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Insight into Cellular Response of Plant Cells Confined within Silica-Based Matrices

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Received October 16, 2009. Revised Manuscript Received December 2, 2009

The encapsulation of living plant cells into materials could offer the possibility to develop new green biochemical technologies. With the view to designing new functional materials, the physiological activity and cellular response of entrapped cells within different silica-based matrices have been assessed. A fine-tuning of the surface chemistry of the matrix has been achieved by the *in situ* copolymerization of an aqueous silica precursor and a biocompatible trifunctional silane bearing covalently bound neutral sugars. This method allows a facile control of chemical and physical interactions between the entrapped plant cells and the scaffold. The results show that the cell–matrix interaction has to be carefully controlled in order to avoid the mineralization of the cell wall which typically reduces the bioavailability of nutrients. Under appropriate conditions, the introduction of a trifunctional silane (ca. 10%) during the preparation of hybrid gels has shown to prolong the biological activity as well as the cellular viability of plant cells. The relations of cell behavior with some other key factors such as the porosity and the contraction of the matrix are also discussed.

Introduction

Nature is composed of a wide collection of complex systems working together in harmony such that interactions between many different components produce patterns or behaviors that are not obtained by combining the discrete actions of each individual part. In particular, living cells can be considered as a powerful tool in the development of novel “green” technologies owing to their sophisticated organization, complex information systems, and elaborate processes.

Recently, numerous studies have been devoted to harnessing the benefits of whole cells in the construction of new devices such as bioreactors,¹ biosensors,^{2,3} and artificial organs.^{4–6} These applications generally require the integration of cells into an abiotic material. Cells, isolated from their native environment, are very fragile. By a smart combination of these biospecies with advanced materials, they can be protected from harsh environments. In addition, this ingenious strategy allows the control of the three-dimensional cellular environment. Exploiting the capability of cells to sense their environment via signal transduction pathways, cells could thus be oriented to achieve specific tasks.

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(1) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Witholt, B. *Nature* **2001**, *409*, 258.
(2) Lei, Y.; Chen, W.; Mulchandani, A. *Anal. Chim. Acta* **2006**, *568*, 200.
(3) Dauner, S.; Barret, G.; Feliciano, J. S.; Shetty, R. S.; Shrestha, S.; Smith-Spencer, W. *Chem. Rev.* **2000**, *100*, 2705.
(4) Read, T.-A.; Sorensen, D. R.; Mahesparan, R.; Enger, P.; Timpl, R.; Olsen, B. R.; Hjelstuen, M. H. B.; Haraldseth, O.; Bjerkvig, R. *Nat. Biotechnol.* **2001**, *19*, 29.
(5) Orive, G.; Hernández, R. M.; Gascón, A. R.; Calafiore, R.; Chang, T. M. S.; De Vos, P.; Hortelano, G.; Hunkeler, D.; Lacik, I.; Shapiro, A. M. J.; Pedraz, J. L. *Nat. Med.* **2003**, *9*, 104.
(6) Carturana, G.; Dal Toso, R.; Boninsegna, S.; Dal Monte, R. *J. Mater. Chem.* **2004**, *14*, 2087.

Particularly, plant cell systems have a bright future in the development of sustainable technologies. Plant cells have some intrinsic properties that make them suitable to designing biosensors⁷ and eco-friendly bioreactors.^{8–10} They can also lend themselves to decontamination applications to clean up polluted soil, water, and air. For instance, their cellular metabolism can be exploited advantageously to decompose toxic chemicals.^{11,12} Moreover, the extraction of heavy metals^{13,14} by living cells can be combined with the biosynthesis of valuable nanomaterials.^{15,16}

The main challenge in designing such kinds of living functional materials is the preservation of cell viability inside the abiotic structures. Silica sol–gel chemistry has emerged as a biocompatible approach to entrap proteins,^{17–20} organelles,²¹ and whole

- (7) Nguyen-Ngoc, H.; Tran-Minh, C. *Anal. Chim. Acta* **2007**, *583*, 161.
(8) Hellwig, S.; Drossard, J.; Twyman, M. R.; Fischer, R. *Nat. Biotechnol.* **2004**, *22*, 1415.
(9) Roberts, S. C. *Nat. Chem. Biol.* **2007**, *3*, 387.
(10) Rao, S. R.; Ravishankar, G. A. *Biotechnol. Adv.* **2002**, *20*, 101.
(11) Yoon, J. M.; Olivier, D. J.; Shanks, J. V. *Chemosphere* **2007**, *68*, 1050.
(12) Vila, M.; Pascal-Lorber, S.; Rathahao, E.; Debrauwer, L.; Canlet, C.; Laurent, F. *Environ. Sci. Technol.* **2005**, *39*, 663.
(13) McGrath, S. P.; Lombi, E.; Gray, C. W.; Caille, N.; Dunham, S. J.; Zhao, F. J. *Environ. Pollut.* **2006**, *141*, 115.
(14) Meagher, R. B.; Heaton, A. C. P. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 502.
(15) Sharma, N. C.; Shahi, S. V.; Nath, S.; Parson, J. G.; Gardea-Torresdey, J. L.; Pal, T. *Environ. Sci. Technol.* **2007**, *41*, 5137.
(16) Gardea-Torresdey, J. L.; Parsons, J. G.; Gomer, E.; Peralta-Videa, J.; Troiani, H. E.; Santiago, P.; Yacaman, M. J. *Nano Lett.* **2002**, *2*, 397.
(17) Luo, T.-J. M.; Soong, R.; Lan, E.; Dunn, B.; Montemagno, C. *Nat. Mater.* **2005**, *4*, 220.
(18) Besanger, T. R.; Brennan, J. D. *J. Sol-Gel Sci. Technol.* **2006**, *40*, 209.
(19) Jin, W.; Brennan, J. D. *Anal. Chim. Acta* **2002**, *461*, 1.
(20) Gill, I.; Ballesteros, A. *TIBTECH* **2000**, *18*, 282.
(21) Meunier, C. F.; Van Cutsem, P.; Kwon, Y.-U.; Su, B.-L. *J. Mater. Chem.* **2009**, *19*, 1535.

cells^{22–26} within silica matrices. Pioneering work on prokaryotic cell encapsulation has shown that bacteria can remain alive for many weeks within a porous silica matrix.²⁷ However, the immobilization of plant and animal cells is more delicate and usually requires a two-step encapsulation procedure in order to avoid deleterious contact of cells with sol–gel byproduct. Cells are typically immobilized into alginate beads which are subsequently coated or entrapped by adding silica precursors.^{6,28} Nevertheless, alginate oligomers can be recognized by plant cells as stress messengers (i.e., an elicitor).^{29,30} Thus, for some applications this immobilization approach is not suitable since high cellular stress can result in unproductive cells.

