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# Phage Dısplay Technology As A Strong Alternatıve To Hybrıdoma Technology For Monoclonal Antıbody Productıon

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## ABSTRACT

Therapeutic treatment of human diseases using human monoclonal antibodies has proven to be effective, though with few side effects. Monoclonal antibodies are specific against antigens. Hybridomas are hybrid cells of myeloma (cancer) cells with antibody-producing cells (lymphocytes from an immunized donor/ animal). Hybridoma technique has been used since 1975 for creating pure and uniform antibodies. The hybrid cell or hybridoma results from the fusion between myeloma cell and spleen cell of immunized cell of the donor. Attempts to use the hybridoma technology for generating human monoclonal antibodies have been hampered by the lack of a suitable human myeloma cell line. However, due to advances in recombinant DNA technology, more sophisticated antibodies can be engineered by combinational approaches such as bacteriophage display libraries, which have been reported as a strong alternative to hybridoma technology for antibody synthesis with desired antigen binding characteristics. Antibody with high affinity and avidity can be produced using bacteriophage display technology which makes use of M13, *Escherichia coli*-specific filamentous bacteriophage (a virus infecting bacteria) to whose gene, gene fragments encoding polypeptides or a peptide library are fused. This fusion protein is thought to become part of the capsid and the heterologous protein is displayed on the surfaces of the phage. The phage, contains a circular single stranded DNA genome. After a large library of mutants for a given antibody gene is made, mutagenesis can be done using error-prone PCR to incorporate random mutation in the gene. The peptide display libraries may be cloned directly into the viral genome so that all five copies of gpIII display the peptide fusion. Phage displaying the desired sequence is finally selected and amplified. This review points out the drawbacks of Hybridoma technology and presents Phage display technology as a strong alternative for monoclonal antibody production.

**Keywords**: *Monoclonal Antibody, Hybridoma Technology, Phage Display Mutagenesis, PCR*

## INTRODUCTION

Advances in Molecular Biology enable the manipulation of DNA and expression of a foreign gene in other organism (heterologous expression). This has made advancement in the process of making changes in proteins at genetic level (Arnold, 1998). Proteins are not always optimized for their properties for various applications and usefulness of a protein may be limited by low stability and /or undesired substrate specificity (Zhao and Arnold, 1997). Protein Engineering is the process of synthesizing proteins with desired function by manipulating stability and specificity of that protein (Kuchner and Arnold, 1997). The ultimate goal for protein engineering is to obtain better or, even, novel molecules with useful functional activities (Smithrud, Benkovic 1997). There are two main approaches for protein engineering, rational design and directed evolution (irrational design). In case of rational design, knowledge of the structure and function of the protein is taken into consideration and a rational gene mutation is planned accordingly. Mostly, this is done by making rationally designed changes in the gene of the protein cloned in expression vector of heterologous expression. The production of protein molecules is altered by site directed or site-specific mutagenesis of their genes. However, in some cases information on protein structure may not be available and thus directed evolution method is required. In this method, random changes (mutation) are made in the protein and a mutant form with desired properties is chosen (McCafferty et al., 1997).

Hybridoma technology has been used for decades to generate human monoclonal antibody with desired characteristics. Antibody secreting Hybridomas are derived from a fusion of myeloma cells that can grow indefinitely and an immune B lymphoblast that expresses a specific antibody gene. Meanwhile, the need for monoclonal antibody for clinical applications is on rise, an attempts to use the hybridoma technology for generating hmAbs have been hampered by the lack of a suitable human myeloma cell line.

Protein engineering applications with antibodies are diverse. Due to advances in recombinant DNA technology, "antibody engineering" is possible. Combinational approaches such as bacteriophage display libraries have been reported as a strong alternative to hybridoma technology for antibody development with desired antigen binding characteristics (Moutel et al., 2009). Studies involving genetic manipulation of mouse monoclonals for producing humanized antibodies and bacteriophage display libraries for Immunoglobulin repertoires have been reported (McArdle, 1992). "Antibody modeling" studies to engineer antibody-like molecules and increase their stability and specificity are common, particularly for humanization of antibodies of animal origin (Antikainen and Martin, 2005). Recently, novel types of proteins have been developed, using combinatorial protein engineering techniques. These binding proteins of non-Ig origin are called "affibody binding proteins". With their high

affinity, these proteins have been used in many different applications such as diagnostics, bioseparation, functional inhibition, viral targeting, and *in vivo* tumor imaging or therapy (Anthonsen et al., 1994).

