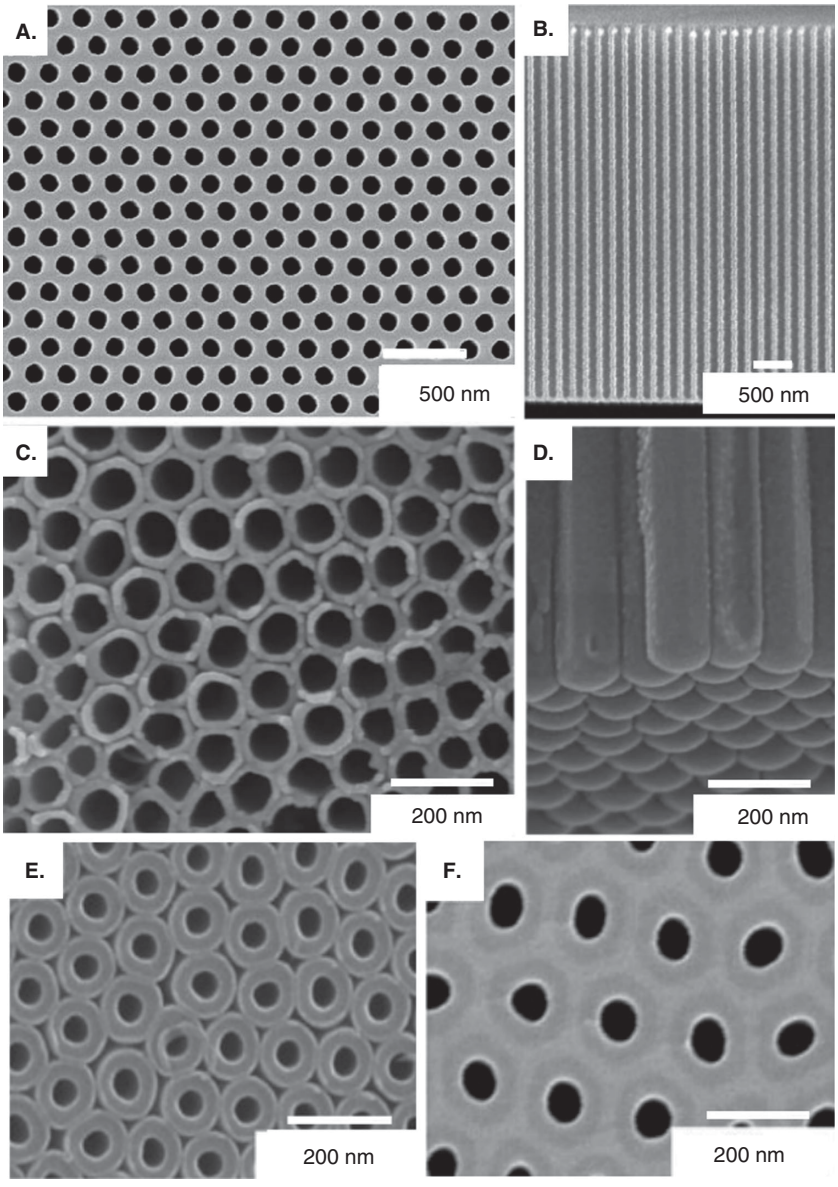

Figure 1. SEM images of electrochemically fabricated nanoporous and nanotube layers. A, B. Pore structures of anodic aluminium oxide, top view and cross-section. **C, D.** Nanotube structures of titania, top view and bottom view. **E, F.** Transition from nanopores to nanotubes, self-ordered anodic oxide structures on TiNb, top view.
A, B. Reproduced with permission from [43].
C – F. Reproduced with permission from [19].

Important features are: fully controllable structural dimensions including pore diameter from 10 to 400 nm; interpore distances from 50 to 600 nm; pore aspect ratio from 10 to 5000; the thickness of porous layer from half a micrometre to several hundred micrometres; pore density 10^9 – 10^{11} cm^{-2}; and porosity (5 – 50%) [18,26,35-37]. Fabrication of porous alumina can be performed on conformal to arbitrary surfaces, either flat or curved surfaces, including bulk Al, foils, wires, tubes, and Al films on Si wafers, glass, titanium; and also on various forms such as porous layer on surface and self-supporting membranes. The fabrication process is simple, low cost, does not need a clean room, and is compatible with existing lithographic and micro-electro-mechanical systems (MEMS) and technologies.

Numerous studies over several decades have explored the anodisation conditions of aluminium, such as voltage, current, electrolyte composition, concentration, temperature and pre-patterning of surface in order to achieve a self-ordering regime and highly ordered AAO pore structures with desired pore diameters [38-41]. In general, three well-studied growth regimes using conventional, so-called mild (MA) or low-field anodisation in H_2SO_4 at 25 V, $H_2C_2O_4$ at 40 V, and H_3PO_4

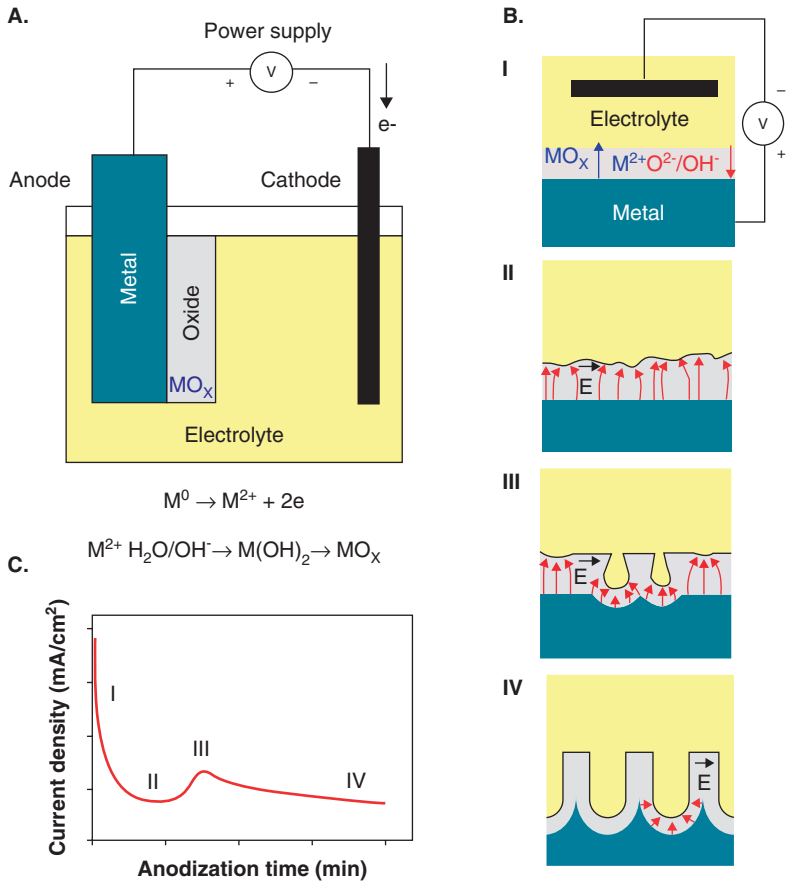

Figure 2. A schematic diagram showing pore formation by electrochemical self-ordering. A. Scheme of electrochemical cell for anodisation and corresponding electrochemical reactions. **B.** Scheme of pore formation, which includes several steps: (I) the formation of oxide layer on metal surface; (II) local field distributions caused by surface fluctuations; (III) the initiation of pore growth by field-enhanced dissolution; and (IV) the pore growth in steady-state condition. **C.** Typical current density curve obtained with anodisation showing these stages [19].

at 194 V, giving 63, 100 and 500 nm interpore distances are accepted as the optimal conditions for fabrication of AAO (**Figure 4B**). It is generally accepted that the pore diameters (*D*p) and interpore distance (*D*c) are linearly proportional to the forming potential of the steady-state growth of anodic porous alumina with a proportionality constant of ~ 1.29 nm/V (*D*p) and 2.5 nm/V (*D*c) [26]. The significant improvement of self-ordering process discovered by Masuda and Fakuda in 1995 relies on self-ordering of pores at the bottom of AAO channels after the first anodisation step [42]. This two-step anodisation approach has been a particularly important advance towards simple fabrication of highly ordered porous alumina [42]. By combining the self-ordering process and pre-patterned surface of aluminium using various imprinting techniques to guide pore initiation, it is possible to obtain not only long ordered arrangements but also change from a hexagonal to a square arrangement [43]. One of the disadvantages of mild anodisation is the low rate, typically (1 – 2 µm/h), which requires a fabrication time of several days. This problem has recently been solved by using a higher current anodisation condition termed hard anodisation (HA). An anodisation rate of 50 – 100 µm/h has been achieved with a considerably increased speed of fabrication [44,45].

