

Hybridoma Techniques

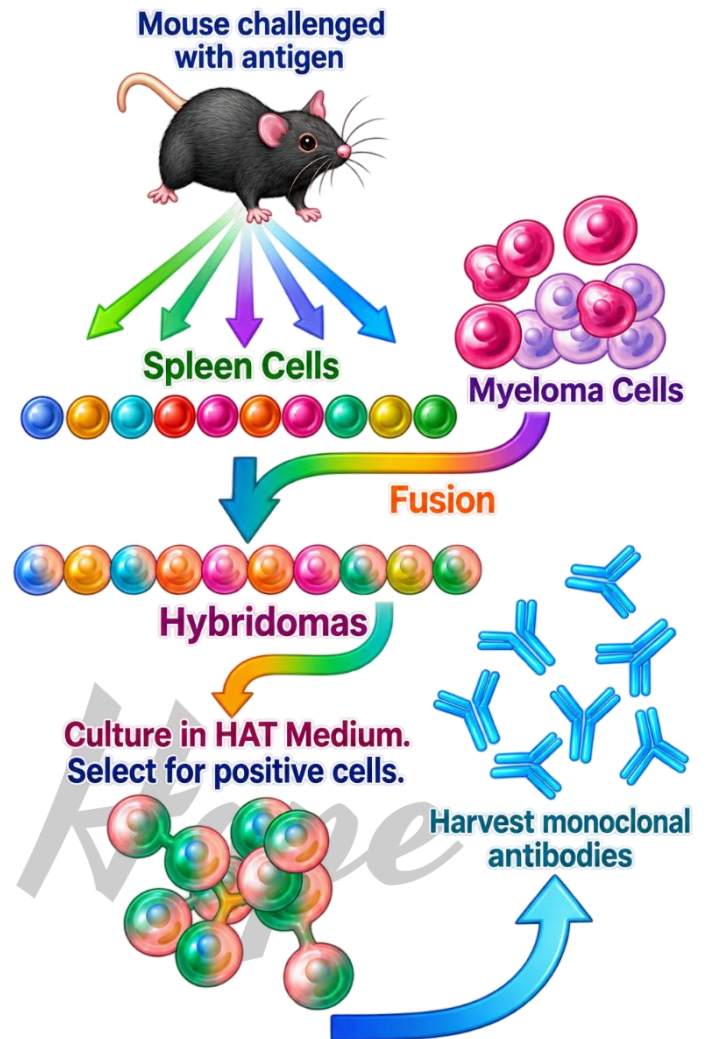
Hybridoma Techniques

Definition of Hybridoma Technology

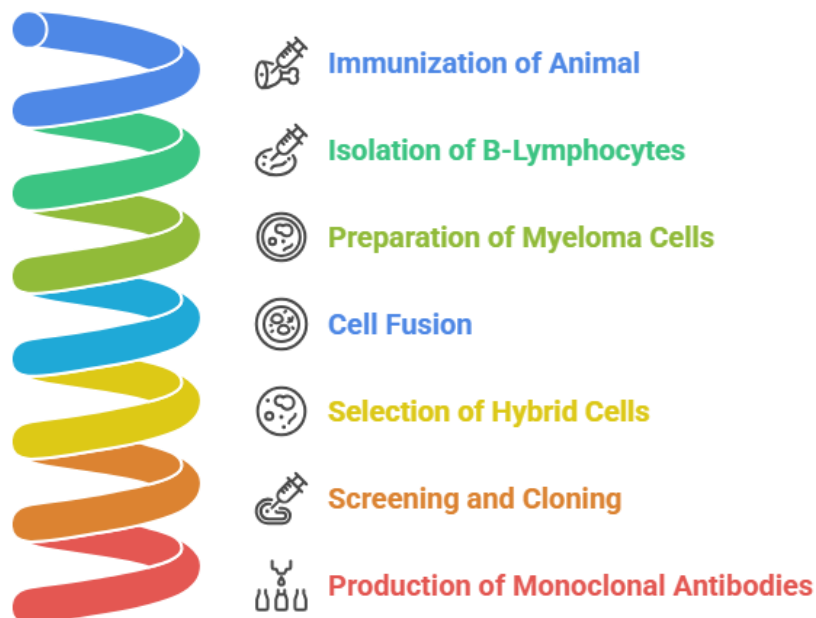
- Hybridoma technology is a method used to produce **monoclonal antibodies (mAbs)** by fusing:
 - B-lymphocytes** (antibody-producing cells)
 - Myeloma cells** (immortal cancer cells)
- The fused cell is called a **hybridoma**, which can both:
 - Produce specific antibodies
 - Divide indefinitely