# HYRIDOMA TECHNOLOGY

Hybridoma technology has contributed to virtually all areas of biology and medicine and has been greatly refined since its introduction in 1975. The two scientist, Georges Kohler of West Germany and Cesar Milstein of Argentina, who jointly with Niels Jerne of Denmark were awarded the 1984 Noble prize for physiology and medicine. Generally, the production of one MAb, using the hybridoma technology, costs between $8,000 and $12,000 (Bretton et al., 1994). In the early days, approaches to produce hmAb included (1) the hybridoma technique, based on the fusion of antibody-producing human B lymphocytes with either mouse or human myeloma or lymphoblastoid cells; (De StGroth and Scheidegger, 1980; Kenneth, 1981) or (2) the EBV immortalization technique, based on the use of Epstein–Barr virus (EBV) to 'immortalize' antigen-specific human B lymphocytes (Steinitz and Klein, 1980; Roome and Reading, 1984 and Robinson et al., 1990) Each method has its advantages and drawbacks (Olssan, 1984). Antibody secreting hybridomas are derived from a fusion of myeloma cells that can grow indefinitely and an immune B lymphoblast that expresses a specific antibody gene. Attempts to use the hybridoma technology for generating hmAbs have been hampered by the lack of a suitable human myeloma cell line. The best results were obtained using heteromyelom as fusion partners. Alternatively, human antibody-secreting cells can be immortalized by infection with the EBV.

## Basic Steps Involved in Hybridoma Technique

The basic steps involved are:
1. Immunization.
2. Generation of β cell hybridomas by fusing prime β cells and myeloma cells.
3. Selection and the screening of resulting clones.
4. Cloning by propagating the desire hybridomas (Vyas and Dixit, 2009)

### 1. Physical Technique

A single-beam gradient force optical trap is combined with a pulsed UV laser microbeam in order to perform laser induced cell fusion (Davis et al., 1982)

### 2. Chemical Technique

Poly ethylene glycol (PEG) is used to induce cell fusions and a high number of cells can be fused in the presence of PEG in a short time (Golestani et al., 2007)

### 3. Electrochemical Technique

In this technique electric potential is applied in the fusion medium to induce cell fusion. This is known as electrofusion. These factors included specific resistance and osmotic strength the ionic composition, of the fusion medium and field strength and proteolytic pretreatment of the cells effect the electrofusion (Kohler and Milstein, 1997; Stenger, 1998)

# METHODOLOGY

A hybridoma, which can be considered as a harry cell, is produced by the injection of a specific antigen into a mouse, procuring the antigen-specific plasma cells from the mouse's spleen and the
subsequent fusion of this cell with a cancerous immune cell called a myeloma cell. The hybrid cell, which is thus produced, can be cloned to produce many identical daughter clones. These daughter clones then secrete the immune cell product. Since these antibodies come from only one type of cell (the hybridoma cell) they are called monoclonal antibodies (Franklin et al., 1996). The advantage of this process is that it can combine the qualities of the two different types of cells; the ability to grow continually, and to produce large amounts of pure antibody. HAT selection medium (Hypoxanthine Aminopterin Thymidine) is used for preparation of monoclonal antibodies. Laboratory animals (eg. mice) are first exposed to an antigen to which we are interested in isolating an antibody against. Once spleenocytes are isolated from the mammal, the cells are fused with immortalized myeloma cells which lack the hypoxanthine-guanine phosphoribosyltransferase gene using polyethylene glycol or the Sendai virus. Fused cells are incubated in the HAT (Hypoxanthine Aminopetrin Thymidine) selection medium. In presence of Aminopterin the myeloma cells die, as they cannot produce nucleotides by de novo or salvage pathway the allows for nucleotide synthesis. Hence, unfused B cell die as they have a short life span. Only the B cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional. These cells produce antibodies (a property of B cells) and are immortal (a property of myeloma cells).The incubated medium is then diluted into multiwell plates to such an extent that each well contains only 1 cell. Then the supernatant in each well can be checked for desired antibody. Since the antibodies in a well are produced by the same B cell, they will be directed towards the same epitope, and are known as monoclonal antibodies. Once a hybridoma colony is established, it will continually grow in culture medium like RPMI-1640 (with antibiotics and foetal bovine serum) and produce antibody (Nelson et al., 2000.). The next stage is a rapid primary screening process, which identifies and selects only those hybridomas that produce antibodies of appropriate specificity. The hybridoma culture supernatant, secondary enzyme labelled conjugate, and chromogenic substrate, is then incubated, and the formation of a colored product indicates a positive hybridoma. Alternatively, immunocytochemical screening can also be used (Nelson et al., 2000). Multiwell plates are used initially to grow the hybridomas and after selection, are changed to larger tissue culture flasks. This maintains the well being of the hybridomas and provides enough cells for cryopreservation and supernatant for subsequent investigations. The culture supernatant can yield 1to 60 ug/ml of monoclonal antibody, which is maintained at 20°C or lower until required (Nelson et al., 2000). By using culture supernatant or a purified immunoglobulin preparation, further analysis of a potential monoclonal antibody producing hybridoma can be made in terms of reactivity, specificity, and crossreactivity (Nelson et al., 2000).

