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Mini-Review Article

Application of Phage Display in Medicine and Pharmaceuticals

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HIGHLIGHTS

- Phage display allows for the production of antibodies without the use of laboratory animals.
- Phage display is used in developing therapeutic and vaccine design by identifying key epitopes.
- Phage display technology utilizes various bacteriophages, including M13, fd, f1, T7, T4, and lambda (λ).

ABSTRACT

Research in medical sciences is vital for improving human health. Timely and accurate disease diagnosis is crucial for effective treatment and public health. Antigens and antibodies are essential for diagnostic testing, making their recombinant production important. Monoclonal antibodies have traditionally been produced using laboratory animals, but this method faces challenges, including the development of anti-mouse human antibodies. Phage display, a revolutionary technique that emerged in the 1980s, allows for the production of antibodies without laboratory animals, eliminating related concerns and enabling the selection of antibodies with optimal affinity for antigens. Phage display has numerous applications in medicine and pharmaceuticals. It aids in the development of therapeutic antibodies for cancer, autoimmune disorders, and infectious diseases. By screening large libraries of antibodies quickly, researchers can identify effective treatment candidates. Additionally, phage display assists in vaccine development by identifying epitopes—specific parts of antigens recognized by the immune system—and plays a role in targeted drug delivery systems. This review emphasizes the transformative potential of phage display in diagnostics and therapeutics, highlighting its role in advancing health outcomes for all.

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Introduction

In the realm of healthcare, the ability to accurately diagnose and treat diseases is paramount to ensuring optimal patient outcomes. The intricate interplay between timely diagnosis, effective treatment, and the utilization of advanced methodologies shapes the foundation of modern medicine. Accurate disease diagnosis is achieved through both clinical assessments and paraclinical tests, with each approach offering unique insights into a patient's health status (Kalt, 2019; Bakhshi Mofrad Kashani et al., 2021). Laboratory diagnostics play a critical role in this process, influenced by factors such as the quality of laboratory equipment and the availability of

reliable test materials. The biological materials used in paraclinical tests - ranging from patient samples to specific antigens and antibodies - are essential for identifying diseases and guiding therapeutic decisions (Zhang et al., 2020).

Antigens and antibodies are prepared using traditional and modern methods, and drugs are available in synthetic (small chemical molecules), solution (plant extracts, etc.), and biological (large proteins such as antibodies, etc.) forms (Wang et al., 2022). Moreover, the evolution of drug formulations, which include synthetic small molecules, natural extracts, and large biological proteins, underscores the diverse strategies employed in treatment. Among these advancements, therapeutic and diagnostic proteins have emerged as vital components in healthcare, facilitating rapid and precise disease detection. One groundbreaking innovation that has transformed the landscape of protein engineering is the phage display. Developed in the mid-1980s by George Smith, this technique allows for the expression of foreign proteins on the surface of bacteriophages, paving the way for new discoveries in diagnostics and therapeutics (Tikunova and Morozova, 2009a).

Phage display technology has revolutionized the landscape of biomedical research and has emerged as a powerful tool with a multitude of applications in medicine. This innovative method allows for the expression of peptides and proteins on the surface of bacteriophages, enabling researchers to screen vast libraries of molecules for specific interactions. The versatility and efficiency of phage display have made it an invaluable asset in various fields, including diagnostics, therapeutics, vaccine development, and antibody engineering. As we delve into the potential applications of phage display in medicine, it becomes evident that this technology is not only reshaping our understanding of disease mechanisms but also paving the way for novel treatment strategies (Hutchings and Sato, 2024).

Monoclonal antibodies

Antibodies are highly specialized proteins that play a crucial role in identifying and neutralizing pathogens as well as pathogenic antigens (Sharma et al., 2023). They are increasingly recognized as valuable therapeutic agents in the treatment of various conditions, including cancer, autoimmune disorders, and infectious diseases (Farajpour et al., 2014). With recent technological advancements, a new generation of engineered antibodies has emerged, designed specifically for therapeutic and preventive applications. In essence, therapeutic antibodies are precision-crafted molecules that offer heightened efficacy and specificity in targeting diseases. The primary objective of antibody-based therapies is to either neutralize or eradicate specific pathogens or disease processes (Sharma et al., 2023) (Fig. 1).

