Review Article **Open Access**

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Mesoporous alumina as a biomaterial for biomedical applications

DOI 10.1515/mesbi-2015-0004
Received June 23, 2015; revised August 6, 2015; accepted August 6, 2015

Abstract: Porous anodic alumina (PAA) is a biomaterial based on a cost-effective electrochemical anodization of pure aluminum with unique geometrical properties, i.e., self-ordering hexagonal pore distribution, tunable pore diameters and interpore distances, and uniformity of the pores in the vertical direction (nanochannels). These remarkable properties have found important applications in several fields such as energy storage, optics, photonics, magnetism, catalysis and, in particular, in the biomedicine field. In this work, we review the current state of research and key issues on cell culture and implants, drug delivery systems with complex release profiles and specific action, and high efficiency and sensitivity biosensors with different biosensing mechanisms, all of them based on PAA. The biocompatibility, morphology of the surface, nanoestructural engineering in-depth, surface functionalization and coatings are discussed and analyzed in detail.

Keywords: porous anodic alumina; biocompatibility; toxicity; *in vivo*; *in vitro*; cell growth; functionalization; drug delivery; micro and nanoparticles; biosensing

1 Introduction

Porous anodic alumina (PAA) is a biomaterial with interesting applications in biomedicine due to its unique properties such as regular pore size, uniform pore density, high stiffness, high mechanical strength, chemical stability and thermal stability. Its pore geometry can be tuned by modifying the fabrication parameters (anodization voltage or current, temperature, electrolyte and post-fabrication etching) and its surface chemistry can be modified by well-established chemical processes [1–3]. PAA is generally considered a bioinert material, but its bioactivity can be achieved by coating its surface with bioactive materials. These remarkable properties have found promising applications as bone and dental implants, immunoisolation devices, scaffolds for tissue engineering, biomolecular filtration, etc. [4–7]. For example, the good mechanical stability, chemical inertness, and tunable surface chemistry have made PAA to be an excellent material for loading large amount of drugs and their controlled release. Other example is biosensing. Its high specific surface area-to-volume ratio (about hundreds of m^2/cm^3) and easy chemical surface functionalization makes PAA a very useful material to target molecules attached inside the nanopores.

In this work, we review recent studies on porous alumina and its application in cell culture, drug delivery and biosensing. We will discuss the biocompatibility and non-toxicity of porous anodic alumina throughout *in vitro* cell culture studies and *in vivo* tests. The effects of the surface morphology of porous alumina on cell growth, cell adhesion, migration, morphology, proliferation, etc, will be also examined for different types of cells. Furthermore, in order to enhance the bioactivity of porous anodic alumina various chemical surface modifications and coatings will be presented. The use of PAA as a drug delivery system will be highlighted. Finally, different examples of nanostructured and functionalized porous alumina for detecting proteins, enzymes, biomolecules, etc, will be overviewed.

2 Biocompatibility and toxicity of porous anodic alumina

The use of porous anodic alumina for potential biomedical uses is closely conditioned by its biocompatibility. It is well known that the structural features of porous alumina do not degrade under *in vitro* and *in vivo* conditions, a very interesting property that has led to the rise of porous

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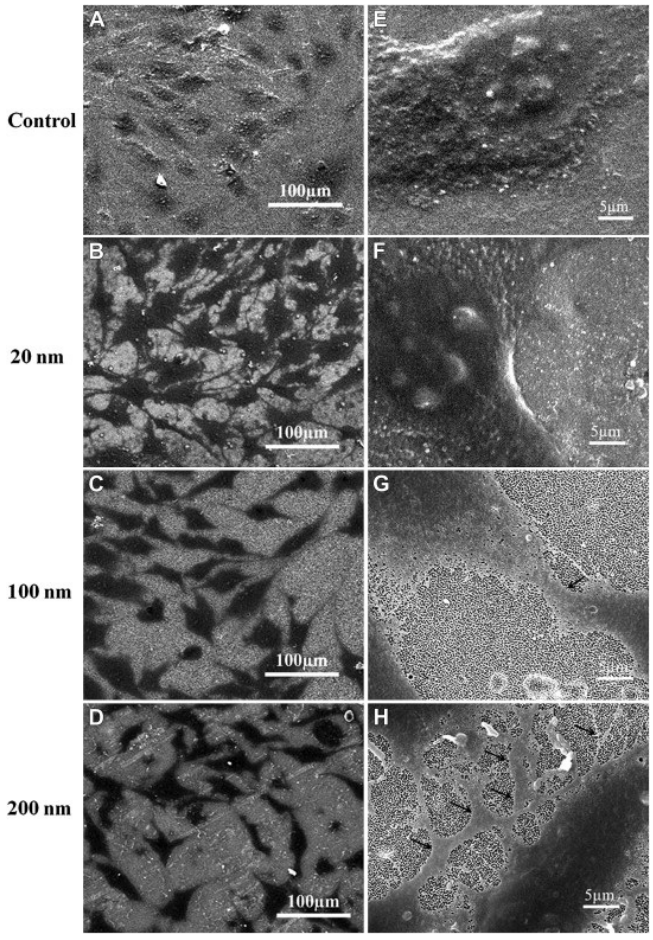

Figure 1: SEM images of the MG63 cells on the nanoporous alumina substrates with different pore sizes or cover glass (control) after incubation for 4 days: (A, E) Cover glass, (B, F) 20 nm, (C, G) 100 nm and (D, H) 200 nm in diameter of nanopore sizes. Dark color in images shows MG63 cells on the different substrates. Black arrows indicate the membrane protrusions. (Ref. [15])

alumina for specific biological applications, such as immunoisolation, biofiltration or biosensing. Nevertheless, the use of porous alumina for medical uses and cell culture requires of cell toxicity and viability studies.

Some *in vitro* immunoisolation studies carried out in PAA membranes suggest that alumina is non-toxic and does not generate significant complement activation. *In vitro* studies showed no toxic effects of porous alumina capsules. *In vivo* studies one week after implantation, showed that there is moderate inflammation of the tissue surrounding both with poly(ethylene glycol) (PEG)-modified or unmodified capsules, and the unmodified ones showed slightly more inflammation). However, after 4 weeks, the reduction in the granulation layer as well as the existence of blood vessels in the tissue nearby the PEG-modified capsules suggests that the inflammation was mainly due to the implantation procedure itself [8]. Such modified membranes exclude antibodies, but permit access of insulin and glucose and therefore may be suitable for the encapsulation of pancreatic islet cells for treatment of type I diabetes.

The release of aluminum ions from PAA structures has also been analyzed on cell activity [9–11]. The results showed a very small aluminum ion leakage corresponding to 0.03% of the original membrane weight into the surrounding media after 9 days. This amount shows no adverse effect on cell activity and some previous works demonstrated that low concentrations of aluminum ion could act directly on osteoblasts to stimulate their proliferation and differentiation.

2.1 Cell growth

PAA is an interesting platform for cell growth applications. It is a suitable vehicle for studying several culture aspects like cell proliferation, cell morphology, cell-to-cell communication or differentiation of cells in cultures. However, the influence of the PAA structure on cell growth is closely related to the type of cell analyzed. For this reason, this section reports on recent studies on several different cells cultured on PAA platforms.

One of the most interesting applications in this field is the use of the PAA for bone engineering and the development of orthopaedic implants. Several studies have demonstrated the suitability of porous alumina for the growth of osteoblastic cells. Kalsson *et al.* used a primary human osteoblast-like (HOB) cell model to study the cell response to PAA with a pore size of 200 nm. The results indicate that a good surface for osteoblastic cell growth, with cells rapidly spreading, flattening and adhering firmly to the PAA [12]. In a similar work, Swan *et al.* evaluated the osteoblast response to porous anodic alumina by studying their adhesion and morphology to human fetal osteoblast cells (hFOB 1.19) [13]. Results showed that after 4 days of culture, osteoblasts exhibited normal phenotype and morphology and were able to extend their processes into the pores. A long term study of the influence of porous alumina membranes on osteoblast adhesion and proliferation is presented by Popat *et al.* Results showed improved osteoblast adhesion and proliferation, and increased matrix production after 4 weeks of study. The results were compared with aluminum, amorphous membranes, commercially available ANOPORE^{TM} membranes and glass [14].

