Encapsulation of Hybridoma Cell Culture for the Higher Secretion of Anti-A Blood Grouping mAbs

Asokan Chinnasamy*
and Otitolaiye. A. Catherine

E Mail: asokan_74@hotmail.com
Mobile Number: +234-8113845248

Department of Biochemistry
Sokoto State University, Sokoto. Nigeria
Blood Group System

Introduction

- Nobel Laureate Karl Landsteiner was discovered both the ABO blood group (1901) and Rh blood group (1937). There are more than 20 genetically determined blood group systems known today.

- These antigens may be Proteins, Carbohydrates, Glycoprotein's, or Glycolipids, depending on the blood group system.
IgM

- First immunoglobulin to be produced after the immune response takes place. IgM is the predominant isotype in the primary response. The role of the IgM is essential as that of the first immunoglobulin to be produced in the humoral response.
IgM
IgG

Immunoglobulin Fragments: Structure/Function Relationships

- **Ag Binding**
- **Binding to Fc Receptors**
- **Complement Binding Site**
- **Placental Transfer**
IgG

- IgG, a monomer, is the predominant Ig classes present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig.

- IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life.
Monoclonal Antibodies (mAbs)

- A monoclonal antibodies (mAbs) is a pure antibody of one molecular type derived from a single clone of B lymphoid cells which hybridized with myeloma cells, invented by Georges Kohler and Cesar Milstein in 1975.

- Influence of different abiotic factors like, medium composition, cultivation conditions and medium osmolarity on mAbs secretion is a matter of interest for many scientists and industries (Chua et al., 1994).
Encapsulation

- Encapsulated cells *in-vitro* prevent the loss of mAbs productivity than cells in high-serum media (Ogata, 1992).
- simplified handling of cells and cells safety against shear stress in the immobilisates
- innovative procedure for the immobilization of biologically active materials which improves long-term stability and minimizes their sensitivity to toxic substances.
Encapsulation

Schematic diagram showing the transport of the low molecular substances between the cells in the gel bead of alginate.
Aim and Objectives

- Develop Hybridoma clone for Anti-A
- To increase Hybridoma cell growth and viability as well as mAbs secretion by Encapsulation technology
  - Abiotic factors shearing forces, pH, osmolarity etc.
- To produce quality mAbs, in-house & Cost effective technology
Materials and Methods

- Immunization
- Hybridoma Technology
Normal Cells

- For Monoclonal Antibodies
  - Animal was Immunized With Antigen 5% A +ve RBC Blood group
- Intraperitoneal Immunization
  - Balb/c Mouse
  - Day 0: 200 µL of Ag In Complete Freund’s Adjuvant
  - Day 14: 100 µL of Ag In Incomplete Freund’s Adjuvant
  - Day 28: 100 µL of Ag In D-PBSA
- Bleed Animal Test Reactivity To Antigen
  - Serum Is Diluted 1:30
  - Final Boost of 50 µL Antigen I.V. 3 days before fusion
- Aseptically Isolate Spleen Cells
Myeloma Cells

- This Cell Line Is Deficient In HGPRT (Hypoxantine Guanine Phospho Ribosyl Transferase)
- Alternatively TK (Thymidine Kinase deficient)
- Cell Line Cannot Survive In Selection Medium
  - Aminopterin Inhibits “De novo Pathway”, “Salvage Pathway” Is Not Possible Due To HGPRT or TK Deficiency
- It Is Also Ig Deficient
  - It can not secret any Immunoglobulins
- Aminopterin (folic acid antagonist) Blocks De novo Pathway
  - SP2/0 cells die in the presence of Aminopterin
  - They cannot utilize the “salvage pathway” because they are HGPRT deficient
Hybridoma Cell Lines

Normal Cells are Fused with a Cancerous Cell Line myeloma SP2/0

- Fusion Is Accomplished with PEG (polyethylene glycol)
- The new Hybrid Cell Exhibits Properties of both Cell Types
  - Unlimited growth
  - Secretes monoclonal antibodies
  - Or Secretes cytokines
Materials and Methods