The present work describes the recent efforts to maintain living eukaryotic plant cells within a biocompatible porous silica matrix. Carturan was the first to report the encapsulation of plant cells in silica, achieved by covering them with a porous silica layer. Even though the enzymatic activity of cells was preserved, no viability data are available.^{31,32} Here, we investigate the cellular response of cells which are confined within porous silica cages. Considering that the cells from mesophyll tissue grow by expansion (and not by division)³³ during the last stage of leaf development, it seems entirely possible that cells can be kept alive within a restricted space. Using a biocompatible synthesis pathway, a robust hybrid silica gel can directly be formed around the plant cells without releasing any byproduct during its construction. As a consequence, the real interactions between cells and matrices can be studied. The results obtained show that the fine-tuning of the host matrix properties based upon the information gathered on cell–matrix interactions is essential when designing living and active functional materials.

Experimental Section

Materials. Protease assay kit, Murashige and Skoog medium, kinetin, 1-naphthaleneacetic acid (NAA), sodium silicate solution, aminopropyltrimethoxysilane, and D-gluconolactone were purchased from Sigma-Aldrich. Amplex Red Hydrogen Peroxide assay kit was obtained from Molecular Probes Co. N-(3-Tri-methoxysilylpropyl)gluconamide (GLTMS) was synthesized from D-gluconolactone and aminopropyltrimethoxysilane as reported elsewhere.³⁴

Cell Culture. Suspension-cultured cells derived from leaves of *A. thaliana* strain L-MM1 ecotype *Landberg erecta* were cultivated in Murashige and Skoog medium (4.43 g L^{-1} , pH 5.7) supplemented with sucrose (30 g L^{-1}), $0.05 \mu\text{g mL}^{-1}$ of kinetin, and $0.5 \mu\text{g mL}^{-1}$ of NAA. Plant cell cultures were maintained under a 16/8 h light/dark photoperiod, at 25°C , on a rotary

shaker at 115 rpm. Cells were diluted 8-fold in fresh medium every 7 days.

Plant Cell Encapsulation. An aqueous sol–gel route was used to encapsulate fragile plant cells into pure (HG0) and organically modified silica matrices (HG10, HG15, and HG20). A sodium free silica sol stock solution was prepared from a sodium silicate solution (1.5 M) as previously described.³⁵ Hybrid silica gel (HG0) was synthesized by mixing 8 mL of silica stock with $800 \mu\text{L}$ of 10-fold concentrated biological medium. The pH value was then adjusted to 5.7 with 0.2 M KOH. Finally, the cell suspension was gently added in order to provide a final cell density of 25 g of fresh weight per liter of gel. Gelation occurred within a few minutes at 25°C .

Organically modified silica matrices were obtained using the same sodium free silica stock solution combined with a GLTMS solution. This organically modified silane stock solution was obtained by mixing 4.2 g of GLTMS into $30 \mu\text{L}$ of 1 M HCl and 10 mL of bidistilled water. After 16 h, the byproduct methanol was efficiently removed by rotary evaporation (up to 95%). To study the effect of this trifunctional silane on the cellular response, different amounts of GLTMS were added into the free sodium silica sol before the addition of cells. Hybrid gels HG10, HG15, and HG20 correspond to molar percentages of 10, 15, and 20%, respectively. All the hybrid gels were aged in the Murashige and Skoog medium (replaced every week) under the same conditions as for the cell suspensions.

Characterization Techniques. Matrix Properties. The morphological and textural properties of the silica-based matrices were acquired using aerogels obtained from the hybrid gels through a process of ethanol dehydration and subsequent critical point drying with liquid carbon dioxide. This procedure prevents shrinkage and preserves the structure yet removes the liquid component from the porous network. Scanning electron microscopy (SEM, JEOL JSM-7500F) observations were made on aerogels sputter-coated with gold. Nitrogen adsorption–desorption experiments were recorded at -196°C with a volumetric analyzer (Tristar 3000 from Micromeritics). Prior to analysis, the aerogels were degassed under vacuum (70 mTorr) for 24 h at 60°C .

The efficiency of the incorporation of the trifunctional silane (GLTMS) into the silica structure was evaluated by ^{29}Si MAS NMR spectroscopy. Hybrid gels (5 mL) were aged for 2 days and washed 3 times with bidistilled water (20 mL) for about 3 h under vigorous stirring. The gels were then dehydrated with ethanol and dried at 60°C . The obtained white powders were subsequently analyzed on a Bruker AVANCE 500 MHz NMR spectrometer.

The kinetics of gel shrinkage was also investigated by following the dimensional changes of bulk samples (aged at 25°C) using a micrometer. The v/v data, compared to the initial volume of hybrid gel, provide the magnitude of shrinkage. It has to be noticed that all measurements were carried out by the same experimenter in order to avoid operator variability.

Biological Activity and Viability of Cells. The physiological functions of entrapped cells were assessed by monitoring the oxygen consumption in a Clark cell vessel (Oxy-lab manufactured by Hansatech Instruments). Typically, 500 mg of monolithic gel pieces (3 mm^3) were added to 1.0 mL of Murashige and Skoog medium (MS medium). The activity of the plant cell suspension was measured just before immobilization and was taken as the reference (100%). Cell viability was determined by gently crushing hybrid gels on the surface of a MS agar medium. The ability of encapsulated cells to grow and form so-called callus tissues (unorganized plant tissues) was used as an indicator of cell viability.

Investigation of Biochemical Interactions. Transmission electron microscopy (TEM, Philips Tecnai 10) micrographs of hybrid gels were taken with an accelerating voltage of 80 kV. Prior to analysis, hybrid gels were cut into small cubes (2 mm^3) and fixed with 2.5% glutaraldehyde in a sodium cacodylate buffer (0.1 M, pH 7.4) for 2.5 h. The samples were rinsed in 0.2 M cacodylate

(35) Meunier, C. F.; Van Cutsem, P.; Kwon, Y.-U.; Su, B.-L. *J. Mater. Chem.* **2009**, *19*, 4131.

buffer and postfixed in a 1% OsO₄ buffered solution overnight. The gels were washed with the same buffer and then dehydrated with increasing concentrations of ethanol. The dehydrated materials were set in propylene oxide before being embedded into an epoxy resin LX112. Ultrathin sections were made with an ultramicrotome and were contrasted with lead citrate and uranyl acetate. All experiments were carried out in triplicate.