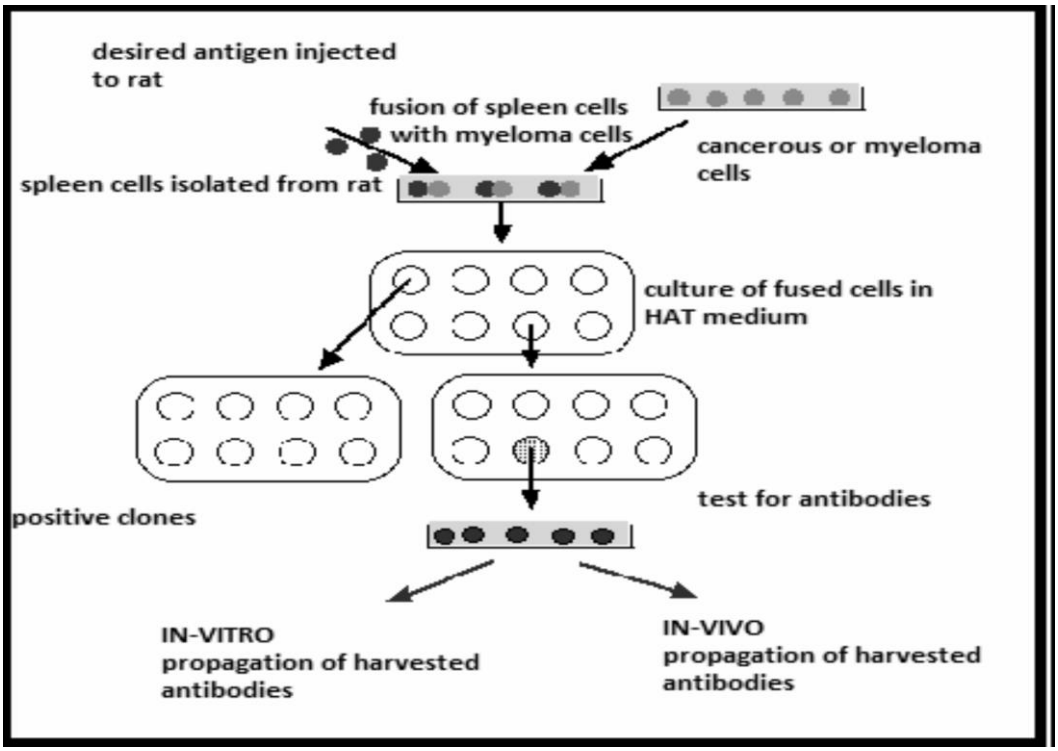


**Figure 1.0: Procedures involved in for Hybridoma Technology**

## Technical disadvantages of hybridoma technology

• No control over the epitopes to which antibodies are formed
• Antibodies must be screened extensively after they are created in the hope that one has been created with characteristics that are desirable to the investigator

## Antigen limitations

• Sensitive antigens (e.g. membrane proteins and nucleic acids) could be inside an animal antibodies are created
• Toxic antigens may kill the host animal before antibodies are produced
• Proteins highly conserved between species may not elicit an immune response

## DNA encoding the antibody is not provided

Hybridoma derived antibodies cannot be improved until they are first converted into
recombinant antibodies

## Time

Hybridoma derived antibodies can take between 4 and 6 months to create (Bretton et al., 1994).

