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# Nanoporous Alumina as an Intelligent Nanomaterial for Biomedical Applications

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***Abstract***

Nanoporous anodized alumina (NAA) is, to date, one of the most widely known and appealing nanomaterials for a diverse range of applications. In recent decades, they have been extensively explored for biomedical research. Multiple abilities and enhanced properties of these nanomaterials were reported continuously in this field. They are engineered by means of a simple, economical, time-effective, and scalable electrochemical anodization process. This chapter focuses on the new generation of this functional nanomaterial for biomedical purpose, highlighting innovative concepts of drug-releasing implants based on NAA directed toward its design progress and forefront features for therapies including orthopedics, dental, coronary stents for cardiac, vasculature, and heart surgeries, tissue engineering, and bio-scaffolds for cell culture. Their attributes as intelligent carriers for drug loading and release by virtue of their nanotubular and nanoporous structures with modifiable physicochemical characteristics were examined, so is the work on their biocompatibility. Lastly, conclusions and future prospects are presented at the end of this chapter, as well as the current challenges that have to be addressed before this biomaterial can be envisaged for real-life biomedical applications.

***Keywords*:** Nanoporous anodized alumina, intelligent, biomaterials, drug delivery, orthopedics, implant, biomedicine

## 6.1 Introduction

Advanced fabrication technologies have enabled a new generation of intelligent nanomaterials such as the emergence of nanoporous anodized alumina

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(NAA) in recent decades for various applications. One of the most appealing areas for its usage is biomedicine and its topic of most relevance, namely, drug delivery (DD). DD is undergoing enormous progress albeit drugs have long been found and used to overcome diseases since former times. Currently, its understanding is focused on the breakthrough of the blood–brain barrier to achieve effectiveness in DD, *viz.*, how drug is channeled through cells and tissues to the targeted sites. Vigorous efforts have been made in the past three decades to create the ideal drug delivery system (DDS) – one that can cross barriers, i.e., gastric/intestinal/lung mucosal, blood-placental, blood-brain, and administer the appropriate dosage of drugs to attain effective therapeutics in due time to targeted sites for action with minimal side effects [1]. The major drawback, however, is the potency which could cause harm to adjacent cells and tissues in close proximity, and the risk of drugs transported to the other areas of the body. This is commonly discerned in traditional DD methods because drugs were delivered over short instances causing overdosage, of which they mostly follow first-order release kinetics [2]. Despite the fact that frequent administration of drugs can now circumvent over- or under-dosing and comply with acceptable toxicity levels, patients often report distress, not to mention the time and financial investment required of them for complicated treatments. Other traditional DD modes include the oral, buccal, subcutaneous or intramuscular, infusion, and inhalation formulation. Nevertheless, these methods suffer from poor selectivity, while some forms can be intrusive and painful [3].

For this reason, nanomaterials have an important role to play in the new generation of DDS for targeted and localized administration of drugs. It is pertinent to adjust the dosing of drugs such that it is within the acceptable therapeutic window to allow for effective treatments in a myriad of diseases, and by introducing nanomaterials as such could assist that process. The original concept for localized DDS was first introduced in the mid-1960s by Folkman *et al*., a Harvard professor and a surgeon at Boston Children's Hospital who was a pioneer in angiogenesis. He proposed the concept of implantable DDS upon discovering the positive outcome of anesthetic gas released from a tube in a rabbit [4]. There and then, the notion of implant as a DD biomaterial was conceived. That also signifies a vital step forward for localized DDS that are based on polymeric, ceramic, and metal implants. They are promising biomaterials by virtue of less frequent dosing, non-systemic drug circulation with better patient compliance and less detrimental effects to patients' overall wellbeing [5, 6].

NAA can be facilely fabricated *via* a simple electrochemical anodization process. In the pursuit for the ideal implant material, it garners much attention due to its multitude of properties that could render it a desirable inorganic nanoporous material. They comprise of its cost-competitiveness,

well-established, easy, and scalable fabrication process, the ease in generating different pore sizes on their surface, in addition to various pore morphology and shapes that are able to be tuned through this approach to tailor to a wide range of drug-releasing characteristics and therapeutic needs [7–12]. In comparison to polymeric materials, NAA could offer better mechanical, chemical, and thermal resistance since they are robust, less likely to destabilize, erode, or degrade over time in any given physiological environment.

The structure of NAA consists of highly ordered and uniform arrays of nanoporous network. As it is fabricated *via* a self-assembling mechanism wherein aluminum is oxidized to alumina nanopores during an electrochemical process. Herein, its specific surface area to volume ratio, pore diameter, interpore spacing, and pore thickness can be precisely controlled [13]. It is also a lithography-free technique that is renowned for its low cost and time-effectiveness suited for large-scale production in industry [14]. In particular, it can contain substantial amount of drugs for release since the nano-scalable dimensions (length, width, and height or thickness) can be predetermined before the synthesis to the end product [15]. In terms of its material properties, NAA boosts of high surface area and porosity, is bioinert, minimally invasive, and minuscule for ease of implantation into the body. As a result, they are extensively researched for both *in vitro* and *in vivo* DD applications by storing and releasing different drug types to address different medical conditions [7, 8, 13].

This chapter outlines the recent progress concerning the role of NAA as an intelligent DD implant in biomedicine spanning several areas, *i.e.* orthopedics, dental, cardio-vasculature stents, tissue engineering, and cell culture as shown in Figure 6.1. The focal point is to describe the intelligent properties imparted by NAA upon structural and chemical modifications made to its porous network, the various drug-releasing concepts associated with NAA and the factors that affect its drug release characteristics, the different types of DDS to cater to various medical needs and the biocompatibility of NAA in biomedicine. Lastly, this review presents a summarized outlook and future prospects, drawing on the challenges and limitations of this material before it can be approved and used in patients.

## 6.2 Nanoporous Anodized Alumina as a Drug Nano-carrier

### 6.2.1 Intelligent Properties of NAA for Drug Delivery

The oldest research pertaining to NAA fabrication began five decades ago [16]. Since then, membranes and substrates with decorative layers

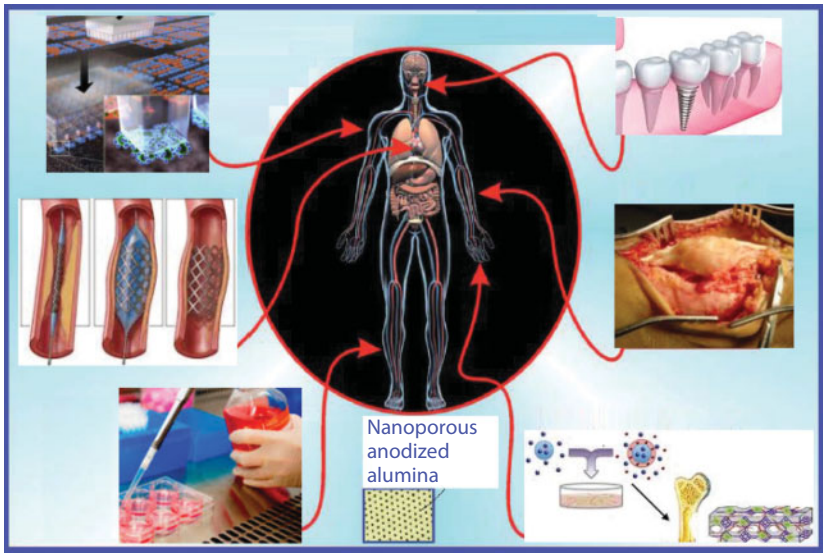


**Figure 6.1** Schematic outline of summary pertaining to the applications of NAA for biomedical applications and delivery of therapeutics, whereby NAA is used as drug-releasing implants, dental stents, base templates for cell growth, and tissue engineering [7].

were constructed *via* electrochemistry based on this material *via* a two-step anodization of aluminum metal immersed in either sulfuric, oxalic, or phosphoric acids as electrolytes with adjustable concentration and bath temperature. Neatly aligned and closely packed hexagonal and cylindrical columns of nanoporous alumina are formed on the Al base metal [13]. A typical NAA architecture is shown in Figure 6.2. The advantage of using this material is that its configuration can be controlled by manipulating the parameters in the anodization process, such as voltage, time, current, and power to tune its pore size ranging from 5 to 300 nm and height from 0.1 to 500 μm. Unique morphologies such as flat, conical, funnel-, symmetrical-, or periodically shaped NAA can also be achieved through this approach [12, 17]. Its structure can be made such that the capturing and releasing of drugs can yield a slow and controlled release profile unlike other nonuniform porous platforms. This is ascribed to its strictly ordered and uniform nanoporous architecture [8, 18]. Moreover, NAA has excellent compressive strength, a low density, is nanoscopic, biologically inactive, compact, wear and corrosion resistant, chemically and mechanically stable. Its construct is versatile on many different metallic surfaces, for instance, on planar or nonplanar 2D platforms regardless of surface texture, curved or flat surfaces, as well as 3D ones such as on tiny, thin wires or on microneedles

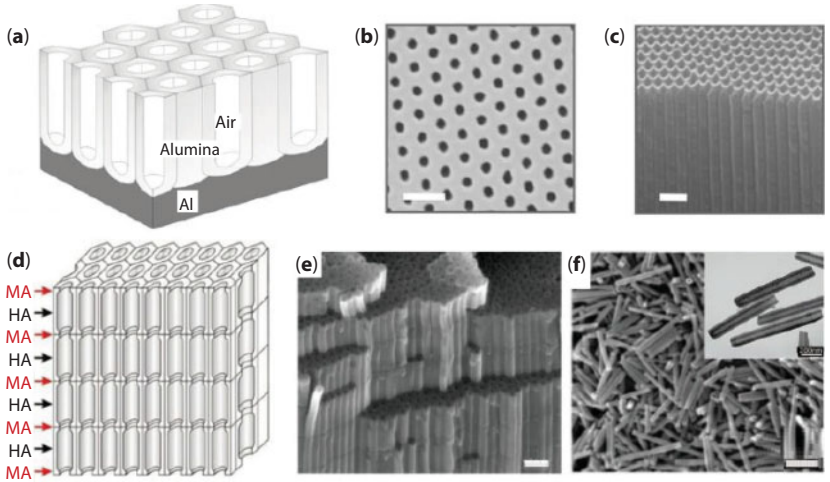


**Figure 6.2** NAA for applications in drug-releasing implants and NNAA as drug carriers. (a) Schematic illustration of the basic structure of NAA. (b) Top and cross section SEM micrographs of NAA (scale bars = 400 and 250 nm, respectively) [adapted from Ref. 2], (c) schematic of cross-sectional view of NAA prepared by pulsing anodization with (d) corresponding SEM image before dispersion of nanotubes (black and red arrows indicate layers created by HA and MA pulses, respectively), (e and f) SEM images of liberated NAA nanotubes with 500 nm length [18–19].

used for surgeries and therefore is implantable for many possible applications [13]. Its fabrication is feasible for mass commercial production due to affordable expenditure for sourcing the raw materials in abundance, purchase of equipment, their setup, and maintenance. Most of all, the process for synthesizing NAA is safe and therefore does not pose detrimental harm or hazards to the environment, health, or ecosystem.

