Fabrication of Nanostructured Microworms for Sensor Applications

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ABSTRACT

Initiated (i)CVD is used to conformally deposit a 50 nm thin layer of p(2-hydroxyl methacrylate (HEMA)-co-ethylene glycol diacrylate (EGDA)) copolymer inside aluminium oxide (AAO) membranes to obtain core/shell cylindrical-shape structures, called microworms. The mesh size of these microworms is studied to determine the entrance availability of ions or small molecules through them for sensor applications. Additionally, the filling process of the microworms with a dye is optimized in order to be used as a sensor for in-vivo glucose detection.

Keywords: iCVD, hydrogel, microworm, sensor, glucose.

1 INTRODUCTION

Sensing devices for in vivo continuous monitoring of specific analytes is a hot topic in medical research. In this sense, main efforts have been addressed towards the implantation of nanoparticles for minimally invasive physiological monitoring. Probes encapsulated by biologically localized embedding (PEBBLEs) combine the fiber optical technology developed for biosensing and a size small enough to perform measurements inside a cell without damage. PEBBLEs incorporate a polymer matrix, which isolate the dye from interaction with cells avoiding cytotoxicity or undesired reactions [1,2]. Furthermore, the nanoscale of these polymeric spheres enable a low detection limit and a fast response time. Nevertheless, the small size also results in them diffusing rapidly away from the desired location. The solution proposed here is the fabrication of core/shell cylindrical-shape sensors (microworms). Microworms combine a long axis (tens of microns), which provides a higher hydrodynamic radius to prevent diffusion, with a nanostructured shell, which facilitate the diffusion of the analyte inside the cylinder. This structure presents a high surface to volume ratio enabling a rapid sensor response. Microworms are fabricated using anodic aluminium oxide (AAO) membranes as template, due to the ideal cylindrical-shape of its pores, and depositing the polymer on its walls by initiated chemical vapor deposition (iCVD). iCVD is a gentle process based on hot-wire CVD. Monomer is introduced into the reactor together with an initiator in the vapor phase. This is decomposed using a resistively heated filament and the polymerization takes place mainly at the surface following the generic free-radical polymerization mechanism [3,4]. Since the energy input is low enough, both monomer and polymer are unaffected at the working temperature, resulting in full retention of pendant functionalities. Moreover, conformal coatings can be achieved by adjusting the fractional saturation of the monomer vapor [5,6]. This conformality permits the formation of the shell structure inside the AAO membrane. Thus, the inner part can be filled with the corresponding optode to measure different analytes. Previous research has successfully demonstrated the application of these sensors for in-vivo sodium detection [7]. Because of this, this process is thought to be valid for other kind of target molecules, such as for in-vivo glucose detection. Tight control over glucose levels can help individuals stave off the devastating side-effects of diabetes, the number one cause of kidney failure, blindness in adults, nervous system damage, and amputations and also a major risk factor for heart failure, stroke, and birth defects.

2 EXPERIMENTAL PART

2.1 Materials

Tert-butyl peroxide (98%, Aldrich), 2-hydroxyl methacrylate (99%, Aldrich) and ethylene glycol diacrylate (98%, PolySciences) were used as received. AAO membranes (60 μm thickness, 0.2 μm pore size, 25 mm diameter) were purchased from Whatman. The filling of microworms was performed using citroflex A6 (Vertellus) as plasticizer and Chromoionophore III (Fluka) as dye.

2.2 Experimental Set-up

iCVD polymerizations were performed in a custom-design reactor, supporting a resistively heated chromalloy filaments and controlling the substrate temperature as described previously [8]. The radical initiator TBPO and the nitrogen gas were fed into the reactor through mass flow controllers. The HEMA monomer was heated to 70ºC and the EGDA cross-linker to 60ºC, both flows were controlled by needle valves. EGDA flow rates varied from 0.05 to 0.25 sccm, for HEMA from 0.50 to 0.70 sccm, TBPO flow rate was fixed at 1 sccm and a patch flow of nitrogen was introduced into the reactor to keep a total flow of 3 sccm for all the experiments. The operating pressure was