However, several studies have demonstrated that pore size is a decisive feature of PAA platforms, with a strongly influence on the morphology and adhesion of the cells.

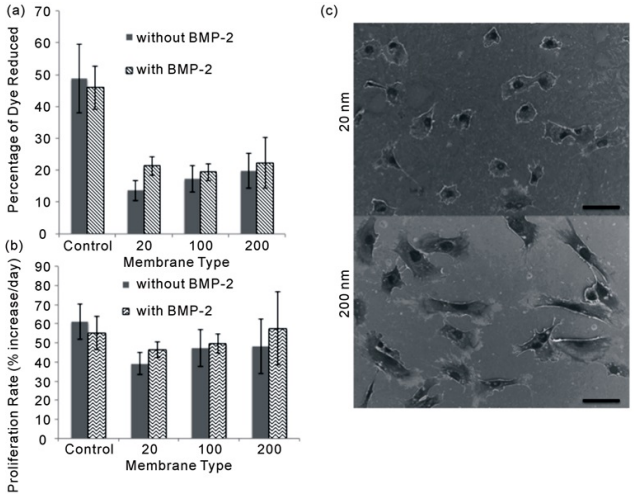

Figure 2: a) Cell proliferation for different substrates after 7 days, b) cell proliferation rate (increment in cell number from 2 days to 7 days), and c) W20-17 cell morphology cultured for 2 days on 20 nm porous alumina. (Ref. [18])

Recently, Song *et al.* investigated the influence of alumina pore size (20, 100 and 200 nm) on the behavior and morphology of human MG63 osteoblast-like cells. Figure 1 shows scanning electron microscopy images of MG63 cells on different substrates after incubation for 4 days. Results demonstrated that different pore size has different effects on the cell's behaviors, and it is possible to control the cellular behaviors with pore sizes [15]. In another work, Ni *et al.* considered PAA of defined pore sizes ranging from 25 nm to 75 nm to investigate the attachment and proliferation of preosteoblast (MC3T3-E1). After 7 days in culture, results showed that nanoporous surfaces did not enhance the initial preosteoblast attachment, whereas preosteoblast proliferation dramatically increased when the pore size was 50 nm and 75 nm. In addition, PAA with relatively larger pore sizes (50 nm and 75 nm) improved roughness and protein adsorption, which might be responsible for improved MC3T3-E1 cell growth [16].

The strong influence of pore size on cell growth has been analyzed/object of study for other types of cells cultured on PAA platforms. One of these studies, developed by Song *et al.*, analyzed the *in vitro* behavior of mesenchymal stem cells (MSC) cultured on PAA substrates with pore sizes of 20 and 100 nm, and evaluated the effect of nanosize on cell adhesion, proliferation, morphology, and osteogenic differentiation. The authors found that the adhesion (expression of integrin $\beta 1$) was enhanced in MSCs cultured on PAA and higher cell viability after incubation for 4 and 7 days was detected in cells cultured on 20 nm-sized porous alumina substrates. In addition, extremely elongated cells and prominent cell membrane protrusions (filipodia) were observed on alumina with the larger pore size (100 nm). Higher levels of osteoblastic differentiation markers such as alkaline phosphatase (ALP), osteocalcin, and mineralization were detected in cells cultured on alumina with 100 nm pores compared with cells cultured on alumina with either 20 nm pores or smooth alumina [17].

In a recent work, Pujari-Palmer *et al.* also analyze the influence of the pore size on cell morphology for different types of stem cells. The authors examined the effect of PAA nanotopography with different pore sizes (20, 100 and 200 nm in diameter) on osteogenic differentiation and drug eluting properties. Stem cells (W20-17, mouse bone marrow stromal cells) were used in the cell cultures. Figures 2 a) and b) show that the cell proliferation and proliferation rate were not significant affected by the pore size, being higher on control surfaces. However, in Figure 2 c) the cell morphology revealed that cells cultured for 2 days on 20 nm pores adopted a rounded shape, while larger pores (200 nm) elicited an elongated morphology. Cell differentiation was determined by analyzing the increase of ALP enzyme activity after 14 days of culture. Results indicate higher levels of ALP activity on 200 nm surfaces compared to 100 nm and control surfaces. The efficacy of PAA to release an osteoconductive agent—bone morphogenic protein-2 (BMP-2) was also investigated. Results indicated that the addition of BMP-2 increased the overall levels of ALP activity and osteocalcin expression as compared to cells without exposure to BMP-2, but was not affected by the pore size [18]. This group has also studied the effects of PAA porosity on inflammatory cell response both *in vivo* and *n vitro*. PAA with two pore diameters, 20 and 200 nm was investigated. *In vitro* cell/alumina interactions were evaluated by observing the adhesion, proliferation, and activation of a murine fibroblast cell line (RAW264.7) and macrophage cell line (NIH 3T3) for 7 days. There was no significant difference between cell adhesion at 24 h and proliferation at 3 and 7 days on the 20 nm compared to the 200 nm membranes for either fibroblast or macrophage cell lines. This indicates that the different porosities had no influence on cell proliferation. The long-term tissue reactivity was investigated by porous alumina membranes implanted subcutaneously in Balb/C mice for 14 days. The extent of inflammatory response to the alumina membranes was analyzed through histology and cytokine protein array analysis. Results indicated that 200 nm pore-size alumina membranes raised a stronger inflammatory response as compared to alumina with 20 nm pores [19]. Similar inflammatory response was obtained for a short-term implantation (16 h) in the peritoneal cavity of mice [20].

The response of HepG2 cells to PAA with different pore diameters (50 to 250 nm) is presented in the work developed by Hoess *et al.* The authors showed that PAA presented no cytotoxic effects and the cells adhered on the membranes even without any further surface modification. Cell proliferation increased with an increase in pore diameter and was highest on substrates with 200 nm pores. In contrast, cell functionality was fostered on membranes with small pore diameters of around 50 nm [21]. These results agree with the results obtained using 50–150 nm PAA and NIH-3T3 fibroblast cells [22], 20–200 nm pore size alumina and human osteoblast-like MG63 cells [23], and 75–300 nm pore size alumina and NIH 3T3 cells [24].

Human mammary epithelial cells (HMEC) have also been grown on PAA platforms. The adhesion and the proliferation of these cells on PAA surfaces as a function of pore size (flat, 30, 40, 45, 50 nm) and depth (0, 50, 90, 130, 180, 240, and 300 nm) has been analyzed. Results showed that the adhesion rate of cells was not affected by the depth of the nanoporous surface whereas the proliferation of cells dramatically increased when the aspect ratio of the nanopore was near unity. They also demonstrated that proliferation was higher when cultured on alumina with 30 nm pores compared with PAA with larger pores and the adhesion rate decreased when the pore size was increased because of a lack of surface area to which the cells could adhere [25]. In a similar work, Thakur *et al.* reported the interaction between vascular endothelial cells (ECV 304) and PAA substrates with respect to the depth of the pores. They found that on shallow pores (500 nm) the cell spread more and the actin cytoskeleton appeared diffuse. In contrast, on deep pores (2000 nm), the morphology and the cytoskeletal arrangement and depended on the pore size [26].

The effect of pore size alumina on human monocytes/macrophages was analyzed in terms of cell adhesion, morphology, and release of proinflammatory cytokines. PAA membranes with two distinct pore sizes (i.e., 20 nm and 200 nm in diameter) were compared. Results showed that few but highly activated cells adhered to the 200 nm membrane in contrast to many but less activated cells on the 20 nm surface. The cells adhering on the 20 nm surface exhibited reduced pro-inflammatory activity [27].

Ferraz *et al.* studied the interaction between PAA with different pore sizes (i.e., 20 and 200 nm), whole blood and platelet rich plasma. The results showed very few platelets on the 200 nm alumina as compared to the 20 nm membrane. The platelets found on the 20 nm membrane showed signs of activation such as spread morphology and protruding filipodia [28]. Furthermore, the procoagulant activity of the two pore sizes was compared by measuring the release of platelet microparticles (PMP). 200 nm pore size alumina promoted PMP generation and adhesion whereas the 20 nm pore did not cause any release or adhesion of PMP. In addition, the 20 nm pores size alumina showed a 100% higher procoagulent activity than the 200 nm alumina membranes [29].

