Preparation and characterization of permeable polymeric membranes by alterned deposition of polyelectrolytes on *Saccharomyces cerevisiae* cells

Ceniceros, M. A.[1], Romero, J.[1],Moya, S.[2], Ledezma, A.[1]
[1]Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna 140, Saltillo, Coah., 25253
[2]Unidad de Biosuperficies CICBiomagune, Paseo Miramón 182 C, 10009 San Sebastián, Guipuzcoa, España.
aledezma@ciqa.mx[1]

1. Summary

Combination of soft matter such as polymers, surfactants and liquid crystals with biological molecules has many important applications in nano and micro fabrication of novel materials and smart devices. Both, soft matter and biological molecules can provide their best characteristics such as specificity and selectivity of biomolecules and physical properties of synthetic polymers (conductivity, mechanical strength, elasticity, etc.). In this study polyelectrolyte multilayer capsules were fabricated using electrostatic Layer by Layer self-assembly technique (L-b-L) of the pairs poly(sodium 4-styrenesulfonate) (PSS)/poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS)/poly(diallyldimethylammonium chloride) (PDADMAC) on *Saccharomyces cerevisiae* cells as a template. In order to dissolve the core of yeast cells we used sodium hypochloride (NaOCl) 4%. Using this technique we accomplished to get capsules prepared with 5 and 15 polyelectrolyte layers. Integrity of this capsules and its morphology were studied by SEM and TEM and was possible to observe a porous and hollow structure with a porous size ranging from 20 to 40nm.

2. Introduction

The interaction of living cells with substrates is an area of intense research due to its relevance for very different applications. Combination of biological molecules and soft matter such as polymers and surfactants has many important applications in nano and micro fabrication of novel materials and smart devices. Both can provide their best characteristics such as specificity and selectivity of biomolecules and physical properties of synthetic polymers. In recent years techniques for nano and micro fabrication using polymers have been developed and a very interesting application of this fabrication is the engineering of smart devices for controlled drug delivery [1, 2, 3]. In this study we present a novel approach based on alterned deposition technique 'Layer by Layer' on biological templates. We used *Saccharomyces cerevisiae* cell as templates, and different polyelectrolytes (PE) charged positively and negatively to form

multilayer capsules. The number of deposited layers was 5, 10 and 15 and then in order to create capsules the template was dissolved with a strong oxidant treatment. While the size of the capsule of PE built with the previous described conditions is between 0.1 and 10μm and is defined for the template size, for the other hand the thickness of this capsules depends on the number of the deposited PE layers [4].

3. Materials and methods

The polyelectrolytes (PE) poly(sodium 4-styrenesulfonate) (PSS), poly(allylamine hydrochloride) (PAH) and poly(diallyldimethylammonium chloride) (PDADMAC) were obtained from ALDRICH, the fluorescent dye fluorescein isothicyanate (FITC) was purchased from SIGMA. For visualizaition of the PE coating, PAH was covalently bound to FITC following the procedure described by Donath et al. [5]. In order to remove the core of cells we use sodium hipochloride (NaOCl) 0.4% wt water from SIGMA-ALDRICH.

We encapsulated bakery yeast cells (*Saccharomyces cerevisiae)* from a liquid culture with different number of layers using the systems of polyelectrolytes PSS/PAH and PSS/PDADMAC. When the desire number of layers were deposited the coated biological templates were exposed to a deproteinizer treatment and the cytoplasmic proteins of the core were dissolved or decomposed. At the same time PE layers were changed by the deproteinizer. The core of cell is dissolve in low molecular-weight products leave the shell interior by diffusing through the shell wall [6]. These products were removed by centrifugation.

The integrity and morphology of the capsule were characterized by Transmision Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) and Laser Scanning Confocal Microscopy (LSCM), and the chemistry of the PE layers was characterized by FTIR.

4. Results and discussion

By means of LSCM of a 15 layer coated yeast we observed the entire coating of the yeast, the 14 layer is FITC labeled PAH. The image shows that the 14 layer covered successfully the cell surface.

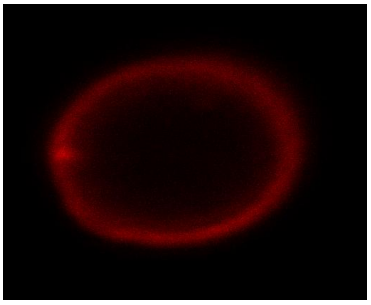

Figura 1. Confocal microscopy scans of a polyelectrolyte shell consisting of 15layers of PSS/PAH templated on a yeast cell. The 14th layer is FITC-labeled PAH.