3.2 Structural and surface modifications of nanoporous AAO

Several new fabrication processes of nanoporous AAO with tailored pore morphologies and different internal pore geometries have been demonstrated for the preparation of branched, dendritic, hierarchical, multilayered and modulated pore structures [46-48]. An anodisation method called cyclic anodisation has been introduced recently by Losic *et al.* for fabrication of pore structures with different pore geometries and complex pore architectures (**Figure 4C**) [49,50]. The nanostructuring of internal pore structures is recognised as a useful strategy for a range of applications, including drug delivery. The nanostructured pores could have a considerable impact on diffusion of drug molecules from the pores and therefore provide sustained drug release. The increase in surface area of porous matrix compared with smooth pores is also useful to

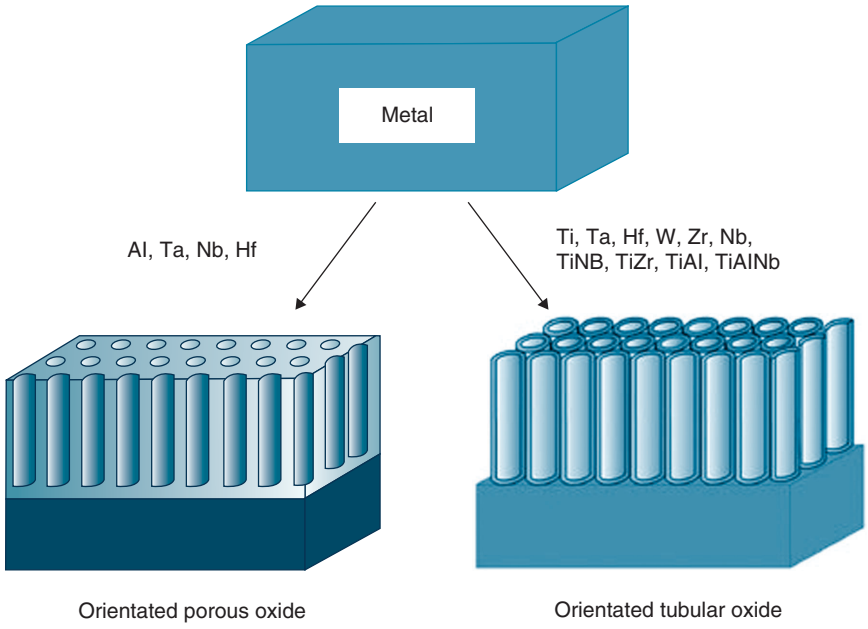

Figure 3. Schematic of two typical morphologies (nanopore and nanotubes) of oxide layers formed by the self-organised anodisation process, which can be obtained from different metals by controlling anodisation parameters such as temperature, applied voltage, electrolyte, pH viscosity and solvent.
Adapted with permission from [19].

increase the drug loading capacity. The authors emphasise the advantages of these new fabrication strategies for offering new opportunities for designing new nanoporous architectures that can potentially have future impact on the development of new drug delivery systems.

The surface of porous alumina is inherently charged as a consequence of the equilibration of charged crystalline lattice defects on the surface [51]. Depending on the net concentration of lattice defects, the surface may be positively or negatively charged with an attendant redistribution of oppositely charged lattice and electronic defects in the near surface region, which can have a significant impact during interaction with biological materials [51]. Therefore, it is highly desirable to modify the surface of nanoporous AAO and improve biocompatibility for applications that involve interaction with biomolecules and cells, such as protein separation, immunoisolation, cell adsorption/growth, tissue engineering and drug delivery. Various solid and soft surface modification techniques of AAO have been explored in the past, including atomic layer deposition (ALD), chemical vapour deposition (CVD), thermal vapour metal deposition, chemical, electrochemical deposition, sol-gel, layer-by-layer deposition and plasma polymerisation [52-55]. A range of surface funtionalisations using organic molecules such as silanes, polythiols, *n*-alkanoic acids, polyelectrolyte layers, poly(ethylene glycol) (PEG), chitosan, poly(sodium styrenesulfonate) (PSS), lipid bilayers and plasma polymers have been reported [54-61]. The fabrication of AAO with layered surface chemistry and multifunctional and tailorable properties inside pores is a particularly important accomplishment towards designing AAO with controlled drug delivery properties (**Figure 4C**) [62]. Unlike with unmodified pore surfaces, drug molecules can be covalently immobilised in the modified pores or trapped between functional layers. Furthermore, drug release can be controlled by triggering an external stimulus such as pH, UV or temperature. These studies suggest that surface modification can be used as a promising strategy to improve the loading and release properties of AAO with a good prospect of adjusting their properties for specific drug delivery applications [63].

3.3 Biocompatibility

Biocompatibility is a prerequisite for the application of new biomaterials and it is defined in terms of cellular response and tissue integration of implantable biomaterials. The bioceramic alumina has been proven as biocompatible and consequently widely used clinically for implants in orthopaedic proteases and as a dental implant material [64]. The main biocompatibility studies were performed on nanoporous AAO related to their applications for orthopaedic and blood (coronary) implants and immunoisolation [65-73].

A major challenge in orthopaedic biomaterials research is to design surfaces that will promote *in vitro* and *in vivo* osteogenesis. However, the influence of surface micro- and nanotopography on osteogenesis is still not well understood. Efforts to use smooth-surfaced alumina have been widely reported in the past, but recent studies have moved towards nanostructured alumina, which shows better bone in-growth [65-70]. The use of nanostructured biomaterials in bone engineering is