Hydrogen peroxide produced by the entrapped plant cells was also measured. Aliquots of gel supernatants (200 μ L) were removed regularly over a period of 24 days. The H₂O₂ assay was performed using the Amplex Red Hydrogen Peroxide Assay Kit according to the supplier's instructions. The absorbance of samples and standards was measured at 570 nm with a microplate reader (Bio-Rad Instruments).

Protease activity in samples was measured using a Protease Fluorescent Detection Kit. Briefly, hybrid gels (or free cells) were ground to a fine powder in liquid nitrogen. Proteins were then extracted with 0.1 M Tris-HCl (pH 7.5) at 4 °C.³⁶ The samples were centrifuged at 20000g for 5 min. The protein concentration of the recovered supernatants was measured using the Bradford method. The protease assays were carried out in 200 μ L microcentrifuge tubes containing the FITC-casein substrate, a phosphate buffer (pH 7.6), and the cell extract. The tubes were gently mixed and incubated at 37 °C for 24 h. The reaction was stopped by the addition of 150 μ L of 0.6 N trichloroacetic acid, with a subsequent incubation at 37 °C for 30 min. Precipitated proteins were pelleted by centrifugation, 15000g for 10 min. 2 μ L of the supernatant was added to 200 μ L of 0.5 M Tris-HCl (pH 8.5). The samples were mixed, and the fluorescence intensity was recorded at 538 nm with an excitation at 485 nm. The protease activity was determined as the change in fluorescence intensity per milligram of protein. All experiments were carried out in triplicate.

Results and Discussion

Matrix Properties: Chemical Environment and Textural Features

Arabidopsis thaliana plant cells were encapsulated within different silica-based matrices. Since biological entities (organelles, whole cells) are very sensitive to modifications of their physiological environment, a biocompatible synthesis pathway was previously designed to allow the formation of a robust silica gel around fragile thylakoids, without releasing any byproduct during the synthesis.³⁵ This method was adapted and used to encapsulate whole plant cells. A trifunctional silane bearing covalently bound neutral sugars (GLTMS) was added to the silica sol in order to study the biochemical properties of the resulting organo-modified hybrid material. The incorporation of GLTMS can tailor the chemical and physical properties of the porous silica cages that formed around the cells. ²⁹Si MAS NMR spectra of thoroughly washed samples give information about the effective modifications of the silica walls. All the spectra of the dry gels possess peaks at -111 (Q₄), -100 (Q₃), and -90 ppm (Q₂) assigned to SiO₄, SiO₃(OH), and SiO₂(OH)₂ units, respectively (Figure 1). These well-known resonance signals of the silica scaffold are accompanied by two additional signals in the case of hybrid gels HG10, HG15, and HG20 which contained different amounts of silyl-modified gluconamide (10, 15, and 20 mol %). The signals at -66 and -55 ppm are characteristic of the formation of Si—O—Si linkages between the trifunctional silane and the silica scaffold since T₃ and T₂ correspond to C—SiO₃ and C—SiO₂(OH), respectively. Brook and co-workers reported that trifunctional silanes bearing polyol moieties (GLTMS) present a lower reactivity compared to tetrafunctional silanes.³⁷ As a

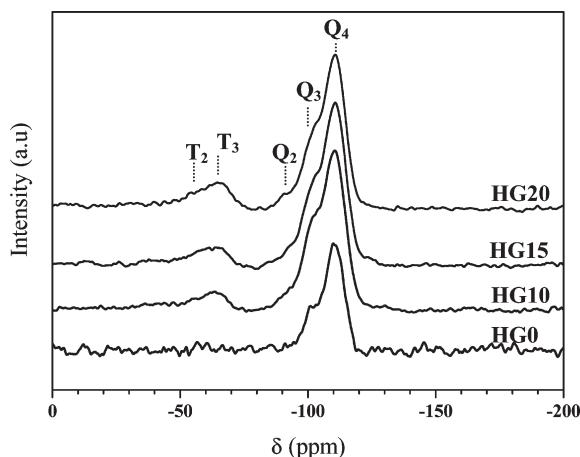


Figure 1. Solid-state ²⁹Si MAS NMR spectra of hybrid gels.

Table 1. Relative Amounts of T₂, T₃, Q₂, Q₃, and Q₄ Units Observed by ²⁹Si MAS NMR of Hybrid Gels^a

gels	T ₂ (%)	T ₃ (%)	Q ₂ (%)	Q ₃ (%)	Q ₄ (%)
HG0				8	22
HG10	3	6	8	20	63
HG15	3	9	8	18	62
HG20	4	11	5	25	55

^a The percentages of T_n and Q_n are given with a 3% error range.

consequence, the addition of GLTMS to the starting silica sol preferentially affects the surface chemistry of the resulting hybrid gel. Table 1 shows that the actual amount of T₃ and T₂ groups, covalently linked to the silica material, increases with the amount of GLTMS added to the silica sol. By modifying the GLTMS/SiO₂ ratio, the quantity of organic groups at the surface of the silica scaffold can be tailored. It is known that the presence of such organic moieties decreases the surface acidity of silica. Moreover, the introduction of sugar alcohols increases the water retention of the matrices by modifying their hydroscopic properties. By controlling the amount of organic groups on the surface of the matrix pores, it is thus possible to control the interactions between the cells and the material.

In order to determine and understand the effect the pore structure of hybrid gels has on the viability of plant cells, SEM and nitrogen sorption experiments were carried out on aerogels obtained by treating hybrid gels under critical conditions of CO₂. This method can keep the silica scaffold intact after removal of the solvent. Figure 2 shows that the texture of all the gels is formed by the aggregation of silica particles. Hybrid gels HG0, HG10, and HG15 present macropores with a large pore size distribution centered at around 150 nm (Figure 2). In the case of hybrid gel HG20, the micrograph highlights a different structure. Whereas hybrid gels HG0, HG10, and HG15 exhibit a continuous tridimensional silica network, hybrid gel HG20 is formed by the packing of individual submicrometric particles (ca. 150 nm). The voids left between these large particles create the macroporous structure. These observations reveal that different morphologies can be obtained depending on the initial GLTMS/SiO₂ ratio. The addition of GLTMS seems to alter the porous properties of the matrix. For example, a molar ratio higher than 15% GLTMS (HG20) causes the collapse of the well-defined macropores observed for the pure silica gel (HG0). At low concentration, the porous structure is well preserved (HG10). This phenomenon could be explained by an increase in the solubility of the sugar-coated colloidal particles, which altered the phase

(36) Swidzinski, J. A.; Leaver, C. J.; Sweetlove, L. J. *Phytochemistry* 2004, 65, 1829.