An intelligent study resulting from this is a recently reported nanoporous–nanotubular anodized alumina (NNAA) that can be used as a drug nano-carrier. Similar to NAA, the dimension of NNAA can be controlled in its anodization process. This is realized by pulsatile mode in which the formation of NAA is converted to nanotubes by the separation of its nanopores as shown in Figure 6.2c and d [19, 20]. This innovation sheds new light on more sophisticated DDS that can be devised based on having simultaneous nanoporous and nanotubular designs in an alumina implant.

In addition to that, the fact that NAA and NNAA are amphoteric and sizeable permit them to contain a variety of drugs. This does not only apply to hydrophilic or hydrophobic drugs but also polar, nonpolar, ionic or non-ionic compounds, as well as small or large biomolecules such as peptides, proteins, DNA, RNA, genes, and polymeric micelles [8, 13, 18]. Drug

release from these NAA and NNAA materials is a diffusion-limiting process and therefore is rate-determining [10]. There are several other external factors that can impact the release rate. This includes the size of the drug, its chemical properties, interfacial interaction between the drug and the alumina nanostructures, drug dissolution, diffusion coefficient, pH of the physiological media, nanopore, and nanotube dimensions. Through various means of modifying the structures and physicochemical properties of NAA and NNAA, these materials can yield varied release types for obtaining an optimal pharmacokinetic profile for specific treatment of diseases. These profiles can be of a zero-order, first-order, combined first- and zero-order rate kinetics, burst or stimuli-responsive, or sustained and extended drug release, or a combination thereof. Furthermore, multiple facets of drug release such as that featuring an immediate, stepwise, delayed, time-programmed, simultaneous or cascading pattern with multiple drugs release, or one that is switchable, can aid in achieving intelligent therapeutics delivery [7, 8, 12, 18].

In order to prolong the time for drug release, a facile method is implemented through the structural modification of the nanopores and nanotubes of alumina [11]. The first ground work was laid when the motion of dyes such as fluorescein isothiocyanate (FITC) bound to dextran with varied molecular weights was studied. This was correlated with the pore size of NAA ranging from 25 to 55 nm [21]. The NAA nanocapsules were applied as biofilters in this work and were loaded with two types of model drugs. It was found that the drug size is inversely proportional to the release rate. For prolonged release of drugs, a type of antibiotics, namely, amoxicillin, which is 8 nm in size on average, was shown to generate a 5-week-long drug release from NAA with a pore size measuring at d = 20 nm [22]. Aside from that, our group had previously studied the release of indomethacin, an anti-inflammatory drug. It was released from NAA with pore sizes of 65–160 nm [11], confirming that with a larger pore size, a larger volume of drug can be stored for longer release times and thus giving rise to prolonged therapeutic effects [11, 23]. These observations are in line with Kang *et al.* who had investigated NAA as potential biomedical stents of different pore sizes and thicknesses to probe their different drug release rates [24, 25]. Besides that, Gultepe *et al.* have also proved similar entailing effects due to the differences in NAA pore sizes on the release of doxorubicin, an anticancer drug [6, 18], while Kwak *et al.* did similar work by investigating the various lengths of the synthesized NAA drug carriers on paclitaxel, a chemotherapy drug for treating ovarian, breast, lung, and pancreatic cancers [26]. Their results show that, despite a 400-h-long investigation, the paclitaxel drug release was still ongoing because the drug was continuously

released from the inner side walls of the NAA channels. These findings are illustrated in Figure 6.3, with the different drug release profiles from NAA of different sizes shown. Consequently, the physical tuning on NAA dimensions, in summary, proves to have a distinct and positive impact on the drug release behaviors for different drug types.

Apart from the physical tuning method, chemical tuning for NAA surface can, likewise, be used to obtain prolonged or shortened drug release profiles [11]. By grafting hydrophobic or hydrophilic functional groups on the surface of the NAA used as drug nano-carriers, changes in its interaction and the binding nature with drugs can ensue, so can the drug loading and release profiles. This concept was initially shown in porous silica capsules used as drug carriers, in which the hydrophobic silica lattice was loaded with ibuprofen, a non-steroidal anti-inflammatory and water-insoluble drug. Drug release was slower in comparison to porous silica of unmodified surface chemistry [7, 10]. In other studies for loading and releasing hydrophilic and hydrophobic drugs, silanes and polymers with active ligands were attached to the nanopores of these NAA drug carriers. However, it is worthwhile to note that for indomethacin, the problem of having an initial burst release of drugs was encountered. Nevertheless, it can be addressed by modifying the NAA surface with amine or pentafluro-terminated silanes to suppress the burst release phenomenon [11]. This measure therefore underscores the importance of surface modifying agents used to tune the chemistry of NAA to control the drug release outcome.

Furthermore, recent advances in particle synthesis have enabled a surge of new and intelligent nanomaterials. Notably, one of them is termed the

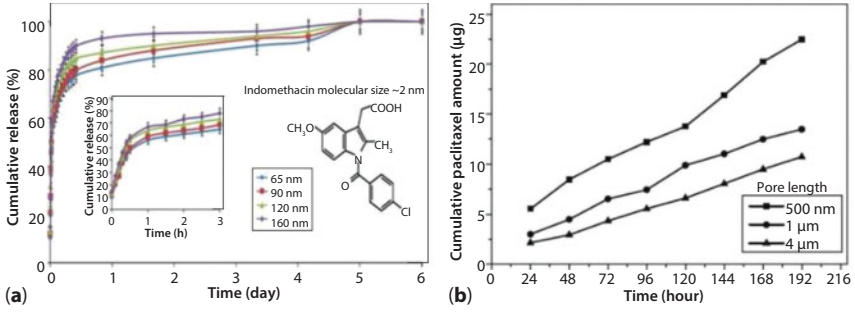


**Figure 6.3** (a) Graph showing the cumulative release (%) of indomethacin (poorly soluble drug) from NAA with different pore diameters (65–160 nm) versus release time (days) [11]; (b) cumulative release of paclitaxel release from NAA as a drug carrier with various pore lengths (0.5–4 μm) [26].

"Janus" nanoparticle, a unique and asymmetric colloid equipped with dual and opposing properties, namely, both hydrophilic and hydrophobic faces for use in tethering with NAA surfaces [27, 28]. It was reported that this effected a change in the pH and ionic strength for NAA and the media it was immersed in, since the incorporation of it resulted in selective functionalization of NAA to an array of drug types. This depends on their affinity to water for varying the drug loading and release rates. In summary, the chemical aspect of surface modification for NAA is underlined here. With the addition of active species or grafting, the drug and drug carrier interactions can be altered, besides the binding strength and interfacial properties of NAA internal walls such as zeta potential and surface charges. The changes that arise can then allow for adequate control in drug loading and release by chemical means.

Considering the fact that intelligent tuning can be achieved by both physical and chemical means, DD from NAA and NNAA implants for biomedicine still experiences some limitations, because a much longer, sustained release timeframe that can be maintained for more than a 3-month period has not been reported. Hence, to examine this issue, a new strategy was devised by coating the top surface of NAA and its opened pores with a layer of biopolymer. The goal is to decrease the pore size of the NAA *via* biopolymer deposition to prolong the drug release period by restricting the channeling of therapeutic molecules through the exit points of the NAA [11, 29–31]. This concept is illustrated in Figure 6.4a and b, in which a layer of plasma polymer formed by the coupling of free radicals was constructed on NAA to decrease its pore size to $d < 5$ nm. Before their stabilized formation, plasma polymers were derived from volatile gases and substances which vaporized in vacuum and later excited by plasma to yield a conformal layer on NAA. It is a simple, one-step process requiring little preparation and merely a few reagents for facile modification of NAA structure in another set of work regarding intelligent implant design [32, 33]. In an experimental study, it was shown that vancomycin, an amphoteric glycopeptide antimicrobial and antibiotic used against resistant strains of *Streptococcus* and *Staphylococcus* bacteria, underwent exceptionally slow and extended drug release from NAA coated with a layer of polyallylamine film. To elaborate, this polymer chain is a cationic polyelectrolyte formed after 50, 120, and 200 s *via* plasma polymerization. The polyallylamine layer grows thicker with longer plasma time. Results are displayed in Figure 6.4c and d, showing that the deposition time is inversely proportional to the drug release amount over time. Results show that without the presence of polyallylamine on NAA, vancomycin release was fully completed in 45 min. However, with 45 min plasma time for the polymer

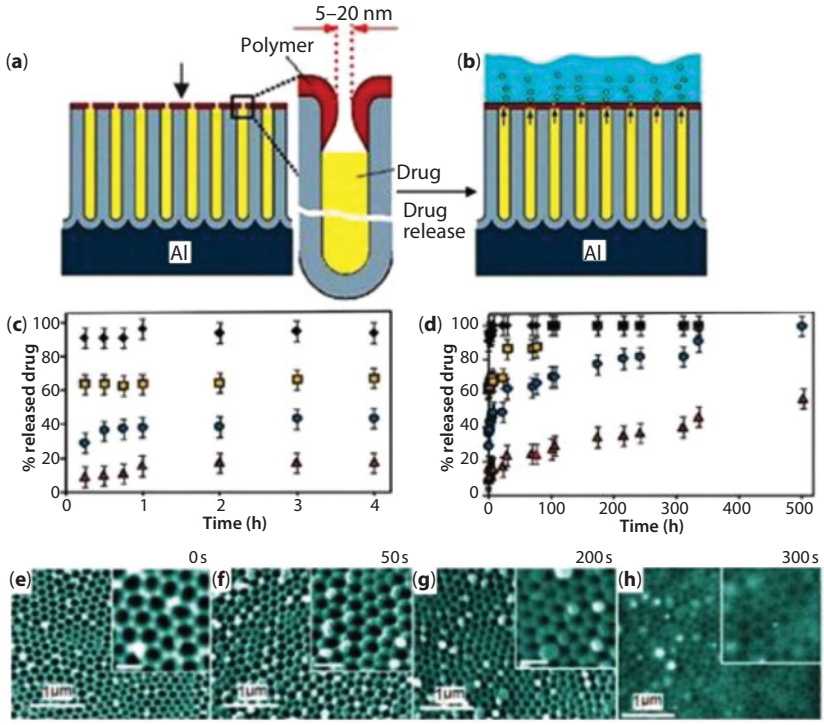


**Figure 6.4** (a) Scheme depicting the concept for controlling drug release from nanopores by reduction of the pore opening (5–20 nm) on the top of NAA using plasma polymerization of biopolymer (polyallylamine); (b) suppressed diffusion of drug molecules from the reduced nanotube structures into the PBS (drug release medium); (c) controlled release of model drug vancomycin from plasma-modified NAA for 4 h and (d) 500 h. The pore opening is precisely controlled by plasma polymerization time from 50 s (squares), 120 s (circles), 200 s (triangles), and the control sample (no plasma deposition, diamonds). SEM images of NAA surface plasma-polymerized with polyallylamine using deposition times of e) 0 s, (f) 50 s, (g) 200 s, and (h) 300 s [29].