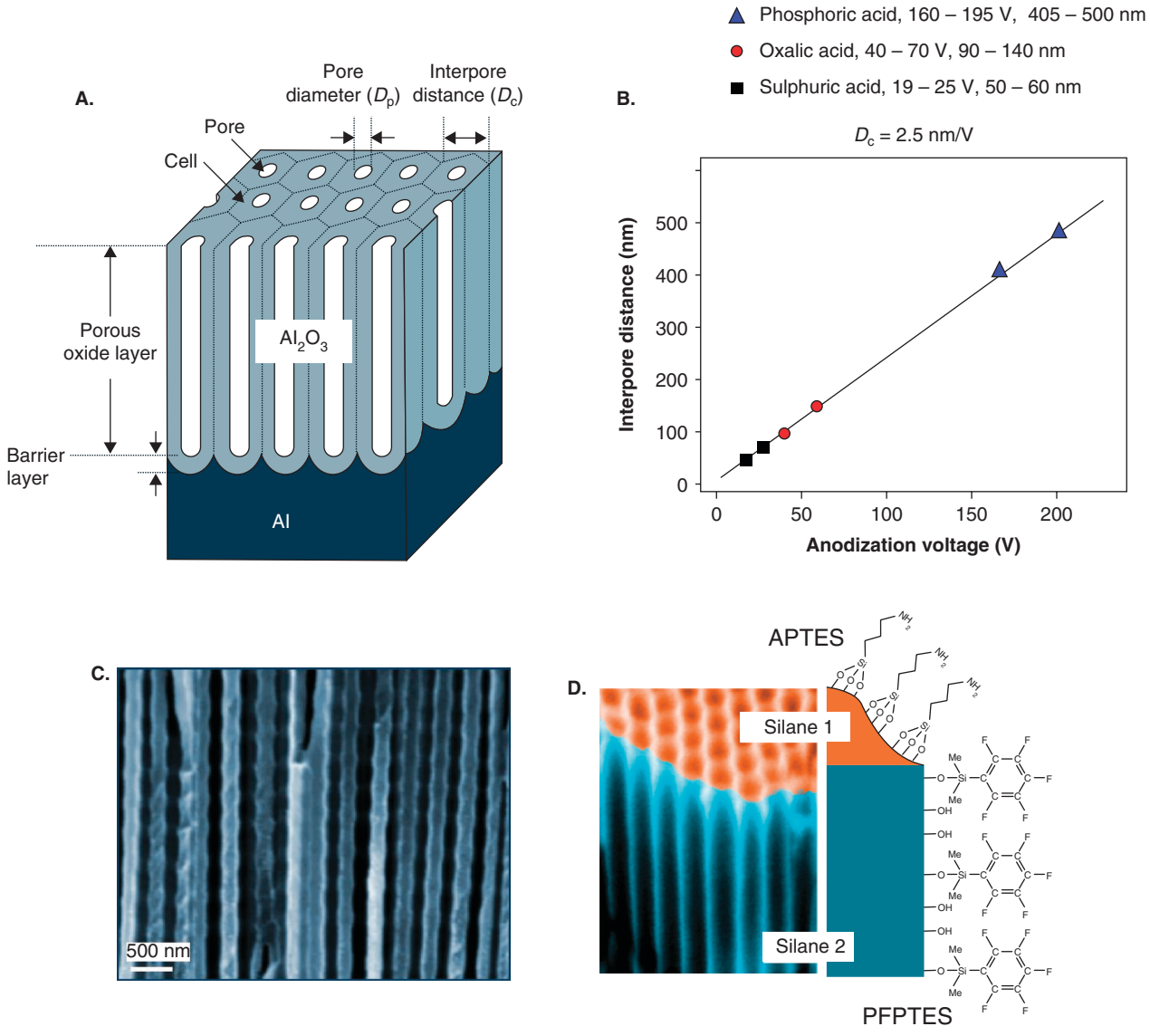

Figure 4. A. The scheme of nanoporous anodic alumina (AAO) structures fabricated by electrochemical anodisation of aluminium. **B.** The dependence of interpore distances and pore diameters of AAO on the electrolyte used (sulphuric, oxalic, phosphoric acid) and anodisation condition (voltage) [44]. **C.** AAO with shaped pore structure fabricated by the cyclic anodisation process, which involves periodic changing of voltage or current during anodisation [49]. **D.** AAO with different surface chemistry on the top and inside of pores [62].

biologically inspired because bone is a naturally occurring porous ceramic material composed of nano-sized organic and mineral phases that form a large macrostructure [74]. Proteins in extracellular bone matrix and calcium phosphate, important constituents of the bone matrix, are nanostructured but the porosities in human bone are predominantly in the range 1 – 100 µm. Earlier studies using oxide ceramics showed that a porous surface with pore diameter of ~ 100 µm is optimal for bone in-growth to maintain blood supply to connective tissue.

Recent studies using nanoprous alumina, however, have revealed that much smaller pores of AAO allow bone in-growth [65-70]. Study of osteoblast response to nanoporous AAO membranes with pore diameters of 30 – 80 nm confirms that extending growth of osteoblasts into the nanopores produced an active matrix in the form of fibrous extensions that contain calcium and phosphorus, typical elements of the bone matrix [66]. A similar conclusion was reached by Popat *et al.*, who reported that nanoporous AAO membranes showed significantly improved osteoblast adhesion and proliferation in comparison with amorphous alumina, aluminium and glass [65]. In this case, a nanoporous surface was revealed as a framework in which osteoblasts produce new bone. Further improved osteoblast adhesion and growth on anodised alumina with the same pore diameters (30 – 80 nm) is reported with modified surfaces by physically adsorbing vitronectin or covalently immobilising a cellular adhesive

peptide arginine-glycine-aspartic acid-cysteine (RGDC) [69]. These encouraging studies show the potential of porous AAO for use as a biocompatible platform for bone growth and orthopaedic implant applications, and more studies are expected in the near future.

The interactions between nanoporous AAO with different pore diameters (20 and 200 nm) and whole blood and platelet-rich plasma were investigated by Karlsson *et al.* [70-73]. The blood–implant contact leads to a series of interlinked events such as protein adsorption, complement activation, platelet and leukocyte adhesion/activation, and activation of the coagulation cascade [71-73]. On blood–implant contact, almost instantly the surface is covered with a layer of plasma proteins. Platelet agonists interact with specific receptors on the platelet plasma membrane, generating physiological responses, including: release of intracellular granule contents, change in shape that promotes adhesion, increased affinity to soluble fibrinogen, rearrangements of the membrane phospholipids into procoagulant surface and platelet microparticle (0.1 – 1 μm) release, often used as markers of platelet activation [73]. Immunocytochemical staining and scanning electron microscopy (SEM) analysis indicate different behaviour of AAO with different pore size in contact with whole blood. AAO membranes with 200 nm pore diameters caused lower complement activation, high platelets and nonsignificant microparticle adsorption. On the contrary, AAO membranes with 20 nm pore diameters caused higher complement activation, negligible platelets and significant microparticle adsorption [72]. It is postulated that different protein adsorption and patterning is created on the surface of AAO depending on the pore size, affecting availability of receptors and binding sites, and also platelet activation. The neutrophile reaction was shown to depend on the type of protein adsorbed on the surface of AAO membrane (pore size 20 and 200 nm) [71]. Neutrophile activation was seen to a higher extent on the uncoated and fibrinogen-coated AAO surfaces in comparison with serum and collagen-coated alumina surfaces. On the contrary, osteoblast development after 24 h was better on collagen-coated AAO in comparison with uncoated, fibrinogen and serum-coated AAO surfaces.

Earlier, *in vivo* biocompatibility investigations of coronary stents with porous AAO layer using a rabbit restenosis model proved biocompatibility and absence of inflammation [75]. However, recent reports on *in vivo* biocompatibility of therapeutic alumina stent coatings investigated using a pig restenosis model indicated inflammatory response [76,77]. The studies on immunoisolation using capsules with AAO membranes with 75 nm pore diameter showed that AAO is non-toxic and without significant complement activation *in vitro* [67]. However, *in vivo* implantation of these capsules into the peritoneal cavity of rats induces a transient inflammatory response, and PEG is useful in minimising the host response to the materials. Blood vessels are seen in the tissue treated with PEG-modified capsules, which suggests that the major amount of inflammation is due to the procedure itself, whereas surrounding tissues are relatively undisturbed. Considering that inflammatory response of porous alumina can be minimised by PEG surface functionalisation, further investigations are required to establish whether surface modifications can eliminate or minimise observed inflammatory/restenosis effect.

3.4 Implantable drug delivery applications of AAO

The use of porous alumina for biomedical delivery applications has been explored in several domains including: therapeutic devices for bone and dental tissue engineering, coronary stent implants and carriers for transplanted cells, that is, immunoisolation.

3.4.1 *Coronary stents implants*

The coronary stent implantation has been established as superior to conventional angioplasty in the treatment of coronary artery disease, but restenosis still remains one of the crucial challenges in interventional cardiology [78,79]. To overcome this major disadvantage, the concept of drug-eluting stents has recently been introduced, where drugs are trapped in polymer films coated on stents, which allows a controlled drug release [78]. Active coating of stents is known to suppress neointima proliferation by releasing anti-inflammatory or antiproliferative therapeutics, for example, immunosuppressive drugs (tacrolimus, sirolimus) and cytostatic drugs (paclitaxel) [78,79]. Although the clinical use of polymer stents has been approved, their inflammatory reaction is still a serious limitation. An alternative approach has been reported recently to design stents with a nanoporous alumina layer filled with anti-inflammatory drugs [75]. The design of active stent coating is one of the most important therapeutic examples for drug release from nanoporous AAO. A SEM image of a stainless-steel stent with a deposited porous alumina layer fabricated by electrochemical anodisation on aluminium film deposited on stainless-steel stent is shown in **Figure 5A, B** [75]. Wieneke *et al.* investigated alumina nanoporous stent coatings loaded with tacrolimus, an immunosuppressive drug [80]. The effect of AAO pore diameter and depth on the release of 2-deoxyadenosine showing sustained release for up to 40 h with pore diameters ~ 20 nm and further delay was achieved by changing the depth from 1 to 4.4 μm (**Figure 5C, D**). These results show that stents with an AAO layer could offer alternative solutions by replacing polymer-coated stents and overcome existing problems of inflammatory response and increased neointima formation. The performance of porous AAO stents can be improved further by designing optimal pore structure and surface modification to increase their compatibility and loading capacity, including controlled release.