(37) Chen, Y.; Zhang, Z.; Sui, X.; Brennan, J. D.; Brook, M. A. *J. Mater. Chem.* 2005, 15, 3132.

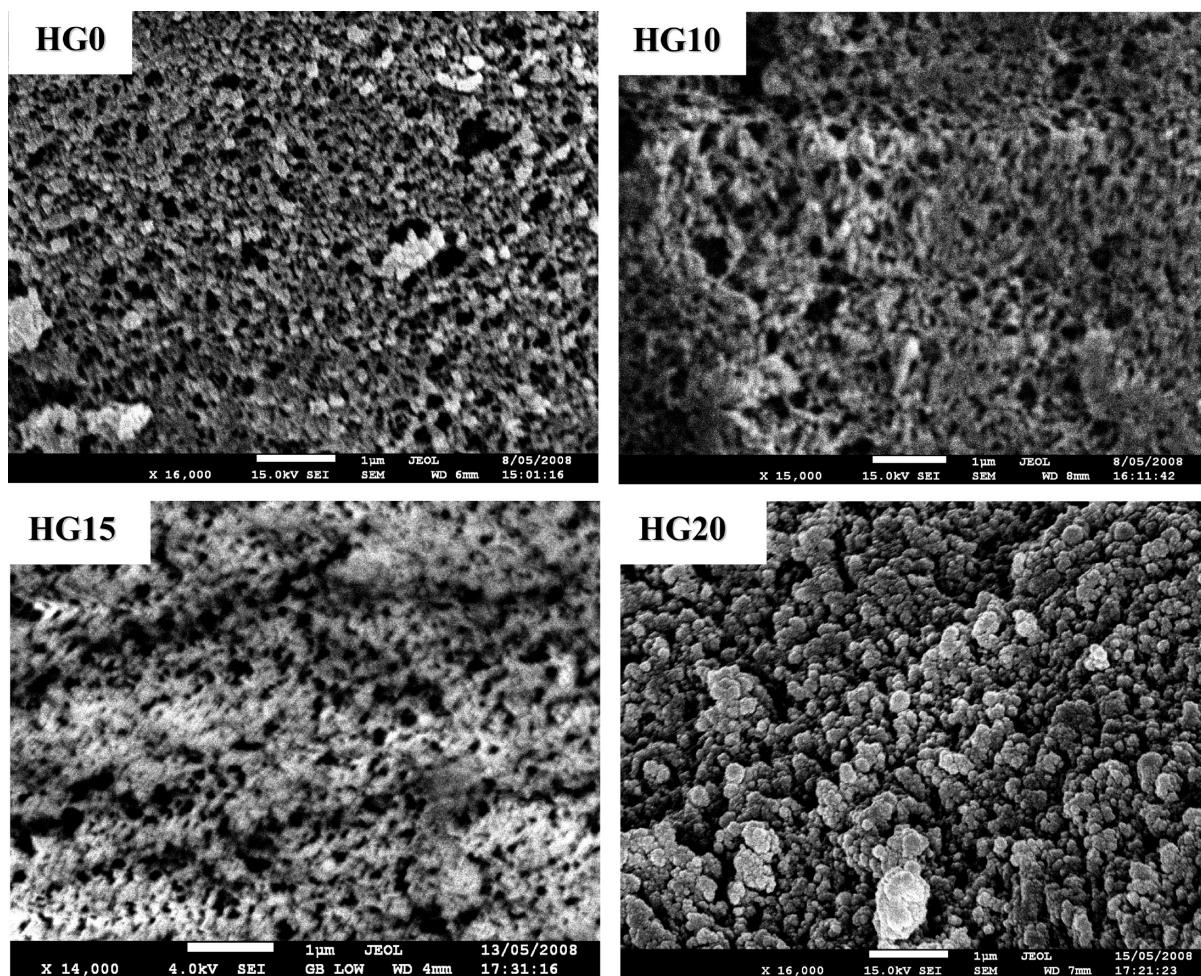


Figure 2. Scanning electron microscopy images from hybrid gels HG0, HG10, HG15, and HG20.

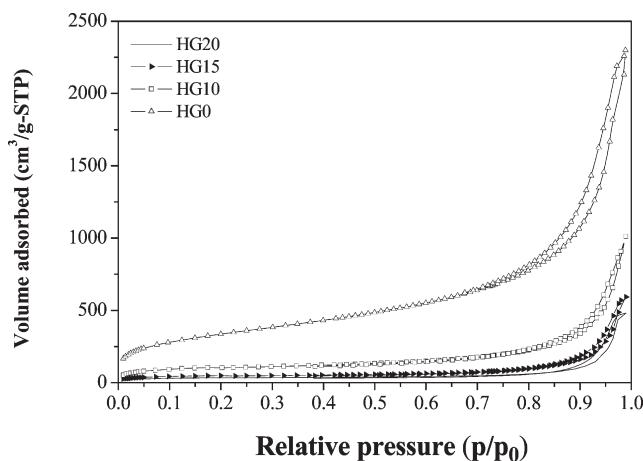


Figure 3. Nitrogen adsorption–desorption isotherms of aerogels derived from hybrid gels.

separation process occurring during sol–gel phase transition, especially for high content of GLTMS. The adsorption–desorption data of all materials revealed type II isotherms which confirmed the macroporous nature of the hybrid gels (Figure 3). Table 2 shows that the addition of trifunctional silanes reduces the surface area, which could be explained by a possible loss of micropores. Indeed, small pores (< 50 nm) are the main factor contributing to high surface areas, generally observed in micro- and

