Optimizing Silica Encapsulation of Living Cells: In Situ Evaluation of Cellular Stress

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The encapsulation of living cells in materials with good optical and mechanical properties often produces death or stress due to the release of toxic byproducts originated during the synthesis. We present here a method to assess the cellular stress that silica entrapment exerts over living cells taking into account the main preparation variables such as the nature of the silica source, protecting functional groups, total solid concentration, or indirect procedures. Measurement of the cellular stress status of genetically modified *Sacharomyces cerevisiae*, a true biological probe, allowed us to perform a quantitative analysis of cellular stress in a short time basis (compared to conventional long-term viability tests), opening the gate for a more sophisticated approach to optimize the synthesis conditions. In addition, the aforementioned findings allowed the preparation of novel materials with enhanced optical and mechanical properties. The relation of cellular stress with initial viability is also discussed.

Introduction

Nowadays, one of the most developing fields in materials science research deals with the encapsulation of biological entities. The entrapment of enzymes, antibodies, and other biomolecules within silica matrixes has been developed for the last two decades.[1–4] Moreover, silica sol−gel chemistry has emerged as an outstanding strategy to obtain whole cell-entrapping biocomposites. This field of research is growing fast with a large number of bioceramics already developed for different applications.[5,6] Pioneer work in whole cell immobilization aimed to exploit the bioactivity of an overexpressed enzyme of interest.[7] In this scenario, the physiological status of entrapped cells was completely irrelevant since enzymatic activity could be retained or even improved by many folds in nonviable cells.[8]

The encapsulation of cells to take advantage of the complex biological activities of living cells in the designing of functional materials is more challenging and has not been so widely exploited, although these systems are a promising tool for the production of low weight proteins or secondary metabolites of interest. Some potential applications to consider are the production of medicinal compounds for the pharmaceutical industry and biofertilizers or biocontrol agents for the agricultural sector. While macromolecules retain their activity within a given range of pH, ionic strength, or water activity (ultimately an on/off response type), living organisms show more sophisticated response patterns. External environmental factors sensed by cells are integrated by signal transduction pathways and converge to regulate cell phenotype, modulating the biosynthesis of metabolic products.[9] Until now, the inherent biocompatibility of the different encapsulation procedures was centered in the measure of the initial viability and the degree of survival of cells submitted to entrapment.[10–12]

For applications in which the entire biosynthetic machinery is compromised, the physiological status of cells becomes of great importance. Viability is not an indicator of biosynthetic capacity: varying any factor that causes cellular stress, a cell may remain well above the tolerance limits but still develop a high stress level, remaining unproductive. Another important highlight of cellular silicate hybrid systems is the design of biosensing devices. A high level of cellular stress can mask the signal to be sensed by the immobilized cell strain.

In standard procedures (i.e., one pot encapsulation) not only are cells exposed to an unnatural environment during the inorganic host synthesis, but also tightly encapsulated cells are kept in permanent contact with the gel's surface silanol groups throughout the immobilization process. An-

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other constraint is the space limitation suffered by confined cells and its immediate consequence: the impossibility to perform cellular division.[13] Since even mild thermal and chemical conditions of sol−gel synthesis may prove harmful for cells of higher organisms, important efforts have been made to improve the biocompatibility of encapsulation procedures in terms of the viability of the cell population submitted to entrapment (i.e., percentage of living cells from the total number of entrapped cells).[14,15]

Recently, our group developed a new procedure that allows cellular proliferation on liquid cavities created inside a silica matrix.[16] Cells are previously immobilized in Ca(II)−alginate beads that are subsequently trapped in the inorganic matrix, avoiding any harmful contact of cells with surrounding precursors.[17] Thus, the inorganic synthesis occurs in the periphery, only affecting closely in-contact cells. Once the silica hydrogel is consolidated, alginate is liquefied by chemical means and cells remain confined into macrocavities containing a liquid medium inside a solid silica monolith. This procedure, herein after referred to as two-step silica encapsulation, is based on inexpensive silica precursors. It has already been employed for yeast (*Sacharomyces cerevisiae*) and bacteria (*Escherichia coli* and *Bacillus subtilis*) and was recently extended to more sensitive plant cells,[18] obtaining not only an extremely high initial viability (ca. 100%) but also allowing cell proliferation inside. This is of particular importance for bioreactor applications that require high cell density for the biosynthesis of value-added proteins.[19]