## PHAGE DISPLAY TECHNOLOGY

Phage-display technology was pioneered by George Smith in 1985. It is a powerful method for engineering proteins with desired binding specificities.. Phage display technology has been used to isolate recombinant antibodies with specificities for autoantigens (Hexham, et al., 1992; Griffith et al., 1993; Chazenbalk; 1993; Portolano et al., 1993; Cha et al., 1993; Barbas et al., 1995; Eggena et al., 1996; Schieret et al., 1996 and Roben et al., 1996) and alloantigens (Burton et al., 1991; Zebedee et al., 1992; Barbas et al., 1992; Williamson et al., 1993 and Sansano et al., 1993) with potential therapeutic and/or diagnostic utility (Barbas et al., 1994; Chai and Garin 1994; Barbas and Burton, 1992; Marks and Marks, 1996; Pareira et al., 1997 and Huls et al., 1999). To date, phage display methods have provided approximately 30% of the 140 monoclonal antibodies currently in clinical development (Kretzschmar and Vonriden, 2002). The most clinically advanced human antibody derived using phage display is the D2E7 tumor necrosis factor alpha (TNFα) antibody currently awaiting approval by the Food and Drug Administration for the treatment of rheumatoid arthritis (Weinblatt et al., 2003). Unlike infliximab (Remicade, Centocor, Malvern, PA), a chimeric human/mouse TNFα antibody obtained by hybridoma technique, D2E7 is fully human, having been derived from a "synthetic" phage display library, the method for which is described below. In transfusion medicine, most applications of antibody phage display have focused on the isolation of human and murine antibodies to human red cells Lynch, 1982, Marks et al., 2003; Chang and Siegel, 1995) and platelets (Griffith, Ouwehand,1995). More recently, hematologic applications have expanded to include the cloning and analysis of human antibodies to clotting factors (van Den Brink et al.,2000; van Den Brink et al., 2002 and

normal (Van der Vuust and Longtenberg, 1999) and abnormal white cells.

## PHAGE DISPLAY PRINCIPLE

It was found that a phage displaying the foreign peptides were able to infect bacteria in the same manner as wild type and fusion protein was functional. In this method M13, *Escherichia coli*-specific filamentous bacteriophage (a virus infecting bacteria) is used. Gene fragments encoding polypeptides or a peptide library are fused to M13 coat protein genes. This fusion protein becomes part of the capsid and the heterologous protein is displayed on the surfaces of the phage. M13 filamentous phage, contain a circular single stranded DNA genome. The genome encodes 10 proteins, 5 of which are virion structural proteins. The genome is enclosed in a protein coat encoded predominantly by 2700 copies of gene protein VIII (gpVIII) which constitute the major coat protein. At the 'tail' end of the phage particle 4-5 copies of the gene VII protein (gpVII) and 4-5 copies of the gene IX protein (gpIX) are found and they are involved in initiating phage assembly and maintaining the stability of the viral particle. At the other end of the phage particle are 3-5 copies of the gene III protein (gpIII) which is required for infection of the host cell and 4-5 copies of the gene VI protein (gpVI) involved in the termination of the viral assembly process. Although gpIII is used for making fusion construct, recently gpVII and gpIX have also been shown to tolerate fusions at their amino termini. After a large library of mutants for a given antibody gene is made mutagenesis can be done using error-prone PCR which incorporates random mutation in the gene. The peptide display libraries are cloned directly into the viral genome so that all five copies of gpIII display the peptide fusion. Phase displaying the desired sequence is finally selected and amplified. The engineered antibody is believed to have high affinity and avidity (Kretzschmar and Vonriden, 2002).