Table 2. Plant Cell Viability and Textural Properties of Porous Hybrid Silica Gels: Specific Surface Area (S_{BET}), Porous Volume (V_p), and Average Pore Size Diameter (D_p)

gels	S_{BET} ($\text{m}^2 \text{ g}^{-1}$)	D_p (nm)	V_p ($\text{cm}^3 \text{ g}^{-1}$)	viability (days)
HG0	990		3.4	3
HG10	354		1.6	5
		> 50		
HG15	159		0.92	< 1
HG20	113		0.74	< 1

mesoporous materials. The *in situ* surface functionalization of the silica matrix with hygroscopic sugars can thus act as a barrier to micropores. All these physicochemical modifications affect the rheological properties of the matrix. The kinetics of gel contraction depends on the GLTMS content as shown in Figure 4. During the first few hours of aging, the rate of shrinkage is very high for pure silica gels (HG0). However, after 1 day of aging, the speed of contraction slows down. In the case of organic–inorganic hybrid gels, the overall shrinkage rate is lower. The initial contraction rate as well as the continued degree of shrinkage (from day 1 to day 25) declines considerably with an increase in the GLTMS/SiO₂ molar ratio. In fact, for the pure silica gel (HG0), the pore-surface silanol groups suffer the most from the further condensation, forming new Si–O–Si bonds with subsequent aging. This phenomenon induces the observed shrinkage of materials combined with the expulsion of the liquid medium from the pores of the gels. By forming a hygroscopic sugar layer

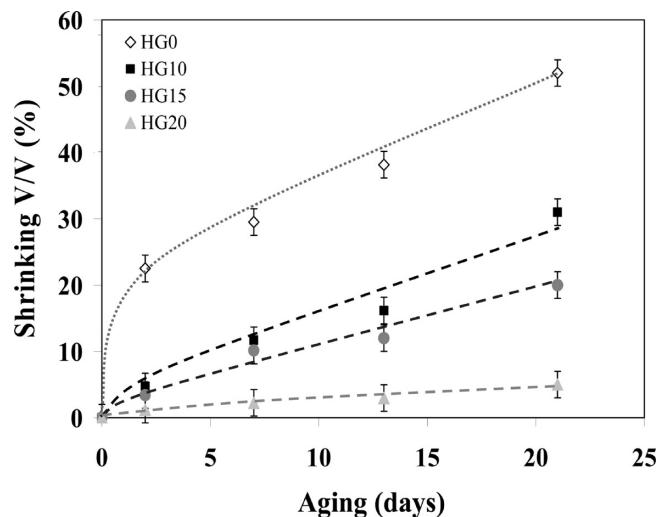


Figure 4. Kinetics of gel shrinkage of hybrid gels at 25 °C.

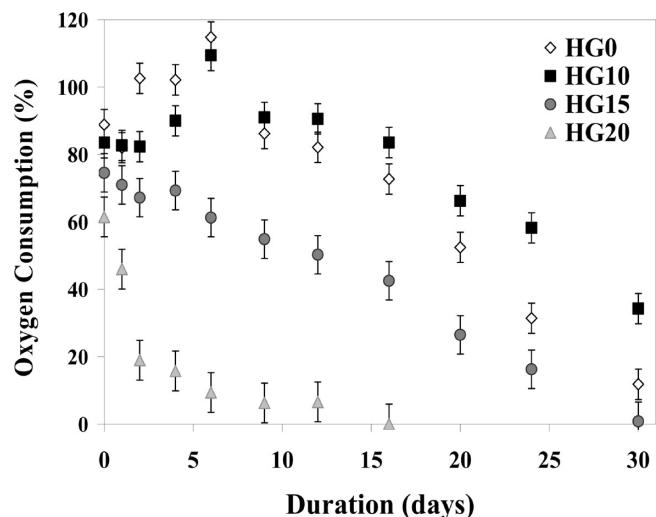


Figure 5. Comparison of oxygen consumption by plant cells (dark respiration) entrapped within either pure silica gel (HG0) or an organic–inorganic hybrid silica gel (HG10, HG15, and HG20). 100% corresponds to the respiratory activity of free cells.

on the silica wall, the subsequent silanol condensation is limited. The ensuing condensation process, known as syneresis, usually provokes deleterious physical constraints on biological species and thus has to be minimized.²¹

Biological Activity and Viability of Immobilized Plant Cells. The physiological functions of entrapped plant cells were determined by measuring the dark respiration activity. The level of oxygen consumption reflects directly on the number of active cells. Figure 5 shows the variation in oxygen consumption of plant cells entrapped within hybrid gels HG0, HG10, HG15, and HG20. During the early stages of the immobilization, the biological activity slightly decreases due to the exposure of plant cells to abiotic environments. The confinement created by the silica cages modifies the cellular metabolism. Nonetheless, after a few days, plant cells seem to adapt to their new surroundings as in the case of HG0 and HG10. The plant cells entrapped in HG0 and especially in HG10 exhibit a high biological activity for over 1 month postencapsulation. However, contrary to this, the physiological functions of cells entrapped within hybrid gels HG15 and HG20 rapidly deteriorate. After 1 month, the number of active cells decreases in the order of HG10 > HG0 > HG15 > HG20.

Even though anisotropic shrinking could disrupt entrapped cells, the biological activity trends, observed in Figure 5, cannot simply be explained by the syneresis phenomenon since HG20 gives the lowest contraction of the gel followed by HG15 and HG0. The bioactivity trend is also different from the variations in surface acidity and the hygroscopic properties since hybrid gel HG20 is characterized by the lowest surface acidity and the highest hygroscopic character due to the highest amount of polyol moieties at surface. The porous characteristics of hybrid gels appear to be a more relevant parameter. The greater and prolonged biological activities of hybrid gels (HG0 and HG10) correspond to the scaffolds with a more defined macroporous structure (Figure 2). Doubtlessly, a good diffusion of nutrients and metabolites through the gels is essential to preserve the physiological function of the cells.

At this stage, it is important to highlight that the data (oxygen consumption) only provide information about the biological activity of plant cells. The cell viability, defined here as their ability to grow and divide, can be lost while certain active metabolic pathways (i.e., respiration in the dark) continue to function due to confined space. For this reason, the viability of immobilized plant cells was assessed by measuring their ability to grow on a solid medium culture (MS medium supplemented with agar) after gently breaking down the hybrid gels. Everyday, a part of each hybrid gel is gently ground, resuspended in MS medium, and finally incubated over the course of 7 days in Petri dishes. This common method highlights if cells are able to grow and divide to form green callus tissue, viz., alive cells. Table 2 shows that cells entrapped into gels HG15 and HG20 rapidly die after just a few hours following the encapsulation step. For gels HG0 and HG10, cells remain alive for 3 and 5 days, respectively. These results show that immobilization of plant cells inside a macroporous matrix can preserve some metabolic activity for over 1 month (HG10) while their ability to grow is rapidly impaired.