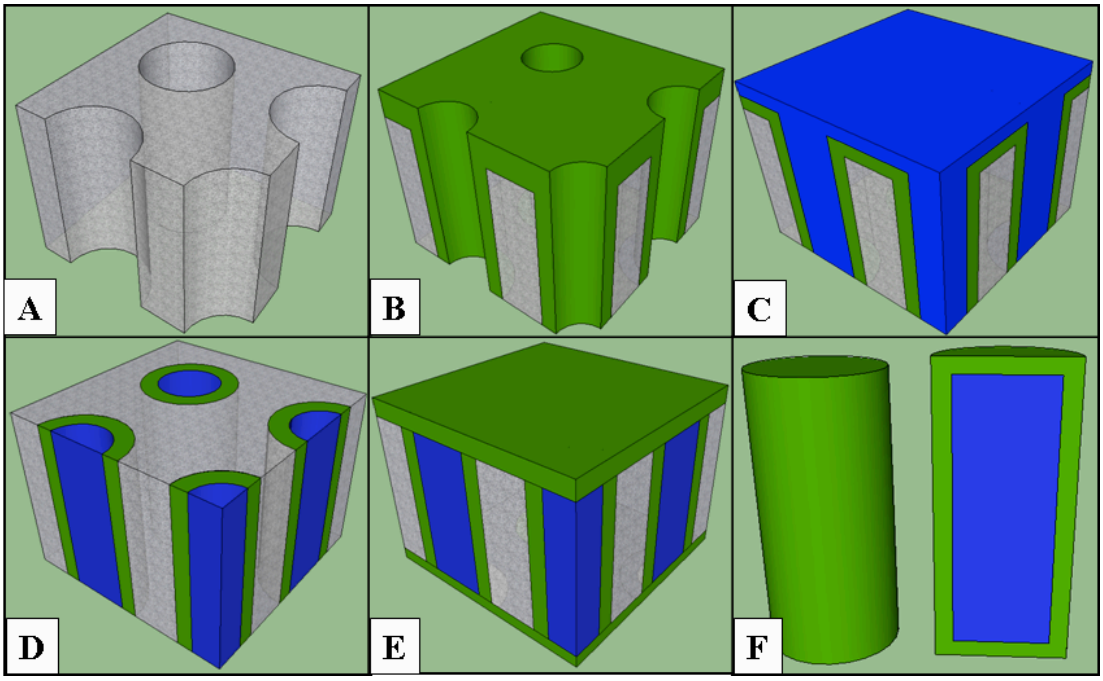

Figure 1. Fabrication steps of the microworms. The inner walls of the AAO template (A) is coated with a thin CVD hydrogel layer (B). The optode forming solution is adsorbed into the pores (C). The excess layers of optode and the hydrogel layer are removed using O_2 plasma (D). Both sides of the membrane are coated with a thin CVD hydrogel layer, capping the nanotubes (E). As the final step, the membrane is removed by etching in solution to release the microworm optode sensors (F).

set to 200 mmTorr and the filament and stage temperature were kept at 250ºC and 30ºC respectively during the depositions. AAO membranes were etched by dipping them in a 1M HCl solution overnight.

2.3 Characterization

Variable-angle ellipsometric spectroscopy (VASE, M-2000, J. A. Woollam) was used to measure film thicknesses. All thickness measurements were performed at a 75 incidence angle using 190 wavelengths from 315 to 718 nm. A nonlinear least-squares minimization was used to fit ellipsometric data of dry films to the Cauchy-Urbach model. The thickness and refractive index coefficients were obtained upon convergence of the algorithm. To measure water-swollen films, a liquid cell accessory was used (J.A. Woollam). The cell clamped over the sample and held the deionized water in a liquid tight seal. The optical properties of the cell windows and water were included in the model. Microworms were imaged using SEM (JEOL, JSM-6060). Confocal images were recorded in a Zeiss LSM 700 confocal microscope.

3 RESULTS AND DISCUSSION

AAO membranes are used as template to fabricate the microworms. The entire process is displayed in Figure 1. The first step is the deposition of the hydrogel on the inner walls of the membranes. Then, the dye solution is filled inside the pores by spin-coating and the excess of dye and polymer remaining on top of the surface is etched by plasma cleaning. Afterwards both sides of the membrane are polymerized again in order to cap the microworms. Finally, the membranes are dipped in 1m HCl to release the microworms. Figure 2 shows the SEM images of the microworms obtained, polymer thickness is approximately 50 nm.