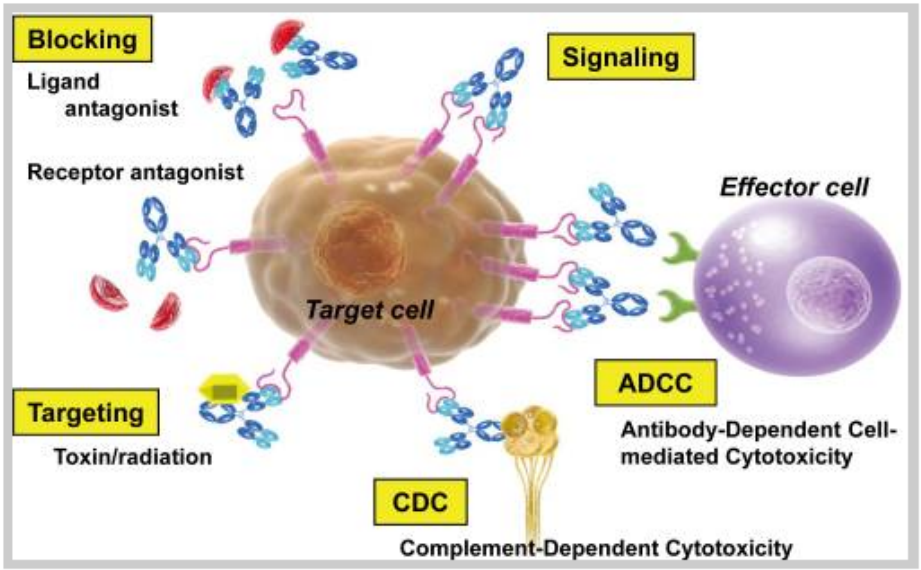

Figure 1: Antibody work model, adapted from (Suzuki et al., 2015). This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-NC-ND) License <http://creativecommons.org/licenses/by-NC-ND/3.0/>.

Antibodies may act through multiple mechanisms, typically illustrated in various models. These mechanisms include a) Inhibiting the specific functions of target molecules, b) inducing antibody-dependent cellular cytotoxicity (ADCC) which recruits immune cells to attack infected cells, c) serving as signaling molecules that facilitate communication within the immune system, d) triggering complement-dependent cytotoxicity (CDC), wherein the complement system is activated to destroy pathogens and e) additionally, antibodies can be utilized as carriers for DNA and antigens, effectively targeting specific cells within the immune system (Suzuki et al., 2015, Merkuleva et al., 2022). This approach can enhance the process of epitope presentation to T cells, thereby stimulating a targeted immune response against particular antigens. A fifth model is the use of antibodies as drug delivery vehicles. The foundation of monoclonal antibody technology dates back to 1975, thanks to the groundbreaking work of Milstein and Kohler (Köhler and Milstein, 1975). Their pioneering research led to the development of hybridoma technology, which revolutionized the production of monoclonal antibodies for both research and therapeutic purposes (Köhler et al., 1976). This innovation has opened the door to a wide array of applications in medicine, transforming the way we approach treatment for a multitude of diseases.

Monoclonal antibodies are specialized antibodies produced by a single clone of immune cells, allowing them to bind specifically to a unique epitope with an exceptional level of precision and specificity (Singh et al., 2018). The production of these antibodies follows a well-defined process that begins with the immunization of laboratory mice with the desired antigen (Fig. 2). Once immunized, the spleen cells are harvested and fused with myeloma cells to create hybridomas. This hybridization is a critical step, as it combines the desired properties of antibody-producing cells with the robust growth capabilities of myeloma cells. Each hybridoma clone is rigorously tested to identify the one that demonstrates the highest affinity for the antigen, ensuring that only the best candidate is selected for further development. Despite their advantages, the production of monoclonal antibodies using this method is not without its challenges. One significant limitation is the time-consuming nature of the entire production process. It can take several weeks to months before a usable antibody is available, which may not meet the urgent needs of research and clinical applications (Parray et al., 2020). Additionally, when these antibodies are introduced into humans, there is the potential for a human anti-mouse antibody (HAMA) reaction to occur. This immune response can interfere with the efficacy of the treatment and complicate the clinical outcome (Mirick et al., 2004). Furthermore, controlling the affinity of the produced antibodies remains a challenge. While hybridoma technology is effective, it does not always guarantee optimal binding characteristics for therapeutic use (Hnasko and Stanker, 2015). However, this issue can be strategically addressed by incorporating a tag at the C-terminal region of the antibody, which can enhance its binding properties and overall performance (Veggiani et al., 2020). Understanding these aspects is crucial for researchers and clinicians who rely on monoclonal antibodies for diagnostics, therapeutics, and various biomedical applications.