The present work was conceived to develop a fast and noninvasive tool for the rational design of immobilization materials with good optical and mechanical properties, based on the stress assessment of entrapped cells;[20,21] the main synthetic strategies developed to date (one pot or two step encapsulation) are revisited in a comparative way. To this aim, here we present a method to evaluate, in a quantitative way, the influence of the principal chemical factors over the cellular stress status of *S. cerevisiae* submitted to different silica encapsulation procedures. The method is based on the encapsulation of a genetic modified strain that encodes for the fusion protein Hsp12:Green Fluorescent Protein (GFP). Heat Shock Protein 12 (Hsp12p) is a small, hydrophilic general stress response protein whose transcription is up-regulated by a wide variety of stresses, including high osmolarity, oxidative stress, heat shock, high ionic strength, the presence of ethanol, and nutrient limitation. Monitoring the expression of this construct under the control of the Hsp12 promoter is easily done by confocal microscopy images, allowing a direct measure of the stress status of cells during the different stages of synthesis. Results obtained allow the optimization of novel synthesis protocols to reach a compromise between mechanical properties and cellular stress.

Materials and Methods

Biological Materials and Culture Conditions. Two commercial strains of *S. cerevisiae* (Invitrogen, Mat*a his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*) were employed in these experiments: one encoding the fusion protein Hsp12:GFPp and the other encoding the fusion protein Gln3:GFPp. The latter strain was used as a control that gives information about the metabolic status of the cells. Yeasts were grown in a 1% yeast extract, 2% peptone, 2% glucose (YPD) liquid medium. All cultures were routinely grown at 30 °C. Liquid cultures were agitated on an orbital shaker to maintain sufficient culture aeration.

Once sol−gel synthesis was completed, Bioceramics were incubated in minimal medium (MM) (0.67% yeast nitrogen based medium (YNB) without amino acids and with ammonium sulfate, Difco) containing 2% glucose and the required auxotrophies.

One Pot Yeast Encapsulation Procedure. *Silicate Ludox Aqueous Route (SLAR).* The silica encapsulation procedure was performed as previously described.[22] An aliquot (2.0 mL) of a culture of exponentially growing cells (A570 nm: 0.6−0.8) was collected by centrifugation and resuspended in 2.0 mL of Milli-Q water (Millipore Corp.). Immediately, 100 μL of this suspension was mixed with 2.5 M sodium silicate, commercial colloidal silica (LUDOX HS40, Aldrich), succinic acid (5% w/w), and HCl (when necessary to adjust pH 6.5−7.5) in glass tubes and vortexed for 30 s. Volumes of the different precursor solutions were adjusted to obtain a fixed SiO_2/water molar relation (4:100) varying the proportion of polymeric to particulate silica precursors (1:5, 1:4, and 1:3) in a final volume of 400 μL. To facilitate microscope observation, an aliquot of this liquid mix previous to reaching the gelation time was spread over a glass microscope slide. Viscosity of this sample undergoing sol−gel transition allows the formation of 1 mm thick gels.

TEOS Alcohol-Free Route (TAFR). Tetraethoxysilane (TEOS) and glycidoxypropyltrimethoxy-silane (GPTMS) (Aldrich) were employed as silica precursor, following the alcohol free procedure described elsewhere.[23] In this case, the cells suspended in 2.0 mL of 0.5 M phosphate buffer, pH 6.5, were mixed with the acid silica sol. Different volumes of pure TEOS (TAFR) based silica sol and buffer cell suspension were mixed to obtain an increasing SiO_2/water molar relation in the final hydrogel. In another series of experiments, GPTMS containing gels (GAFR) were prepared, keeping constant the SiO_2/water molar relation in the final hydrogel.

Both SLAR and TAFR silica hydrogels were prepared in a range of compositions that allows handling without gel disruption.