PAA membranes have also been used for co-cultures of primary cells. In the system proposed, the membrane is a physical barrier between different cell types, whereas the cell-to-cell communication is provided by the diffusion of soluble molecules through the nanopores. The co-cultures were carried out with primary mouse hepatocytes and human adipose-derived mesenchymal stem cells (hASCs). The results showed that the mRNA expression of hepatogenic genes was induced in hASCs under static culture conditions [30].

2.2 Surface modification and functionalization

Cellular behavior can be strongly influenced by modifying the surface chemistry of PAA platforms. The functionalization of the porous alumina surface with different functional molecules provides the material with interesting biological properties, i.e., improves its bioactivity, the adhesion, proliferation of cells, and biocompatibility.

Swan *et al.*, studied the improvements in bone cell adhesion and proliferation by chemical modification of porous alumina surface with vitronectin and a cellular adhesive peptide (i.e., arginine-glycine-aspartic acid-cysteine, RGDC). The results demonstrated the improvement of hFOB 1.19 cell adhesions and the production of extracellular matrix [31]. Recently, a phosphorylation treatment of PAA has been proposed for inducing bone-like apatite formation and their application as bioactive surface coatings on implant metals and alloys [32]. If phosphoric acid is used in the anodization process then the remaining phosphate incorporated into the alumina surface may improve biomineralization.

BMP2 has been demonstrated to be able to stimulate osteogenic differentiation and promote bone formation [33, 34]. In a recent study, BMP2 was immobilized onto porous alumina substrates with different pore sizes (20 and 100 nm) in order to investigate the proliferation and osteogenic differentiation of MSCs. Figure 3 shows the immunofluorescence images of MSCs adhered to different substrates without and with BMP2, and the enhanced integrin $\beta 1$ expression after 2 incubation days. The results also showed that the BMP2-alumina substrate was able to

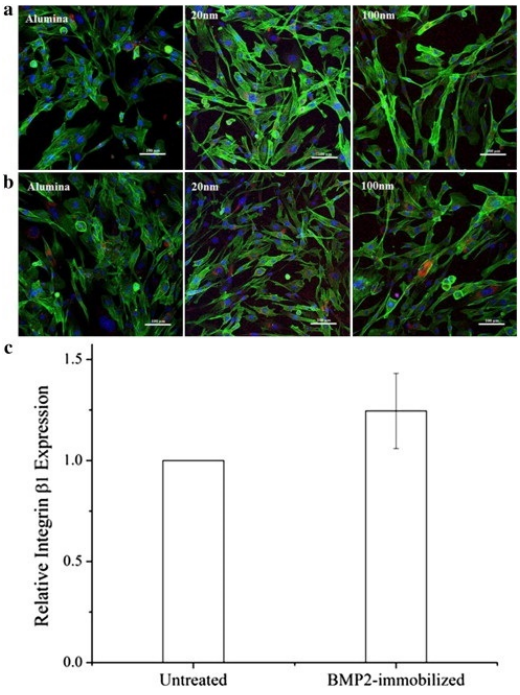

Figure 3: Immunofluorescence images of MSCs adhered to different substrates (smooth and porous alumina with 20 and 100 nm pore diameters) for 2 days: (a) without and (b) with BMP2. Cells were triple stained with actin filaments (green), cell nuclei (blue) and integrin $\beta 1$ (red). (c) Quantitative PCR analysis of integrin $\beta 1$ gene expression. MSCs were cultured on 20 nm sized alumina substrate for 2 days. (Ref. [35])

promote adhesion and spreading of MSCs. Compared with those of untreated alumina substrates, significantly higher ALP activities and mineralization were detected in cells cultured on BMP2-alumina substrates. Regarding the increasing pore size of the PAA membranes, the cell viability was decreased gradually in both BMP2 and untreated alumina, and MSCs cultured on 100 nm sized alumina showed a relative higher ALP activity as compared with 20 nm sized group in both BMP2 and untreated alumina substrates [35].

2.3 Porous anodic alumina coating

The versatility of the PAA is one of the great advantages of this material. A clear illustration/example of this versatility is that a thin layer of PAA can be fabricated on the surface of other materials and transfer its physical and chemical properties such as its high surface area and biocompatibility.

For this purpose, a thin layer of aluminum can be deposited on any metal surface and completely converted to porous anodic alumina through a well-known anodization process [36–38]. Aluminum can be deposited using an electron beam evaporator on titanium implant surfaces [39]. For more complicated surfaces like cardiovascular stents, physical vapor deposition process can be used for deposition of aluminum [40]. Following this approach and in order to improve the bone implants, porous alumina coating produced by anodization of aluminum on a titanium substrate has been proposed. The porous coated system was demonstrated by MG63 osteoblastic cell cocultured on different substrates. Results showed the biocompatibility of this system and it is comparable to that of conventional bioinert implant materials such as titanium and fully dense, sintered, alumina. Moreover, this porous coated system was loaded with silica nanoparticles and can be used to deliver infection combating and bone regenerating materials to the implant site and secure fixation between bone and implant through bioactive fixation [41].

The versatility of PAA has been recently demonstrated by the fabrication of aluminum wire implants composed of porous layers on the surface using the electrochemical anodization process [42]. Porous anodic alumina-aluminum wires were utilized as drug-releasing implants in bone using an *ex vivo* bone reactor. The thin PAA layer present pore diameters of 30–35 nm and pore lengths of 10–60 μm. The biocompatibility study of the implants evaluated using human osteoblast cell culture showed strong growth of cells, their spreading and their adhesion to the implant surface. These results are in agreement with previous studies on PAA on the planar surface showing that porous anodized aluminum oxides are a biocompatible substrate and support the growth of osteoblast cells [13, 14, 41, 43].

3 Drug delivery

Many are the physical and chemical properties of PAA that make of this material a versatile and interesting vehicle for biological applications. Among them, its porous geometry with a highly ordered distribution endows porous alumina a high effective surface and an attractive structure for drug delivery applications. In this regard, pores of PAA can be considered nanocontainers or reservoirs with regular and precisely controlled structural features for loading active agents like drugs or molecules. Likewise, the surface of porous anodic alumina can be easily chemically modified in order to regulate the bonding and release of the drug and the access to the pores can be covered with biodegradable, chemical or pH responsive agents to regulate their drug release.

The need to develop drug release therapies has emerge to overcome the drawbacks of conventional treatments (generally oral or intravenous administrations), that have major side effects due to their nonspecific action. A new generation of drug release platforms with sustained and complex release profiles, specific action on localized areas and reduced therapeutic dose is the main objective of investigation of interdisciplinary research groups nowadays, and porous alumina is one of the most promising materials for that purpose.

The geometry of pores is one of the main determinant factors of the amount of drug loaded inside porous alumina reservoirs. The formation process of PAA is a well known electrochemical technique described in several works [44, 45]. For this reason, pore length, pore diameter (selectable from few nm to hundreds of nm), pore geometry (one or several different diameters in depth), and pore distribution (porosity) of alumina layers can be precisely controlled to meet the applications requirements.

Certainly, the volume associated to the pore geometry is highly decisive for the amount of drug loaded, but also important is the size of the agent to be loaded inside the pores and its diffusion rate. As expected, drugs are not exclusively loaded inside the pores of PAA, but the surface itself can also withhold a part of this load, that can be considerably high, promoting a different stage release [46]. Generally, thermogravimetric analysis is used for determining the quantity of drug loaded on porous platforms [47, 48].

Alumina and drug are generally linked in an electrostatical way resulting in a quite strong bonding based on the attraction between opposite signal charge elements. Drug loading into alumina pores is usually performed through capillary action by either immersing alumina into concentrated drug solutions or dropping the solution slowly on the alumina surface [49, 50]. Although this is a fast and easy process, its major drawback is the nonspecific adsorption that becomes an important impediment for the development of diagnostic and monitoring or drug screening and testing.

To overcome these limitations, functionalization, that is the chemical modification of porous alumina under convenience, has revealed to be the most effective way for selective and covalent binding of molecules to alumina platforms. Recently, Baranowska *et al.* evaluated the effectiveness of three different functionalization processes for collagen and bovine serum albumin molecules binding to alumina platforms: silane-PEG-NHS (triethoxysilane-polyethylen-glycol–N-hydroxysuccinimide), APTMS (3-aminopropylotrimethoxysilane) and GTA-activated APTMS (3-aminopropylotrimethoxysilaneglutaraldehyde) [51]. Whereas APTMS is an electrostatic linker, the rest are covalent linkers. These experiments have concluded that although APTMS and APTMS-GTA are more commonly used linkers, silane-PEG-NHS is the most suitable functionalization path for alumina as a highly homogeneous attachment of the proteins to alumina is obtained for any pore size.