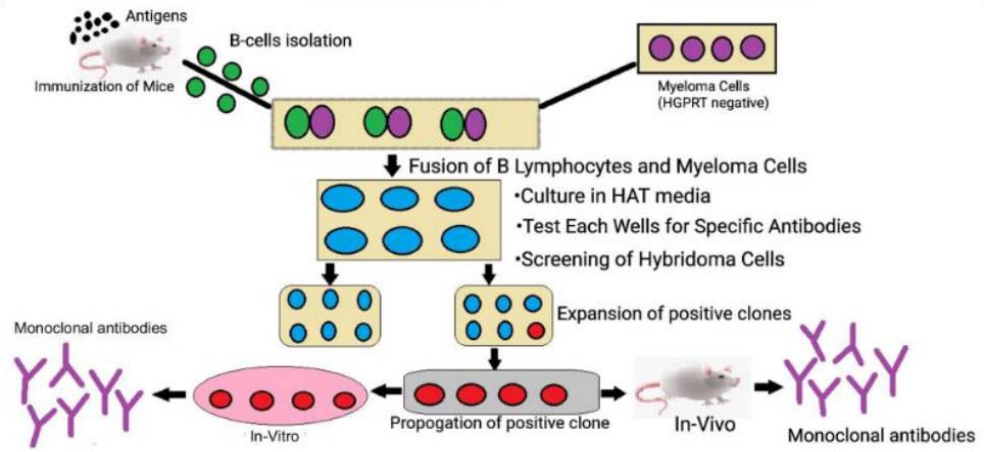

Figure 2: Preparation of monoclonal antibody, adapted from (Mitra and Tomar, 2021). This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In 2018, George P. Smith and Gregory P. Winter received the Nobel Prize in Chemistry for their pioneering work in phage display technology for peptides and antibodies. That same year, James P. Allison and Tasuku Honjo were honored with the Nobel Prize in Physiology or Medicine for their discoveries in cancer immunotherapy by blocking T-cell inhibitory receptors (CTLA-4 and PD-1) to improve anti-tumor responses (Alfaleh et al., 2020).

Antibody phage display libraries

To mitigate the issues of hybridoma technology, chimeric or humanized antibodies have been developed, combining murine variable regions with human constant regions. Fully human antibodies are produced using transgenic mouse models like HuMabMouse and XenoMouse (Lu et al., 2020).

Antibody phage display libraries offer a valuable alternative to hybridoma methods. This technique isolates fully human-derived mAbs from extensive immunoglobulin gene repertoires displayed on bacteriophages. Phage display technology was first described by George P. Smith in 1985, and the process of "panning" was introduced to isolate specific peptide-phage fusions (Alfaleh et al., 2020). M13, a filamentous bacteriophage of *Escherichia coli*, is commonly used for antibody phage display. It infects *E. coli* via its F pilus and contains a circular single-stranded DNA genome, with proteins that facilitate display. Most systems utilize pIII-antibody fusion proteins for their flexibility and functionality (Ledsgaard et al., 2018).

Antibody Engineering

Phage display has also made significant contributions to antibody engineering, particularly in the development of monoclonal antibodies for therapeutic use. The technology allows for the rapid screening and selection of antibodies with high affinity and specificity against various antigens. This process streamlines the development of therapeutic antibodies used in treating conditions such as autoimmune diseases, cancers, and infectious diseases (Frei and Lai, 2016). Moreover, smaller recombinant antibody formats, such as variable domains (Fv) and single-chain variable domains (scFv), are easier to express in bacteria than full antibodies. Combinatorial scFv libraries can be constructed by linking VH and VL domains with a flexible linker. These innovations in phage display have greatly enhanced antibody discovery and development (Singh et al., 2023).