layer to be formed on NAA, the release lasted for 200 h, while 120 s plasma times yielded 500-h-long therapeutic release [29]. The modification of the top NAA layer is evidenced in Figure 6.4e and f, whereby the scanning electron microscopy (SEM) micrographs display the smaller NAA pore size due to polyallylamine that was adhered on its surface as a result of the said plasma polymerization process. Zero-order release kinetics resulted from this study. This kind of release is a predictable and consistent drug release pattern that can be adequately monitored over time. Additionally, an alternative approach to plasma is the use of dip-coating, yet another simple method to deposit a polymer coating on NAA. For example, by

using chitosan, the most important derivative of chitin, also a dietary fiber component derived from the shells of crustaceans with a strong affinity to hydrophobic molecules, in the dip-coating process, NAA pore size can be decreased. The only difference between plasma polymerization and dip-coating is the different mechanisms involved in these approaches for attenuating the drug release for prolonged release times. In this approach, it is achieved by the biodegradation of the chitosan layer that was 0.5–2 μm in thickness covering the NAA top surface [11, 34]. Chitosan and the plasma-induced polyallylamine thin layers on NAA could result in drugs having different aggregating properties in different types of solutions and pH values due to their individual and differing properties and film thickness, and thus are potentially useful to cater to various therapeutic needs depending on the drug types to be delivered and synthesized NAA structure for loading and release.

Aside from implementing a layered NAA surface for prolonged release patterns, another type of intelligent concept is a stimuli-induced DDS with the aid of external sources. The reason for devising this DDS is that it is pivotal for controllable therapeutics to be on standby mode and only take action, when or if need be, because in medical diagnosis of unforeseen circumstances, every individual responds to a range of diseases in various ways, depending on their inherent genetic disposition, environmental, growth factor, background, and history [7, 10]. From mild to severe, temporary to permanent, occasional or irregular to chronic pain and suffering, the need for releasing drugs in programmable, or multiple doses with incremental or constant amount, or at arbitrary times under unpredictable conditions, to this day, has not been formulated to cater to personalized needs or onset of action. As a result, DDS equipped with intelligently rapid and instant functions is necessary to be developed, hence the concept for stimuli-responsive DDS as a new research direction. To realize this goal, NAA endowed with special triggers has to respond discretely to a particular stimulus to undergo certain changes to permit the release of drugs. There are numerous avenues for such systems to control drug loading and release, such as inducing discriminative effects for drugs to undergo protonation, hydrolytic cleavage, or supramolecular changes to release therapeutics. Exogenous or endogenous stimuli including electromagnetic field, pH, thermal, solar energy for spatial, amount, and temporal maneuverings of drug motions can be used for stimuli-responsive biomedical therapies. An example as such was demonstrated by Joen *et al.* whereby NAA was proved to generate an electrifying effect toward drugs. The ingenuity lies in doping polypyrrole with dodecylbenzenesulfonate anion (PPy/DBS) and electro-polymerized it on the top of NAA. The pores of NAA can thus

be opened or closed simply by changing the PPy/DBS volume, which in turn, varying its electrochemistry. Figure 6.5a and b depicts the electropolymerized NAA device, while Figure 6.5c and d shows the atomic force microscopy (AFM) images of electrically actuated opened or closed pores. Another drastic measure for on-call release of drugs was shown by using bovine serum albumin (BSA), also termed as "Fraction V" – a serum albumin protein isolated from cows that is ideal for enzymes reactions and stabilization during storage. It was used as a model protein doped with FITC. It can produce a very brief drug release moment that lasts <10 s, and allow for impromptu commands to be made to release drugs at any time with a high concentration. This particular type of control for drug flow can be used to treat urgent attack of diseases such as that of angina pectoris and discrete episodic impairment, for instance, seizures and cardiac arrhythmias [35, 36]. Moreover, it is worthwhile to mention that NAA and NNAA with wider pore size distributions offer more options over single pore size distribution for drug release with diversified profiles and storage of drugs with varying stabilities that are more complex [7, 36, 37]. In addition to that, an alternative technique was to apply electromagnetic energy, such as that sourced from magnetic field with the aid of iron nanoparticles, as well as radiofrequency generating systems equipped with transducer and amplifier, with the aid of gold nanoparticles to demonstrate this transmittance

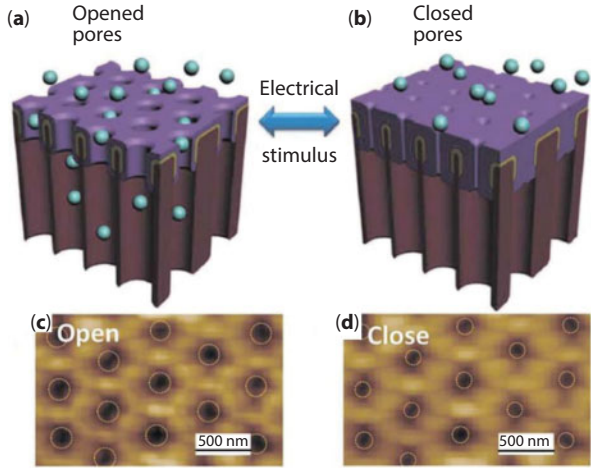


**Figure 6.5** Drug-releasing NAA nano-carrier based on electrical stimulus. (a) Schematic diagram showing the mechanism of an electrical stimulated NAA nano-carrier for drug release with the incorporation of PPy–DBS for opening and closing of the pores, (b) AFM showing the morphology of the NAA pores before and after voltage charges [35].

effect [11, 12, 38–41]. In summary, further development of intelligent NAA based on sensory detection to target multiple and simultaneous delivery of drugs is an attractive key topic to be researched on.

## 6.3 Biocompatibility of NAA and NNAA Materials

The fundamental criterion of an approved and safe biomaterial is its capability of being present alongside living tissues or organisms without being recognized as a threat and a foreign object, nor rejected by the immune system, at the same time not inducing toxic, injurious, or immunological reactions to patients' body. Regardless of how effectively or intelligently a nanomaterial can function, in order for it to be of value for real-life biomedical applications, biocompatibility is the underlying basis and the deciding factor. By having the biomaterial tested positive for cellular response and tissue integration is an obligatory legislative protocol for it to gain approval for its usage.

NAA can be used as nanostructured implants due to their topography, increased wettability, high surface area, and numerous similarities to extracellular matrices for cellular recognitions at the interface between implants and host bone or tissue. Due to these outstanding attributes, research on NAA is currently associated with bones and orthopedics-related therapeutics. Owing to the fact that NAA is chemically stable, it is highly unlikely that they destabilize to release inorganic and toxic nanoparticles or fragments to cause danger to the body. Instead, their topography containing nanofibers has shown to encourage calcium deposition and alkaline phosphatase (ALP) synthesis in osteoblast culture [42]. In order to guarantee safety over the long run for extended periods of implants inside the body, it is crucial to understand the biohazards associated with nanosized wear particles at the bone–prosthesis interface. Since implant structure can be precisely designed *via* parametric control in electrochemical anodization for NAA, the behavior of tissues and organs can be studied with regard to the function and properties of adsorbed protein to establish the surface properties and cellular response, and address any arising toxicity by enveloping the inorganic materials with biocompatible organic substances for harmonious bonding between an implant and adjacent bones. NAA were shown to exhibit these properties and thus has been reported to be explored in orthopedic proteases and dental stents [42].

For more in-depth and detailed comparison of biocompatibility among various implants, Desai and co-workers have conducted experiments studying the adhesion and proliferation of osteoblast on NAA relative to

amorphous alumina, bare aluminum, and glass to probe the interaction between osteoblasts and these materials [43–47]. Results confirmed that NAA improved the adhesion of osteoblasts as compared to the other material types. This also highlights the significance of size on the nanoscale and surface chemistry on cell culture and proliferation. Other types of cell line such as that procured from the epithelial cells called "Vero" cells from the kidney of a mammalian, namely, *Cercopithecus aethiops* (African green monkey), can be cultured on NAA, because NAA is suitable as a support stand/base template for cell culture [48]. In this study, it was shown that after 24 h of cell cultivation on NAA, excellent cell adhesion was observed, as the Vero cell coverage on the surface was extensive and the cells had actively produced mammalian extracellular matrix (ECM) for constructive remodeling of tissues. This occurrence can be seen in the optical microscopic images in Figure 6.6a [48]. This indicates that NAA can be beneficial in clinical translation and in improving patient care for the development of regenerative medicine strategies in tissue and organ replacement. In Figure 6.6b and c, SEM micrographs show the presence of filopodia distributed on the NAA surface and at the cell boundary. NAA and ECM displayed outstanding adherence to the cells as the nanopores served as a template and site for its habitation and growth. Figure 6.6d and e depicts the AFM images of the cells in addition to many filopodia which are highly dynamic cell surface protrusions for probing their external surroundings. Cell proliferation assays were conducted on the glass piece and on NAA, specifically using a

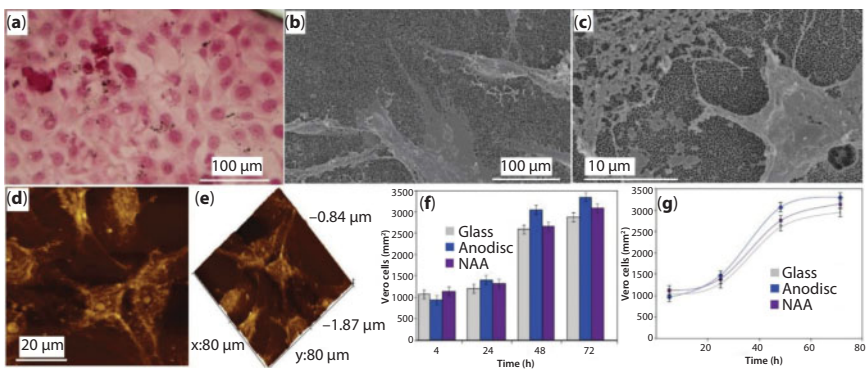


**Figure 6.6** (a) Optical microscopic image of Vero cell morphology, (b) its SEM micrograph, (c) cell attachment on NAA with the presence and growth of filopodia, (d) AFM images of cells and filopodia on NAA, (e) angled, close-up view of NAA surface obtained from AFM, (f) cell proliferation on Vero cells cultured on three different materials in 72 h, and (g) comparison among NAA, Whatman Anodisc and glass control based on viable Vero cell count over the said time period [48].

commercially approved, non-deformable, crack-free, uniform honeycomb-structured nanoporous membrane called Whatman® Anodisc made from NAA. It consists of epitaxial aluminum oxides and possesses the ability to coat nanosized substrates in a conformal manner. Figure 6.6f and g displays the outcome of a 72-h cell proliferation assay to attest to the stability of Vero cell lines over a 3-day period as there were no traces of infection or toxicity on the cells. To further elaborate, the greatest amount of live cells was found on the NAA substrate surface according to Figure 6.6g; meanwhile, the least amount was on the glass surface. Hence, in-house NAA is feasible as a safe and effective cell culture base for the Vero cell line, confirming its biocompatibility with it; in particular, coarser NAA texture could stimulate better cell growth due to more pitting as potential sites for cells to proliferate and a greater ease of controlling the pore size distribution.