3.4.2 *Biocapsules for immunoisolation*

The encapsulation of living cells, that is, cellular immunoisolation using microfabricated capsules with semipermeable barriers, has been investigated over the past few decades as a

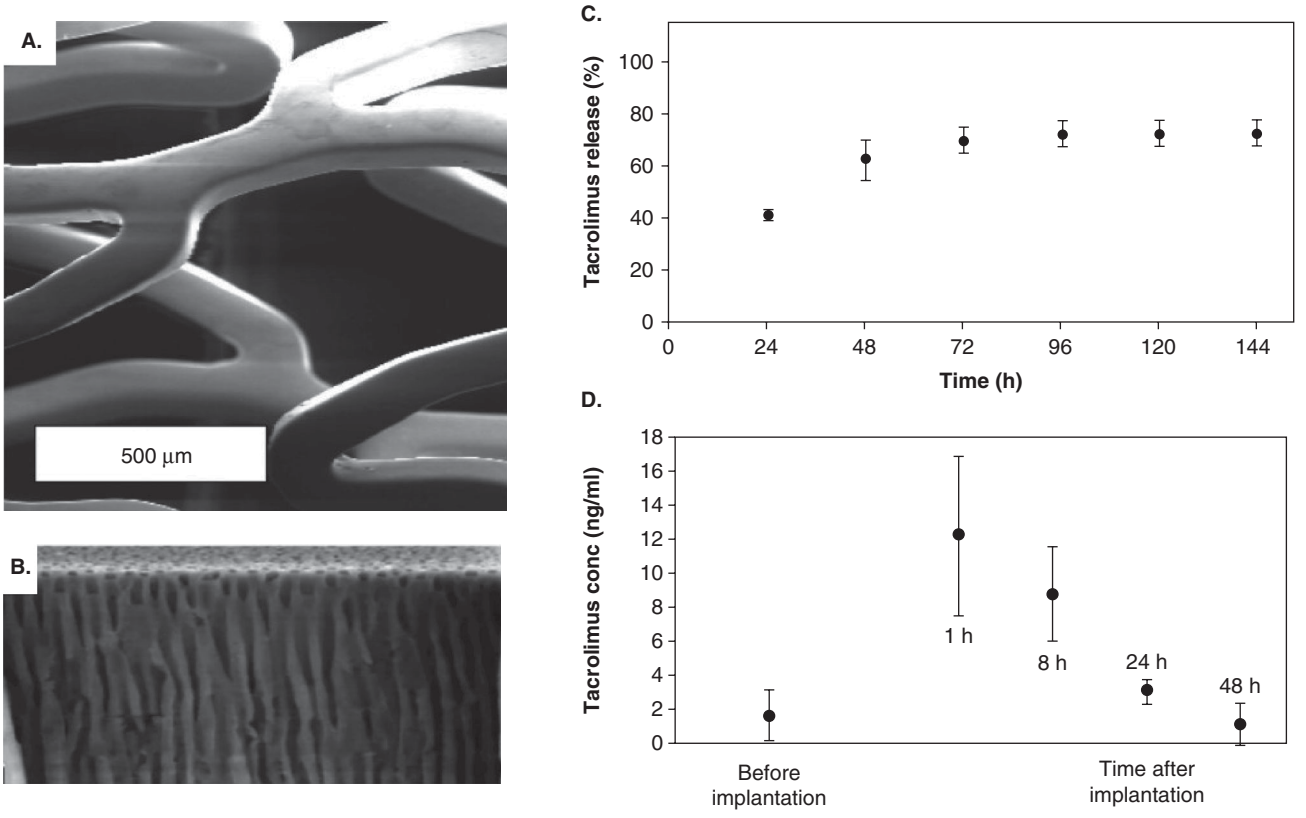

Figure 5. A, **B.** SEM image of stents coated with nanoporous alumina oxide (cross-section of AAO layer on the top) with pore size between 5 and 15 nm and pore density 10^{12} cm^{-2} allowing sufficient drug loading with tacrolimus. **C.** *In vitro* drug release. Cumulative tacrolimus release within the first 144 h. After 72 h, ~ 75% of the loaded 60 µg tacrolimus has been eluted. About 25% is still trapped in the nanoporous AAO coating. **D.** *In vivo* drug release. Time course of whole blood tacrolimus concentration after stent implantation in the carotid arteries of rabbits.

A, **B.** Adapted with permission from [75].

D. Adapted with permission from [81].

potential treatment of several diseases, such as diabetes mellitus, Parkinson's and Alzheimer's [67,81,82]. The encapsulation of living cells is a promising future therapy because it has been proved that immunoprotected cells such as pancreatic islets or hepatocytes can respond physiologically both *in vitro* and *in vivo* to appropriate stimuli [83]. Therapeutic cells are encapsulated within a semipermeable membrane that provides the exchange of insulin, oxygen, nutrients and cellular waste; however, larger entities such as immunoglobulins are prevented from penetrating the membrane and destroying the cells. Microfabrication techniques have been applied to create a biocapsule for effective immunoisolation of transplanted cells for treatment of diabetes [81-86]. Typical immunoisolation devices consist of a polymeric membrane, but there are several issues associated with these designs, including poor chemical resistance, inadequate mechanical strength and broad pore size distributions [82,83]. Nanoporous AAO membranes offer many advantages in comparison with polymer membranes in designing implantable biocapsules, such as better mechanical and chemical stability, adjustable pore size and surface chemistry, and easy incorporation into microfabricated silicon devices.

The use of therapeutic nanoporous AAO biocapsules with controlled drug release for immunoisolation was first demonstrated by Gong *et al.* [86]. **Figure 6A** shows a capsule fabricated by electrochemical anodisation, consisting of a porous layer with uniform pores with diameters of 25 – 55 nm. To prove the controlled release capacity of these capsules, initial study using model molecules such as fluorescein (molecular mass 400 Da), fluorescein isotiocyanate and dextran conjugates of varying molecular mass as a function of time (molecular mass 4, 20, 70 and 150 kDa) has been performed. The study demonstrates that it is possible to control the diffusion of nutrients and low-molecular-mass proteins (~ 3.5 nm diameter) unhindered, while transport of larger molecules (> 30 nm diameter) was prevented. Their later work demonstrates the feasibility of using nanoporous AAO capsules for the encapsulation of cells. AAO capsules (pore diameter 46 – 75 nm) incorporated with insulin-secreting MIN6 cells could act as effective semipermeable devices,