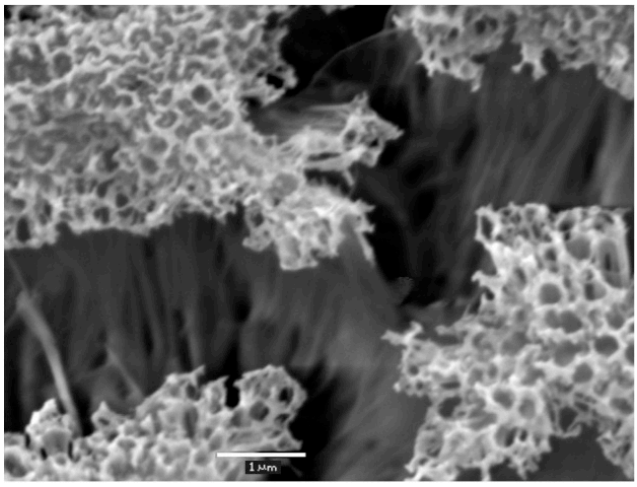

Figure 2. SEM image of the microworms before the filling.

Controlling the polymer mesh size is a key point in the fabrication of the medical device. The mesh size must be large enough to allow the entrance of the desired analyte inside the microworm to interact with the optode, yet while it must be small enough to prevent the entrance of undesired substances or to avoid the optode leaking out of the polymeric structure. The mesh size can be controlled adjusting the amount of cross-linker in the polymerization reaction. Peppas and Merril developed equation 1 to study cross-linking from the equilibrium swelling [9].

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\left(\overline{\upsilon}/V_1\right)\left[\ln\left(1-\upsilon_{2,s}\right)+\upsilon_{2,s}+\chi\upsilon_{2,s}^2\right]}{\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3}-\frac{1}{2}\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)\right]} \quad (1)$$

Where $\overline{M}_c$ is the number average molecular weight between cross-links, $\overline{M}_n$ is the number average molecular weight of the polymer before cross-linking, $\overline{\upsilon}$ is the specific volume of pHEMA, V_1 is the molar volume of the swelling agent (18 cm^3/mol), $\upsilon_{2,r}$ and $\upsilon_{2,s}$ are the polymer volume fractions before and after swelling and χ is the Flory polymer-water interaction parameter and can be calculated as a function of the polymer volume fraction [10]. Then M_c is used to calculate the end-to-end distance of the unperturbed (solvent-free) state, $(r_0^2)^{1/2}$.

$$\left(\overline{r}_0^2\right)^{1/2} = l\left(\frac{2\overline{M}_c}{M_r}\right)^{1/2} C_n^{1/2} \quad (2)$$

Here l is the C-C bond length, 1.54 Å, M_r is the molecular weight of the HEMA repeating unit (130 g/mol) and C_n is the characteristic ratio of the chain (for HEMA 6.9). Finally $(r_0^2)^{1/2}$ is used to estimate the mesh size (ξ):

$$\xi = \upsilon_{2,s}^{-1/3}\left(\overline{r}_0^2\right)^{1/2} \quad (3)$$

Figure 3 shows the mesh size distributions of the polymer deposited on the walls of the microworms depending on the amount of monomer and cross-linker introduced into the reactor. It seems that the minimal size that can be obtained is close to 5 Å, then there is an exponential growth as long as the HEMA/EGDA ratio increases, until reaching a maximum of 21 Å for a ratio of 5.6. When this ratio is increased, the amount of cross-linker is so small that the hydrogel is not able to maintain its structure when performing the swelling experiments. At the first stage, when the low cross-linked sample is exposed to water, the polymer swells as usual. However, when the solvent continues diffusing into the polymer and reaches a critical values, chain disentanglement occurs causing the polymer dissolution [11], which explains the decay observed after the maximum value. The mesh size obtained is large enough to enable small ions or molecules, such as sodium (1 Å) or glucose (5 Å), diffuse trough the polymer to reach the optode. Additionally, it would not allow the entrance of proteins or macromolecules that could affect to the sensor performance. For these reasons, a 20 Å large mesh size is chosen for further experiments.