For human bones, a biocompatibility test was carried out on human osteoblasts (HOBs) to examine the feasibility of NAA working with the cells. Two sets of assay comprising (i) Alamar Blue (Figure 6.7a) and (ii) DNA analysis (Figure 6.7b) were prepared. Initial high levels of tritiated thymidine, [3H]-TdR incorporation (per mg DNA) were observed for both the control sets and the NAA as shown in Figure 6.7c. The maximum growth was observed on the third day. Besides that, in the modulation of ALP activity, the osteoblastic phenotype was maintained on the NAA as shown in Figure 6.7d. A confluent cell layer was found after a week of investigation, while the ALP expression was most pronounced after 2 weeks. Cell filopodia were present on the NAA as evidenced from SEM and transition electron microscopy (TEM) micrographs in Figure 6.7e and f, respectively. A zoomed-in view indicates that the cells in contact with the NAA had multiplied in numbers and bred onto the NAA pores according to Figure 6.7g [49]. This work shows positive osteoblastic response wherein HOB cells were nurtured on NAA with good cellular activity for 7 days and DNA growth for 14 days [49–51]. In a separate work, NAA was also shown to be nontoxic to cells when functioned as a bone implant [52], while some studies have reported the same results when NAA existed as a coating layer [21, 36, 53]. On the contrary, there remain controversies and debatable areas pertaining to the biocompatibility of NAA. To address this, it was reported that by applying the atomic layer deposition technique, a conformal coating consists of titania to passivate NAA can prevent corrosion and improve its biocompatibility in dental, orthopedic, and cardiovascular applications [43–45, 54, 55]. This claim was supported by cell growth on titania surface decorated with trace amounts of NAA that showed distress cell signals; this shows that NAA could hamper the ability of cells to produce mineralized matrices and hinder the development of bones [53]. This shows despite that

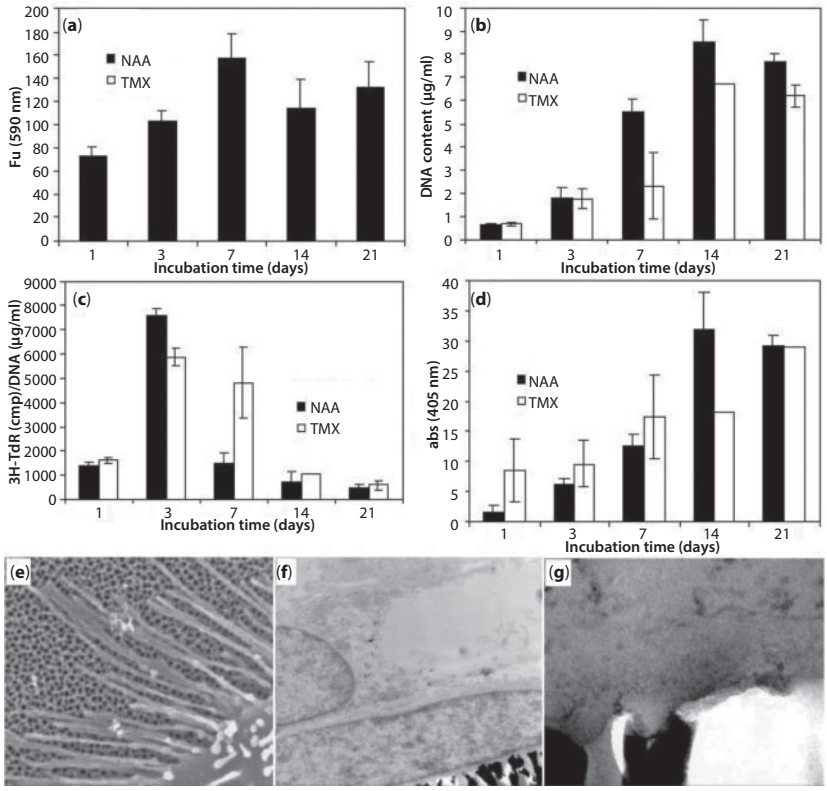


**Figure 6.7** (a) Alamar Blue assay results from HOB cells cultured on NAA for up to 21 days, (b) DNA content for HOB cells cultured on NAA and control thermonox for up to 21 days, (c) [3H]-TdR incorporation/DNA for HOB cells cultured on NAA and control thermonox for up to 21 days, (d) ALP activity of HOB cells cultured on NAA and control thermonox for up to 21 days, (e) SEM micrograph of HOB cells cultured on NAA for 1 day, (f) TEM micrograph of cross section of HOB cells cultured on NAA for 21 days, and (g) higher magnification of the image showing filopodia entering the NAA nanopores [49].

alumina and NAA material are reported to be biocompatible in many cases, there is nonetheless a cause for concern since it was shown recently that NAA with a 1D construct that is longer (L >20 μm) was shown to have a higher potency as compared to its shorter counterparts (L <10 μm) [56], inferring that the likelihood of toxicity stems from having a longer configuration or exists as fiber or particulate [57, 58].

To investigate the feasibility and biocompatibility of NAA in the form of nanotubes for cancer therapy, 600-nm-long NAA nanotubes were studied on breast cancer cells (MDA-MB231-TXSA) and macrophage cells (RAW264.7 macrophages) [18, 59]. Optical microscopy in

Figure 6.8a and b displays the visualized images of RAW 264.7 and TXSA cell lines incubated with NAA nanotubes at 100 μg $mL^{-1}$. After 5 days, cells proliferated immensely for both cell lines, indicating that NAA nanotubes did not obstruct cell growth but was shown to be biocompatible to them instead, even as they were placed in close contact with the surrounding cells without negatively impacting them. TEM analysis was carried out to probe the interactions between the NAA nanotubes and cells after incubation overnight. It can be observed in Figure 6.8c and d that the NAA nanotubes were evidently internalized by immune response and breast cancer cells. For macrophage cells, all the visible nanotubes were localized in the autophagic vacuoles. The blending of autophagosome with autophagic vacuoles is detected from the micrograph, while autophagic vacuoles containing cellular debris were also seen based on Figure 6.8c and d. This

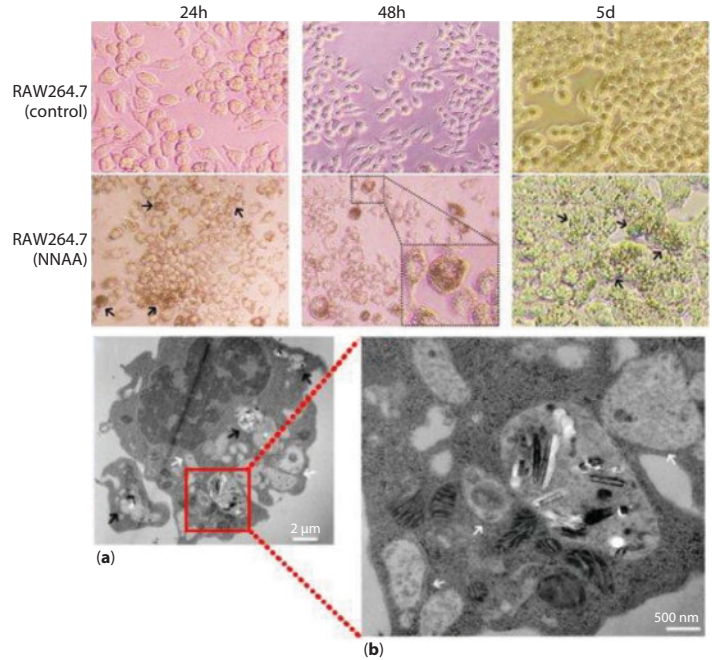


**Figure 6.8** (Top left) Optical microscopic images of NNAA (100 μg $mL^{-1}$) incubated with RAW264.7 macrophages cell lines. Black arrows indicate that cells are closely surrounded by AANTs. Note that the cells covered by NAA nanotubes have darker color due to the optical light refraction and adsorption of anodized alumina. No cell viability inhibition during the 5-day experiment. Insets show magnified views of individual cells after 48 h. (Right) (a and b) TEM images of NAA nanotubes (100 μg $mL^{-1}$) internalized by RAW 264.7 macrophage cells. The black arrows identify nanotubes inside cells; white arrows indicate the fusion of autophagic vacuoles, in which cellular debris were located inside [18].

proves the positive cell uptake from NAA nanotubes. An in-depth study was also carried out to probe the effects of NAA nanotubes with a series of length measuring at 0.7, 2.5, and 5.8 μm to investigate their interaction with macrophage cells and breast cancer cells [59]. It was found the least toxicity level was recorded for cells placed with NAA with the shortest nanotubes. For further expansion from a laboratory level, real-life investigation is required of NAA to probe its safety with chemical composition of bone tissue and human bone's nanostructures in addition to abide to public health requirements when in contact with the articulation and entire validity of the implant system with body fluids.

