**St Peter’s Institute of Pharmaceutical Sciences**

**Course: Bachelor of Pharmacy**

**Subject: Pharmaceutical Biotechnology**

**Subject Code: BP605T**

 **HYBRIDOMA TECHNOLOGY**

**Monoclonal antibodies (mAb** or **moAb)** are antibodies that are made by identical immune cells (B cells) that are all clones of a unique parent cell. Monoclonal antibodies can have monovalent aifinity, in that they bind to the same epitope.

This has become an important tool in biochemistry, molecular biology, and medicine.

**Production of monoclonal antibodies by Hybridoma technology:**

1. Selection of an animal: a healthy mouse is taken for the preparation.
2. Immunization of the mouse: antigens for which monoclonal antibodies required are injected into the mouse, to stimulate the B cells for the production of antibodies. Booster doses can be given for proper stimulation.
3. Separation of B cells from mouse: animal is sacrificed; spleen cells are separated, macerated and centrifuged to obtain the B cells.
4. Fusion of cells: B cells separated from mouse are fused with myeloma cells in presence of fusing agents like PEG. Here B cells are HGPRT+ and myeloma cells are HGPRT- (HGPRT: hypoxanthine phospho ribosyl transferase enzyme). By this fusion three type of cells are obtained: normal B cells which have not fused (HGPRT+), fused B cells (hybridized cells) which are HGPRT+ and with myeloma property and un-fused myeloma cells (HGPRT-). But we require only hybridized B cells.
5. Culturing on HAT media: HAT stands for Hypoxanthine, Aminopterine and Thymidine. This is a selective media which allows the growth of only hybridized cells. Aminopterine inhibits the de novo synthesis of DNA. Hypoxanthine and thymidine are required for salvage pathway synthesis of DNA. Only HGPRT+ cells can survive on this media i.e. hybridized B cells and normal B cells. But normal B cells are lost because they do not have the myeloma property and cannot survive long invitro.
6. Subsequent culturing of Hybridoma cells: Hybridoma cells which are screened on HAT media are transferred onto a mesh media.
7. Screening of the clones: now the hybridized B cells are screened for appropriate antibody production. Techniques like ELISA can be used for this purpose. Other hybridized B cells which do not produce required antibodies are discarded.
8. Hybridized B cells are genetically unstable so they are frequently analysed for their capability of antibody production.
9. Hybridized B cell clones are expanded on large scale for the production of antibodies. This is done invitro or invivo.

In invitro method: hybridized B cells are cultivated in a bioreactor. Now antibodies produced are separated from the spent media.

Invivo method: hybridized B cells are implanted in peritoneal cavity of mouse (isogenic animal). Antibodies produced from the peritoneal cavity are extracted. 

Application of monoclonal antibodies: Monoclonal antibodies are mainly used for:

1. Research and analytical purpose
2. Diagnostic purpose
3. Therapeutic purpose
4. **Research and analytical:**
	1. Studies of isolated genes and their products.
	2. Labelling and precise identification of specialized cells and its structures EX: neurons and cell membranes.
	3. In the preparation of specific vaccines i.e. isolation of specific antigens required for vaccine production.
	4. serogenetic classification of infectious agents.
	5. enzyme genetics and enzyme purification
	6. Immunopurification of medicinally important substances.
5. **Diagnostic purpose:**
	1. in ELISA for diagnosis of many diseases like HIV.
	2. Blood grouping i.e. A B 0 and Rh grouping
	3. For grafting of tissues and transplants.
	4. In RIA for detection of antigens
	5. In serology, diagnosis of many diseases both infectious and non-infectious diseases.
		1. immuofluroscence: here antibodies conjugated with fluorescence material are used for detection of specific antigens.

(g) In pregnancy tests and hormonal level detection.

(i) Diagnostic imaging (immune scintigraphy): like myocardial infarction, deepvein thrombosis, atheroscleroris, cancers, deep infections etc.

1. **Therapy purpose:**
	1. To suppress the immune system:
	2. To kill or inhibit malignant cells:
	3. Angiogenesis inhibitors: (for dissolving clots).

**References**

1. U.Satyanarayana.(2005). Text book of Biotechnology, pp.213-225.