Other applications of phage display technology

Phage display is a crucial technology in modern drug discovery that facilitates the identification of a diverse array of biologics, including peptides, antibodies, and other proteins. This technology allows researchers to fine-tune essential properties such as potency, specificity, and cross-species binding. Unlike in vivo technologies, the production of antibodies derived from phage display is not constrained by immune tolerance. Antibody drugs characterized by high affinity and specificity are particularly effective and safe for treating challenging diseases, including cancers and autoimmune disorders (Nagano and Tsutsumi, 2021; Jahandar-Lashaki et al., 2024).

Phages have significantly contributed to drug discovery and currently represent eight of the top twenty pharmaceutical products globally in terms of sales. Phage display plays an important role in developing therapeutic peptides and peptidomimetics. By utilizing randomly generated DNA sequences along with molecular biology techniques, researchers can create diverse peptide libraries that are displayed on the surface of phages (Hamzeh-Mivehroud et al., 2013). This capability has garnered attention in targeted therapy, as phages can identify potential therapeutic candidates that specifically bind to disease targets. Through this process, peptides exhibiting high affinity and specificity can be discovered for further therapeutic or diagnostic applications. Phage libraries are also valuable for rapidly screening and identifying novel antibodies for immunotherapy development (Jahandar-Lashaki et al., 2024).

In recent years, phage display technology has become essential in clinical research, aiding in the generation of antibodies that can specifically bind to complex antigens. This specificity is critical for identifying biomarkers and enhancing diagnosis and treatment strategies (Sadraeian et al., 2024). The phage display technique was first introduced by George Smith in 1985, who demonstrated that DNA fragments could be inserted into gene III of filamentous phage, resulting in a fusion protein incorporated into the virions (Smith, 1985). These virions remain infectious and present amino acids in an immunologically accessible form. The affinity of this fused phage for antibodies is up to 1,000 times greater than that of normal phages. Each phage contains a

specific antigenic determinant of the target antigen, allowing it to be distinguished from other virions. This technique is also beneficial for epitope mapping and the isolation of antibodies from immunized rabbits (Parmley and Smith, 1989).

Tens of millions of small peptides can be screened for strong binding to an antibody or receptor using an epitope library, which consists of a mixture of multiple filamentous phage clones, each displaying a different peptide on its surface. Phage replication occurs in *Escherichia coli*, and the sequence of the displayed amino acids is determined by analyzing the gene sequences present in the virions (Farajpour et al., 2013). Phage display is an exceptionally powerful tool for drug discovery, enabling the identification of new ligands, such as enzyme inhibitors and receptor antagonists or agonists, for target proteins (Pouyanfard et al., 2012). Unlike the hybridoma technique, phage display allows researchers to select specific antibodies against both biological and non-biological antigens without the need to immunize animals (Mitra and Tomar, 2021).

Diagnostics

One of the most significant applications of phage display lies in the field of diagnostics. The ability to identify biomarkers associated with specific diseases is crucial for early detection and effective management. Phage display facilitates this process by enabling researchers to create libraries of peptides that can bind selectively to disease-related targets. This capability has led to the development of diagnostic tests that can detect specific biomarkers in patient samples, thereby improving diagnostic accuracy and patient outcomes (Song et al., 2024).

Moreover, phage display is instrumental in epitope mapping, which involves identifying the specific regions of antigens that are recognized by antibodies. Understanding these epitopes is essential for developing targeted immunotherapies and vaccines. By mapping B-cell and T-cell epitopes, researchers can gain insights into immune responses, which is particularly important in the context of infectious diseases and cancer (Moreira et al., 2018).

Therapeutics

In therapeutic applications, phage display has proven to be a game-changer in drug development. The technology allows for the identification of bioactive peptides and proteins that can serve as potential drug candidates. Researchers can screen extensive peptide libraries to discover those that bind specifically to disease-related targets, such as receptors or enzymes involved in pathological processes (Omidfar and Daneshpour, 2015). This technology has shown promising application in biomarker discovery of diseases (Sadraeian et al., 2024).

One notable application is in enzyme inhibition, where phage display has been used to discover peptide inhibitors for enzymes implicated in various diseases. This approach enables the development of novel therapeutic agents that can modulate enzyme activity, potentially leading to new treatments for conditions such as cancer or metabolic disorders (Hyde-DeRuyscher et al., 2000). Additionally, phage display plays a critical role in targeted drug delivery systems. By utilizing peptides identified through this technology, researchers can create delivery vehicles that specifically target diseased tissues or cells. This targeted approach enhances the efficacy of treatments while minimizing side effects, a significant advancement in personalized medicine (Sioud, 2019).