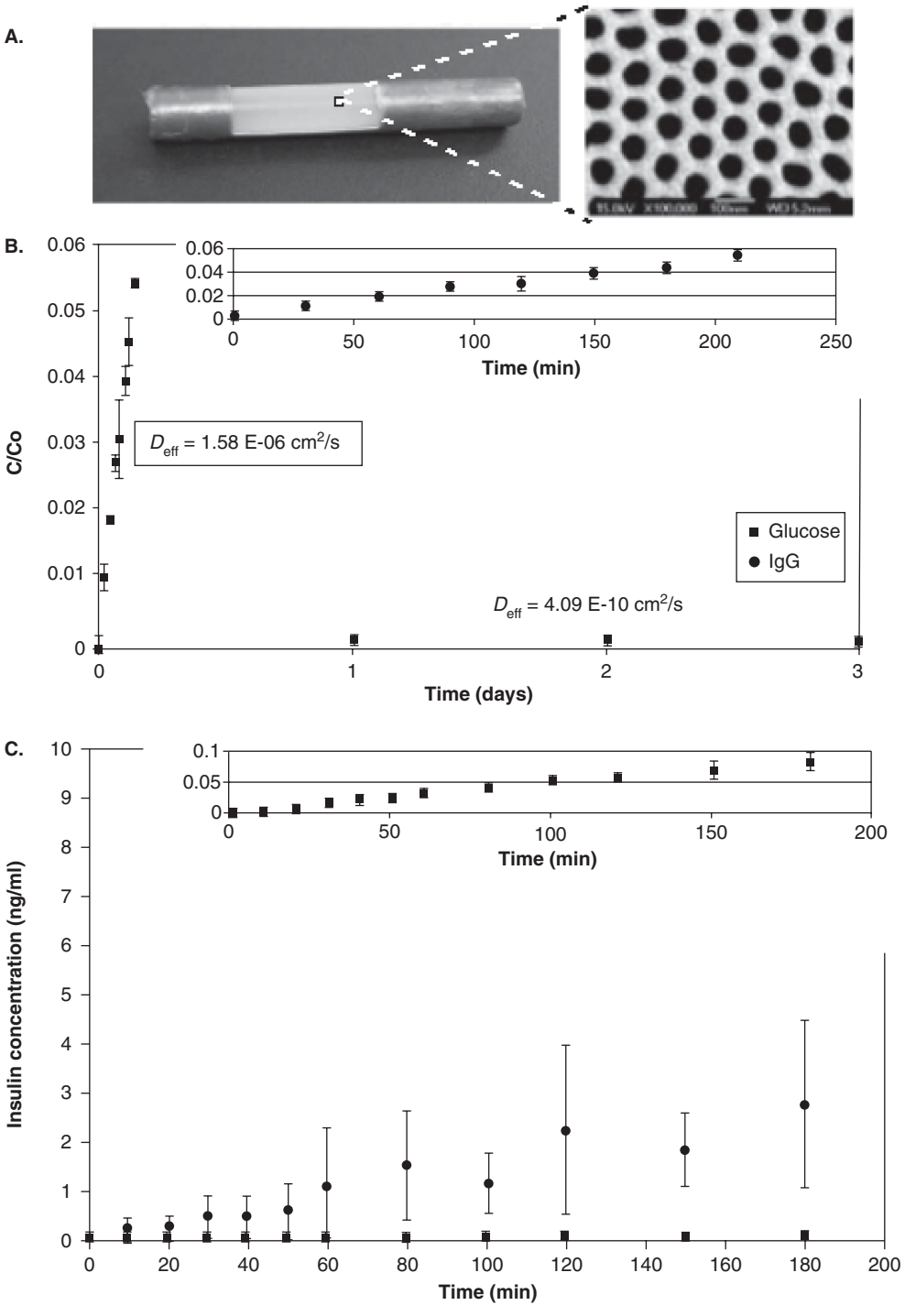

Figure 6. A. Photos of completed nanoporous alumina capsule. **B.** Normalised release of glucose (squares, $D_{eff} = 1.58 \times 10^{-6}$ cm^2/s) and IgG (diamonds, $D_{eff} = 4.09 \times 10^{-10}$ cm^2/s) through a nanoporous alumina membrane with a nominal pore size of 75 nm. *C* is the concentration at time *t*, Co is the loading concentration. Inset: glucose release on a 210-min timescale. **C.** Insulin release from encapsulated (experimental, squares) and unencapsulated (control, diamonds) MIN6 cells following a step increase in glucose. The nominal pore size used for the encapsulated cells was 75 nm. Inset: enlarged graph of experimental data.
Reproduced with permission from [86].

allowing transport of glucose and insulin while impeding the transport of larger proteins such as immunoglobulin G [84-86]. **Figure 6B** shows fast release of glucose and hindered release of IgG over 4 days. The release profile of insulin through porous alumina from encapsulated MIN6 cells in response to an increase in glucose level is shown in **Figure 6C**. The experimental trials suggest not only that encapsulated cells do release preformed insulin in response to glucose, but that they are also able to produce new insulin. Furthermore, it is important to emphasise that cells retain their viability in the close proximity of AAO membrane, showing future promise of this approach for clinical immunoisolation.

4. Nanotubular titania

4.1 Fabrication and properties

Titania nanotube arrays fabricated by a self-ordering process with electrochemical anodisation have also attracted remarkable attention in recent years owing to their unique combination of wide band gap semiconductor properties, nanotube geometry, biocompatibility and large surface area. This material has been used for diverse applications, including photocatalysis for hydrogen production, solar cells, energy storage, catalysis, water purification, sensors, membranes, tissue engineering, implants and drug delivery [19-20,24-25]. Titania nanotube structures, shown schematically in **Figure 7A, B**, are composed of vertically orientated, highly ordered nanotubes with hexagonal arrangement and controllable nanotube diameters (10 – 300 nm) and thickness (0.5 – 300 μm). The lengths of the nanotubes correlate with the efficiency of film formation, with the longest nanotubes and the highest efficiency being found for nanotubes formed under controlled voltage. The reason for separation into tubes, as opposed to a nanoporous structure, is not yet entirely clear. A possible explanation is that owing to the electric field and local-heating-enhanced dehydration, the titania nanotubes could separate from each other, where the directions of volume contraction of the hydroxide layer are normal to the walls. Titania nanotubes can be prepared in several forms, including nanotube layer on bulk titanium, self-supporting nanotube arrays and nanotube membranes, and their typical structures are summarised in **Figure 7D – G**. It is apparent from the cross-sectional SEM images in the inset of the figure that the nanotubes are well separated into individual entities, with an average tube diameter at the top of the layer in the range 160 – 200 nm.

The electrochemical fabrication of self-ordered titania nanostructures was introduced in 1999 by Zwilling *et al.* by anodisation of titanium in a fluoride electrolyte [87]. Since then, several anodisation approaches, mainly focused on finding the optimal electrolyte and anodisation conditions, have been explored to achieve a self-ordering regime for titania nanotube growth [19-20,24-25,88-93]. These studies demonstrated that structural parameters of TiO_2 nanotubes, including inner diameter, wall thickness, length and TiO_2 crystallinity, can be controlled by adjusting electrochemical conditions such as composition of Ti substrate, electrolyte, pH, temperature, anodisation voltage, current and anodisation time (**Figure 7C**). In general, anodic TiO_2 nanotube layers can be formed in aqueous and non-aqueous electrolytes containing small amounts of fluoride ions (HF or HF mixtures, NaF or NH_4F). When anodisation is carried out in acidic, neutral, or alkaline aqueous electrolytes, short tube lengths were formed (500 nm to 2 μm), which are described as the first generation of titania nanotubes [20,24]. The next generation of nanotubes were grown with thicknesses of > 250 μm in non-aqueous (such as glycerol-based) fluoride-containing electrolytes [19-20,24]. To keep dissolution low and grow long tubes, low electrolyte acidity and low fluoride concentration are desired. The highest tube lengths were obtained in organic viscous electrolytes. This condition under anodisation voltage of 80 – 120 V yields vertically orientated, hexagonally close-packed TiO_2 nanotube layers, as shown in **Figure 1C, D** and **Figure 7**. New self-ordering titiana morphologies, such as bamboo-type nanotubes, nanolace, branched tubes, inner tubes and multilayer nanotubes were shown by altering the voltage during the formation of nanotubes [94,95]. Although there is considerable research on surface modification of sister materials such as porous silicon and AAO, surprisingly, the surface modifications of TiO_2 nanotubes have not yet been widely explored [96].

4.2 Biocompatibility

As biocompatible materials and because of their excellent mechanical properties and chemical resistivity, titanium and its alloys, particularly Ti-6Al-4V, have been used extensively in orthopaedic and dental implants since 1970 [97-99]. Most biocompatibility studies on nanotubular titania were focused towards their potential applications for tissue implant engineering, vascular implants and stem cells, where the importance of nanometric scale topography and surface modifications (wettability) were studied.

Several studies by Desai *et al.* have demonstrated that the nanotubular titania surface is a favourable template for bone cell growth and differentiation, and provides clear evidence that osteoblast activity can be significantly enhanced using controlled nanotopographies [100-102]. These surfaces supported higher cell adhesion, proliferation and viability for up to 7 days of culture when compared with plain titanium surfaces [98,101]. Cells cultured on nanotubular surfaces also demonstrated higher alkaline phosphatase activity (ALP) without causing adverse immune response under *in vivo* conditions. *In vivo* biocompatibility was proved by implanting surfaces subcutaneously in rats and performing histological analysis for 4 weeks; chronic inflammation or fibrosis was absent [100]. Increased chondrocyte adhesion on anodised titanium with nanotube structures compared with unanodised titanium has also been reported [103]. Furthermore, the calcium and phosphorous concentrations were 50% higher on these surfaces, suggesting that matrix deposition was

