MethodsX 2 (2015) 24–32

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

# A simple and effective method to encapsulate tobacco mesophyll protoplasts to maintain cell viability☆

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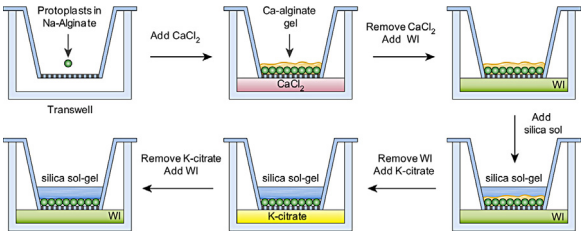
## GRAPHICAL ABSTRACT




## ABSTRACT

Protoplasts have been widely used for genetic transformation, cell fusion, and somatic mutation due to the absence of a cell wall. However, without the protection of a cell wall, protoplasts are easy to rupture and aggregate during washing, collecting, and gene transfection. In this work, we propose a simple and effective silica/alginate two-step method to immobilize protoplasts with advantages in experimental manipulation and microscopic imaging, as well as in potentially studying cell biological processes such as secretion and metabolism. The proposed two-step immobilization method adopts Transwell with clear tissue culture-treated membrane to support protoplasts in the form of uniform thin layer, which has three unique properties.

*Abbreviations:* Tris, tris(hydroxymethyl)aminomethane; MES, 2-(*N*-morpholino)ethanesulfonic acid; PET, polyethylene terephthalate; ETAF, extra thin alginate film; TAL, thin alginate layer; FDA, fluorescein diacetate; CLSM, confocal laser scanning microscope.



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http://dx.doi.org/10.1016/j.mex.2014.11.004

- The tissue culture-treated membrane has a good affinity for the plant cell; thus, protoplasts can spread evenly and form a very thin layer.
- There are more choices for membrane pore size, depending on the application.
- It is very convenient to change or collect the solution without mechanically disturbing the protoplasts. This simple and effective silica sol–gel/alginate two-step immobilization of protoplasts in Transwell has great potential for applications in genetic transformation, metabolite production, and migration assays.



ARTICLE INFO
*Method name:* Protoplast immobilization
*Keywords:* Protoplasts, Immobilization, Silica sol–gel, Alginate, Thin layer
*Article history:* Received 8 August 2014; Accepted 27 November 2014; Available online 28 November 2014

## Method details

In this silica/alginate two-step immobilization procedure, we use Transwell as a support for immobilized protoplasts, which has three unique properties. (1) The tissue culture-treated membrane has a good affinity for the plant cell; thus, protoplasts can spread evenly and form a very thin layer. (2) There are more choices for membrane pore size, depending on corresponding applications. (3) It is very convenient to change or collect the solution without mechanically disturbing the protoplasts. We hope this simple and effective silica sol–gel/alginate two-step immobilization of protoplasts in Transwell will have great potential for applications in genetic transformation, metabolites production and migration assays.

*Preparation of the plant protoplasts*

*Materials*

- Tobacco plant *Nicotiana tabacum* cv. White Burley, grown in a temperature and moisture-controlled growth chamber with a 12 h-light/12 h-dark cycle at 24–27 °C, RH 75%.
- Celluase "ONOZUKA" R-10 and macerozyme®R-10 were purchased from Yakult Pharmaceutical Industry Co., Ltd. (Tokyo, Japan). 2-(*N*-morpholino)ethanesulfonic acid (MES), mannitol, potassium citrate, fluorescein diacetate (FDA), sodium alginate, sodium silicate, acid ion exchange resin (Amberlite® IR120), LUDOX® HS-40 colloidal silica (40 wt.% suspension in water), tris(hydroxymethyl) aminomethane (tris), PEG 4000 (Fluca, cat. no. 81240) and succinic acid were obtained from Sigma–Aldrich. KCl, NaCl, and $CaCl_2$were purchased from Beijing Chemical Works (Beijing, China). 12 mm polyethylene terephthalate (PET) membrane with 0.3 μm pore size transwell-clear inserts (Costar®) was purchased from Corning (Corning, USA). All solutions were prepared using Milli-Q water (resistivity = 18.2 Ωcm).

*Reagent setup*

- The pH of 0.2 M MES solution was adjusted with 1.0 M tris solution to 5.8.
- Protoplast medium (PM): prepare 20 mM MES (pH 5.8) containing 0.5 M mannitol, 20 mM KCl, and 10 mM $CaCl_2$.
- Enzyme solution: prepare 1% (wt/vol) cellulase Onozuka R-10 and 0.5% (wt/vol) macerozyme R-10 in PM solution. Warm the solution at 55 °C for 10 min to enhance solubility. Cool to room temperature (25 °C). Filter the final enzyme solution through a 0.22-μm syringe filter device into a Petri dish ($100 \times 25\,mm^2$ for 10 ml enzyme solution).
- Washing and incubation solution (WI) solution: prepare 4 mM MES (pH 5.8) containing 0.5 M mannitol and 20 mM KCl.
- FDA stock solution: dissolve 5 mg of FDA in 1 ml acetone, and store in refrigerator.
  *Note*: The protoplasts medium (PM) and the enzyme solution were filter sterilized. The enzyme solution should be freshly prepared.