Vaccine development

The potential of phage display extends to vaccine development as well. The technology is employed to design and optimize vaccine candidates by identifying antigens that elicit strong immune responses. For instance, researchers can use phage display to screen libraries of peptides derived from pathogens to identify those that stimulate robust immune reactions. This capability is particularly valuable in developing peptide-based vaccines against infectious diseases and cancer. By focusing on specific antigens that provoke an immune response, phage display enables the creation of vaccines that are more effective and tailored to individual patient needs (Aghebati-Maleki et al., 2016).

Research applications

Beyond its clinical applications, phage display serves as a powerful tool in research settings. It enables detailed studies of protein-protein interactions, helping researchers understand complex biological systems and signaling pathways. This knowledge is crucial for developing new therapeutic strategies and elucidating disease mechanisms (Jo et al., 2023).

In addition, phage display contributes to functional genomics by allowing scientists to identify genes encoding proteins that interact with specific peptides or antibodies. This information enhances our understanding

of gene function in health and disease, providing insights that are essential for advancing biomedical research (Sadraeian et al., 2024).

Types of phages for display

Filamentous phage display system

The filamentous phage display system is a powerful method for displaying peptides and proteins on the surface of filamentous bacteriophages. This approach is particularly effective for selecting peptides and protein domains, including antibodies. One key advantage is the direct physical link between phenotype and genotype since both the analyzed polypeptide and its corresponding DNA fragment are contained within a single phage particle (Tikunova and Morozova, 2009b, Wu et al., 2016). Filamentous phages of *Escherichia coli*, such as f1, fd, and M13, are commonly used for phage display. Most antibodies and peptides are displayed on the PIII protein (406 amino acids) and the PVIII protein (3,000 copies) (Jaroszewicz et al., 2022).

T4 bacteriophage display system

T4 bacteriophages and their related strains have been fundamental in shaping many biological concepts. These include the recognition of nucleic acids as genetic material, defining genes through subtle structural mutations, and demonstrating the tripartite nature of the genetic code. Key discoveries associated with T4 include mRNA, the importance of recombination, and analyzing molecular events in *E. coli* infected with T4 (Gamkrelidze and Dąbrowska, 2014). The display system of this phage operates using a two-part T4 HOC/SOC system, which is designed for cDNA expression and can display large proteins at high copy numbers. Proteins can be displayed either at the C-terminal end of the SOC (small outer capsid) protein, with 810 copies, or at the N-terminal end of the HOS (highly antigenic outer capsid) protein, with 155 copies (Wu et al., 2007).

T7 phage display system

As a phage display platform, M13 comprises single-stranded DNA, while T7 phage is made up of double-stranded DNA, offering greater stability and reduced mutation rates during replication. T7 phage has additional advantages; it does not rely on the protein secretion pathway during the lytic cycle. The peptides and proteins expressed are typically located in the C-terminal region of the gp10B capsid protein, which helps to avoid issues related to steric hindrance. Both phage and T7 particles exhibit high stability under various extreme conditions, such as high temperatures and low pH, facilitating efficient high-throughput affinity washing (Deng et al., 2018). T7 phage is widely used in phage display to showcase small peptides (up to 50 amino acids) at high copy numbers and large proteins at lower copy numbers (Hashemi et al., 2012).

Lambda phage display system

Bacteriophage lambda (λ) has played a crucial historical role in advancing our understanding of molecular genetics. Its lytic nature and the incorporation of its major capsid protein, gpD, into capsid assembly, provide several advantages for phage display applications. The unique conformation of the λ capsid and the potential to employ gpD for controlled phage decoration design offers exciting opportunities for future applications (Nicastro et al., 2014). Lambda phage can display high molecular weight proteins as fusion proteins with either the C-terminal or N-terminal regions of the PD head protein (405 copies) or the C-terminal region of the PV tail (6 copies). Compared to filamentous phage systems, lambda phage tends to induce a stronger immune response, despite displaying proteins at high copy numbers (Beghetto and Gargano, 2011).