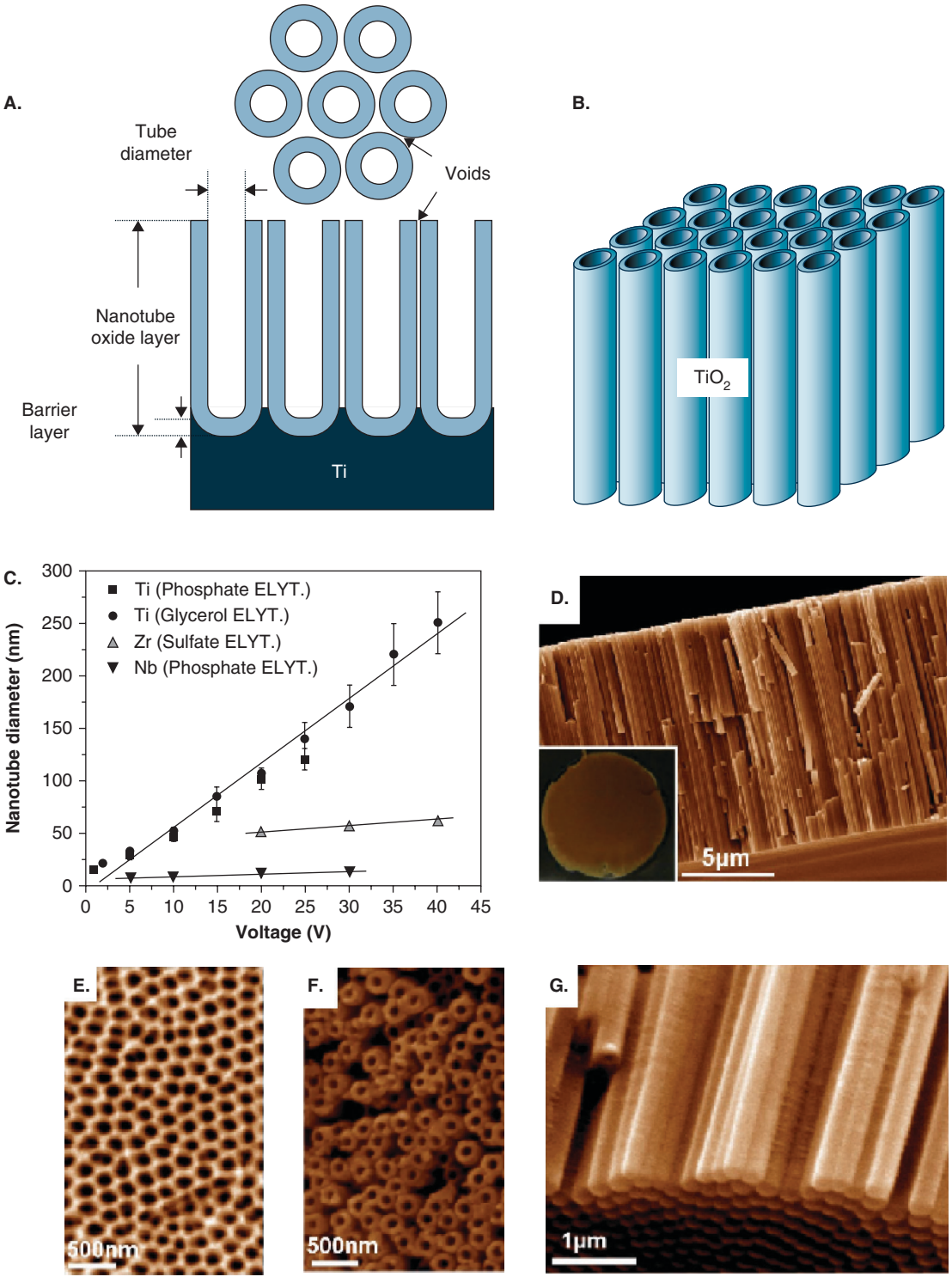

Figure 7. A. Typical structures of nanotube titania fabricated by electrochemical anodisation of titanium. **B.** TiO_2 nanotube structures fabricated by electrochemical anodisation in NH_4F/ethyleneglycol electrolyte showing the cross-sectional image of self-supporting TiO_2 nanotube layer and the entire structure (nanotube film) not detached from titianium substrate (inset). **C**, **D.** The top of the nanotube surface showing porous and nanotube morphology obtained after removing of the top porous layer. **E.** The top surface showing the nanopores and nanotube structures. **F.** The bottom surface showing the nanotube structures detached from underlying titanium substrate.

upregulated on nanotubular surfaces. The significant adhesion/propagation of the osteoblast by the topography of the TiO_2 nanotubes, with the filopodia of growing cells actually going into the nanotube pores, producing an interlocked cell structure, has been described by Oh *et al.* [104,105]. The growth rate of osteoblast cells is significantly accelerated, > 300 – 400% higher than with Ti surface [104]. *In vivo* studies using implants with 30 nm nanotube diameters placed in the front scull of a domestic pig confirmed the enhanced osteoblast function and bone development [106]. These studies suggest that the incorporation of such nanoarchitectures on implant surfaces has the potential to facilitate the differentiation of cells and promote long-term osteointegration. Owing to their ability to mimic the dimensions of constituent components of natural bone and the possibility to serve as a gene and drug delivery carrier, nanotubes seem to be a promising coating for medical implants.

Two groups recently demonstrated that nanotopography, that is, dimensions of nanotube diameters, of culture media prepared from titania nanotubes can influence adhesion, spreading, growth, and differentiation of human and rat mesenchymal stem cells (MSC) [107-109]. Culturing hMSC on a range of nanotube diameters between 30 and 100 nm, Oh *et al.* found that cell stretching and expression of osteogenic differentiation markers are highest on 100 nm nanotubes [107]. Conversely, Park *et al.* reported different mesenhimal cell response to the nanoscale topography [108]. This study reports the highest adhesion, spreading, growth and differentiation of mesenchymal stem cells on 15 nm nanotubes and dramatically decreased cell functions on 70 and 100 nm nanotubes. Furthermore, Park *et al.* reported similar behaviour of both bone-forming cells and bone-resorbing cells [109]. Considering the same size-dependent response to both bone and mesenhimal cells to TiO_2 nanotubes, it has been suggested that a surface geometry with a lateral spacing of ~ 15 nm that corresponds to the dimension of integrin heads is preferentially recognised by cells. Additionally to nanotube dimeters, the surface wettability was also found to be an important parameter for interaction with cells. Bauer *et al.* found considerably enhanced mesenchymal cell attachment to the superhydrophobic titania nanotube surfaces modified by self-assembled monolayers (octadecylphosphonic acid) [110]. The findings of opposite effects of nanotube diameter on osteogenesis of MSCs by two groups motivated more systematic studies on the influence of TiO_2 nanotube geometry, processing parameters, surface chemistry, crystal structure and other differentiation approaches on the response of various stem cells.

As noted previously, vascular prostheses such as stents or vascular grafts are used as common treatments of coronary artery disease. However, such interventions are associated with major complications, such as narrowing of the prosthesis due to vascular smooth muscle cell (VSMC) proliferation and thrombosis as a result of injury and dysfunction of endothelial cells (EC). Active stent coatings with antiproliferative drugs not only inhibit smooth muscle cells prolifereation, but also disturb re-endothelisation, which increases the risk of trombosis. TiO_2 nanotubes represent a unique approach where a stent surface can promote re-endothelialisation and decrease VSMC proliferation [102]. When the endothelial layer is denuded, vascular smooth muscle cells may undergo proliferation and intimal hyperplasia, which is the principal mechanism behind restenosis (vessel blockage). The unique effects of titania nanotubes are improved proliferation and function of endothelial cells, decreased proliferation of vascular smooth muscle cells and enhanced production of prostaglandin I_2 (ability to blunt thrombosis and restenosis). The reason for such effects is still unclear, but it is hypothesised that restriction of cell size may play a role. Further studies with different tube dimensions and topographies are warranted in order to understand mechanisms of cell behaviour. These results show that titania nanotubes have various desirable effects on cells involved in repair after vascular injury and are a promising candidate for next generation vascular materials and stent applications.

4.3 Drug delivery applications of titania nanotube material

Nanotubular titania for drug delivery applications has been explored mainly for medical implants, such as drug-eluting coating for orthopaedic implants, dental implants and vascular (coronary) stents, where not only the controlled release of drugs, for example, antibiotics or growth factors, is desired, but also appropriate biointegration required [100-106,111]. More recently, stimuli-responsive therapeutic systems based on titiania nanotubes have been reported as another promising application.

4.3.1 Therapeutic bone and stent implants

Most medical implant procedures such as hip replacements, dental implants, or vascular stents require subsequent drug therapy to prevent infection, control clotting, or decrease inflammation [112]. Delivery of drugs locally from an implant surface rather than systemically can reduce side effects. A common strategy is drug elution through a drug-loaded polymer coating. Although effective in delivering a drug for long periods, polymer degradation may induce an inflammatory response, activating phagocytes and increasing vascular smooth muscle proliferation, which can lead to implant failure [78]. To address these problems, the newest developments in drug-eluting surfaces are focused on non-polymer-based drug delivery implant platforms, such as nanoporous surfaces (AAO or titania) [102]. These surfaces are capable of eluting drugs and are effective in reducing intimal hyperplasia [113].