Cell–Matrix Interactions and Cellular Response. Further investigations were carried out to understand the behavior of plant cells entrapped within restricted environments. Transmission electron microscopy was employed to investigate the interactions between the cells and the matrices after 2 days of aging. The micrographs in Figure 6 show that plant cells are immobilized within a confined space. After the encapsulation step, the structural organization of cells is well preserved. The organelles as well as the plasma membranes remain intact within the gels. Particularly, the large central vacuole of entrapped cells is clearly preserved (Figure 6). Generally, hyperosmotic stress can typically provoke the retreat of the cytoplasm from the cell wall via a large reduction in the vacuole volume. This feature is not observed in any hybrid gels examined. This observation suggests that plant cells are confined within a biocompatible environment.

TEM images also highlight that the formation of a sugar layer covalently linked to the silica scaffold modifies the cell–matrix interactions. The TEM images in Figure 7 show that all silica-based gels are formed by the aggregation of nanoparticles which interact with the cell surface and can even diffuse through the cell wall (see arrow in HG0 of Figure 7). The introduction of GLTMS during the formation of hybrid gels strongly affects the cell surface. The exterior part of the wall (distal layer) of the entrapped cells appears to increase in darkness with increasing GLTMS incorporation, i.e., HG10 < HG15 < HG20 (Figure 7). This darkening indicates the presence of organically modified silica oligomers within the distal layer of the cell wall. In fact, during the overall encapsulation process, plant cells are directly in contact with sol–gel precursors. The cell wall constitutes the boundary layer between the cells and their surrounding. The properties of this porous semirigid structure, composed of

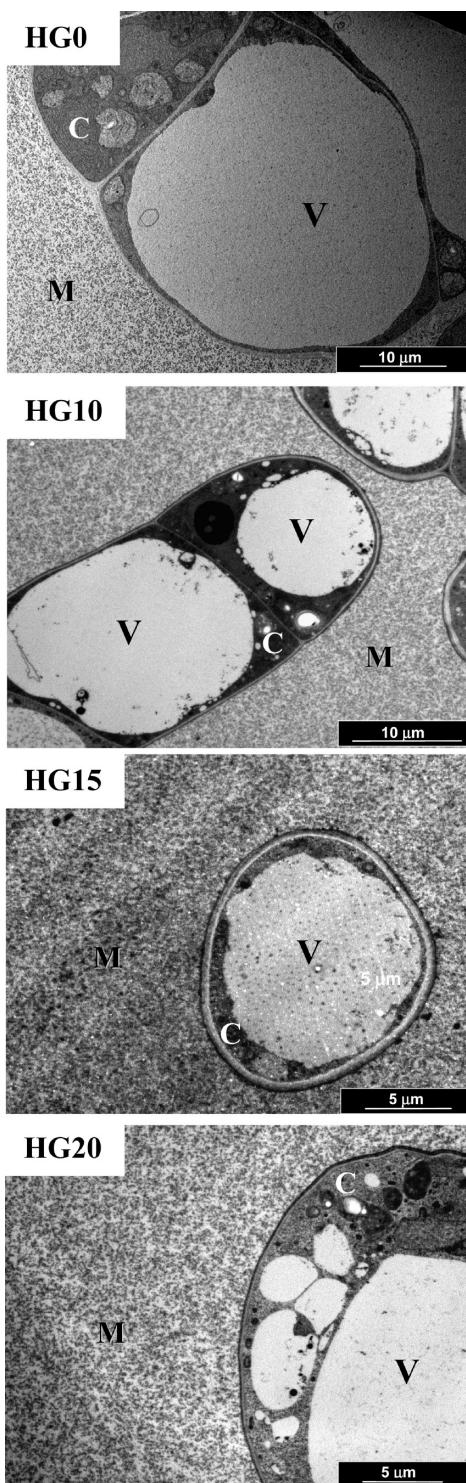


Figure 6. Transmission electron microscopy images of immobilized plant cells within pure silica gel (HG0) and organically modified silica gels (HG10, HG15, HG20) after 2 days of aging. V, C, and M stand for vacuole, cytoplasm, and matrix, respectively.

polysaccharides, glycoproteins, phenolic compounds, and enzymes, could be affected by immobilization. Even though the silica and the cell wall are globally negatively charged at the physiological pH, colloidal silica particles have strong interactions with the cell walls. The addition of increasing amounts of GLTMS into the sol modifies the electric charge on the polymeric silica chains. Consequently, hybrid organic–inorganic oligomers diffuse more easily through the cell wall since the electrostatic

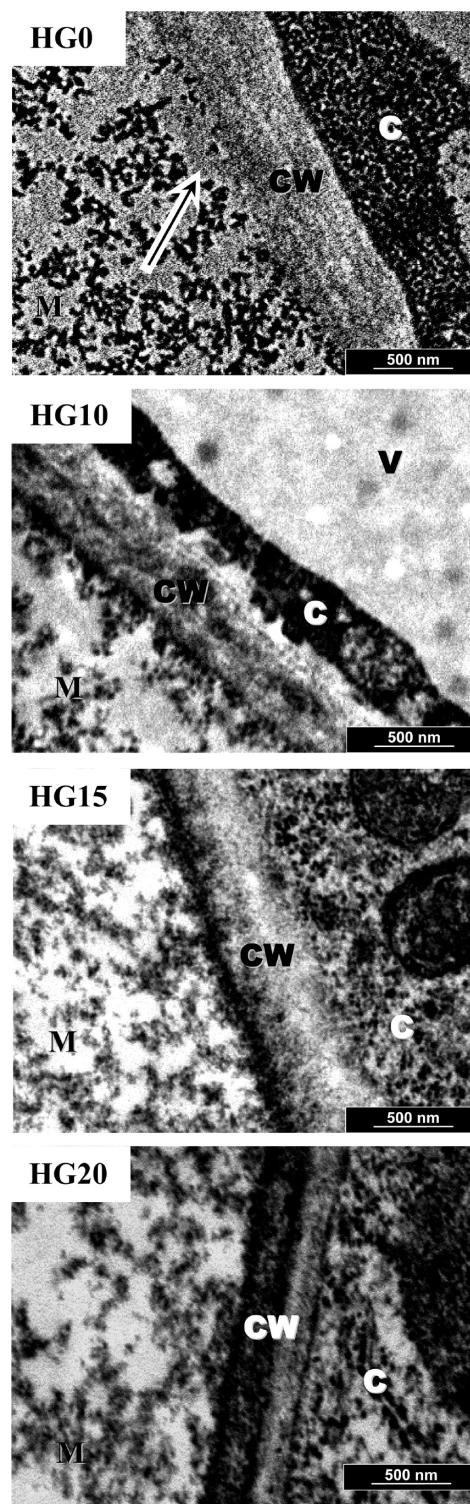


Figure 7. Cell wall micrographs (TEM) of encapsulated plant cells within pure silica gel (HG0) and organically modified silica gels (HG10, HG15, HG20) after 2 days of aging. V, C, CW, and M stand for vacuole, cytoplasm, cell wall, and matrix, respectively.