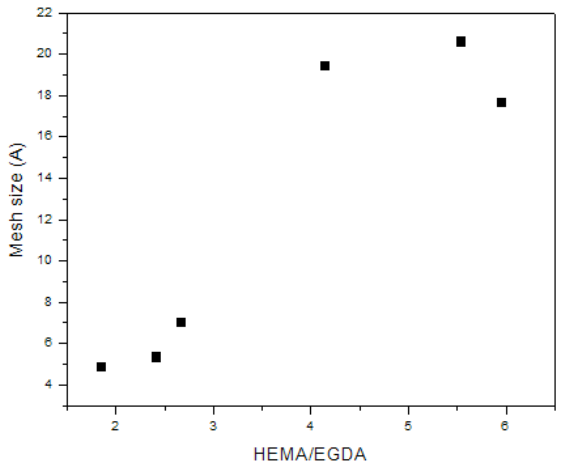

Figure 3. Mesh size distribution (Å) in the microworm structure as a function of HEMA and EGDA flows.

The polymer structure plays also a very important role in the filling of the microworms. Figure 4 shows the confocal microscopy images of the study of the filling. It should be note that when there is not the polymeric shell, the filling does not retain the expected structure, resulting in a shapeless conglomeration (1). To enhance the filling, the plasticizer and the dye were diluted in different concentrations in THF. The 1:2 dilution corresponds to image 2, where part of an AAO membrane without total releasing of the microworms can be observed. It is clearly seen that the filling is achieved. Nevertheless, there is a considerable amount of dye spread in the solution. On the

other hand, the 1:7 dilution (3) presents an optimal filling and practically no dye can be observed in the solution. The images suggest that a proper amount of dye must be charged in the solution, otherwise when adding the solution on the AAO membrane, the microworms are not able to incorporate all of it and the excess remains on top of the surface. Moreover, a larger dilution decreases viscosity and helps the diffusion into the tubes. However, when drops are added on top of the membrane, it is found that at one of the end of the microworms there is more dye remaining, which could be explained by a capillary effect when the THF evaporates. This accumulation could prevent a correct capping of the microworm. Therefore, to solve this problem, the filling is performed by spin-coating (4), so it makes the diffusion easier and avoid the problem of capillarity. The microworms obtained are approximately 30 μm in length and show an accurate filling. In addition, no leaching out is observed in any of the samples.

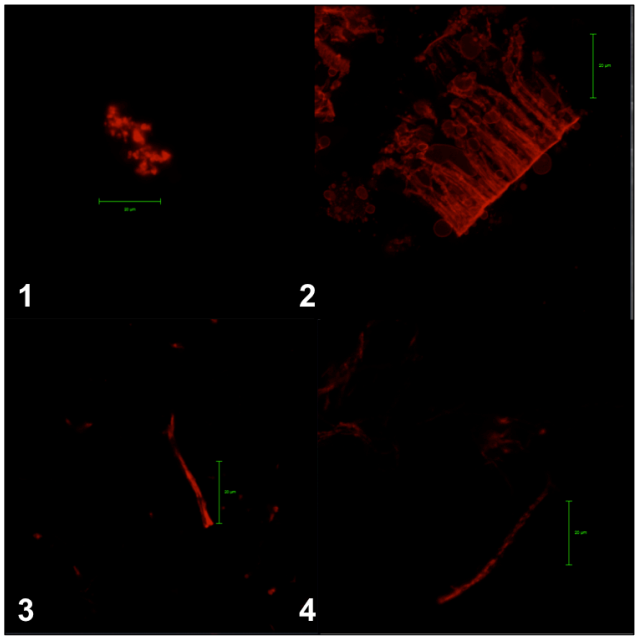

Figure 4. Conformal microscopy images of the microworm filling. Image 1 corresponds to a control sample, in which the filling is performed without the polymeryc shell. Image 2 and 3 display the filling with 1:2 and 1:7 dilutions in THF. In image 4 microworms were filled with a 1:7 dilution in THF by spin coating. Scale bar in all images is 20 μm.

4 CONCLUSIONS

A core-shell cylindrical shape structure has been obtained depositing a HEMA and EGDA copolymer by iCVD on AAO membranes. The mesh size for these microworms has been studied, ranging from 5 to 21 Å. Moreover, the dye filling process performed using spin coating has shown 30 μm long microworms with a homogeneous filling. Next experiments are being addressed towards the in vitro and in vivo testing of the microworms for glucose monitoring.

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