Moreover, the optimization of the drug-surface interaction with a suitable chemical functionalization is not only relevant for the drug loading process, but it is also the most effective way to control the subsequent release rate. The absence of alumina surface modification generally provokes a high initial drug release that can be up to 75% in the first hours [52]. However, the main challenge of new generation drug delivery systems is the development of complex, controlled and long term release profiles that should reduce the therapeutic drug dose in long-term therapies, avoiding overdoses at early release stages.

In this regard, Kapoor *et al.* functionalized porous alumina with various hydrophilic and hydrophobic surface chemical groups and studied their influence on the delivery of ibuprofen [53]. The functionalization with hydrophilic groups resulted in a significantly higher drug payload (21%–45%) and slower rate of release (12%–40% over a period of 5 h), whereas hydrophobic groups resulted in low degree of drug loading (approximately 20%) and fast rate of release (85% over a period of 5 h).

In another approach to develop long term drug delivery systems, Law *et al.* incorporated chitosan coatings of different thicknesses on the top of the alumina surface to extend the release of the protein loaded inside the pores over long periods of time [52]. The application of chitosan coatings on the surface of alumina wires significantly minimized the initial burst release to approximately 20%.

Simovic *et al.* deposited a thin polymer film via plasma polymerization on top of alumina after drug loading [54]. This is a one-step, dry technique which prevents elution of the loaded drug by solvents and excludes contamination. In addition, this technique does no compromise the amount of drug loaded.

Plasma polymerization has also been used by Aw *et al.* for controlling the release of indomethacin in their two-step releasing control system [55]. In this work, PAA is used for developing an implantable drug delivery system. The pores of alumina are loaded with polymer micelles that in turn are the nanocarriers of the drug [56] (Figure 4). To achieve extended release of micelles from nanopore or nanotube structures, ultrathin polymer film of allylamine was deposited via plasma polymerisation. This way, a complex release profile was achieve with an ex-

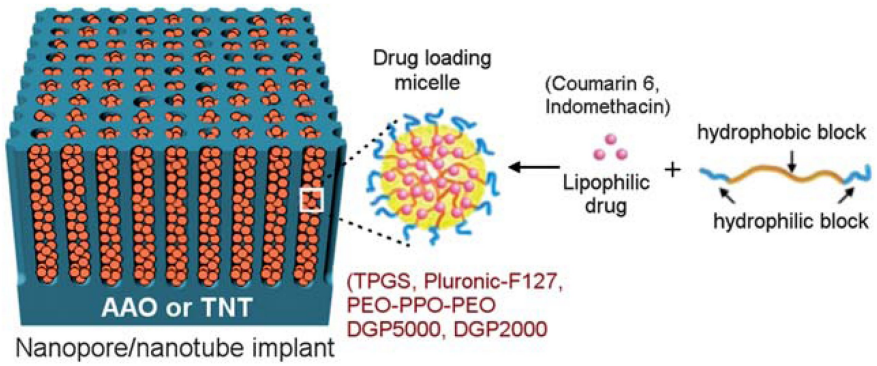

Figure 4: Schematic illustration of the multistep release therapeutic device developed by Aw *et al.* (Ref. [55])

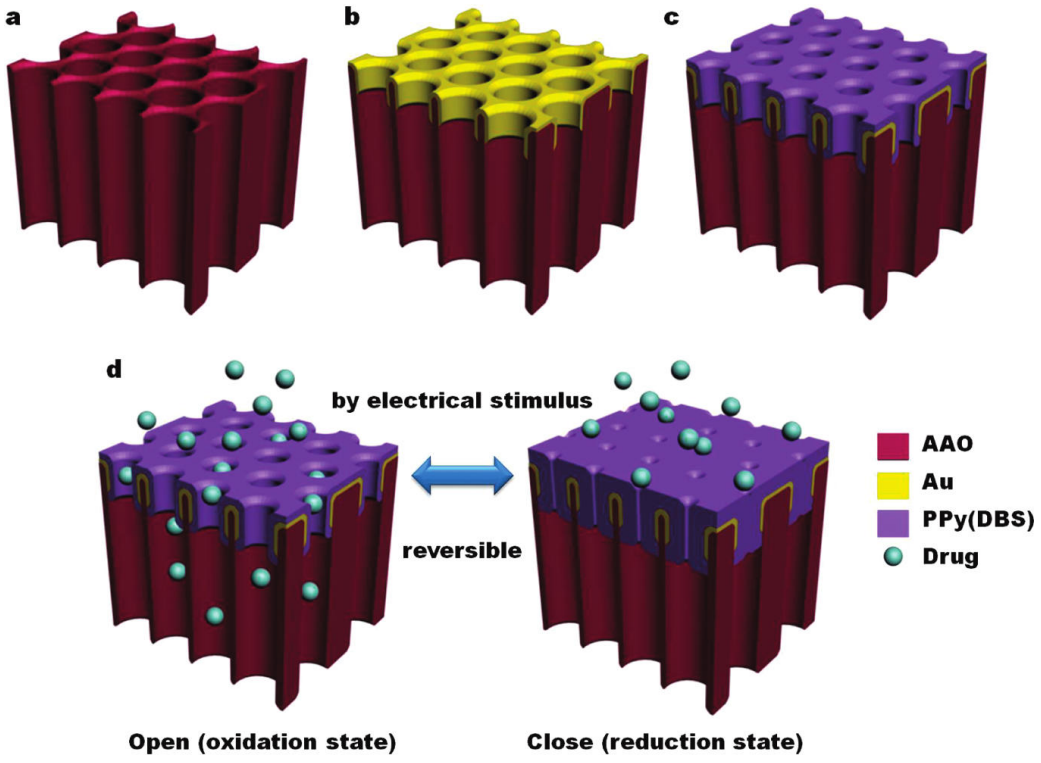

Figure 5: Scheme of the electrically responsive nanoporous membrane developed by Jeon *et al.* (Ref. [57])

tended time release of 27–31 days, and a significantly lowered burst release of 12–15%.

Beyond chemically controlled release from porous alumina structures, Jeon *et al.* presented an electrically responsive nanoporous alumina membrane [57]. In this work, the model drug is released on-demand using electrical stimulus with a fast switching time of a few seconds and a high flux of PAA drug. Figure 5 shows the schematic of the drug release system where the loaded alumina pores are covered onto their top and upper side wall with electropolymerized polypyrrole doped with dodecylbenzenesulfonate anions (PPy/DBS). As a result, the pore size of the PPy/DBS membrane was reversibly actuated by the electrochemical state.

The quantification of drug released from alumina layers can be performed by different methods, most of them closely dependent on the properties of the drug used. This is the case of all the methods based on spectrometry where UV-Vis or NIR absorbance spectrum is measured for detecting the delivery of fluorescent drugs or molecules labeled with fluorescent groups [58, 59].

Recently, reflectometric interference spectroscopy (RIfS) has been used to monitor in real-time the diffusion of drug from nanoporous alumina implants [60].