## 6.4 NAA for Diabetic and Pancreatic Applications

Aside from the application of NAA for DD, Desai and co-workers spearheaded the first work on NAA for the transport of glucose, immunoglobulin G (IgG) – a type of antibody and protein complex to combat diabetes [60–62]. Figure 6.9a shows electrochemically anodized NAA that were made in the form of nano-biocapsules (d = 46–75 nm). They were

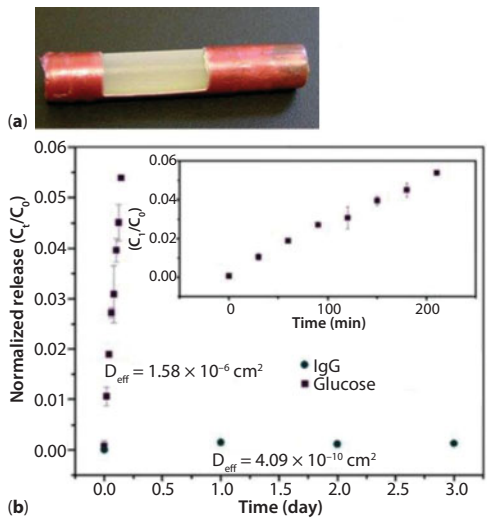


**Figure 6.9** (a) True image of prepared NAA biocapsules, and (b) normalized release of glucose IgG (circles) and (squares) over 3 days through NAA biocapsules with a nominal pore size of $d$ = 75 nm. $C_t$ = concentration at a specific moment in time $t$, $C_0$ = loading concentration at $t$ = 0. Inset displays glucose release on a much shorter, *i.e.* 210-min time scale [60].

loaded with insulin-secreting MIN6 insulinoma cells embedded in a collagen matrix. Figure 6.9b shows that glucose was released, but there was restricted release for IgG. This frontier study reported low diffusion coefficients for their release from NAA in comparison to free diffusion of both substances in water. To elaborate, the diffusion was lowest for IgG due to restricted nutrition supply to the inner channels of the biocapsules which in turn, impeded insulin release. Nevertheless, the cells embedded in the matrix were capable of generating a new supply of insulin for release, and for this, the release of insulin from the NAA biocapsules was recorded for up to 3 h long. Alternatively, NAA biocapsules can be inserted in micromachined silicon substrates for the immunoisolation of transplanted pancreatic islet cells [63]. The nanopores ($d$ = 10 nm) can effectively inhibit cellular and humoral immune species from advancing through the NAA channels at the same time allowing ample supply of oxygen and nutrients to sustain cell activity. Unfortunately, these NAA biocapsules are not safe over the longer term, and it is compulsory to surgically withdraw them from the body upon completion of therapeutics release [60].

## 6.5 NAA Applications in Orthopedics

Bone implants are, in essence, manufactured from Ti and its Al alloys in the form of screws or plates to mend broken bone fragments and direct bone growth. There is a growing attention to address bone infections as they are prevalent clinical complications, besides reports on rising implant failures and rejection [7, 8, 12]. NAA is a notable candidate to serve as an implant for treating conditions such as osteoporosis, osteoarthritis, Paget's disease, bone infections, primary and secondary cancers [63], in addition to explore the structural and biomechanical requirements, as well as its interaction with osteoblasts, osteoclasts, and osteocytes in bones, be it compact (or cortical) or spongy (cancellous) bone types. This is because they are robust biomaterials, can promote bone mass and microstructural integrity in a steady state as well as be developed for replacement in arthritic hip joint and eliminate the damaged bearing surfaces that are causing pain [64]. In addition to that, highly adherent NAA was successfully coated on Ti-containing substrates and applied as a functional bone implant as reported by Briggs *et al.* [65].

The use of NAA for prosthesis and total hip replacement has been much explored in terms of its technical aspects such as its material strength, design robustness, and the risk of fracturing. The extremely low generation of wear debris and its outstanding tolerance proved promising for a

sustainable implant life for young and active patients. There is a rising need for NAA to be studied to prove that HOBs cultured on NAA can induce the growth of physiological phenotype cells [47, 49]. A murine bone marrow stromal cell line, W20-17 was selected to probe the behavioral studies of osteogenic differentiation on NAA. Results were shown to be positive for a range of NAA substrates when pore sizes of $d$ = 20, 100, and 200 nm were used [65, 66]. *In vitro* studies exhibited the intelligent feasibility of NAA to release bone morphogenic protein-2 (BMP-2) for inducing cell proliferation as well as osteogenic differentiation and gene expression [66]. Behavioral studies of cell in response to NAA and their structural changes were carried out. It was observed that cells were laid out in different formation on NAA. For NAA with a pore size of $d$ = 200 nm, cells grew and assembled in elongated forms. On the other hand, cells on NAA with a pore size of 20 nm were noticed to have more spherical shapes. This implies that with greater pore size, there was greater volume for cells to occupy and proliferate, and thus they would utilize this space for greater growth as shown in Figure 6.10 [67]. Proliferation was observed to be higher on the control surfaces (wherein tissue culture polystyrene, TCPS, was used) compared to nanoporous surfaces (Figure 6.10c). Figure 6.10d shows that there was no significant difference in terms of cell amount among the different pores, whereas in Figure 6.10e, the total ALP values were normalized with intracellular protein content showing higher levels of ALP activity on 200 nm surfaces compared to 100 nm and control surfaces by 14 days of culture. A strong trend was found by day 14 with samples cultured with BMP-2, indicating higher levels of ALP activity with an increase in pore size as compared to the control. Figure 6.10f indicates an increased differentiation rate on 200 nm surfaces was found compared to 100 nm surfaces ($p < 0.05$) on substrates without the addition of BMP-2 was found. Differentiation rate seemed to even out for all surfaces, with the addition of BMP-2. Higher levels of osteocalcin (OC) gene expression on the 200 nm surfaces compared to 20 nm surfaces ($p < 0.05$) was observed in Figure 6.10g, and the same trend can be seen with cells exposed to BMP-2 [67].

*In vitro* HOBs cell culture on NAA shows no negative effect on the cell activity. Enhanced osteoblast adhesion (up to 52%) can be obtained on NAA (with smaller grain size < 49 nm) as compared to NAA (with a larger grain size > 67 nm) after 4 h of cell incubation. Minimal amount of aluminum ions (0.03 wt.%) leached out after 9 days, confirming its overall stability [50]. On the subject of metal leaching, residual phosphate ions were found left inside the nanopores of the NAA upon the completion of its etching process, when phosphoric acid was used as the electrolyte for the anodization of aluminum metal. It is beneficial in that the ions, which

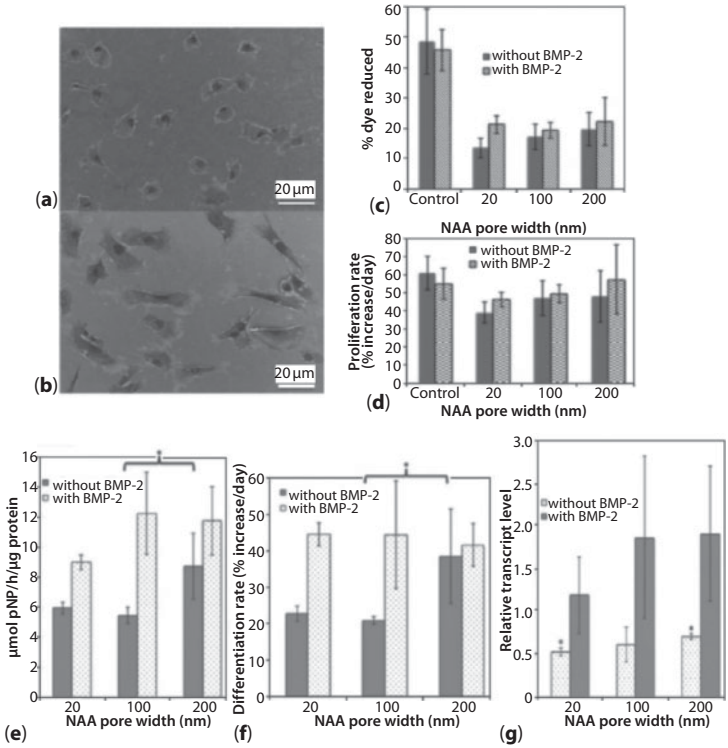


**Figure 6.10** Cell morphology: W20-17 cells cultured for 2 days on (a) 20 nm NAA and on (b) 200 nm NAA. (c) Cell proliferation was analyzed at day 7 using Alamar Blue. (d) Cell proliferation rate was determined by analyzing the increase in cell number from 2 to 7 days. (e) ALP activity of polystyrene (control) and NAA surfaces (20, 100, and 200 nm) with and without the addition of BMP-2 was measured after 14 days of culture. (f) Cell differentiation rate was determined by analyzing the increase in ALP enzyme activity for 12 days from day 2 to day 14. (g) OC gene expression with and without BMP-2 was analyzed at 14 days [67].

are the main constituents of hydroxyapatite, contribute to the strengthening of bone mineral and promote the embedding of the NAA implant in orthopedics. After all, bone is made up of 80% of the body's overall supply of phosphorus, aside from its niche in human cell culture, due to its ability to construct bone and aid in its maintenance. NAA is perceived as one of the excellent materials to be combined with ceramics, calcium phosphates, silica, zirconia, and titania due to their positive interactions with the human tissues [68]. A study has shown much feasibility to construct biomedical composites with diverse mechanical properties, water absorption strength, and bioactivity behavior by reporting the synthesis of alumina in combination with bioactive tricalcium phosphate, colloquially

coined as "bone ash", to form thermoplastics *via* the injection molding process. This ceramic–alumina material was used to reinforce hybrid, liquefiable polymers consisting of soybean proteins and casein. It was shown that the incorporation of tricalcium phosphate into the soybean weakened the thermoplastic. However, upon contact with simulated biofluids, the formation of a bioactive calcium–phosphate film due to nucleation was found on the thermoplastics. Zirconate, a common ceramic perovskite that is piezoelectric was added to the synthesized material. It was noticed that with an amount as low as 1%, zirconate significantly promoted the nucleation and growth of the bioactive films on the hybrid surface. Later, stability test was conducted by placing the said material in an isotonic saline solution to examine any disintegration for a 2-month period by monitoring their weight and water absorption. However, it is observed that after 30 days of subjecting the alumina-based biomaterial in biofluids, up to 30% of weight loss was found, indicating chemical degradation. As a result, there is much room for improvement with regard to its mechanical strength and the hydrolytic stability of the alumina–ceramic composite [69].