Two-Step Yeast Encapsulation Procedure. *First Step: Encapsulation in Ca(II)−Alginate.* An aliquot (500 μL) of a culture of exponentially growing cells (A570 nm: 0.6−0.8) was collected by centrifugation and resuspended in 2.0 mL of sodium alginate (concentrations of sodium alginate: 0.5%, 1.0%, 2.0%, and 4.0%). This suspension was dropped into 0.1 M $CaCl_2$ with the aid of a

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syringe provided with a needle 0.5 mm in diameter. After 10 min of stirring, Ca(II)−alginate beads containing the immobilized yeast cells were collected by filtration. Ca(II)−alginate beads were cut in thin slices (0.2−0.3 mm thick) using a surgical blade and placed onto a microscope glass slide.

Second Step: Encapsulation of Ca(II)−Alginate in Silica Hydrogel. Different precursor mixtures, either SLAR (SiO_2:H_2O molar relation 4:100; ratio of polymeric to particulate silica precursors 1:4) or TAFR (SiO_2 6−16% p/p) were spread over the Ca(II)−alginate beads deposited at the bottom of an eppendorf tube. For stress assessment measurements, 1 mm thick silica hydrogel was spread over the Ca(II)−alginate slide to obtain a 1 mm thick gel as described above. Samples were exposed for 15 min to 0.05% potassium citrate to solubilize the Ca(II)−alginate matrix. After redissolution of Ca(II)−alginate, slides were rinsed twice with Milli-Q water and introduced into a flask containing liquid YNB medium. The flask was maintained under orbital agitation (120 rpm) at (30 ± 1) °C for 2 h.

Fluorimetry and Confocal Microscopy (CM) Studies. Preliminary studies of the time dependency of Hsp12:GFPp expression were carried out using a Stationary QuantaMaster QM1 spectrofluorimeter (excitation wavelength = 466 nm; emission wavelength range of integration 490−525 nm). Cell suspensions were directly measured in a spectrofluorimeter cuvette.

For CM studies, samples were withdrawn at different stages of the synthesis procedures and observed after 30 min of incubation at 30 °C. Images were acquired with a confocal microscope model Olympus FV300 (laser source: Ar, 488 nm) equipped with a software controlled unit that allows a continuous variation of intensity. For liquid suspensions, a drop of sample was spread over a glass microscope slide for direct observation. For all the samples, a series of confocal microscope images were acquired at different *z* planes. For each cell, the green intensity corresponding to the plane of maximum fluorescence was quantified using the *Image J* (version 1.37v) software.[24]

Na(I) Quantification. Direct measurements of Na(I) concentration in the outer 0.1 M $CaCl_2$ solution as a function of elapsed cross-linking time were carried out by atomic absorption spectroscopy (AAS). Concentrations of Na(I) inside the alginate bead are calculated from the difference between Na(I) present in the initial drop of Na(I)−alginate solution and the above-mentioned measurements, taking into account the volume change of the bead as a function of time.

Mechanical Properties. A series of TAFR hydrogels, with increasing silica contents, were cast in 3 mm height−3 cm diameter molds, during 2 h. The resulting gel samples were immersed in excess of phosphate buffer (pH 6.5, 0.1 M) for 24 h at 298 K, before compression tests. A mechanical evaluation of the resulting hydrogels was performed at 298 K by means of a Stable Microsystems TA-XT2i Texture Analyzer using a cylindrical probe (P/36R 36 mm diameter). The samples were incrementally compressed, from 10 to 30% of the initial height, at a compression rate of 0.5 mm/s, until the observation of fractures.