Basic Principle

- B cells produce **specific antibodies** but have a short life span.
- Myeloma cells can **divide indefinitely** but do not produce antibodies.
- Fusion combines the advantages of both:
 - Specific antibody production + unlimited growth**



Steps in Hybridoma Technique



1. Immunization of Animal

- Animal (usually mouse) is injected with antigen
- Immune system produces B cells specific to that antigen

2. Isolation of B-Lymphocytes

- Spleen is removed from immunized animal
- B cells are isolated

3. Preparation of Myeloma Cells

- Myeloma cells are cultured in laboratory
- These cells are selected to lack HGPRT enzyme

4. Cell Fusion

- B cells and myeloma cells are fused using chemical or physical methods

5. Selection of Hybrid Cells

- Only fused cells survive in selective medium

6. Screening and Cloning

- Hybridomas producing desired antibody are identified
- Selected cells are cloned

7. Production of Monoclonal Antibodies

- Hybridomas are cultured to produce antibodies

Characteristics of Hybridoma Cells

- Immortal growth (from myeloma cells)
- Ability to produce specific antibodies (from B cells)
- Stable and continuous antibody production

Fusion Methods for Myeloma Cells and B-Lymphocytes

Overview of Cell Fusion

- Fusion is the process of combining **two different cells into one hybrid cell**.
- Essential step in hybridoma technology.