In summary, most of the literature reports on the applications of NAA pertaining to *in vitro* DD and the construction of biomaterials as *ex vivo* scaffolds or proliferation sites for the culture of bone-forming cells (osteoblasts). Notwithstanding, for pragmatic applications, there is a considerable gap to fill before NAA-based biomaterials can arrive to actual clinical settings and applied in real-life biological environment. The release characteristics with time and space profiles of drug molecules from NAA implant to the bone is an area not fully examined before, due to the limitations of real-time *in situ* and *in vivo* settings. To tackle this challenge, an unconventional approach was initiated by employing the Zetos™ bone bioreactor for the investigation of 3D drug release in bones from NAA synthesized on thin wires which was performed in an external environment closely mimicking the natural conditions. The drug-loaded implant was embedded at the center of a bovine bone core whereby the movement of drug molecules for its release from the wires over time could be monitored and was fluorescently imaged (Figure 6.11, left) [70]. The concentration profiles obtained are an overall indication of the drug distribution in bones from all angles depicted on and around the NAA wire. This study also demonstrated that diminutive versions of NAA such as that anodized on wire or needle rather than flat aluminum surface with reduced invasion in the body can be used as bone implants to render sustained release of therapeutics over time in orthopedics. The primary outcome is that no bone cells suffer from damage or harm, and that the NAA implants induced no negative effects on the cell viability of surrounding bones, while retaining

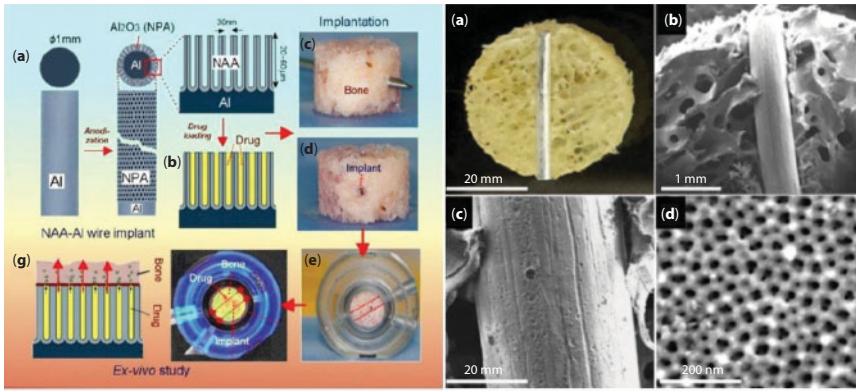


**Figure 6.11** (Left) Scheme of prepared Al wire implants with NAA layer on the surface showing their implantation into *ex vivo* bone model and *in situ* fluorescence drug release measurement using bone reactor Zetos™. (Right) (a and b) SEM micrographs of porous bone structure with placement of the NAA–Al wire implant showing (c and d) stable and unchanged surface morphology and nanopores structures of NAA [70].

the robustness of the material *per se* (Figure 6.11, right). Since the wire is millimeters thin and easy to penetrate into the skin, this technique is invasive only to an alleviated extent. It is envisaged that by using the implantable wires, NAA can be used in a clinical context without having patients to undergo major surgical operations in hospitals. Fewer complications would ensue as compared to current drug-releasing implants which are surgically invasive and valid only for transient use. This work leads to a myriad of possibilities for NAA to be used as drug-releasing implants in clinical applications, namely, cancer- and infection-related bone or skeletal conditions, metastatic bone disease (MBD), osteoporosis, Paget's disease, and bone deformities [7, 71, 72].

## 6.6 NAA Applications for Heart, Coronary, and Vasculature Treatment

The dawn of medical stents (expandable metal mesh tubes) in cardiology and vasculature has dramatically revamped the way heart and arteries-related diseases are treated. Traditionally, angioplasty was a stand-alone method. Nonetheless, on numerous accounts, medicines cannot clear blocked arteries, for instance, a very narrow coronary artery would require more advanced assistance to lower the risk of a heart attack. Hence, the introduction of percutaneous coronary intervention (PCI) – a widely and

currently used revascularization technique in the treatment of ischaemic heart disease to open up blocked coronary arteries (caused by coronary artery disease), such as balloon angioplasty was added into the picture. From then on, plaque can be shattered with a balloon and consequently unclog the blocked artery. However, too often there were complaints on patients experiencing restenosis, i.e. tissue regeneration that, unfortunately, re-obstruct the arteries again. Stents can retain the opening of an artery after angioplasty and overcome restenosis. However, new problems have continually and notoriously surfaced even with the use of angioplasty and stents, of which all would require new solutions. Herein, NAA is a suitable candidate for propping open an artery, and even very narrow ones, while preventing the formation of clots. Although polymer-coated stents have been clinically approved, they can cause inflammation over the longer term, not to mention recurring reports of late stent thrombosis (up to 1 year) and even later stent thrombosis (after 1 year) that have been reported due to delayed re-endothelialization. As a result, drug-loaded NAA implants were considered as an alternative option. Specifically, for interventional cases, coronary stent implantation has been perceived as more outstanding than conventional methods for tackling angioplasty. NAA is promising in that no reports claim that this material destroys the local arteries, or scar the tissues and have replacement ones that would grow over it. Neither was there any report made on NAA inducing the formation of clots on arterial walls. In-stent restenosis (ISR) is a subject that nevertheless demands considerable attention [72, 73]. Treating patients with ISR remains a pivotal clinical problem despite the fact that ISR occurrence has been decreased. Also, inflammatory infiltrations in the tissue surrounding struts, in addition to thrombogenesis and hyperplasia of intima are matters to look into in post-implantation [74]. Stent designs and coatings can vary; however, it is pertinent to coat stents in medication to keep a blocked artery from re-closing. This is an effective way as it was shown in NAA by imparting a layer of passive coating or to prepare an active layer on the stents for better biocompatibility and for restraining neointima proliferation through DD of anti-proliferative and immunosuppressive drugs in the stents [75]. Besides NAA synthesized on wires, for its role in heart, vasculature and coronary arteries, NAA is more commonly used in the coated form and also in *in vivo* DD [73, 76]. In one study, NAA was used to coat stainless steel coronary stents and loaded with an immunosuppressive drug, tacrolimus for implantation in a typical carotid artery of New Zealand white rabbits to treat restenosis. A layer of aluminum was laid across the surface of 316L stainless steel stents prior to the intended anodization process to alumina. It was found that the tacrolimus level in the rabbit's blood increased and the concentration

peaked after 1 h of implantation. However, the drug level subsided slowly in the next 48 h. Moreover, an evident decline in the neointima thickness for the drug-loaded and NAA-coated stents was also observed. *In vivo* studies in porcine models have demonstrated that the shedding of particle debris released from the NAA nanoporous coatings produces a significant increment of neointimal hyperplasia, as compared to bare stainless steel stents. Also, with NAA coated rather than bare-metal stents, the likelihood of restenosis (re-narrowing) would be relatively lower, even more so for patients receiving drug-loaded NAA stents. Drug-loaded stents report much milder inflammation as compared to the uncoated ones, independent of the drug loading. Regardless, a more thorough investigation on the biocompatibility and re-endothelialization in tissues after injuries are necessary before these implants can proceed to clinical trials to test in patients. NAA can be modified to become a self-expanding stent for urgent usage in an emergency situation. The intelligent function lies in its self-expanding ability via balloon angioplasty. Owing to the vast areas that stents can be put into use, such as in the brain for treating aneurysm, or in conjunction with multidrug DDS whereby emergency surgical needs are called for, to tend to coronary artery blockage when a blood vessel has been blocked by plaque, it holds abundant potential. In summary, for angina treatment, instead of repetitive and heavy medications, or arranging for a major bypass surgery, addressing coronary artery plaques can be much more convenient and less complicated with NAA. Besides blood vessels, with the many intelligent properties of NAA such as its nanoscaled size and inertness, NAA-coated stents can be used to literally open up a multitude of passageways throughout the human cardiovascular system, such as bile ducts, bronchi, or ureters.

## 6.7 NAA in Dentistry

Another branch of biomedicine that cannot be overlooked relates heavily to periodontal ligament health and dental implant osteointegration. They are indicative of a special type of orthopedics but not related to the skeletal system. Rather, it is confined to the oral research area in which dentistry can be secondarily referred to as "dental orthopedics", because teeth are after all not a mineralized extension of the bone structures in joints or limbs, in a general sense, but rather a living and thriving organ in the form of an array of living teeth with sensitivity in connection with pulp and nerves, also surprisingly one of the most complex ones in the human body. It is essential to have higher elastic modulus and higher fracture toughness to support teeth and any cavities associated with it. As a result, alumina is one of the most

used ceramic and composite materials due to its high chemical and thermal stability, high specific area, and controllable microstructure. With regard to the fracture toughness, alumina present values that are three to four times higher than for silicate or glass commonly used in composite resins. NAA biofillers can fulfill that requirement. When in need of reinforcement, dental resin composite with high elastic modulus can increase the modulus of the composite which partially comprises of NAA to approach the modulus of tooth dentine (12–20 GPa) [77]. For work on DD, a novel coupling-agent-free dental restorative resin composite based on fillers made from NAA was an intelligent effort carried out in the *in vitro* regime [78]. The NAA biofillers were synthesized *via* milling with silver nanoparticles inserted inside their nanopores. It was then loaded with standard resin at 50 wt. % loading (maximum amount) to form a composite. The fillers were prepared by milling NAA membranes, followed by composites prepared with standard resin at a maximum loading of 50 wt.%. Stability tests showed that these NAA-containing composites are more long-lasting and age slower than commercial materials. The underlying cause for this is ascribed to the extremely close and well-interconnected nanoporous network in the alumina. Excellent mechanical interlocking between the fillers and NAA without involving any chemical interactions can be achieved and because of this advantage, the problem arising from the release of coupling agent that was often reported as chemical degradation can be circumvented. These NAA-containing composite fillers can also be activated *via* pore filling using bio-agents with twofold intelligent functions, to enhance the biocompatibility of this composite, as well as in an effort to annihilate any possible chemically triggered deterioration of its robustness. By virtue of its tunable nanopore size *via* electrochemical anodization, again, drug-releasing function can be assimilated in the dental biofillers for different drug types and drug release patterns to treat inflammation, periodontal and tooth diseases, tooth disorders, or dental pathology, aside from it being used as dental fillers. Structural and surface treatment of experimental fillers can be made by NAA to fortify resin composites or resin-modified glass ionomer cements [79, 80]. A novel synthesis approach for preparing porous alumina monoliths with high specific surface area was by treating them with a functionalized organometallic silane coupling agent called trimethyletoxysilane (TMES) *via* a simple and brief impregnation process. Calcination at 1300 °C was carried out to consolidate the biomaterial to generate stronger bonds and increase the porosity. Figure 6.12 displays the SEM micrograph of the alumina surface morphology. Apparently, the silanization of the mineral phase in the NAA monoliths with a polymer-modified silane and combining them with methacrylate or polyalkenoate as additives is required before

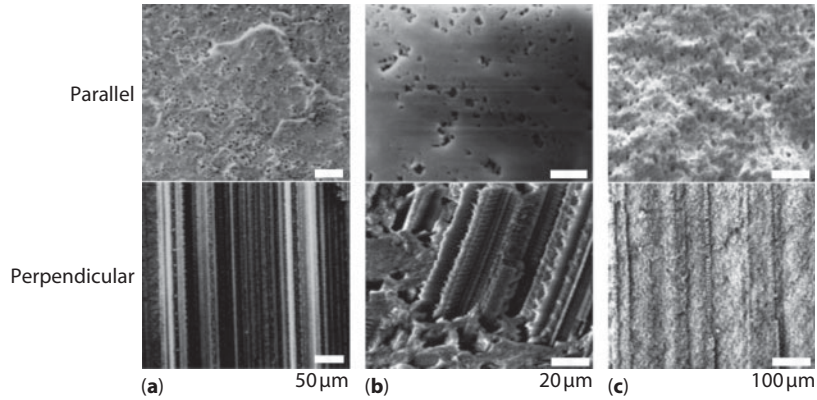


**Figure 6.12** SEM micrographs of the NAA monolith samples with controlled and specific surface (at least 10 times higher than that of typical biofillers) for the synthesis of dental composites, with the parallel and perpendicular images (top to bottom) shown for NAA-based materials (a) annealed at 1150 °C, (b) at 1300 °C, and (c) to the alumina block growth direction [79].

they underwent mechanical testing and were used as fortifying agents in resin composites, resin-modified glass ionomer cements, or cortical bone replacement in dentistry [79, 80]. These studies show that the fabrication of dental restorative composites with NAA as one of the building blocks could enhance the elasticity, stability, morphology, hardness, and surface properties for dental applications. In recent years, intelligent composites that are worthy of notice are NAA matrices engineered with high interfacial area and high dispersion of fine nanoparticulate in the structuring of biofillers. In a more recent work, NAA membranes were further processed by ball-milling, to increase the elastic modulus. Upon thermal treatment, the filler micro-particles of ball-milled porous alumina render better elastic modulus stability than commercial restorative materials. This is ascribed to a mechanical interlocking phenomenon due to the nanopores infiltrated by resin through mixing and sonication [81, 82].