Results and Discussion

In agreement with previous studies,[25] no GFP fluorescence was observed when exponentially growing yeasts, grown in glucose containing YPD medium, were examined using a fluorescent microscope. In order to study the time response of the induced expression of the fusion protein Hsp12:GFP in our system, a preliminary study was carried out with one factor known to stress yeast cells: ionic strength. The latter expressed as NaCl concentration is taken as reference for stress stimuli. Yeast cells incubated with NaCl (0, 200, 400, or 800 mM) were collected by centrifugation and resuspended in YNB medium after the specified incubation time. GFP fluorescence in whole-cell suspensions was immediately measured as described in the previous section. In the absence of added NaCl, a low fluorescence signal was observed which was not detected in fluorescence microscope observations. It may be attributed to a basal level of expression of the construct under the control of the Hsp12 promoter. Nonetheless, these levels are low enough to allow the observation of a significant increase in GFP expression with ionic strength. Confirming published results,[21] a high level of expression of Hsp12-GFPp is reached after 30 min of induction, and the up-regulation occurs in a concentration-dependent manner except for the highest concentration of NaCl assayed (i.e., 800 mM), that is postulated to stress severely the cells, causing an inhibition of transcriptional and translational machinery (with the possible exception of systems involved in Na(I) efflux). From the analysis of the data, it can be concluded that the high level of expression of Hsp12-GFPp is maintained for at least 30 min (data shown as Supporting Information). Once the time dependency of Hsp12:GFPp induction was established, further studies with the different treatments were carried out at a fixed time of (30 ± 5) min.

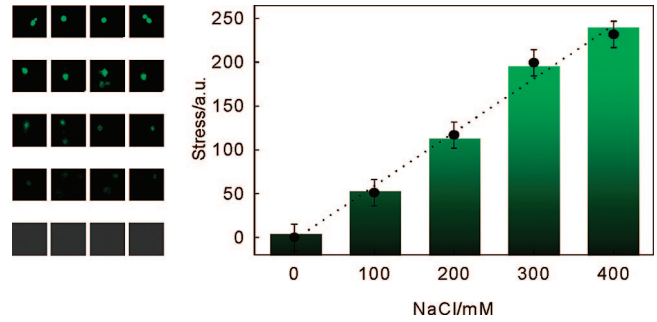

Figure 1. Left: representative fluorescence confocal microscope images corresponding to each ionic strength treatment of the yeast cells (0, 100, 200, and 400 mM NaCl, from bottom to top). Right: curve of GFP fluorescence (in arbitrary units) vs ionic strength of the medium (NaCl).

Figure 1 shows the GFP fluorescence intensity taken from CM images, defined as stress (arbitrary units), as a function of NaCl concentration for aliquots of the previous cultures incubated for 30 min. These results indicate that for moderate stress signals (i.e., below 250 au), a proportional relation between the observed stress and the stress stimuli can be assumed.

Stress Assessment of One Pot Encapsulation. Concerning the one step entrapping procedures, the main strategies to construct silica hydrogels under biocompatible conditions can be defined in two main groups: the alcoxide route and the silicate one, depending on the nature of the silica source employed. The so-called alcoxide route typically employs hydrolyzed tetramethylorthosilicate (TMOS) or tetraethylorthosilicate (TEOS) as sodium-free silica precursors; the harmful byproducts methanol or ethanol, respectively, must be removed by controlled distillation after the gel forma-

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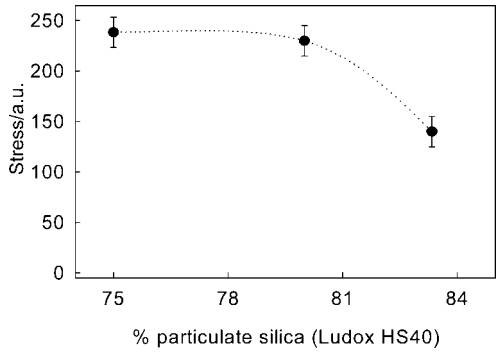

Figure 2. Measured stress as function of the Ludox HS40 silica content in SLAR hydrogels (total SiO_2 content = 12.7%).

tion.[23] On the other hand, the aqueous route, based on the neutralization of sodium silicate solutions, offers a cheaper and straightforward strategy to form silica hydrogels.