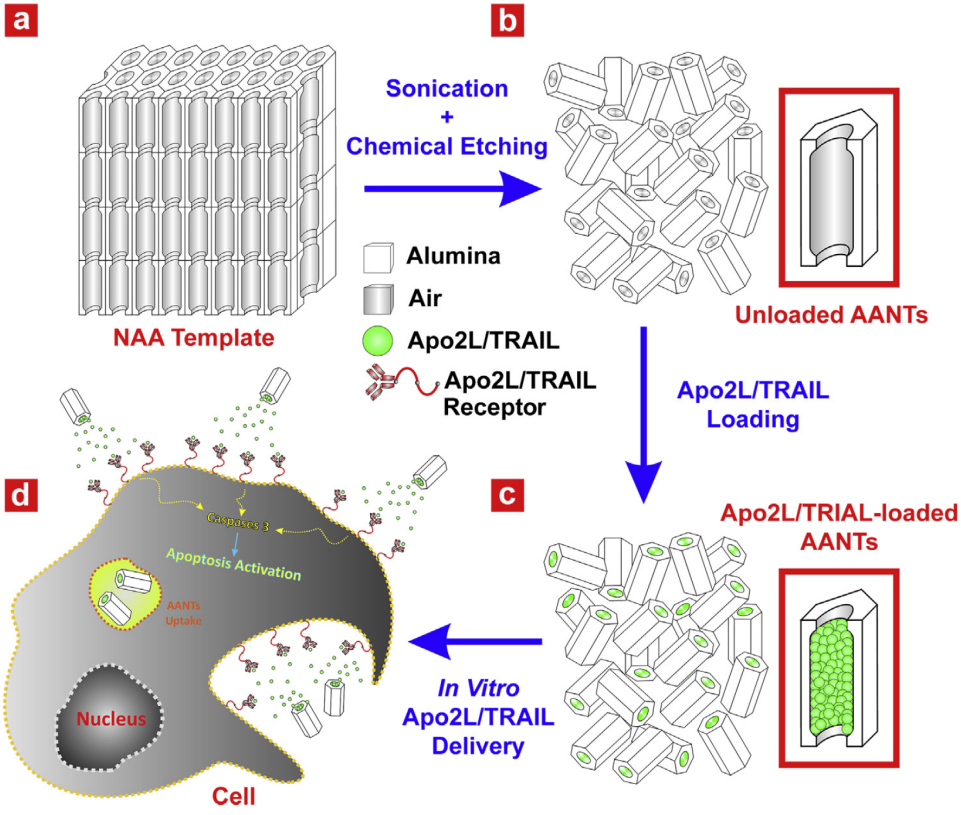

Figure 6: Scheme of the fabrication, loading and drug delivery of alumina nanotubes loaded wih Apo2L/TRAIL developed by Wang *et al.* (Ref. [59])

In this work, Kumeria *et al.* assessed the release of anti-inflammatory drug indomethacin using RIfS. Its detection principle is based on changes in the effective medium of the alumina layer that is monitored by measuring the effective optical thickness of the layer. The release process is carried out in a microfluidic chamber under selectable flow rates. Advantageously, this setup enables the study of drug release under the physiological conditions that porous alumina implants will experiment inside a host body. This study concluded that under dynamic flow conditions the release is much faster and the results are more accurate and reliable than under conventional static conditions.

All these studies have verified the great capabilities of PAA for controlled long-term drug releasing. However, the interesting potential of porous alumina increases significantly when the material is presented in the form of nanometric particles. These particles, with a variety of shapes, have the same physical and chemical properties as bigger alumina templates but with the added advantage of their tiny size. This way, PAA nanoparticles are nano-vehicles with functionalized surface and encapsulated drugs with great potential for active targeting. Their nanometric size allows the particles to be uptaken by the cells and release its load inside the target ones and to flow over the bloodstream to reach the target area [61]. This localized drug delivery has many important advantages over conventional therapies like the reduction of the doses -the losses on the different body barriers are dramatically minimized-, and the decrease of healthy cells damage due to the high localization of the therapeutic release. Recent investigations against cancer are focused on the development of nanoparticles for local active therapeutics, opposite to the high damaging conventional therapeutic treatments like radiotherapy and chemotherapy, with significant secondary effects.

Very recently, Wang *et al.* reported on porous alumina nanotubes loaded with Apo2L/TRAIL for studying its antitumour capability [59]. Porous alumina nanotubes of 100 nm outer diameter and 600 nm length were obtained by modified pulse anodization process under galvanostatic control [62, 63] (Figure 6). Cell toxicity was evaluated for breast cancer cells (MDA-MB231-TXSA) and immune response cells (RAW264.7 macrophages) cultured with several concentrations of nanotubes (from 1.56 to 100 mg/ml) for 1 and 5 days. The results demonstrated

that porous alumina nanotubes were not toxic for both cell lines and for all nanotube concentrations. In addition, nanotubes were loaded with Apo2L/TRAIL and cell viability was studied for MDA-MB231-TXSA cancer cells. Taking advantage of the high drug loading capacity of nanoparticles (104 ± 14.4 μg/mg) the viability of these cells highly sensitive to Apo2L/TRAIL-mediated apoptosis was studied. This study revealed that cell viability decreased significantly in a dose-dependent pattern after 165 min of incubation with Apo2L/TRAIL-loaded nanotubes.

In addition, PAA structures with several shapes and sizes have been developed for drug delivery. Poplausks *et al*. developed sharp aluminum probes coated with a porous alumina layer to delivery DNA into the cytoplasm of selected plant cells in order to investigate cell communication problems [64]. Law *et al*. reported aluminum wires with diameter 1 mm and a length of 1 cm covered by a porous alumina layer [52], and Gong *et al*. capsules with porous alumina for molecular transport [65].

Porous alumina has revealed to be a suitable platform for delivery of several types of drugs. Anti-inflamatories like ibuprofen, indamethacin or naproxen that are orally administrated require high doses for correct absorption and affect other organs like liver or heart, have been successfully loaded and released from alumina reservoirs [53, 55, 60, 66, 67]. Other types of drugs like antibiotics, ophthalmic and immunosuppressive drugs or anticancer drugs like doxorubicin, have also been controllably delivered from alumina templates [40, 68, 69].

4 Biosensing

PAA is an outstanding material for biosensing for several reasons. First, it shows a great stability under many different environmental conditions, which avoids drifts and uncertainty in the measurement of the transduction sensing parameters. Another important feature of PAA in biosensing is its great surface-to-volume ratio, which offers an increased possibility of interaction of the analytes with the chemical species intended for the detection. The surface chemistry of PAA has been also deeply studied, which permits a great variety of functionalizations to target the specific analytes with sensitivity and selectivity. Finally, the technology for obtaining the PAA has reached a level of maturity that permits the modulation of pore diameter, pore length and interpore distance over a wide range. In addition, technology also permits the engineering of the pores, that is, the variation of pore geometry in the depth of the PAA layer, which confers the structure with further optical and geometrical properties with special interest in biosensing. In this mini-review we aim at offering an overview of the literature-reported applications of PAA to biosensing, with special attention to the latest achievements. We have classified the biosensing applications of PAA into two main sections: those based on optical transduction mechanisms and those based on electrochemical mechanisms. For more detailed information of each of the subjects there exist more extensive reviews, such as refs. [2, 70–72].

PAA oxide has been traditionally a material of interest from the point of view of its optical properties [73]. Even in its application as a protective coating for aluminum, it is known that by controlling the thickness, the colour can be tuned in a restricted range [74]. These optical properties arise from different aspects of the material. The aluminum oxide obtained by anodization has a different structure and stoichiometry than standard bulk crystalline aluminum oxide and consequently, it possesses a different refractive index. Furthermore, the anodization process (under certain conditions) results in the incorporation of electrolyte ions in the alumina matrix or in the formation of oxygen vacancies or of singly- and doubly-ionized F-centres [75]. This has a direct incidence on the extinction coefficient and thus in the absorption and photoluminescence of the material. The porosity is also determinant in the optical properties, since it contributes to modify the refractive index and extinction coefficient of the porous oxide matrix. In the case of PAA with small interpore distances, such as those produced with oxalic or sulphuric electrolytes, the combined pore-matrix geometry results in an effective medium with a refractive index that can be modeled by any of the different effective medium theories (Bruggeman, Maxwell-Garnett, etc.). Instead, for interpore distances larger or in the order of light wavelength, the structure optical properties are modified in a more complex way giving, origin to light scattering effects. Finally, optical properties depend also on other geometrical parameters of PAA such as the degree of ordering of the pores [76] or the in-depth variation in the pore diameter [77, 78]. Structures with pores ordered in an hexagonal array can be produced with the two-step anodization procedure introduced by Masuda and Fukuda [79]. Such ordering can be perfect in a long range if nanoimprint techniques are used previously to the anodization; in this case, photonic crystal band gaps can be observed [80]. Even in the naturally produced alumina, in which the order is broken into domains of several hundred of pores in diameter and with random orientations, photonic stop bands can also be found [76]. By adequately controlling the pore morphology in the depth of the PAA using variable anodiza-

tion conditions pore diameter variations or controlled pore branching are obtained. Such structures constitute optical structures such as Fabry-Pérot (F-P) cavities [81] or distributed Bragg-reflectors (DBR) [77, 78, 82].