## 6.8 Conclusions and Future Prospects

This review has outlined the recent findings in the present-day and ongoing research on electrochemically anodized NAA deemed as a progressive nanomaterial for biomedical applications. NAA offers many advantages and have been proved in numerous reports to be a promising bio-device ascribed to advanced intelligent functions based on nanotechnology research. It holds tremendous potential for manufacturing and translation

into clinical, commercial, pharmaceutical as well as surgical usage, as the anodization-based, electrochemical synthesis for NAA is a well established and scalable, simple and low-cost technique. NAA coating, layer, composite, or fillers can be generated on existing medical implants such as orthopedic plates, dental screws, coronary, implantable wires in brains, vasculature stents with a wide range of adjustable shapes and sizes based on anodization parameters. Specifically, for the delivery of therapeutics, NAA with intelligent drug-releasing ability is capable of eluting drugs for sustained, long- or short-term, pulsatile, stepwise, rapid, or continuous release and thus is tailorable to different conditions and patient needs. The drug release characteristics are governed by the tunable NAA surface area, nanopore dimensions, geometries, morphology, and surface chemistry. Due to this, drug-loading and drug release kinetics can be controlled. NAA has shown to have many outstanding properties, including chemical and corrosion resistance, mechanical and thermal stability, rigidity, and proved biocompatibility that have been explored for broad medical applications presented in this review including orthopedic and dental implants, heart/coronary/vasculature stents, tissue engineering and cell culture. Nevertheless, to achieve successes of concepts presented throughout this review, more in-depth research is required to be carried out in order to make NNA-based drug-releasing implants and biomedical devices feasible for real-life clinical applications. Although NAA and NAA nanotubes are reported to be bio-inert and non-toxic, more biocompatibility characterizations are required before their translation to clinical and human trial stages. More studies are also required to confirm the efficiency and suitability of NAA for their applications as biomedical devices such as nanoguide wires, stent/vascular grafts, and orthopedic implant insert-able into different parts of the body, e.g., esophagus, trachea, arteries, or brain. Finally, several exciting future developments are anticipated. This includes the integration of digitized NAA implants with microchips, the development of advanced sensory-based drug release, the application of implants for combination therapy, and potential for localized therapy of primary solid cancers or secondary bone cancers. Lastly, scientists are currently investigating whether there is an increased risk of stent thromboses with certain drug-eluting stents.

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## References


1. Mainardes, R.M., Silva, L.P., Drug delivery systems: past, present, and future. *Curr. Drug Targets,* 5, 449–55, 2004.
2. Drews, J., Drug discovery: A historical perspective. *Science,* 287, 1960–4, 2000.
3. Hoffman, A.S., The origins and evolution of "controlled" drug delivery systems. *J. Control Release,* 132, 153–63, 2008.
4. Folkman, J., Mark, V.H., Diffusion of anesthetics and other drugs through silicone rubber: Therapeutics implications. *Transac New York Acad. Sci.,* 30, 1187–95, 1968.
5. Wolinsky, J.B., Colson, Y.L., Grinstaff, M.W., Local drug delivery strategies for cancer treatment: Gels, nanoparticles, polymeric, films, rods, and wafers. *J. Control Release,* 159, 14–26, 2012.
6. Gultepe, E., Nagesha, D., Sridhar, S., Amiji, M., Nanoporous inorganic membranes or coatings for sustained drug delivery in implantable devices. *Adv. Drug Deliv. Rev.,* 62, 305–15, 2010.
7. Santos, A., Aw, M.S., Bariana, M., Losic, D., Drug-releasing implants: Current progress, challenges and perspectives. *J. Mater. Chem. B,* 2, 6157–82, 2014.
8. Losic, D., Simovic, S., Self-ordered nanopore and nanotube platforms for drug delivery applications. *Expert Opin. Drug Deliv.*, 6, 1363–81, 2009.
9. Roy, P., Berger, S., Schmuki, P., $TiO_2$ nanotubes: Synthesis and applications. *Angew. Chem. Int. Ed. Engl.,* 50, 2904–39, 2011.
10. Bauer, S., Schmuki, P., *et al*. Engineering biocompatible implant surfaces, Part I: Materials and surfaces. *Prog. Mater. Sci.,* 58, 261–326, 2013.
11. Aw, M.S., Kurian, M., Losic, D., Non-eroding drug-releasing implants with ordered nanoporous and nanotubular structures: Concepts for controlling drug release. *Biomater. Sci.,* 2, 10–34, 2014.
12. Losic, D., Aw, M.S., Santos, A., Gulati, K., Bariana, M., Titania nanotube arrays for local drug delivery: Recent advances and perspectives. *Expert Opin. Drug Deliv.,* 12, 103–27, 2015.
13. Md Jani, A.M., Losic, D., Voelcker, N.H., Nanoporous anodic aluminium oxide: Advances in surface engineering and emerging applications. *Prog. Mater. Sci.*, 58, 636–704, 2013.
14. Masuda, H., Fukuda, K., Ordered metal nanohole arrays made by a two-step replication of honeycomb structures of anodic alumina. *Science,* 268, 1466–8, 1995.
15. Lee, W., Ji, R., Gosele, U., *et al.*, Fast fabrication of long-range ordered porous alumina membranes by hard anodization. *Nat. Mater.,* 5, 741–7, 2006.
16. Wood, G.C., Sullivan, J.P.O., Vaszko, B., The direct observation of barrier layers in porous anodic oxide films. *J. Electrochem. Soc.,* 115, 618–20, 1968.
17. Santos, A, Kumeria, T, Losic, D, Nanoporous anodic aluminium oxide (AAO) for chemical sensing and biosensors. *TrAC*, 44, 25–38, 2013.
18. Gultepe, E., Nagesha, D., Casse, B.D.F., Sustained drug release from non-eroding nanoporous templates. *Small,* 6, 213–6, 2010.

19. Wang, Y., Santos, A., Kaur, G., Evdokiou, A., Losic, D., Structurally engineered anodic alumina nanotubes as nano-carriers for delivery of anticancer therapeutics. *Biomaterials,* 35, 5517–26, 2014.
20. Wang, Y., Santos, A., Evdokiou, A., Losic, D., Rational design of ultra-short anodic alumina nanotubes by short-time pulse anodization. *Electrochim. Acta.,* 154, 379–86, 2015.
21. Gong, D., Yadavalli, V., Paulose, M., *et al*., Controlled molecular release using nanoporous alumina capsules. *Biomed Microdevices,* 5, 75–80, 2013.
22. Noh, K., Brammer, K.S., Choi, C., *et al.,* A new nano-platform for drug release via nanotubular aluminum oxide. *J. Biomater. Nanobiotechnol.*, 2, 226–33, 2011.
23. Losic, D., Velleman, L., Kant, K., *et al*., Self-ordering electrochemistry: A simple approach for engineering nanopore and nanotube arrays for emerging applications. *Aust. J. Chem.,* 64, 294–301, 2011.
24. Kang, H.-J., Kim, D.J., Park, S.-J., *et al.*, Controlled drug release using nanoporous anodic aluminum oxide on stent. *Thin Solid Films,* 515, 5184–7, 2007.
25. Kipke, S., Schmid, G., Nanoporous alumina membranes as diffusion controlling systems. *Adv. Funct. Mater.,* 14, 1184–8, 2004.
26. Kwak, D.-H., Yoo, J.-B., Kim, D.J., Drug release behavior from nanoporous anodic aluminium oxide. *J. Nanosci. Nanotech.,* 10, 345–8, 2010.
27. Das, S.K., Kapoor, S., Yamada, H., *et al*., Effects of surface acidity and pore size of mesoporous alumina on degree of loading and controlled release of ibuprofen. *Mesopor. Micropor. Mater.,* 118, 267–72, 2009.
28. Perro, A., Reculusa, S., Ravaine, S., *et al.*, Design and synthesis of Janus micro- and nanoparticles. *J. Mater. Chem.*, 15, 3745–60, 2005.
29. Simovic, S., Losic, D., Vasilev, K., Controlled drug release from porous materials by plasma polymer deposition. *Chem. Commun.,* 46, 1317–9, 2010.
30. Simovic, S., Losic, D., Vasilev, K., Controlled release from porous platforms. *Pharm. Technol.,* 35, 8, 68–71, 2010.
31. Gulati K., Aw, M.S., Losic D., Controlling drug release from titania nanotube arrays using polymer nanocarriers and biopolymer coating. *J. Biomater. Nanobiotechnol.*, 2, 477–84, 2011.
32. Losic D., Cole M.A., Dollmann B., *et al*. Surface modification of nanoporous alumina membranes by plasma polymerization. *Nanotechnology,* 19, 245704 (7 pg), 2008.
33. Vasilev K., Poh Z., Kant K., Chan J., Michelmore A., Losic D., Tailoring the surface functionalities of titania nanotube arrays. *Biomaterials,* 31(3), 532–40, 2010.
34. Gulati K., Ramakrishnan S., Aw M.S., Atkins G.J., Findlay D.M., Losic D., Biocompatible polymer coating of titania nanotube arrays for improved drug elution and osteoblast adhesion. *Acta Biomater.,* 8, 449–56, 2012.
35. Jeon G., Yang S.Y., Byun J. *et al*., Electrically actuatable smart nanoporous membrane for pulsatile drug release. *Nano Lett.,* 11, 1284–8, 2011.