The TEM image (Fig. 2) shows a capsule formed with 15 PE layers using the PSS/PDADMAC system. The image shows the capsule as an empty structure once the core was removed from the shell interior. The covering it's observed like uniform layer and the dark fields are assumed to be 'folds' of the capsule or polymeric aggregates.

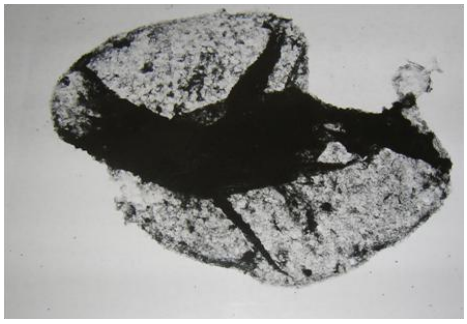

Figura. 2. TEM image of a capsule prepared of 15 layers of PE using yeast cell as template

Topography of the capsules was studied by SEM. The micrography (Fig. 3a) shows capsules prepared with 5 PE layers using PSS/PAH system. The first image present flat structures due to the volume are lost, so the capsules are empty and the shape of the template is preserved. Also it is possible to observe folds created during dry preparation of the material.

At high resolution (Fig 3b) we can see that the porous of the membrane has 70nm as maximum, however most of them are in a range of 20 and 40nm. The size of the porous may have influence in the permeability of the capsule and will define the molecular weight of the material that can pass through the walls. Besides of the porous we can observe inorganic

crystals, which can assign to NaCl, traces of NaOCl and the washed treatment with saline solution.

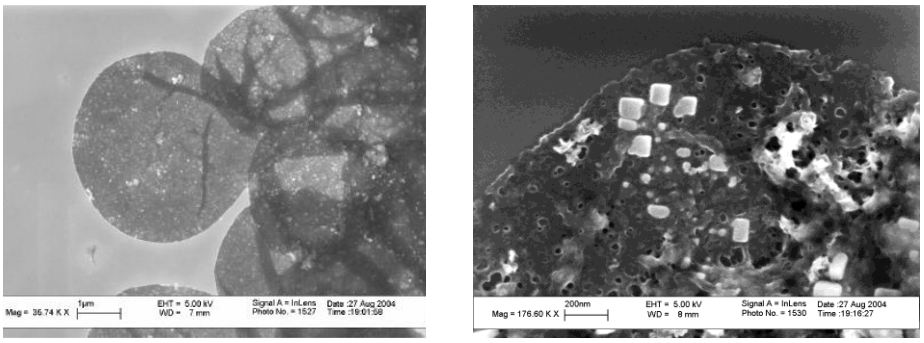

Figura 3. SEM images of capsules fabricated with 5layers of PSS/PAH system at different magnifications

5. Conclusions

In this investigation we use the systems of polyelectrolytes PSS/PAH and PSS/PDADMAC in order to create capsules using *Saccharomyces cerevisiae* cells as template. By means of oxidation treatment was possible to dissolve the core and form capsules using the systems PSS/PAH and PSS/PDADMAC templated on yeast cells (S. cerevisiae). It is demonstrated by TEM, SEM and LSCM that we have and empty and continuous structure with porous of 20 to 40nm. Finally, according to FTIR PE layers were affected chemically by the oxidation treatment and several functional groups were lost.

6. References

1. S. Krol, O. Cavalleri, P. Ramoino, A. Gliozzi and A. Diaspro, *Journal of Microscopy,* 212, 239-243, **2003.**

2. S. Moya, L. Daehne, A. Voigt, S. Leporatti, E. Donath, H. Möhwald, *Colloids and Surfaces Aspects,*183, 27-40, **2001**.

3. A. Diaspro, D. Silvano, S. Krol et al., *Langmuir*, 18, 13, 5047, **2002.**

4. R. Georgieva, S. Moya, M. Hin, R. Mitlöhner, E. Donath, H. Kiesewetter, H. Möhwald and H. Bäumler, *Biomacromolecules* 3, 517-524, **2002.**

5. Donath, E. Sukhorukov, G. B., Caruso, F., Davis, S. A. & Möhwald, H., Angew. Chem. Int. Ed. 37, 2202-2205, **1998.**

6. E. Donath, unpublished video microscopy observations.