- Cell Culture Development
- Encapsulation of Cells
  - Chitosan beads (Ogata 1992)

Fig. 1. Beads preparation
Materials and Methods

Fig. 2. Encapsulated cell

Schematic diagram showing the transport of the low molecular substances between the cells in the gel bead of alginate.
Materials and Methods

- Encapsulated Cells

Fig. 3. Encapsulated Cells
Materials and Methods

- Avidity (antigen antibody agglutination)
- Titer Analysis (serial dilution)
- Total Protein Estimation (Lowry et al 1951)
- Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli 1970)
Results

- Avidity:
  - C1—Commercial product
  - C2-Control
  - T-Encapsulated culture supernatant

Fig. 1. Antigen antibody agglutination test.
Results

- Titer Analysis

- Fig. 2. Antigen and Antibody button formation
Results

- Titer Analysis
  1. Control (RPMI-1640+5% FBS)
  2. Encapsulated Anti-A Clone (RPMI-1640+5% FBS)

Table 1. Titer analysis
## Results

- **Total Protein Estimation**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Protein (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI-1640+5%FBS (Control)</td>
<td>1.859 ± 0.15 mg/mL</td>
</tr>
<tr>
<td>Anti-A Encapsulated Culture</td>
<td>2.931 ± 0.25 mg/mL</td>
</tr>
</tbody>
</table>

Table. 2. Total protein estimation analysis. p < 0.001
Results

- **SDS-PAGE Analysis**

  - Fig. 3. SDS-PAGE profile of IgM, IgG and BSA

  - IgM 900 kDa
  - IgG 150 kDa
  - BSA 66 kDa
Results

- Lane 1 - BSA
- Lane 2 - Control (RPMI-1640 + 5% FBS)
- Lane 3 - T1 Encapsulated Anti-A clone
- Lane 4 - T2 Encapsulated Anti-A clone
- Lane 5 - T3 Encapsulated Anti-A clone
- Lane 6 - T4 Encapsulated Anti-A clone
- Lane 7 - RPMI-1640 + 5% FBS Medium
Conclusion

- **Avidity:** The Encapsulated + 5% FBS samples shown good agglutination in 2-3 seconds.

- **Titer analysis,** Encapsulated + 5% FBS sample has shown the 4+ value up to 1:128 (7th tube) serial dilution.

- **Total protein:** Encapsulated sample + 5% FBS sample has shown highest total protein (2.931 ± 0.25 mg/mL)
Conclusion

- SDS-PAGE: performed using 8% separating gel to confirm the high secretion of IgM antibody by comparing with the control.

- Present study concludes that yield of mAbs is maximized by using encapsulation method.

- Anti-A mAbs can be used for Diagnostic use.

- In-house Technology may Facilitate the High Quality and Economy of the mAbs
Further Study

- Replacement of Fetal Bovine Serum/Fetal Calf Serum (FBS/FCS) with Soy Hydrolysate as supplement for the cell culture

- Advantages and Importance as follows
Fetal Calf Serum

- Serum is non-physiological fluid for the cells, provides undefined low molecular weight nutrients.
- It is not reproducible & there are safety issues (TSE/BSE).
- It interferes in the purification of mAbs (James Babcock et al., 2007).
- Generally, in cell culture 5% to 15% serum is used as supplement in the standard basal media in addition to salts and amino acids for healthy cell growth (James Babcock et al., 2007). Expensive.
Soy Hydrolysate

- Soy Hydrolysate is one of the supplement and replacement of fetal calf serum for the growth of the mammalian cells and secretion of the biological molecules.
- It contains low molecular weight nutrients, peptides and free amino acids which are necessary for cell culture. (James Babcock et al., 2007).
- Cost effective.
Acknowledgements

- DR. N. John, Antibody Solutions, Sunny Vally, CA, USA.
- Prof. G Sudhandirran, Department of Biochemistry, University of Madras, Chennai, India.
- Ms. Mamatha Pillai, Department of Biochemistry, University of Madras, Chennai, India.
THANK YOU ALL