All these properties have been exploited in applications to detect analytes in a biological fluid. Optical biosensors based on PAA rely on the change of one or more of such properties and on its transduction in a detectable signal by means of light probing. The change of the optical property can be of different kinds: the presence of an analyte in the fluid can produce a change in the refractive index of the fluid, which in turn produces a change in the refractive index of the effective medium constituted by the filled PAA. This approach is extensively used in order to evaluate the sensitivity of PAA-based biosensors on the basis of glucose/sucrose solutions of different concentrations [83, 84]. If a low limit of detection is intended, then the analyte concentration to be detected is very small. This means that the refractive index of the solution differs very slightly from the refractive index of the same solution without the analyte, and that a change in the refractive index of the fluid cannot be used as the measurement strategy. In such case, it is necessary to perform a preconcentration of the analyte on the PAA inner pore walls. Such pre-concentration is achieved with a functionalization of the inner pore surface that promotes the grafting of the desired species. This pre-concentration creates a thin film on the pore surface with its own refractive index that influences in a certain amount the optical properties of the whole porous structure. With this, different analytes have been detected such as immunoglobulin (IgG) [85], or mercury or gold ions [86, 87]. The change in the optical properties is measured using one of several different techniques, or in some cases, a combination of them. These techniques can be classified into for main groups: i) using the PAA in optical waveguide spectroscopy (OWS), ii) taking advantage of the nanostructuring in combination with metal coating or infiltration four surface-enhanced Raman scattering (SERS) or localized surface plasmon resonance (LSPR), iii) measuring changes in the material photoluminescence (PL), and iv) evaluating the spectral changes in RIfS.

Optical waveguide spectroscopy consists of coupling light to the PAA thin film through a prism and registering the changes in the guided mode wavelength as the different species attach to the pore wall. With such a strategy, highly sensitive measurements have been reported such as the investigation in the absorption/desorption dynamics of bovine serum albumin (BSA) within the pores [88], the complexation of bathophenanthroline (Bphen) with Fe(II) ions [89], or the hybridization of DNA-DNA after conformal covering of the pores with polyelectrolyte bilayers [90, 91]. The PAA can also be used as a template for structuring other materials such as curable polymers, which are subsequently used in OWS experiments. In ref. [92] polycyanurate nanorod arrays are fabricated with the help of PAA, obtaining a high surface-to-volume structure that is mounted on the coupling prism surface within the flow cell. With this setup, the researchers investigated the formation of a taurine monolayer on the polycyanurate rod arrays surface. In a different approach, light coupling with the prism can be overcome by taking advantage of the photoluminescence emission of the material. As such emission is produced within the PAA layer, it is naturally coupled and the changes in the resonant mode wavelength can be evaluated from the measurement of the edge emission. Following this, in ref. [93] the detection of the pesticide chlorpyrifos has been demonstrated.

The high tunability of the PAA geometrical properties (pore diameter, interpore distance and pore length) on the nanometer scale, provides a basis for its use in SERS and LSPR sensing applications. The possibility of adjusting these dimensions to the adequate values and to depositing metals such as gold in the inner surface of the pores as well as in the PAA upper surface permits the design and optimization of different SERS substrates. For instance, in ref. [94], a study on the fabrication of disposable SERS biosensors based on PAA and the thermal evaporation of gold within the pores is presented. In this study, an enhancement factor of up to 103 to 104 in Raman spectroscopy with respect to a flat gold surface is reported, using intrinsically fluorescent molecules such as cresyl violet, rhodamine of green fluorescent protein. In the work by Shaban *et al.* [95], PAA is covered with a gold coating resulting in an hexagonal array of gold nanodots intended for the detection of heavy metal ions such as Hg^{2+}, Cd^{2+} or Pb^{2+}. The structure of PAA is also used as template to obtain nanostructured metal surfaces as in ref. [96], in which a Ag nanowire array is obtained by AC electrodeposition within the pores with a subsequent aluminum dissolution. Templates with different pore diameters and aspect ratio were obtained to study this influence when used as SERS substrates in the detection of 4-mercaptopyridine (4-Mpy). SERS detection is also combined with other techniques: in ref. [97] the previously described OWS is used to excite the plasmon resonance within a PAA film decorated with Ag nanoparticles. With this strategy, a highly sensitive immunoassay is reported. Instead, in refs. [98] and [99] the PAA is covered with a gold thin film to form a structure of nanodomes distributed in an hexagonal array. By measuring the change in reflectivity at the localized surface

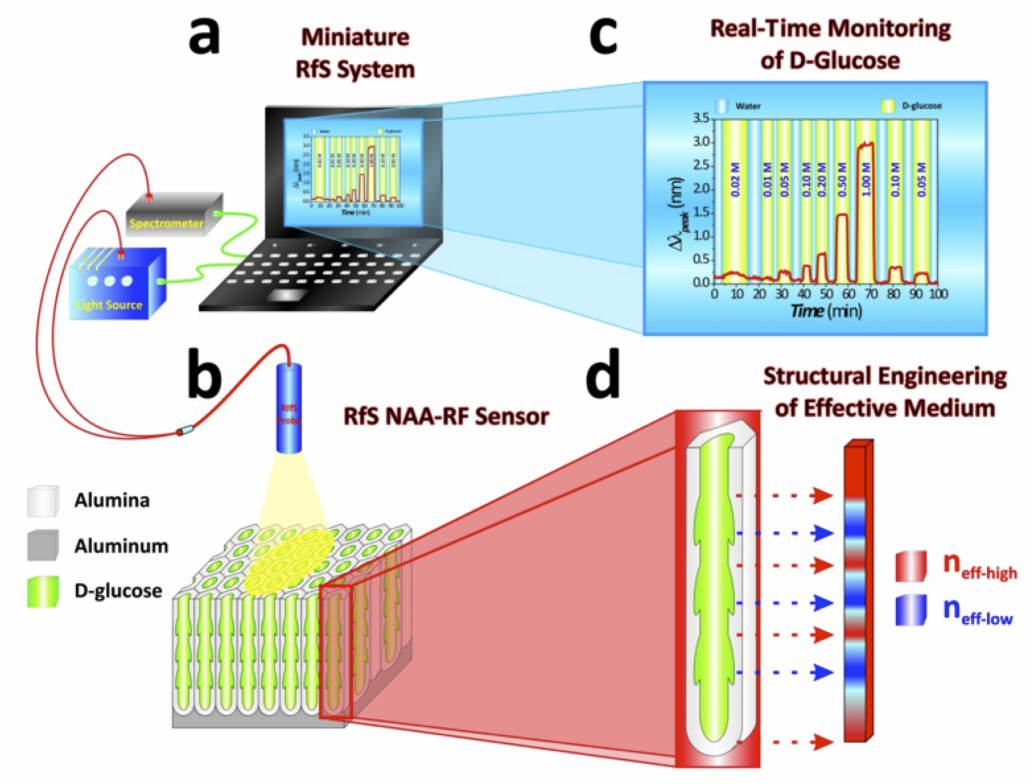

Figure 7: Basic elements of a Reflectance Interference System (RIfS). (Ref. [83])

plasmon resonance the authors are able to detect DNA or aptamer-protein interactions in a label-free approach.

Although photoluminescence is not extensively used in the application of PAA to biosensing, some works can be found. A selective and highly sensitive enzymatic photoluminescent biosensor is presented in ref. [100]. It highlights for its accurate detection and quantification of the analyzed sample, trypsin. The sensing technique for this biosensor is based on the change in the wavelength of the oscillation maxima in the photoluminescence spectrum. This sensing principle, highly sensitive to the presence of small quantities of analyte, allows the qualitative detection of the immobilized enzymes. In another approach, Li *et al.* [101] have developed micropatterns of PAA on indium tin oxide (ITO) glass substrate that can enhance the fluorescence signals of the fluorophores and labelled biomolecules on the substrate up to 2 or 3 orders of magnitude compared to the glass substrate. This would significantly reduce the consumption of the biosamples for fluorescence-based sensing, imaging, and analysis, and would allow the detection of biomolecules of ultralow concentration. The authors investigated the influence of the nanopore size in the fluorescence enhancement.

