Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supporting Information

Correlating biological methods to assess *Escherichia coli* bacteria viability in silica gels

M. Blondeau, R. Brayner, F. Guyot and T. Coradin

Bacteria culture

The bacteria *Escherichia coli* (CIP-54125) from the Collection of the Institut Pasteur (Paris, France) were grown in Luria Bertani medium up to stationary phase at 37 °C under agitation. The bacteria were then inoculated (0.6 ml) in 100 ml of minimum phosphate buffer (pH 7) supplemented with 20 mM of glucose at 37 °C with stirring (150 rpm). After 4 hours of incubation, the culture in the exponential phase was then centrifuged at 4400 rpm for 15 min at 10 °C. The pellet was washed three times with phosphate buffer and suspended in phosphate buffer containing 10% of glycerol to a 10^9 CFU/ml cell density.

Encapsulation of bacteria in silica gel

Silica gels were prepared from a precursor of sodium silicate solution (27 wt% SiO_2, 10 wt% NaOH) from Sigma Aldrich. A mixture of sodium silicate (0.8M, 1ml) and glycerol (10 wt%, 1 ml) was neutralized with hydrochloric acid (4 M, 0.155 ml) under stirring. After 5 seconds, 1 ml of bacterial suspension (10^9 CFU/ml) was added and the mixture was stirred for 5 minutes. The gels were formed after 5-6 min at room temperature. The gels were then kept at 20 °C for up to 15 days. For viability tests (plate count, metabolic activity and ATP production), the bacteria gels were dispersed for 1 hour in 5 ml of phosphate buffer under magnetic stirring.

Plate counting

The number of viable culturable cells was determined after resuspension of the gel in 5 ml of phosphate buffer. A series of 10-fold dilutions were realized in phosphate buffer and 0.1 ml of the diluted suspension was added to the plate containing LB agar medium. Petri dishes were then placed at 37 °C overnight. The number of colonies (CFU) was determined by counting and correlated to initial CFU/ml.

Alamar Blue test[1]

Metabolic activity was determined using the Alamar Blue (resazurin) test. The amount of reduced dye can be directly correlated to the number of cells in suspension. 2 ml of the suspended gel were

mixed with 2 ml of phosphate buffer without nutrient. The mixture was removed and successively diluted 2-fold in phosphate buffer. For each dilution, 0.2 ml of resazurin (0.01 wt%) was added and the suspensions were then stirred and incubated at 30 °C under mild stirring (130 rpm) for 2 hours in the dark. In parallel, 2 ml of phosphate buffer were incubated with 0.2 ml of resazurin to have a control. After 2 hour of incubation, the suspensions were filtered and the optical densities of each sample were determined at two wavelengths : 570 nm to quantify the reduced Alamar Blue and 600 nm for the Alamar Blue. The percentage of reduced Alamar Blue can be determined using the following calculation:

$$\text{Reduction (\%)} = \frac{A_{570} \times (\varepsilon_{OX})_{600} - A_{600} \times (\varepsilon_{OX})_{570}}{A'_{600} \times (\varepsilon_{RED})_{570} - A'_{570} \times (\varepsilon_{RED})_{600}} \times 100$$

ε_{OX} = molar extinction coefficient of Alamar Blue

ε_{RED} = molar extinction coefficient of reduced Alamar Blue

A = absorbance of sample

A' = absorbance of negative control. The negative control should contain media and Alamar Blue without cells.

A standard curve was obtained based on the percentage of reduced Alamar Blue as a function of the number of bacteria determined by plate counting. The equation of the standard curve was used to determine the cell number (up to 10^8 cell/ml).

Extraction and quantification of ATP[2,3]

- **ATP extraction**

The resuspended gel was diluted 10-fold in phosphate buffer and 0.1 ml was added in 0.9 ml of extraction buffer (0.1 M Tris-HCl, 2mM EDTA - pH 7.8) previously heated 3 min at 100 °C. The suspensions contained in the extraction buffer were vortexed and placed at 100 °C for 3 minutes to release ATP of the cells and to inactivate enzymes such as bacterial ATPases. In this step, the samples can be stored in ice or placed at -20 °C. The extraction was then completed by centrifugation of 0.9 ml of sample with 0.5 ml of 0.4 mm glass beads at 5000 x g for 15 min at 4 °C. The supernatant was collected and stored at -20 °C before the quantification of ATP.

- **ATP quantification**

After extraction, the samples were incubated on ice until the measures.
The reagent solution containing luciferin and luciferase was diluted 20-fold in the specific buffer (FLAA 1KT-kit from Sigma Aldrich). 0.1 ml of each sample and blank (extraction buffer) were placed in a microplate kept in ice. 0.1 ml of reagent were then added with the samples and blanks. The plate was placed in an EnVision plate reader from PerkinElmer. The samples were stirred at 900 rpm for 3

min at room temperature (20-25 °C) then the quantity of light released (RLU - Relative Luminescent Units) was measured. A standard curve is performed in parallel using an ATP standard solution of known concentration.

SEM observations

The gel samples were fixed in glutaraldehyde (2.5%) for 1 hour and then with osmium (2%) for 1 hour. A series of dehydration baths were made and then the samples were impregnated in resin. After polymerization and hardening of the resin (3 days), the blocks containing the samples were cut using an ultramicrotome. SEM observations were realized on sections of 0.1 microns placed on carboned copper grids in secondary electron detection mode, using a Zeiss Ultra SEM-FEG working at 2 kV.

1. P. Goegan, G. Johnsson and R. Vincent, *Toxic. In Vitro*, 1995, **9**, 257
2. C. Lee, J. Kim and S. Hwang, *Biodegradation,* 2006, **17**, 347
3. J. Filipic, B. Kraigher, B. Tepus, V. Kokol and I. Mandic-Mulec, *Bioresource Technol*., 2012, **120**, 225.