One Pot SLAR Route. The use of sodium silicate solutions as the source of silica was recently reported for whole-cell encapsulation.[26] In order to improve biocompatibility, Livage and co-workers proposed to incorporate part of the SiO_2 in the form of nanoparticles (Ludox HS40), diminishing the concentration of free sodium ions and/or silicates.[27] We are interested in knowing to which extent the ratio of polymeric to particulate silica is affecting the level of cellular stress. For this system, the molar relation SiO_2/water is the main factor controlling gel rigidity. It was fixed at 4:100 (total SiO_2 12.7% w/w) since with this relation high cell viability is obtained, maintaining good mechanical properties. As can be seen in Figure 2, for all the polymeric SiO_2 to particulate SiO_2 ratios assayed a high level of stress is observed. For the lowest ratio, stress is comparable to that observed when cells are submitted to a concentration of NaCl in the 200−300 mM range. As expected, raising the proportion of sodium silicate increases the cellular stress, making it comparable to that experienced by yeast in 400 mM NaCl. This stress can be explained due to the concentration of Na(I) and silicates. Different proportions of precursors were not investigated since lower polymeric to particulate ratios led to the formation of gels with poor mechanical stability, while higher concentrations of sodium silicate greatly affected cell viability.

One Pot TAFR-GAFR Route. As a counterpart of the sodium silicate route, the so-called alcoxide one is commonly used to prepare silica hydrogels. After a proper alcohol separation from the precursor's solution, silica hydrogels with good optical and mechanical properties can be obtained (see Supporting Information).

The one pot entrapment in these TAFR hydrogels presents an increase of stress and decrease of viability, with increasing SiO_2 content. In Figure 3, the initial viability (expressed as the percentage of remaining CFU) is contrasted with the measured stress (expressed as the percentage of maximum GFP fluorescence intensity observed) after one pot entrapment in TAFR hydrogels, as a function of the total silica

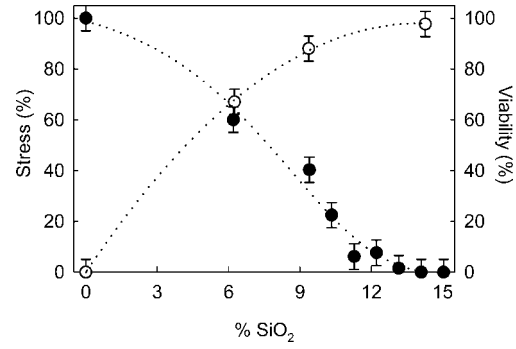

Figure 3. Percentage of initial viability (●) and stress (○) of *S. cerevisiae* after one pot entrapment in TAFR-based hydrogels, as function of the total silica content.

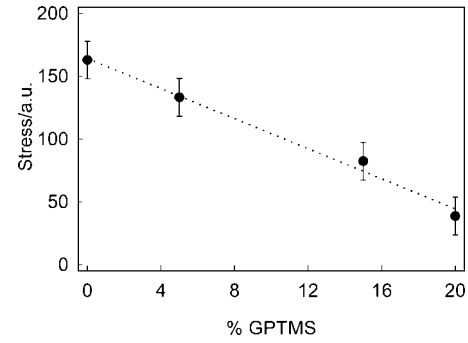

Figure 4. Stress as function of the GPTMS percentage in GAFR based hydrogels (total SiO_2 content = 9.4%).

content. The cellular stress quickly rises to a saturation value for silica contents of 9% (w/w) or higher. Notwithstanding, in accordance with previous reports, high viability is observed for silica contents up to 9% (w/w); however, this value dramatically drops to total cell death for silica contents higher than 12% (w/w). From this it can be concluded that the stress signal gives additional valuable information on the status of the remaining living cells.

It was claimed that the TAFR route can be improved including $R'-Si(-OR)_3$ precursors in the formulation, were $-R'$ is a nonhydrolizable organic function, giving rise to $R'-$ functionalized silica hydrogels with enhanced biocompatibility.[23] The addition of small fractions of GPTMS (less than 20%) has a noticeable effect in reducing the inherent stress of the matrix, confirming the valuable role of organically modified silica additives, reported for viability tests.[23] These results are presented in Figure 4. It is worth mentioning that all the GAFR based hydrogels, in contrast with the TAFR based ones, exhibit poor mechanical properties and developed massive cracks after a month of aging. In addition, optical properties are severely distorted for GPTMS contents higher than 10% (see Supporting Information).