repulsions are weaker. The darkening of the cell walls (Figure 7) clearly shows that the modification of parietal properties increases with the amount of GLTMS. However, the inner part of cell wall in contact with the plasma membrane seems not to be modified. These results provide evidence that modified silica oligomers diffuse and polymerize from the bulk solution surrounding the cells toward the primary cell wall. During the condensation step,

there could be reactions between the reactive species of the sol and the functional groups on the cell wall. In general, orthosilicic acid has a strong affinity to the organic polyhydroxyl compounds, which participate in the biosynthesis of the cell wall.³⁸ Moreover, some parietal proteins containing a proline rich sequence were able to catalyze the polymerization of silica species. Even though, the deposition of silica through the cell wall contributes to the reinforcement of its mechanical properties,³⁹ this process can affect the plant cell physiology. In addition to their role in maintaining the shape and size of cells, plant cell walls play an important part in the transport, absorption, and secretion of different chemicals. The precipitation of organo-modified silica inside the cell wall certainly reduces its permeability, especially for high GLTMS/SiO₂ molar ratios. The transport of nutrients and metabolites through this boundary structure could thus be altered, inducing a rapid death of entrapped plant cells. This hypothesis is supported by the rapid decrease of oxygen consumption observed for HG20 hybrid gels (Figure 5). The TEM picture clearly showed that the corresponding parietal properties of entrapped plant cells are irreversibly changed (darkening of cell walls shown in Figure 7). For a GLTMS/SiO₂ molar ratio lower than 10%, the oxygen consumption by plant cells stays stable for several weeks (HG0 and HG10, Figure 5), suggesting that the rate of diffusion at the cell–matrix interface is sufficient to maintain the metabolic activity of the plant cells. The control of the interactions between the plant cells and their abiotic surroundings combined with a matrix of well-defined porosity are indispensable in the preservation of short-term cellular viability and activity.

Long-term preservation of active cells requires more than an improvement to the nutrient diffusion process; it demands the understanding and control of more complex cellular responses. The space limitation inhibiting cell division together with the modification of parietal properties contribute to inducing abiotic cellular stress. In an effort to explain the sharp contrast between the viability (Table 2) and the biological activity (Figure 5) of entrapped plant cells, a kind of cell death pathway was investigated. Plant cells can die in different ways, as for instance by necrosis or by programmed cell death (PCD). Necrosis is typically an uncontrolled type of cell death which happens when cells are very far from their ideal physiological conditions. Placed under these conditions, cells would lose their ability to osmoregulate leading to the release of their cellular content into the aqueous phase of the hybrid gels. Conversely, PCD is an orderly process of cellular suicide that requires the active gene expression which brings about the controlled disassembly of cells. One stress marker that triggers plant development, stress adaptation, and cell death is hydrogen peroxide. H₂O₂ is known as a key signaling molecule generated by various environmental stimuli in response to cellular stresses.^{40,41} Figure 8 shows the total concentrations of accumulated hydrogen peroxide in the cell culture medium (control) and in the gel supernatants. In all cases, the hybrid gels do not significantly accumulate H₂O₂ with time. Compared to free cells, the hydrogen peroxide concentration is lower. The observed cell death within gels would therefore appear not to be triggered by the production of H₂O₂. Since protein degradation is a well-recognized feature of cells that undergo PCD,⁴² the increase of proteolytic activity can be used to screen the cellular response and determine the kind of death undergone by entrapped cells.

(38) Perry, C. C.; Keeling-Tucker, T. *J. Biol. Inorg. Chem.* **2000**, *5*, 537.

(39) Kauss, H.; Seehaus, K.; Franke, R.; Gilbert, S.; Dietrich, R. A.; Kröger, N. *Plant J.* **2003**, *33*, 87.

(40) Gechev, T. S.; Hille, J. *J. Cell Biol.* **2005**, *168*, 17.

(41) Apel, K.; Hirt, H. *Annu. Rev. Plant Biol.* **2004**, *55*, 373.

(42) Beers, E. P.; Woffenden, B. J.; Zhao, C. *Plant Mol. Biol.* **2000**, *44*, 399.

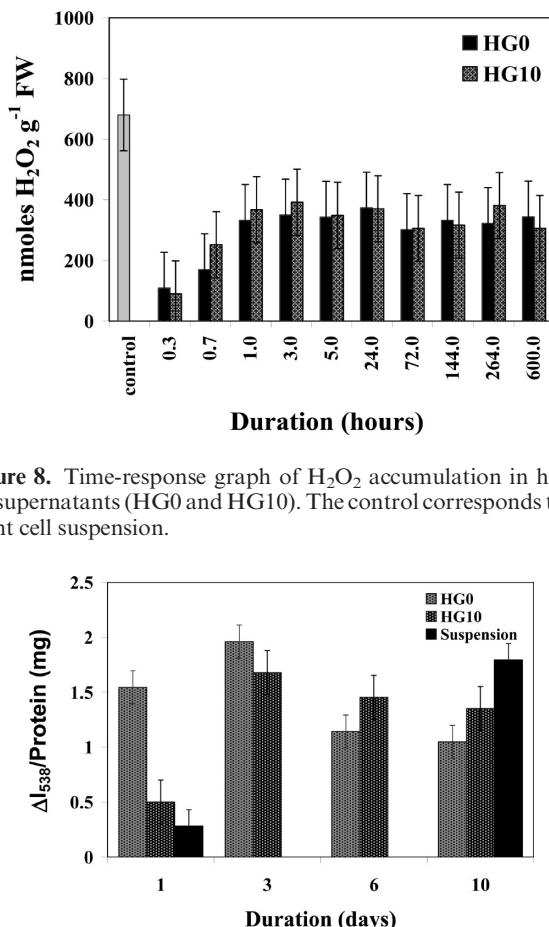


Figure 8. Time-response graph of H₂O₂ accumulation in hybrid gel supernatants (HG0 and HG10). The control corresponds to the plant cell suspension.

