Cell-templated Biosilicification for Encapsulation of Single Cells and Multicellular Islets

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INTRODUCTION AND OBJECTIVE

Transplantation of pancreatic islets or β cells from pluripotent cell sources is a potential therapy to treat type 1 diabetes; however, in humans a majority of the cells die after transplantion. One of the primary goals of bioencapsulation of pancreatic islets is to protect the β cells from attack by the immune system.

Biologically formed porous silica is an attractive bioencapsulation material for transplantation. Silica is found naturally in many living organisms, is well tolerated by the human body and is an essential component of normal human hard tissue formation. Silica is bioactive, which could be used to achieve long-term stability of transplants. Multifunctional composites are produced by a wide range of chemical, physical, and biological modification. Biologically formed silica has a high pore volume with pores in the mesoscale (5-50 nm) range, which may be ideal for size-exclusion of medium to large proteins (e.g. cytokines, antibodies) but transport of small proteins (e.g. insulin). Mesoporous silica is a proven material in precision separation applications (Mekawy 2011).

We present a silica bioencapsulation method that is inspired by biomineralization in natural organisms both extinct and modern. Cell and tissue surfaces are studded with proteins and carbohydrates containing hydroxyl (-OH) groups. These hydroxyls can participate in hydrogen and covalent bonding with silica precursor molecules, which then serve as a site for silica polycondensation. The result is a cell/tissue specific silica coating that can vary from 1-100's μm and that forms to the geometry of the cell or tissue. Previously we demonstrated the bioencapsulation of bacterial biofilms to reduce cell detachment in bioreactor applications (Jaroch 2011). This paper demonstrates the bioencapsulation of single pluripotent and β cells and intact mouse islets.

MATERIALS AND METHODS

Cell Culture. Pluripotent P19 embryonic carcinoma cells (ATCC CRL-1825) were cultured in α-MEM media supplemented with 7.5% bovine calf serum and 2.5% fetal bovine serum. Insulin producing INS-1 β cells were cultured in RPMI 1640 medium, 10% fetal calf serum, 50 μmol/l mercaptoethanol, 10 mmol/l HEPES, 1 mmol/l sodium pyruvate, 2 mmol/l L-glutamine, 100 U/ml of penicillin, and 100 μg/ml streptomycin. Mouse islets were harvested from 8-12 week old CD-1 mice using the procedure described in (Gotoh 1990). Islets were incubated in RPMI 1640 with 10% fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml streptomycin.

Encapsulation. Tetramethyl orthosilicate (TMOS) was hydrolyzed in a 1:16 mol ratio (TMOS:H_20) deionized water solution using 2μl of 0.04 molar hydrochloric acid initiator per 1g of solution. The mixture was stirred vigorously for 10 minutes until clear. Methanol was removed by rotary evaporation under vacuum at 47°C (30% reduction in solution volume). For encapsulation of adherent INS1 or P19 cells, 100 – 500 μL silica sol was added to 10 mL of a warm media/PBS solution. Culture media was removed and replaced with the silica media solution for a range of 5 – 20 minutes before removing the silica media and replacing with culture media. For encapsulation of P19 or islet cell suspensions, cells were centrifuged either into a pellet or into AggreWell plates and culture media was again replaced with silica/media/PBS solution for varying time.

Microscopy and Element Analysis. P19 cells were stained for membrane integrity (propidium iodide), and cellular viability (CellTracker Green CMFDA) and imaged using a Radiance 2100 MP Rainbow on a Nikon TE2000 inverted microscope. SEM images were taken using a FEI NOVA nanoSEM high resolution FESEM and elemental analysis was conducted by energy dispersive X-ray spectroscopy (EDS) on both point sources and 10x10μm scan areas using an OXFORD INCA 250 detector.

RESULTS AND DISCUSSION

Silica layer thickness was dependent on Si concentration in mineralizing solution, exposure time and cell type. Figure 1 shows suspended P19 cells after a 15 min. exposure to two different silica sol concentrations. The silica peak in the EDS spectra at the cell surface grows with increasing sol concentration. Figure 2 shows fluorescent imaging using CellTracker (Invitrogen) stain, indicating that the P19 cells remain viable after encapsulation with silica. Bright field/fluorescent composite images of cell mediated encapsulated P19 cellular clusters (300μl sol, 15 min exposure) after 4 hour of incubation reveals refractive edges that are a result of the optical properties of the sol. Pluripotent P19 cells displayed more biosilicification compared to INS-1 β cells. Figure 3 shows INS-1 cells treated with 300μl and 400μl sol for 15 minutes. P19 cells treated with 300μl of silica for 15 min display much higher levels of silica than INS-1 cells. We hypothesize that cells that produce more extracellular matrix are better

surfaces for templating silica. The silica encapsulant on mouse islets varies in thickness depending on encapsulation conditions. Figure 4 shows that the physical environment of the islet can dramatically influence the thickness of the encapsulant.