Stress Assessment of the Two Step Encapsulation Procedure. *First Step: Suspension in Na(I)-Alginate Solution and Ca(II)−Alginate Encapsulation.* In order to evaluate the inherent stress induced by Na(I)−alginate, yeast cells collected by centrifugation were suspended in Na(I)−alginate solutions, with concentrations ranging from 1% to 4% (w/v). Once the cells were exposed for 30 min, the observed stress trend as a function of Na(I) concentration of the medium (as determined by AAS experiments) can be superimposed

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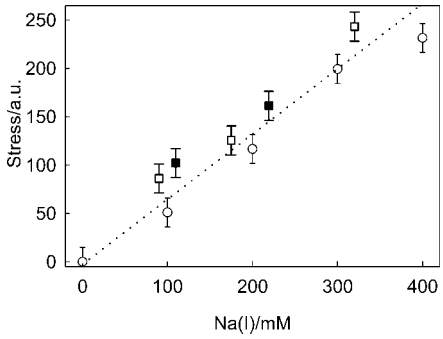

Figure 5. Stress in 1% and 2% Na(I)−alginate solution (■) and 1%, 2%, and 4% Na(I)−alginate cross-linked in 0.1 M $CaCl_2$, at 298 K (□) as a function of Na(I) concentration, superimposed to the NaCl reference curve (○).

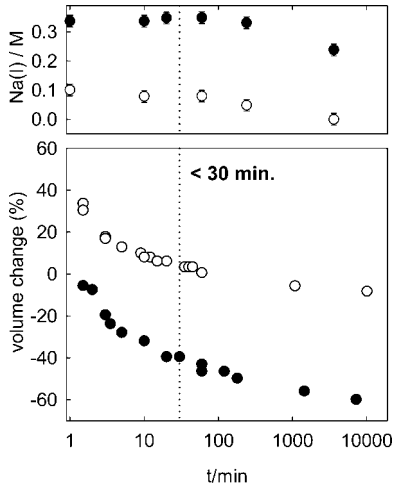

Figure 6. Inner Na(I) concentration (top) and change in volume (down) of Na(I)−alginate drops, 1% (● and 4% (○), submitted to cross-linking in 0.1 M $CaCl_2$, at 298 K.

to the NaCl response, as shown in Figure 5. This result suggests that the sole suspension in sodium alginate causes a strictly Na(I) concentration-dependent stress. The highest concentration (4% Na(I)−alginate) is not included since it was out of the linear range.

The following step consists of gelation of the alginate polymer with Ca(II). Freshly prepared Na(I)−alginate cell suspensions were dropped in 0.1 M $CaCl_2$ and after 30 min of stirring, and the resulting Ca(II)−alginate beads were collected by filtration. This second step implies the coordination of Ca(II) by the negatively charged alginate polymer forming a cross-linked gel.[28] Stress of cells submitted to this immobilization in Ca(II)−alginate (data included in Figure 5) follow the same trend as a function of Na(I) concentration of the medium as in the previous case.

Figure 6 presents the evolution of the bead volume change (expressed as volume percentage, taking the initial drop's volume as the reference) against aging time in the presence of 0.1 M $CaCl_2$. With the exception of the highest alginate concentration, all the samples evolve with a strictly contracting regime (see Supporting Information). Once a drop of alginate cell suspension makes contact with the Ca(II) solution, a cross-linked Ca(II)−alginate shell develops around

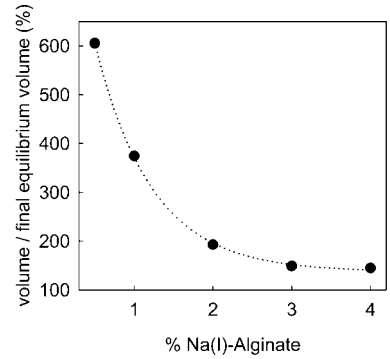

Figure 7. Volume of Na(I)−alginate solution submitted to cross-linking in 0.1 M $CaCl_2$ at 298 K at a sampling time of 10 min with respect to the estimated final volume of cross-linked alginate at infinite time, as a function of the Na(I)−alginate concentration.