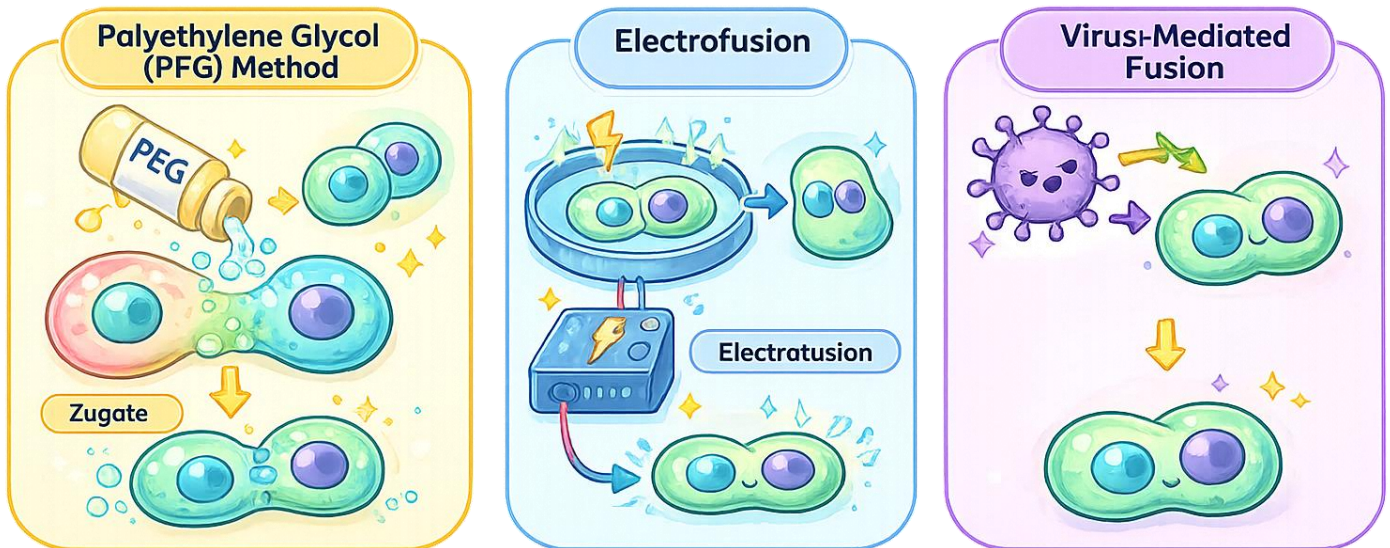
Purpose of Cell Fusion

- **To combine:**
 - Antibody-producing ability of B cells
 - Immortality of myeloma cells

Requirements for Fusion

- Viable B-lymphocytes from immunized animal
- Suitable myeloma cells (non-antibody producing, HGPRT deficient)
- Fusion agent

Methods of Cell Fusion



1. Polyethylene Glycol (PEG) Method

- Most commonly used method
- PEG acts as a fusogen

Mechanism:

- PEG disrupts cell membranes
- Promotes merging of adjacent cells
- Leads to formation of hybrid cells

Advantages:

- Simple and efficient
- Widely used in laboratories

2. Electrofusion

- Uses electric pulses to induce fusion

Mechanism:

- Cells are aligned using electric field
- Short electric pulse creates pores in membranes
- Membranes fuse to form hybrid cell

Advantages:

- More controlled and efficient
- Higher fusion specificity

3. Virus-Mediated Fusion

- Uses viruses (e.g., Sendai virus) to induce fusion

Mechanism:

- Viral proteins help in membrane fusion
- Cells merge to form hybrid cells

Limitations:

- Safety concerns
- Less commonly used

Types of Cells Formed After Fusion

1. Unfused B cells

- Short-lived → die naturally

2. Unfused Myeloma cells

- Cannot survive in selection medium

3. Hybridoma cells (desired)

- Survive and grow
- Produce specific antibodies

Types of Fusion Outcomes

- **Homokaryons:** fusion of same cell types (B+B or myeloma+myeloma)
- **Heterokaryons:** fusion of different cell types (B cell + myeloma) → desired hybridomas

Importance in Immunotechnology

- Critical step for monoclonal antibody production
- Determines efficiency and success of hybridoma formation

Selection Techniques

Definition of Selection in Hybridoma Technology

- Selection is the process of identifying and isolating successfully fused **hybridoma cells** from a mixture of cells after fusion.

Cell mixture after fusion includes:

- Unfused B-lymphocytes

- Unfused myeloma cells
- Fused hybridoma cells (desired)

HAT Medium Selection Technique

- HAT medium is a selective culture medium containing:
 - **H**: Hypoxanthine
 - **A**: Aminopterin
 - **T**: Thymidine

Principle of HAT Selection

- Cell survival depends on DNA synthesis, which occurs via two pathways:
 - **De novo pathway** → Blocked by aminopterin
 - **Salvage pathway** → Requires **HGPRT enzyme**

Role of HAT Medium

A. Aminopterin

- Blocks the **de novo pathway** of DNA synthesis
- Cells must use the **salvage pathway** to survive

B. Hypoxanthine and Thymidine

- Provide substrates for the salvage pathway

Cell Behavior in HAT Medium

A. Unfused Myeloma Cells

- Lack **HGPRT enzyme**
- Cannot use salvage pathway
- Die in HAT medium

B. Unfused B-Lymphocytes

- Have HGPRT enzyme
- Can survive temporarily
- Die naturally after short time (limited lifespan)

C. Hybridoma Cells (Desired Cells)

- Obtain HGPRT from B cells
- Obtain immortality from myeloma cells
- Survive and multiply in HAT medium

Outcome of Selection



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