The most applied technique in optical biosensing based on PAA is the RIfS. This technique is based on the analysis of the reflectance spectrum of a PAA thin film and of its variation upon a change in the reflective index of the filling medium or upon the event of grafting the analyte molecule on the inner PAA walls. This technique is usually carried out by an optical system, as the one depicted in Figure 7. The light from a wideband source is directed to the sample by means of an optical fibre and focusing optics, and the reflected light is collected by the same optics and driven to a spectrometer. A further data processing is needed to obtain the desired information. This technique can be applied to both PAA single layers (also known as Fabry-Pérot interferometric structures) and to PAA films with in-depth variations of the pore diameter [84]. Such variations are achieved by an adequate pore engineering technique [73]. If single-layer PAA films are employed, the reflectance spectra show an oscillating behaviour that can be translated to an effective optical thickness value through a Fourier transform procedure. A first example of this application is found in ref. [85], where the change in PAA optical properties is used to study the grafting of protein A to the inner pore walls, the subsequent capture of an immunoglobulin antigen (rabbit anti-sheep IgG) and the selectivity between rabbit and chicken IgG. This approach has since then been extended to a wealth of different analytes, for instance in refs. [102]

Table 1: Minimum measured concentration, sensitivity and limit of detection (LOD) of the different reported optical biosensing methods based on PAA.

Analyte	Method	Minimum Concentration	Sensitivity	LOD	Ref.
D-glucose	RIfS-DBR		4.93 nm M^{-1}	0.01 M	[83]
glucose	RIfS-DBR		34% RIU^{-1}	0.04 RIU	[84]
	RIfS-FP		15% RIU^{-1}	0.08 RIU	
IgG	RIfS-FP	1 mg mL^{-1}			[85]
Hg^{2+}	RIfS-DBR		72 nM	1 µM	[86]
Au^{3+}	RIfS-FP			0.1 µM	[87]
BSA	OWS	50 µM			[88]
Bphen+Fe^{2+} complexation	OWS	265 mM, [Fe^{2+}]			[89]
DNA-DNA hybridization	OWS	100 nM			[90]
BSA	OWS	1 µM			[91]
Taurine	OWS	8 mM			[92]
Chlorpyriphos	OWS	10^{-4} M			[93]
Cresyl Violet dye	SERS	3.5 µM			[94]
Hg^{2+} Pb^{2+} Cd^{2+}	SERS	1 ppb [Hg^{2+}]			[95]
4-Mpy	SERS	10^{-3} M			[96]
IgG				0.1 mg mL^{-1}	[97]
DNA	LSPR-FP	10 µM			[98]
Thrombin	LSPR-FP	10^{-10} M			[99]
Trypsin	PL-FP	0.1 mg mL^{-1}			[100]
H_2 gas	RIfS-FP	2%			[102]
H_2S gas	RIfS-FP			5 %	[103]
Tumour cells (PANC-1)	RIfS-FP			< 1000 cells mL^{-1}	[104]
IgG	RIfS-Polarimetry	1 mg mL^{-1}			[105]
Mercaptoundecanoic Acid (MUA)	RIfS-FP	10 µM			[107]

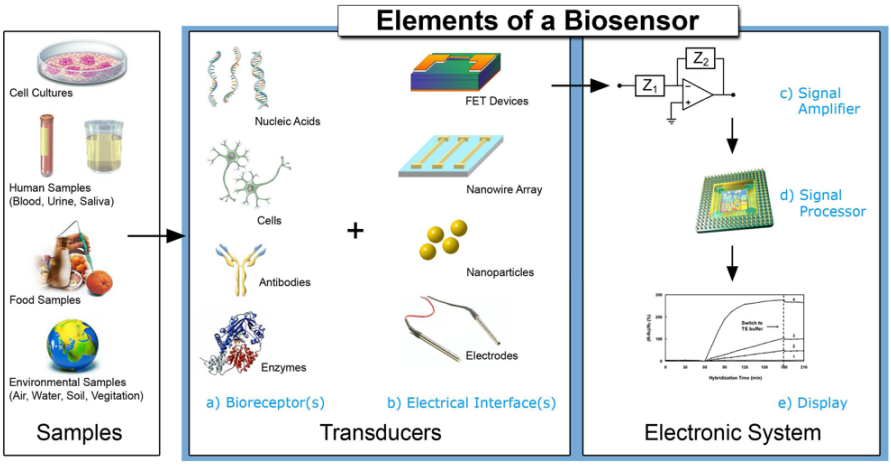

Figure 8: Depicts the main elements and components of a typical electrochemical biosensor. (Ref. [111])

and [103], where the RIfS method is applied to sense sulphur volatile gases by coating the top of the inner pore walls with a thin film of Au, which shows affinity to the gas molecules. The same structure and strategy has been also applied to the detection of circulating tumour cells by adding a self-assembled monolayer of the adequate antibodies by means of biotin-streptavidin attachment [104]. The functionalization of the PAA inner pore walls with 3-mercaptopropyl-triethoxysilane (MPTES) permits ultra sensitive detection of Au3+ ions [87]. A similar approach, although not based on reflectance but on polarimetry, can be applied [105] to define an immunoassay for anti-(beta)-lactoglobulin with selectivity towards anti-rabbit IgG. Finally, there exist also studies on the optimization of the Fabry-Pérot structures to obtain a maximum sensitivity, such as in refs. [106] and [107].

In order to improve the sensitivity of the RIfS method, a bilayer PAA structure has been also proposed [81]. The bilayer structure is composed of an upper layer with bigger diameter pores which at a given depth decrease diameter into a lower layer. The Fourier transform analysis of the reflectance spectrum of a bilayer yields three effective optical thicknesses, two of them corresponding to the two physical layers and the third one to their union. Provided the pore diameters are adequately designed, the lower layer can preclude the penetration of certain species, providing a reference for their detection at the upper layer. Furthermore, it is also shown that a gold coating on the top of the pores increases the sensitivity. More elaborated pore engineering leads to the incorporation of complex optical functions to the PAA structure. If a cyclic potential with the adequate amplitude and period are applied, photonic stop bands for light propagating parallel to the pores are created [108]. The position in the reflectance spectrum of such bands can be used as the transduction mechanism for sensing, without the need of the Fourier transform step. The sensitivity of this strategy has been assessed from different kinds of solution such as glucose [82, 83] and [109] or alcohols and alkanes [110]. Table 1 summarizes the optical biosensing methods reported in this review, indicating, where appropriate, the minimum measured concentration, the sensitivity or the limit of detection.

Porous anodic aluminum oxide is also a material of great interest in the electrochemical sensing of biological substances. Its stability under most of biological media conditions together with the high surface-to-volume ratio offer the possibility of creating highly dedicated electrodes for the different existing bio-electrochemical detection techniques [111]. Figure 8 depicts the main elements and components of a typical electrochemical biosensor. In such applications, PAA can be used as the substrate for the electrode, which is then obtained as a thin film in the inner pore walls, or it can also be used as a template to obtain nanostructured electrodes of other materials [112, 113]. In both cases the electrodes need to be adequately functionalized in order to provide the necessary selective electrochemical reactions that lead to the detection of the desired species. However, when PAA is used as a substrate for the electrode, the pores can act also as nanochannels [114] modifying the transport of molecules or ions and thus affecting the electrochemical signal and providing an additional information in the sensing procedure. Electrochemical biosensors are generally divided into two kinds [115], biocatalytic sensors and affinity sensors. Biocatalytic sensors primarily use enzymes (although other biocatalytic species such as organometallic compounds, whole cells or tissue slices are also reported [115]) to trigger specific biochemical reactions that are then detected

by the electrochemical transducer system [116]. In the case of affinity sensors the electrochemical signal is produced upon the selective strong binding of biomolecules such as antibodies, membrane receptors or oligonucleotides [117, 118]. Electrochemical techniques can also be classified depending on the signal measured by the transduction system [111]: amperometric if the signal is a measurable current, voltammetric if it is a voltage or an accumulation of charge or conductometric (also impedimetric) if the measurable magnitude is the change in conductance or impedance of a material.