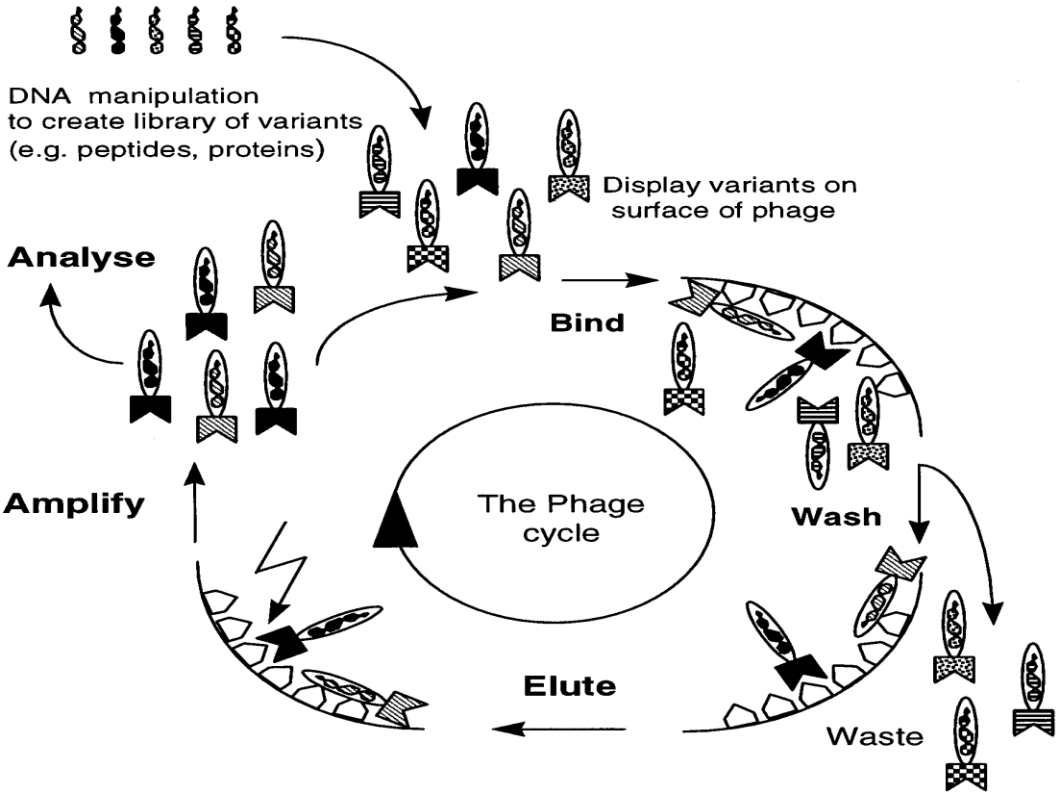


**Figure 2: The phage display cycle. DNA encoding for millions of variants of certain ligands (e.g. peptides, proteins or fragments thereof) is batch-cloned into the phage genome as part of one of the phage coat proteins (pIII, pVI or pVIII) (Abelson et al., 1996)**

## USES OF MONOCLONAL ANTIBODY

Monoclonal antibodies or specific antibodies, are now an essential tool of much biomedical research and are of great commercial and medical value. For instance, ABO blood groups could be earlier identified with the help of human sera carrying antibodies of known specificity.These human sera have been replaced by monoclonal antibodies produced by hybridomas, for the identification of ABO blood groups. Thus the diagnostic and screening value of the monoclonal antibodies through serological tests has been demonstrated. Besides the use of monoclonal in identification of blood groups, monoclonal antibody is used in diagnosis (including ELISA test for detection of viruses and imaging), immunopurification and therapy (Akon et al., 2004).

Some therapeutic monoclonal antibodies approved for use in oncology are:

**Rituximab (Rituxan):** In the form of chimeric IgG1, targets CD20 and indicated for the treatment of Non-Hodgkin lymphoma (Scott et al., 2007)

**Trastuzumab (Herceptin):** In the form of humanized IgG1, targets HER2 and indicated for the treatment of Breast cancer (Vogel, 2002)

**Alemtuzumab (Campath-1H):** In the form of humanized IgG1, targets CD52 and indicated for the treatment of chronic lymphocytic leukemia (Lundin et al., 2002)

**Cetuximab (Erbitux):** In the form chimeric IgG1, targets EGFR and indicated for the treatment of colorectal cancer (Van Cursem et al., 2009).

**Bevacizumab (Avastin):** In the form of humanized IgG1, targets VEGFand indicated for the treatment of colorectal, breast, lung cancer (Hurwitz et al., 2004)

**Panitumumab (Vectibix):** In form of human IgG2, targets EGFR and indicated for the treatment of colorectal cancer (Weiner et al., 2008)

**Ofatumumab (Arzerra):** In form of human IgG1, targets EGFR indicated for the treatment of chronic lymphocytic leukemia (Wierda et al., 2010)

**Immunoconjugates**

**Gemtuzumab ozogamicin (Myelotarg):** In form of humanized IgG4, targets CD33 Acute myelogenous leukemia (Witzig et al., 2002)

**90Y-Ibritumomab tiuxetan (Zevalin):** In the form of Murine, targets CD20 Lymphoma (Witzig et al., 2002)

**Tositumomab and 131I-tositumomab (Bexxar):** Murine, targets CD20 indicated in Lymphoma (Witzig et al., 2002)

## CONCLUSION

The promises of Hybridoma technology and its drawbacks have been documented and a better alternative, that is the Bacteriophage display technology which utilizes recent advancement in recombinant DNA technology to produce human monoclonal antibody with higher affinity and avidity presented.

## RECOMMENDATION

Review of advances in phage display technology and its limitations is needed to explore its emerging potential applications

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