the liquid Na(I)−alginate drop (naked eye observation).[29] Given the concentration gradient, the outer Ca(II) ions diffuse toward the center of the bead and the inner gel achieves an increasing degree of cross-linking, up to the equilibrium form, while the remaining Na(I) diffuses to the outer solution. If the rate of cross-linking prevails, the bead results in a net volume contraction. Interestingly, as shown in Figure 6, for the highest alginate concentration during the first hour of aging, the beads remain strictly expanded with respect to their initial volume. This fact indicates the occurrence of an osmotic gradient between the liquid core (330 mM Na(I)) and the outer liquid phase (100 mM $CaCl_2$), which promotes the uptake of solvent from the latter phase toward the former one. This means that the cells are exposed to high Na(I) concentrations after the bead formation, explaining the occurrence of stress even when no physical shrinkage exists. The time evolution of the Ca(II)−alginate beads was extrapolated to find the equilibrium volume (see Supporting Information). From these values of equilibrium volume, the time evolution of cross-linking degree can be inferred. Figure 7 presents the bead volume after 10 min (typical time for silica entrapment) in contact with 0.1 M $CaCl_2$, with respect to the predicted volume of the thermodynamically stable structure (total contraction). At this time, cross-linking degree is highly dependent on initial Na(I)−alginate concentration. After alginate trapping, the viability inside the beads decays to 80% and 50% for alginate concentrations of 1% and 4%, respectively. In both cases, the CFU value inside the bead remained constant during the time scale of the experiment. Both results are in accordance to previous reports.[30,31]

Second Step: SLAR Encapsulation. To study the effect of the following step involved in the this synthesis procedure, slices of Ca(II)−alginate beads were covered with a precursor mix as described in the Materials and Methods section. This colloidal solution underwent a rapid sol−gel transition, and Ca(II)−alginate slices were quickly surrounded with a nanoporous silica hydrogel, preventing the cell exposition to free silicates. Once silica gelation was completed, Ca(II)−alginate was wrapped by complexation of Ca(II) with

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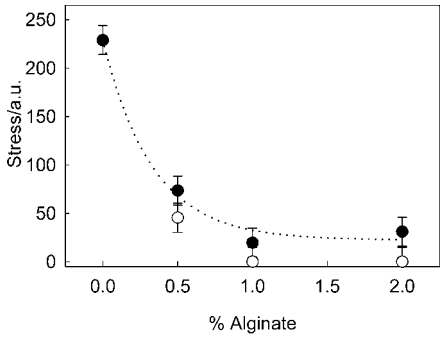

Figure 8. Stress after SLAR two step procedure silica synthesis (total SiO_2 content = 12.7%) as a function of the alginate concentration used in the first step (alginate 0% corresponds to the direct encapsulation procedure). Measurements were performed 30 min after silica synthesis (●) and for samples incubated in liquid YNB medium at (30 ± 1) °C for 2 h (○).

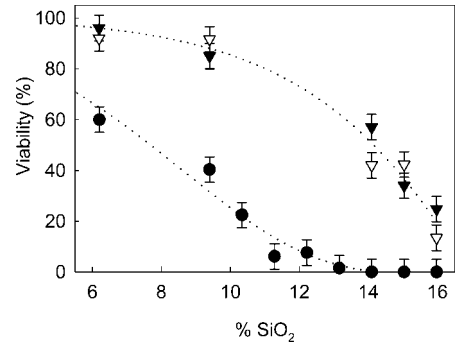

Figure 9. Percentage of initial viability of *S. cerevisiae* after one pot (●) and two step TAFR procedure (alginate 1% (▼) and 4% (▽)) entrapment, as a function of the total silica content.

citrate, leaving cells inside a millimeter-scale liquid cavity. During the encapsulation process cells were starved for more than 30 min. [After 15 min, potassium citrate was rinsed with distilled water, and slides were placed into flasks containing liquid YNB culture medium; however, diffusion of nutrients through the silica matrix is not immediate.] The stress observed after this stage is presented in Figure 8; as expected, for alginate concentrations above 1%, the stress level was significantly lower than that observed in cells submitted to the direct synthesis procedure. Herein, immobilized cells were kept in near-standard culture conditions, as each volume containing liquid MM culture medium can be regarded as a millimeter-scale bioreactor. Samples were withdrawn after 90 min of incubation in the MM medium and analyzed for GFP fluorescence detection (data in Figure 8). The level of stress descends to an extremely low value, being much lower than the optimum one achieved with the one pot entrapment (sample 20% GPTMS). These results are in line with the extremely high values of viability previously observed.[16,18] Noteworthy, the higher level of stress is detected for the lowest alginate concentration (i.e., 0.5% Na(I)−alginate), in which stress induced by Na(I)−alginate is minimum. This fact is associated to the properties of the resulting Ca(II)−alginate encapsulating phase. Taking into account the inferred low cross-linking degree (see Figure 7), the observed stress can be attributed to the permeability of the Ca(II)−alginate matrix, which cannot prevent the diffusion, before gelation, of outer soluble Na(I)−silicate toward the entrapped cells.