Among the different electrochemical transduction techniques, the most used is clearly the cyclic voltammetry (CV). In this technique, the voltage between a reference electrode (usually Pt) and a working electrode based in PAA is cycled between two values (V1 and V2) at a fixed rate. As the voltage is swept, the current between the working electrode and the counter electrode is registered. In this way, a current-voltage diagram is obtained, where the different redox potentials of the different reactions taking place during the cycle are indicated by peaks in the curve. Recent examples of the use of this technique can be found in literature. For instance, in ref. [119] CV is used to monitor the different steps in the preparation of lipid membranes in PAA films and their further use as sensors of the non-ionic detergent Triton X-100. Cyclic voltammetery is also used in ref. [120] to monitor the correct functionalization of Ag nanotube electrodes produced using PAA as a template. Another example of the assessment of electrode performance by means of CV is in ref. [112], where PAA is used as a template to obtain polythiophene nanotube arrays that are employed on a glassy carbon (GC) surface to detect riboflavin.

A close approach to CV is differential pulse voltammetry (DPV), in which voltage is swept in a limited range and varied in defined differential steps. The changes in current are recorded to yield current-voltage diagram where the peak current change and peak voltage are characteristic of the chemical reaction being studied. This is a more sensitive method as compared with CV and thus it is preferably used after electrodes have been assessed by CV. For instance, in ref. [121] DPV is used to evaluate antibodies related to the West Nile virus as they are adsorbed in the inner walls of a PAA membrane on a platinum disk electrode. Most remarkable are the works by Merkoçi and co-workers, in which DPV is used in combination of PAA pore blocking to detect different kinds of analytes such as cancer biomarkers [114], DNA hybridization [122], or thrombin-thrombin aptamer affinity [123, 124]. In a similar approach, Li and co-workers [125] exploit the hindered diffusion of $[Fe(CN)_6]^{3-}$ through PAA pores caused by DNA hybridization into the nanopores to obtain a specific DNA sensor.

The second mostly cited transduction technique is the electrochemical impedance spectroscopy (EIS). This is an impedimetric method in which through the application of a sinusoidally varying potential, the resulting varying current is registered. By sweeping the frequency of the signal, the complex impedance of the electrode can be obtained and their characteristics related with the condition properties of the material. One interesting example of this strategy is found in ref. [126] in which a silane-modified PAA membrane is at the bottom of microwells in a poly(ethylene glycol) (PEG) hydrogel-based microchip. The well has hydrophobic (PEG) and hydrophilic (PAA) regions with differential functionalizations. With this procedure, *E. coli* bacteria are trapped in the bottom of the wells. EIS is used to detect the attachment. In another example, the pH sensitive characteristics of PAA membranes functionalized with poly(acrylic acid) are studied [127]. A PAA membrane can also be used as both working and counter electrode by coating both sides with a submicron layer of Pt. This permits to design a simple diagnostic device for the Dengue virus [128]. Finally, the measurement of the ionic impedance of electrolytes through the PAA nanopores permits the detection of pathogenic bacteria (E. coli O157:H7) in whole milk [129]. Other impedimetric techniques have been also reported, of which it is remarkable the application of electrode-separated piezoelectric sensing (ESPS). In this technique, changes in fluid conductivity caused by an enzymatic reaction (urea-urease in this case) are measured as resonant frequency changes of a system consisting of a piezoelectric transducer coupled to the flow cell where the enzyme is immobilized into the PAA pores [130, 131].

Amperometric biosensors rely on the measurement of the variation of current with time as different redox reaction stake place as recognition events and contribute differentially to current. Porous anodic alumina is used in several ways in amperometric biosensors. In ref. [132] PAA is used as a template to obtain a gold nanotube array by electroless deposition, which is then applied as an electrode for the sensing of glucose in a enzyme-free approach. The oxidation of glucose is catalyzed by the gold forming the nanotubes. Similarly, in an enzymatic approach [113] Au nanowire arrays are grown on a silicon substrate through PAA and then functionalized with glucose oxidase. The same authors report also a similar application to the growth of single-walled carbon nanotube (SWCNT) electrodes enhanced with Pd nanotubes and Pt nanospheres [116], grown in-situ from a PAA template. Then, these structures are converted to glutamate

Table 2: Linear range, limit of detection (LOD) and sensitivity of the reported electrochemical biosensing methods based on PAA.

Analyte	Method	Linear range	LOD	Sensitivity	Ref.
Riboflavin	Voltammetry	0.01 – 65 µM	3 nM		[112]
Glutamate	Amperometry	50 nM – 1.6 mM	4.6 nM		[113]
Cancer biomarker CA15-3	DPV		52 U mL^{-1}		[114]
Glucose	Amperometry	1 – 21 mM	0.1 mM	23.9 nA $mM^{-1}cm^{-2}$	[116]
D-glucose	Voltametry			1.5 mV mM^{-1}	[117]
D-fructose				3.5 mV mM^{-1}	
E. coli O157:H7	EIS		10 CFU mL^{-1}		[118]
Cholesterol	Amperometry	0.28 mM – 33 mM	0.18 mM		[120]
West Nile virus protein domain III	Voltammetry	> 50 viral	53 pg mL^{-1}		[121]
West Nile Viral particle		> 53 pg mL^{-1} particles per 100 mL	> 50 viral particles per 100 mL		
DNA hybridization	DPV	5 mg $µL^{-1}$	42 ng $µL^{-1}$		[122]
Thrombin	DPV		2 ng mL^{-1}		[123]
IgG	Amperometry		100 µg mL^{-1}		[124]
DNA hybridization	Amperometry	1–100 nM	1 pM		[125]
pH	EIS	2-6			[127]
Dengue virus 2	EIS		0.23 PFU mL^{-1}		[128]
Dengue virus 3			0.71 PFU mL^{-1}		
E. coli O157:H7	EIS		1 CFU mL^{-1}		[129]
Urea	Piezoelectric		0.2 µM		[130]
Glucose	Amperometry	1 – 42.5 mM	10 µM		[132]

sensors by functionalizing the SWCNT with glutamate oxidase. More recently [133], this amperometric approach has been applied for real-time monitoring of the enzyme reaction kinetics. In this case, the inner pore surface of PAA is functionalized with the glucose oxidase and the PAA membrane is attached to an Au disk that acts as the working electrode. With this, the effect of ionic strength, amount of immobilized enzyme and pore diameter on reaction kinetics were demonstrated. Table 2 summarizes the electrochemical biosensing methods reviewed in this paper, indicating, where reported, the linear range, the limit of detection and the sensitivity.

5 Conclusions

In summary, porous anodic alumina has proved to be an efficient and versatile biomaterial for cell culture, tissue engineering and implants. The biocompatibility study of the implants evaluated using human osteoblast cell culture showed strong growth of cells, their spreading and their adhesion to the implant surface. Porous anodic alumina has also interesting physical and chemical properties for drug delivery, such as high effective surface area, easy surface chemical modification and pores with controllable geometry that are used as reservoirs. In the emerging field of drug release, porous alumina has revealed to be a suitable and efficient structure allowing sustained and complex release profiles for several types of drugs. Anti-inflammatory, antibiotics and anticancer drugs are some of the therapeutic agents successfully loaded inside the porous alumina pores so far, but the range of possible biological agents to be loaded is very wide. Nanometric carriers based on porous alumina have been developed with interesting results in the drug delivery field. Although still in the early stages, porous alumina nanometric particles and nanotubes that flow over the body bloodstream and are able to reach a target area are being developed for localized treatment. The first results have been very promising; however, more exhaustive fundamental research must be carried out. An important challenge to be addressed for *in vivo* applications of porous alumina is the origin of the inflammatory response observed in the few studies published about its *in vivo* biocompatibility. Many more *in vivo* assessments need to be conducted for elucidating its origin and finding ways to avoid it. Finally, porous alumina biosensors provide high sensitivity measurements based on either optical transduction mechanisms or electrochemical mechanisms. Optical properties such as the inherent photoluminescence and tunable reflectivity spectrum of porous alumina structures have demonstrated to be highly sensitive to the presence of chemical species such as IgG, mercury or gold ions. The same way, porous alumina biosensors based on electrochemical techniques have revealed to be high sensitive for detecting biological agents such as riboflavin, antibodies, membrane receptors or oligonucleotides. In summary, this review has demonstrated that there are promising opportunities for further advances and developments of biomedical systems based on PAA.

Acknowledgement: This work was supported by the Spanish Ministry of Economy and competitiveness (MINECO) under grant number TEC2012-34397, the ICREA under the ICREA Academia Award and by Catalan authority under project AGAUR 2014 SGR 1344.

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