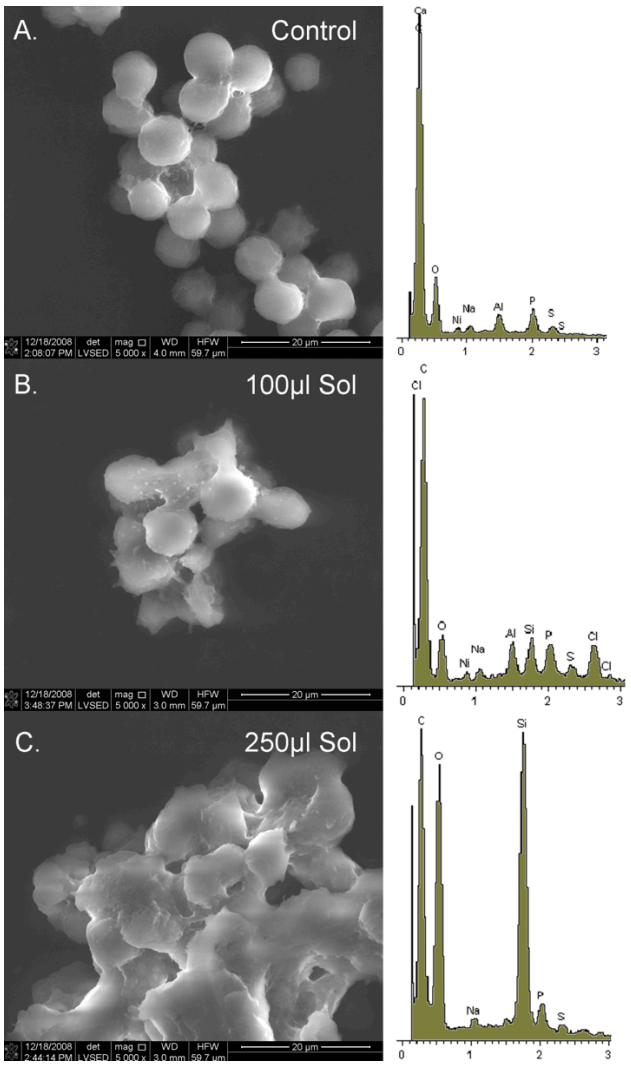

Figure 1. SEM images and EDS spectra of (a) unencapsulated control P19 cells, (b) cells encapsulated with 100 µl sol solution for 15 min, and (c) cells encapsulated with 250 µl sol solution for 15 min.

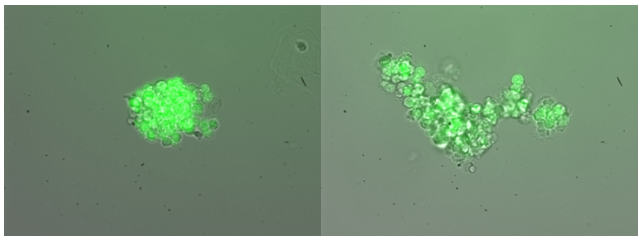

Figure 2. Composite bright field and live cell stain fluorescence image of suspended pluripotent P19 cells after encapsulation.

While not show here, we have also successfully encapsulated human islets. Both encapsulated mouse and human islets survive and function for 2-4 weeks in culture. Additional data shows that the silica is porous, islets and β cells retain good glucose and oxygen flux, and as well as glucose stimulated insulin release, and that the silica shell is bioactive resulting in secondary biomineralization in certain physiological conditions.

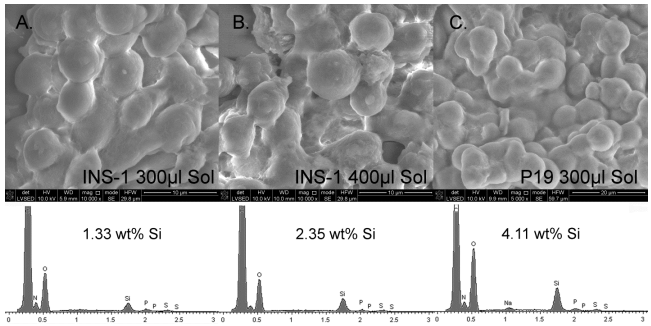

Figure 3. INS-1 and P19 cells after encapsulation with varying silica sol concentrations.

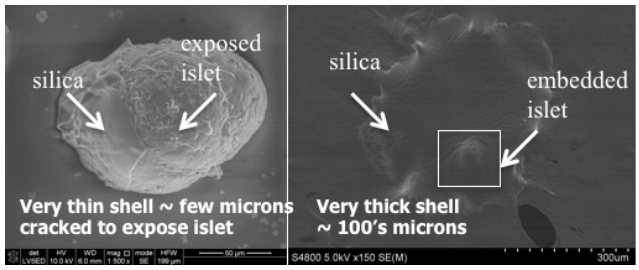

Figure 4. Mouse islets encapsulated after centrifugation into a pellet (left) and into a well (right).

CONCLUSIONS

Bioencapsulation of single cells and multicellular islets can be achieved by a simple solution phase cell-templating process with encapsulant properties that depend on both cell type and synthesis conditions.

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