Two-Step TAFR Encapsulation Procedure. The stress and viability observations exposed in the preceding sections suggest that the two step procedure can be improved, taking the advantage of stress minimization of alginate pre-encapsulation in combination with the better mechanical and optical properties exhibited by the TAFR based hydrogels (see Supporting Information, Figures 7 and 9). Then, the natural question that emerges is how much can the silica based entrapping material be improved, preserving acceptable level of cell viability/growth. To this aim, alginate beads were submitted to encapsulation within TAFR hydrogels with increasing silica contents. Figure 9 compares the viability observed when the probe cells are entrapped with or without alginate. As might be expected, for low (nonharmful) silica contents, no significant differences are observed; for silica contents higher than 14%, the two step procedure preserves more than 50% of the cell viability while the one pot method results in total cell death. The response seems to be independent from the alginate concentration employed; the viability decay observed for the highest silica contents can be interpreted in terms of free silicate diffusion toward the inner space of the alginate bead, due to the higher gradient existing during gelation. One last question remains concerning the transport properties of these biomaterials, since the effective diffusion coefficients of silica hydrogels are strongly dependent on the silica content.[32] Cell growth experiments indicate that all the samples follow a common trend, irrespective from the silica and/or alginate concentration employed. Then, despite the difference in initial cell viability, in the explored conditions the transport properties of the matrix are not a limiting constrain for cell growth.

Conclusions

We present here a method to assess the influence of the principal synthesis parameters over the cellular stress status of *S. cerevisiae*, submitted to a wide range of silica encapsulation procedures. In principle, these results can be extended to other cell types since yeasts have been used as a model for the study of stress response in eukaryotic cells.[33] Moreover, Hsp12p can be replaced by any other gene to assess different types of stresses, in a more specific fashion.[34] A battery of genes can be used to unmask the specific type of stress caused by the different synthesis conditions or even to evaluate the possibility to produce a specific biosynthetic product.

Among the straight (one pot) encapsulation procedures, the GAFR route offered the best biocompatibility results. However, this additive affected the physical properties of the hydrogels. In addition, the inherent opalescence of these hybrid matrices restricts their potential use in optical based biosensing devices. Among the analyzed methods, the two step strategy offers an optimum response in diminishing the stress level. The use of adequate concentrations of Na(I)−alginate in the starting cell suspension grants a minimal stress

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before and after the Ca(II)−alginate bead formation. Using high Na(I)−alginate concentrations in the bead formation generates ionic stress while the lax structures that low concentration of Na(I)−alginate produces cannot confer an effective protection to immobilized cells during the next step of silica synthesis. The two step method opens the gate for the preparation of novel biomaterials with improved mechanical and optical properties, preserving maximum cell growth rates.

In contrast to the straight observation of cell damage by means of ex situ or destructive methods (cryo-SEM textural observation),[11] the CM based method is a noninvasive technique, allowing the immediate quantification of stress both of an isolated cell or colony, as function of time or external stimuli. Due to the inherent fast response and the low sampling volumes required, this technique arises as the gate for a combinatorial approach to the formulation of optimized cell entrapping biomaterials.

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Supporting Information Available: Details about preliminary studies of GFP time response and gelation of the alginate polymer with calcium cations as well as figures showing fluorimetry assays, volume change and relative volume of Na(I)−alginate solution, confocal microscope images, viability of *S. cerevisiae*, growth rate of alginate entrapped *S. cerevisiae*, absorbance of TAFR-based and GAFR-based hydrogels, stress−strain curves, and evolution of compression modulus (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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