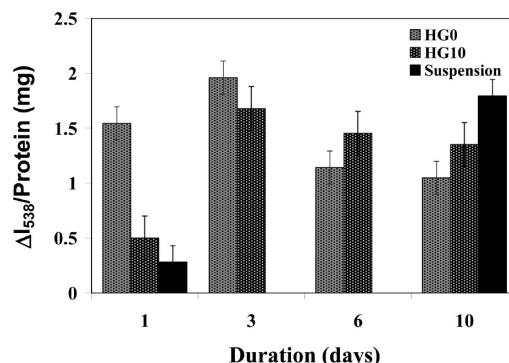


Figure 9. Change in proteolytic activity of entrapped cells (HG0 and HG10) and free *Arabidopsis* cell suspension cultures.

In fact, PCD naturally occurs in plant cell suspensions by allowing the batch culture to senesce.⁴³ If the biological medium is not replaced after 7 days of cultivation, the nutrient shortage induces the senescence of cells. Compared to day 1 of cultivation, the proteolytic activity of free plant cells after 10 days of cultivation significantly increases as shown in Figure 9. For hybrid gels, the biological medium is replaced every 6 days in order to avoid nutrient starvation. Nevertheless, Figure 9 highlights that protease activity rapidly increases after 1 and 3 days for gels HG0 and HG10, respectively. Thus, plant cells have not undergone necrosis after a few days but have the ability to respond to their new environment by an increase of protease activity that seems to lead to a PCD. Compared to the life span of cells (3 and 5 days for HG0 and HG10, respectively), a high content of proteolytic enzymes is not systematically accompanied by the death of cells. If the cells are removed from their confined environments (gentle breakdown of the matrix) within the 2 days following the sharp increase of protease activity, they are still able to grow. One possible explanation could be a decrease in nutrient availability for entrapped cells owing to mass transfer limitations. However, the diffusion processes between hybrid gels HG0 and HG10 seem to be similar (Figure 2), and thus the viability trend cannot be easily explained. The major difference between these two hybrid gels is the surface chemistry of the silica scaffold. As already mentioned, silica is negatively charged under ideal physiological conditions. The presence of these fixed charges around the cells creates a Donnan potential. The membrane potential that

(43) Swidzinski, J. A.; Sweetlove, L. J.; Leaver, C. J. *Plant J.* **2002**, *30*, 431.

determines the equilibrium concentrations of ions in the cytosol could be affected in a detrimental way by the charged silica matrix. The addition of trifunctional silanes bearing covalently bound neutral sugars decreases the surface charge of the matrix (HG10). This modification to the silica wall seems to be beneficial in delaying the detrimental increase in proteolytic enzymes, observed after 24 h in the case of pure silica gel HG0. Moreover, porous hybrid organic–inorganic gels have the desirable feature to undergo less shrinkage compared to pure silica gel (Figure 4). Since hybrid gel HG10 shrinks more slowly than pure silica gel (HG0), the critical contraction value, which affects the cellular integrity, is reached faster for the gel HG0. The bioactivity trends of hybrid gels HG0 and HG10 (Figure 5) could thus be explained by these different cell–matrix interactions. Nevertheless, the molar ratio of GLTMS/SiO₂ must not be too high in order to avoid the deleterious precipitation of organo-modified silica observed inside the cell wall (Figure 7). Therefore, cellular viability is better preserved within organo-modified silica gel with a molar percentage of 10% (HG10).

Conclusions

Arabidopsis thaliana plant cells were encapsulated into different silica-based matrices. The preservation of cell viability within artificial structures is a real challenge. This work demonstrates that the synthesis of hybrid gels containing enzymatically active plant cells requires not only a biocompatible synthesis pathway of the host support but also one that results in a matrix that minimizes any adverse interactions between the cell wall and the surface functionalities found on the matrix. The results have shown that the integration of physical, chemical, and physiological parameters is crucial to preserve plant cell activity and viability.

The biological activity of entrapped cells essentially depends on the diffusion properties of the matrix. The introduction of controlled amounts of trifunctional silane (GLTMS) is especially crucial during the preparation of the hybrid silica sol. If the GLTMS/SiO₂ molar ratios used were too high, the porosity and the cell wall properties were affected in a detrimental way. In fact, the mineralization of the cell wall has been observed and could

thus reduce the bioavailability of nutrients. For lower molar percentages of GLTMS ($\leq 10\%$), the activity and viability of entrapped plant cells can be well preserved. In fact, the long-term preservation of cellular viability remains an obstacle since additional parameters have to be taken into account. Even though plant cells were not able to divide within the host matrix, they remain alive for several days. No oxidative stress has been detected. However, the overexpression of protease activity has been highlighted. These results suggest that cells respond to their new environment by an orderly process of death (PCD). This phenomenon seems to be linked with the Donnan potential created by the fixed charges on the silica matrix and also shrinkage of the matrix. A careful control of the surface chemistry of the silica wall proved to be crucial to mitigate the PCD process observed in pure silica gel (HG0). Particularly, the *in situ* functionalization of the silica scaffold with 10% GLTMS (HG10) really improves the biological activity and viability of the entrapped plant cells. Based on these results, new encapsulation approaches can be targeted that prolong the life and bioactivity of the cells even further. Such hybrid gels could play an important role in the development of sustainable technology. Particularly, it is believed that the encapsulation of photosynthetic plant cells could be used to target a bioreactor.^{44,45}

Acknowledgment. C. Meunier and A. Léonard thank the Belgian FNRS (Fonds National de la Recherche Scientifique) for their Research Fellow position and postdoctoral position, respectively. This work was realized in the frame of Inanomat, a Belgian federal government (Belspo) PAI-IUAP (6/17) project and an Interreg IV (France–Wallonie) “Redugaz” project financially supported by the European Community and Wallonia region. B. L. Su acknowledges the Chinese Central Government for an “Expert of the State” position in the program of “Thousands Talents” and the Chinese Ministry of Education for a “Changjiang Scholar” position at the Wuhan University of Technology.

(44) Meunier, C. F.; Dandoy, Ph.; Su, B.-L. *J. Colloid Interface Sci.* **2010**, *342*, 211.

(45) Meunier, C. F.; Rooke, J. C.; Léonard, A.; Van Cutsem, P.; Su, B.-L. *J. Mater. Chem.* **2010**, *20*, 929.