Bacterial infection is the most common complication after orthopaedic implant surgery [113]. Bone marrow swells during infection and reduces blood supply because of the compression of blood vessels in the bone marrow. The infection can also spread to the surrounding muscle tissues. To reduce the

chances of serious complications, antibiotic therapy is indicated 6 – 8 weeks after surgery. Systemic antibiotic applications (intravenous, intramuscular, oral) can be toxic and ineffective at reaching infected tissues near the implant. Design of therapeutic implant devices that contain antibiotics (e.g., gentamicin) has several advantages, such as fewer side effects and efficient therapeutic effects. To address the problem of bacterial infection after orthopaedic implant surgery, Popat *et al.* explored titania nanotubular arrays for local delivery of antibiotics off-implant at the site of implantation [102]. A titania nanotube (80 nm pore diameter and 400 nm length) was loaded with gentamicin (70 – 85% loading efficiency). Drug release kinetics are dependent on initial loading; and there was a sustained release, for 45, 90 and 150 min for loadings 200, 400 and 600 µg. Bacterial adhesion on the surface of gentamicin-loaded titania nanotubes is reduced significantly (in comparison with titanium surface and unloaded nanotubes), whereas normal osteoblast adhesion and proliferation is retained [103-111]. Antoci *et al.* came to a similar conclusion, showing that vancomycin covalently tethered to a Ti alloy surface prevents bacterial colonisation and biofilm formation [114,115]. Moreover, the tethered antibiotic is stable, maintains its activity even when challenged multiple times with bacteria and does not foster resistance. Covalent attachment of antibacterial molecules on implant surfaces is advantageous owing to the permanent defence from infection and implant surgery complications.

Peng *et al.* recently reported elution kinetics for two model drugs, paclitaxel (l1.3 kDa drug with a small (< 0.5 nm) hydrodynamic radius) and bovine serum albumine (66 kDa and hydrodynamic radius 2 – 3 nm) from titania nanotubes with varying length and pore diameter [111]. This study demonstrated that TiO_2 nanotubes can control small molecule delivery in the order of weeks, and larger molecules in the order of months. The total drug elution was mostly affected by nanotube length, and maximum paclitaxel elution was reached at ~ 2 weeks. Comparison between nanotube arrays with the same diameter (100 nm) but different lengths (1 µm versus 5 µm) revealed that on average nanotubes of 1 µm held less than half the amount of drug trapped by 5 µm nanotubes. While nanotubes controlled the elution of small molecules for 7 - 14 days (**Figure 8A**), they were capable of controlling the delivery of larger molecules such as bovine serum albumin (BSA) for > 30 days (**Figure 8B**). Data fitted in Fick's first law of diffusion suggest that elution can be described in a two-phase kinetic model, with an initial rapid phase within the first 24 h and a slower phase thereafter. At size scales of 100 nm and larger, diffusion of both types of molecule was largely insensitive to tube diameter, but total drug elution was dependent on tube length, with longer tubes performing better than shorter ones [111].

To demonstrate the efficacy of using titania nanotubes as drug-eluting coatings for implantable devices, Popat *et al.* studied the loading with various amounts of drugs such as BSA (negatively charged) and lyzozyme (LYS) (positively charged), and their controlled release by varying the tube length, diameter and wall thickness [102]. The authors showed that the release rates of BSA and LYS can be controlled by varying the amount of proteins loaded into the nanotubes. Furthermore, by changing the nanotube pore diameter, wall thickness and length, the release kinetics can be altered for each specific drug to achieve a sustained release. The release kinetics was slower and sustained (up to 65 and 110 min) when loading level was increased and electrostatic interaction lyzozyme–titania surface operative [102].

4.3.2 *Stimuli-responsive therapeutic systems*

In conventional porous systems described in the previous sections, the release of adsorbed molecules usually follows a sustained kinetic mechanism that can be expressed in terms of diffusion of adsorbed molecules. It is desirable for certain applications to control release by environmental stimuli such as pH, temperature changes, light or magnetic field [12]. The nanoscale encapsulation of ferromagnetic structures has received a great deal of attention because of the exciting possibilities of using these materials in various applications that range from new electromagnetic to biomedical devices. For example, nanoscale magnetic entities could be transported and concentrated at pre-targeted locations or organs within the human body by means of an external magnetic field in order to exert a specific function with high local and temporal precision. Shrestha *et al.* reported fabrication of magnetically guided titania nanotubes and demonstrated the use of these nanotube layers as magnetically guided photocatalysts important in responsive stimulus release [116]. The drug release concept is based on the fact that the UV-induced hole generation in the valence band of the TiO_2 will lead to chain-scission of a monolayer attached to TiO_2. As outlined in **Figure 9A**, the cleavage takes place at the anchoring siloxane groups, which causes the release of the model drug. It should be pointed out that this release approach is almost unique to TiO_2, as its bond-breaking ability for attached linker molecules occurs because of the position of the TiO_2 valence band relative to the water redox levels. Fluorescence microscopy images demonstrated that UV-induced photocatalytic activity of the magnetic TiO_2 nanotubes may be used to kill cancer cells (**Figure 9B, C**). Another extraordinary example of using titania nanotubes as a therapeutic agent for the treatment of cancer cells opens many new opportunities for drug delivery applications, which need to be explored in the future [117]. Song *et al.* reported the fabrication and use of an amphiphilic TiO_2 nanotubular structure that provides a highly controllable drug release system based on a hydrophobic cap on a hydrophilic TiO_2 nanotube [96]. This hydrophobic cap prevents uncontrolled leaching of the hydrophilic drug into an aqueous environment. By exploiting the photocatalytic nature of TiO_2 for UV-induced chain-scission of attached organic monolayers, the cap can be removed and a highly controlled release of drugs and proteins can be achieved. The chain-scission-induced release from TiO_2 surfaces

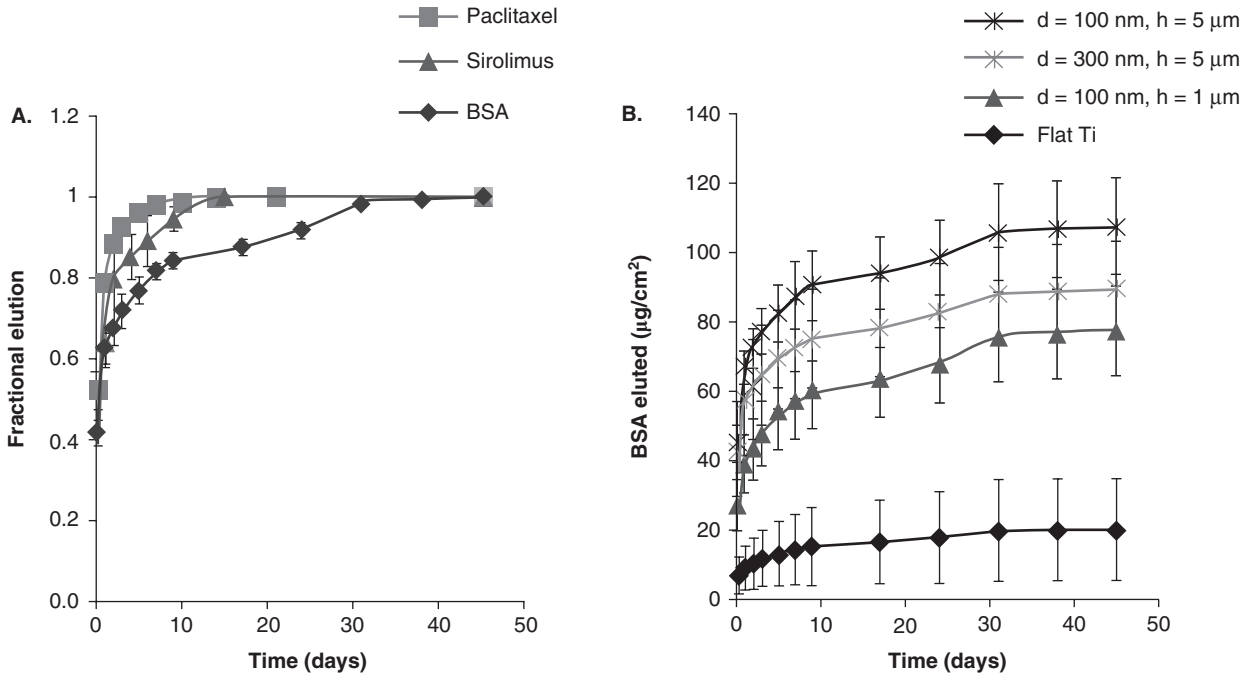

Figure 8. Small molecules and protein elution study from titania nanotubes. A. Fractional elution of paclitaxel, sirolimus and BSA. **B.** BSA elution from titania nanotube arrays of various dimensions.
Reproduced with permission from [111].

can be triggered not only by UV light but also by suitable X-ray radiation.