36. Kapoor S., Hegde R., Bhattacharyya A.J., Influence of surface chemistry of mesoporous alumina with wide pore distribution on controlled drug release. *J. Controlled Release,* 140, 34–9, 2009.
37. Abelow A.E., Persson K.M., Jager E.W.H., *et al.,* Electroresponsive nanoporous membranes by coating anodized alumina with poly(3,4-ethylenedioxythiophene) and polypyrrole. *Macromol. Mater. Eng.,* 299, 190–7, 2014.
38. Aw M.S., Addai-Mensah J., Losic D., A multi-drug delivery system with sequential release using titania nanotube arrays. *Chem. Commun.,* 48, 3348–50, 2012.
39. Aw M.S., Simovic S., Addai-Mensah J., Losic D., Polymeric micelles in porous and nanotube materials as a new system for extended delivery of poorly soluble drugs. *J. Mater. Chem.* 21, 7082–9, 2011.
40. Aw M.S., Addai-Mensah J., Losic D., Magnetic-responsive delivery of drug-carriers using titania nanotube arrays. *J. Mater. Chem.,* 22, 6561–3, 2012.
41. Bariana M., Aw M.S., Moore E., Voelcker N.H., Losic D., Radiofrequency-triggered release for on-demand delivery of therapeutics from titania nanotube drug-eluting implants. *Nanomedicine,* 9, 1263–75, 2014.
42. Sedel L. Evolution of alumina-on-alumina implants. *Clin Orthopaed Relat Res,* 379, 48–54, 2000.
43. Popat K.C., Swan E.E.L., Mukhatyar V., Chatvanichkul K.I., Mor G.K., Grimes C.A., Desai T.A., Influence of nanoporous alumina membranes on long-term osteoblast response. *Biomaterials* 26, 4516–22, 2005.
44. Swan E.E.L., Popat K.C., Grimes C.A., Desai T.A., Fabrication and evaluation of nanoporous alumina membranes for osteoblast culture. *J. Biomed. Mater. Res Part A,* 72A, 288–95, 2005.
45. La Flamme K.E., Popat K.C., Leoni L., Markiewicz E., La Tempa T.J., Roman B.B., Grimes C.A., Desai T.A., Biocompatibility of nanoporous alumina membranes for immunoisolation. *Biomaterials* 28, 2638–45, 2007.
46. Popat K.C., Chatvanichkul K.I., Barnes G.L., Latempa T.J., Grimes C.A., Desai T.A., Osteogenic differentiation of marrow stromal cells cultured on nanoporous alumina surfaces. *J. Biomed. Mater. Res. Part A,* 80A, 955–64, 2007.
47. Swan E.E.L., Popat K.C., Desai T.A., Peptide-immobilized nanoporous alumina membranes for enhanced osteoblast adhesion. *Biomaterials,* 26, 1969–76, 2005.
48. Poinern G.E.J., Le X.T., O'Dea M., *et al.*, Chemical synthesis, characterisation, and biocompatibility of nanometre scale porous anodic aluminium oxide membranes for use as a cell culture substrate for the Vero cell line: A preliminary study. *BioMed. Res. Int.,* Article ID 238762, 10 pgs, 2014.
49. Karlsson M., Pålsgård E., Wilshaw P.R., *et al.*, Initial *in vitro* interaction of osteoblasts with nano-porous alumina. *Biomaterials,* 24, 3039–46, 2003.
50. Karlsson M., Johansson A., Tang L., Boman M., Nanoporous aluminum oxide affects neutrophil behaviour. *Microsc Res Tech,* 63, 259–65, 2004.
51. Karlsson M., Tang L., Surface morphology and adsorbed proteins affect phagocyte responses to nano-porous alumina. *J. Mater. Sci. Mater. Med.,* 17, 1101–11, 2006.

52. Lee S., Park M., Park H.-S., *et al.*, A polyethylene oxide-functionalized self-organized alumina nanochannel array for an immunoprotection biofilter. *Lab Chip.,* 11, 1049–53, 2011.
53. Canabarro A., Diniz M.G., Paciornik S., *et al.*, High concentration of residual aluminum oxide on titanium surface inhibits extracellular matrix mineralization. *J. Biomed. Mater. Res.,* 87A, 588–97, 2008.
54. Cameron M.A., Gartland I.P., Smith J.A., *et al.*, Atomic layer deposition of $SiO_2$ and $TiO_2$ in alumina tubular membranes: Pore reduction and effect of surface species on gas transport. *Langmuir,* 16, 7435–44, 2000.
55. Narayan R.J., Monteiro-Riviere N.A., Brigmon R.L., *et al.*, Atomic layer deposition of $TiO_2$ thin films on nanoporous alumina templates: Medical applications. *JOM,* 61, 12–6, 2009.
56. Kostarelos, K., The long and short of carbon nanotube toxicity. *Nat. Biotechnol.,* 26, 774–6, 2008.
57. Poland C.A., Duffin R., Kinloch I., *et al.*, Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat. Nano.,* 3, 423–8, 2008.
58. Hart,G.A., Kathman, L.M., Hesterberg, T.W., In vitro cytotoxicity of asbestos and man-made vitreous fibers: roles of fiber length, diameter and composition. *Carcinogenesis,* 15, 971–7, 1994.
59. Wang Y., Kaur G., Zysk A., Liapis V., Hay S., Santos A., Losic D., Evdokiou A., Systematic *in vitro* Nanotoxicity Study on Anodic Alumina Nanotubes with Engineered Aspect Ratio: Understanding Nanotoxicity by a Nanomaterial Model, *Biomaterials,* 46, 117–30, 2015.
60. La Flamme K.E., Popat K.C., Leoni L., *et al.*, Biocompatibility of nanoporous alumina membranes for immunoisolation. *Biomaterials,* 28, 2638–45, 2007.
61. La Flamme K.E., Gopal M., Gong D., La Tempa T., Fusaro, V.A., Grimes C.A., Desai T.A., Nanoporous Alumina Capsules for Cellular Macroencapsulation: Transport and Biocompatibility. *Diabetes Technol. Therapeutics,* 7, 684–94, 2005.
62. Desai T.A., Chu W.H., Rasi G., Microfabricated biocapsules provide short-term immunoisolation of insulinoma xenografts. *Biomed. Microdevices,* 1, 131–8, 1999.
63. Walczak R.J., Boiarski A., West T., *et al.*, Long-term biocompatibility of NanoGATE drug delivery implant. *Nanobiotechnology,* 1, 35–42, 2005.
64. Webster T.J., Siegel R.W., Bizios R., Osteoblast adhesion on nanophase ceramics. *Biomaterials,* 20, 1221–7, 1999.
65. Briggs, E.P., Walpole, A.R., Wilshaw, P.R., Karlsson, M., Palsgard E., Formation of highly adherent nanoporous alumina on Ti-based substrates: A novel bone implant coating. *J. Mater. Sci. Mater. Med.*, 15(9), 1021–9, 2004.
66. Habraken W.J.E.M., Wolke J.G.C., Jansen J.A., Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. *Adv Drug Delivery Rev,* 59, 234–48, 2007.
67. Pujari-Palmer S., Lind T., Xia W., *et al.*, Controlling osteogenic differentiation through nanoporous alumina. *J. Biomater. Nanobiotech.,* 5, 98–104, 2014.

68. Hoess A., Teuscher N., Thormann A., *et al*., Cultivation of hepatoma cell line HepG2 on nanoporous aluminum oxide membranes. *Acta Biomater.,* 3, 43–50, 2007.
69. Parkinson L.G., Rea S.M., Stevenson A.W., The effect of nano-scale topography on Keratinocyte phenotype and wound healing following burn injury. *Tissue Eng. Part A,* 18, 703–14, 2012.
70. Vaz, C.M., Fossen, M., van Tuil, R.F., de Graaf, L.A., Reis, R.L., Cunha, A.M., Casein and soybean protein-based thermoplastics and composites as alternative biodegradable polymers for biomedical applications. *J. Biomed. Mater. Res. Part B: Appl. Biomater.,* 87, 455–60, 2008.
71. Rahman S., Atkins G., Findlay D., Losic D., Nanoengineered drug releasing aluminium wire implants: a model study for localized bone therapy. *J. Mater. Chem. B,* 2015, DOI: 10.1039/c5tb00150a.
72. Gulati K., Aw M.S., Findlay D., Losic D., Local drug delivery to the bone by drug-releasing implants: perspectives of nano-engineered titania nanotube arrays. *Ther. Deliv.,* 3, 857–73, 2012.
73. Karoussos I.A., Wieneke H., Sawitowski T., *et al*. Inorganic materials as drug delivery systems in coronary artery stenting. *Materialwissenschaft und Werkstofftechnik,* 33, 738–46, 2002.
74. Wieneke H., Sawitowski T., Wnendt S., *et al*., Stent coating: a new approach in interventional cardiology. *Herz,* 27, 518–26, 2002.
75. Kollum M., Farb A., Schreiber R., *et al*. Particle debris from a nanoporous stent coating obscures potential antiproliferative effects of tacrolimus-eluting stents in a porcine model of restenosis. *Catheter. Cardiovasc. Interv.,* 64, 85–90, 2005.
76. Park, G.E., Webster, T.J., A review of nanotechnology for the development of better orthopedic implants. *J. Biomed. Nanotechnol.*, 1, 18–29, 2005.
77. Wieneke, H., Dirsch, O., Sawitowski, T., *et al*., Synergistic effects of a novel nanoporous stent coating and tacrolimus on intima proliferation in rabbits. *Catheter. Cardiovasc. Interv.,* 60, 399–407, 2003.
78. Augker, L., Swain, M.V., Kilpatrick, N., Micro-mechanical characterisation of the properties of primary tooth dentine. *J. Dent.,* 31, 261–7, 2003.
79. Thorat, S.B, Diaspro, A., Salerno, M., *In vitro* investigation of coupling-agent-free dental restorative composite based on nano-porous alumina fillers. *J. Dentistry,* 42, 279–86, 2004.
80. Azevedo, C., Tavernier, B., Vignes, J.L., *et al*., Design of nanoporous alumina structure and surface properties for dental composite. *Key Eng. Mater.,* 361–3, 809–12, 2007.
81. Azevedo, C., Tavernier, B., Vignes, J.L., *et al.,* Structure and surface reactivity of novel nanoporous alumina fillers. *J. Biomed. Mater. Res. B Appl. Biomater.,* 88, 174–81, 2009.
82. Adiga, S.P., Jin, C., Curtiss, L.A., Nanoporous membranes for medical and biological applications. *Wiley Interdiscip Rev Nanomed. Nanobiotechnol.*, 1, 568–81, 2009.

83. Salerno, M., Diaspro, A., and Thorat, S., Combined Material Including Anodic Porous Alumina and a Polymer Matrix, and Its Use for the Dental Recondition. Available at: http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2014053946A1&KC=A1&FT=D&ND=3&date=20140410&DB=EPODOC&locale=en_EP, 2014.