5. Other nanoporous and nanotubular metal oxides

The strategies described previously for self-ordering conditions for growth of oxide with porous and nanotubular morphology have been described for several transitional and valve metals, such as Al, Ti, Ta, W, Nb, Hf, Zr and their alloys TiNb, TiAl, TiZr, Ti6Al7Nb, Ti6Al4V, and so on [19]. The formation of both morphologies is essentially governed by the same self-ordering process. For titanium and another valve metals, in contrast to aluminium, a low pH (acidic electrolyte) is not sufficient to create a porous layer, and the presence of fluoride ions is required to form soluble metal fluoride complex. A chemical dissolution process (in the case of Ti, the HF electrolyte) is suggested to play a key role for separation of nanopores and the formation of nanotubes rather than a nanoporous structure. Extensive research is now underway and more metals can be expected to be fabricated by changing electrolytes and anodisation conditions in order to obtain nanoporous, nanotubular or even mixed morphologies [19].

It has been reported that aluminium and vanadium ions can be dissolved from Ti alloy (Ti–6Al–4V), which is a concern because aluminium is a growth inhibitor of bone and a possible cause of Alzheimer's disease and vanadium has strong cytotoxicity [101,118,119]. Matsuno *et al.* reported the biocompatibility and osteogenesis of several refractory metal implants (Ti, Hf, Nb, Ta and Re) by both histological examination and imaging methods in *in vivo* animal implantation tests [98]. Titanium, hafnium, niobium, tantalum and rhenium wires were implanted in the subcutaneous tissue of the abdominal region and in femoral bone marrow of rats for either 2 or 4 weeks [119]. No inflammatory response was observed around the implants, and all the implants were encapsulated with thin fibrous connective tissue. No dissolution of these metals was detected by X-ray scanning analytical microscope (XSAM) in the soft tissue [119]. Histological examination of the hard tissue showed that the amount of new bone formation decreased slightly from the second to the fourth week after implantation, and that the percentage of bone in contact with the implant increased markedly over the same period. These results indicate that titanium, hafnium, niobium, tantalum and rhenium have good biocompatibility and osteoconductivity.

The striking possibility for future application of therapeutic titania and other metal oxide nanotubes lies in non-invasive anticancer therapy [116,117]. One of the longstanding problems in medicine is how to cure cancer without harming normal body tissue. The real benefit would be finding a way selectively to kill cancer cells and not damage healthy ones. New devices based on stimuli-responsive titania nanotubes (e.g., magnetically guided, UV, X-ray responsive) represent a promising approach in chemotherapy.

Furthermore, nanopore and nanotube platforms can be prepared in a variety of forms (foils, films and bulk), with capacity to load a large amount of drugs. Titania nanotube

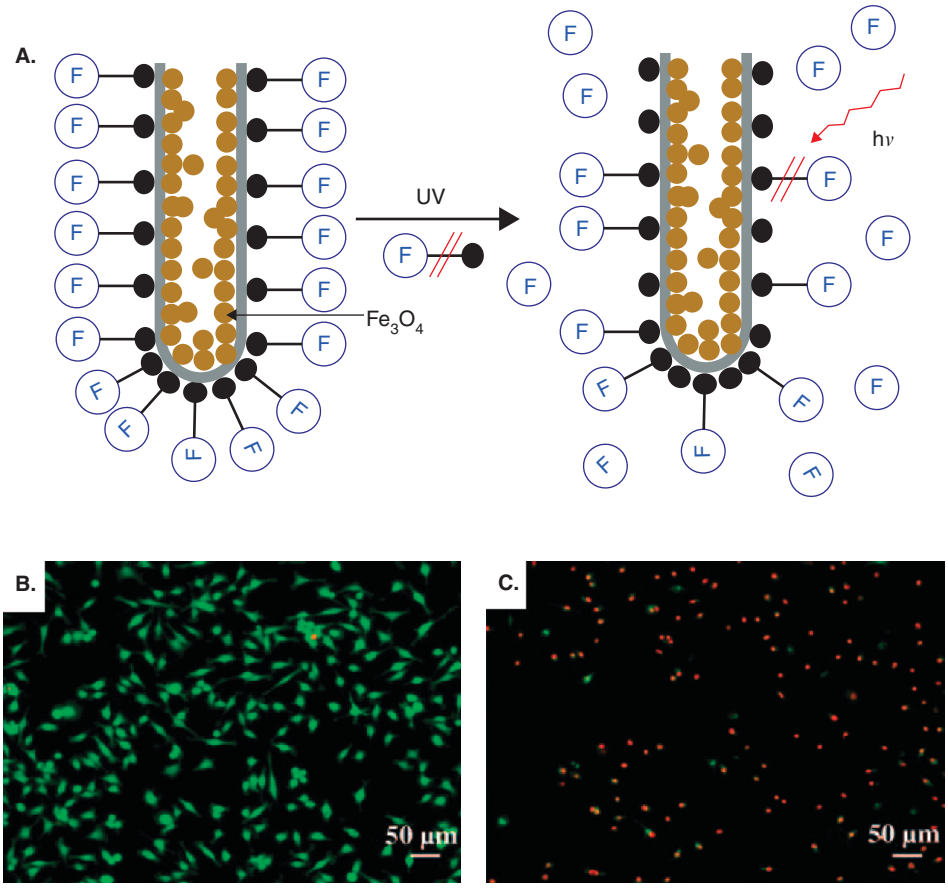

Figure 9. A. Diagram showing the release principle of active molecules (model drug) from the functionalised magnetic TiO_2 nanotubes on irradiation with UV light. A fluorescent dye (active molecule) was attached to the TiO_2 nanotubes with a siloxane linker. Fluorescence microscopy images demonstrate UV-induced cancer cell killing by using HeLa tumour cells cultured on TiO_2 nanotube layers. The image shows cells **(B)** without and **(C)** after UV light irradiation. Live cells were detected with calcein and dead cells were detected with EthD-1.
Reproduced with permission from [116].

films, for example, could potentially provide simple and effective alternatives for dermal/transdermal delivery of problematic drugs. Many candidates for dermal/transdermal application (e.g., vitamins, antioxidants) have poor stability against light, oxidation or physiological pH, and consequently poor bioavailability [7]. To overcome this problem, liposomes or emulsions are often applied, which are unstable systems and offer only limited solution, and the use of nanotube platforms could provide many advantages.

6. Expert opinion

This review has presented the recent progress on electrochemically engineered nanopore/nanotube materials such as nanoporous alumina and nanotubular titania for implanatable drug delivery systems. These new nanomaterials have been shown to have many favourable properties for drug delivery, including high surface area, and controllable pore and nanodube dimensions, geometries and surface chemistry. Owing to their ability to mimic the dimensions of constituent components of natural bone and the possibility to serve as gene and drug delivery carriers, nanotubes are seen as a promising coating for medical implants. Therefore, it is not surprising that research on applications of porous alumina and nanotubular titania for therapeutic implant devices has been increasing considerably in the past few years. This new field can be described as one of the best examples of multidisciplinary approaches that combine the areas of nanotechnology, biomaterial chemistry, controlled drug delivery and biomedical engineering. Although still in the early stages, a few *in vivo* studies clearly show the potential of these materials for drug delivery devices in orthopaedic implants, dental implants and vascular stents, where not only the controlled release of drugs such as antibiotics or growth factors is desired, but also appropriate biointegration is needed. This is only the beginning of further research in terms of correlating biomaterial chemistry and tissue responses and new clinical approaches required not only for orthopaedics, but also for the treatment of several other

diseases (hearth, cancer, diabetes, Parkinson's, Alzheimer's, etc.). The remarkable catalytic and anticancer therapeutic properties of some of these materials, such as titania nanotubes, are expected to attract more research in the near future.

Future research potentially has multiple directions, including: i) the increasing biocompatibility by covalent immobilisation of therapeutic molecules on implant surfaces, for example, antibacterial and anti-inflammatory drugs; ii) the achievement of controlled and sustained drug elution over a long period of time using specific surface modification, structural design of pores and combining with biocompatible polymers; and iii) the development of stimuli-responsive systems such as magnetic field, light, temperature, pH is particularly important for highly toxic drugs. The implementation of nanoporous and nanotube membranes or films into microfabrication devices for creating chip-based drug delivery platforms is also a future direction. Therapeutic systems that possess a combination of structural, mechanical, biosensing and electronic features may overcome challenges associated with conventional delivery therapies. Dermal (topical) or transdermal delivery systems are another field of research where these materials could contribute significantly to medical applications.

Declaration of interest

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