Conclusion

The versatility of phage display technology continues to drive innovation across multiple domains within medicine. Its applications range from enhancing diagnostic capabilities to facilitating the development of novel therapeutics and vaccines. As advancements in this field progress, phage display is poised to play an even more significant role in addressing healthcare challenges globally. In fact, phage display represents a transformative approach that not only enhances our understanding of biological interactions but also leads to practical solutions for diagnosing and treating diseases. As researchers continue to explore its full potential, we can anticipate exciting developments that will further integrate this technology into clinical practice, ultimately improving patient care and outcomes worldwide.

In this mini-review article, the use of phage display in medicine and pharmaceuticals and its advantages were presented. This technique provides researchers with a newer approach to the preparation of monoclonal

antibodies that have anticancer and antiviral uses in medicine than the discovery of Kohler and Milstein (Mustafa and Mohammed, 2024). The use of laboratory animals to produce monoclonal antibodies has problems that are overcome by phage display (Rahbarnia et al., 2017). The development of phage display has made it possible to present a wide variety of peptides, attached to one of the bacteriophage capsid proteins. The use of this system has allowed for the achievement of enormous advantages in the selection of bioactive molecules. In fact, phage display technology has been applied in numerous fields such as biotechnology, immunology, and biomedicine (both in diagnostics and therapy), the formation of new materials, and many others (Pierzynowska et al., 2024).

Ethical Statement

This work did not contain any animal or human studies performed by the authors.

Competing Interests

The authors have no conflicts of interest to declare.

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Data Availability

All relevant data has been reported in the manuscript.

Authors Contribution

M. Bandehpour and B. Kazemi contributed to the manuscript drafting. The final manuscript was reviewed and confirmed by both authors.

References

Aghebati-Maleki, L., Bakhshinejad, B., Baradaran, B., Motallebnezhad, M., Aghebati-Maleki, A., Nickho, H., Yousefi, M. and J. Majidi, (2016). "Phage display as a promising approach for vaccine development." *Journal of Biomedical Science,* **23**(1): 66. DOI: https://doi.org/10.1186/s12929-016-0285-9.

Alfaleh, M. A., Alsaab, H. O., Mahmoud, A. B., Alkayyal, A. A., Jones, M. L., Mahler, S. M. and A. M. Hashem, (2020). "Phage display derived monoclonal antibodies: from bench to bedside." *Frontiers in Immunology*, **11**: 1986. DOI: https://doi.org/10.3389/fimmu.2020.01986.

Bakhshi Mofrad Kashani, A., Asalani Mehr, M. and P. Abedi Elkhichi, (2021). "Challenges of laboratory sampling and diagnosis of sars-cov-2 virus of disease (covid-19)." *Studies in Medical Sciences*, **32**(3): 156-174.

Beghetto, E. and N. Gargano, (2011). "Lambda-Display: a powerful tool for antigen discovery." *Molecules*, **16**(4): 3089-3105. DOI: https://doi.org/10.3390/molecules16043089.

Deng, X., Wang, L., You, X., Dai, P. and Y. Zeng, (2018). "Advances in the T7 phage display system (Review)." *Molecular Medicine Reports*, **17**(1): 714-720. DOI: https://doi.org/10.3892/mmr.2017.7994.

Farajpour, Z., Rahbarizadeh, F., Kazemi, B. and D. Ahmadvand, (2014). "A nanobody directed to a functional epitope on VEGF, as a novel strategy for cancer treatment." *Biochemical and Biophysical Research Communications*, **446**(1): 132-136. DOI: https://doi.org/10.1016/j.bbrc.2014.02.069.

Farajpour, Z., Rahbarizadeh, F., Kazemi, B., Ahmadvand, D. and M. Mohaghegh, (2013). "Identification and in vitro characterization of phage-displayed VHHs targeting VEGF." *Journal of Biomolecular Screening*, **19**(4): 547-555. DOI: https://doi.org/10.1177/1087057113514275.

Frei, J. C. and J. R. Lai, (2016). "Chapter three - protein and antibody engineering by phage display." In: Pecoraro, V. L., (ed.) *Methods in Enzymology,* Vol. 580, Academic Press. pp. 45-87. DOI: https://doi.org/10.1016/bs.mie.2016.05.005.

Gamkrelidze, M. and K. Dąbrowska, (2014). "T4 bacteriophage as a phage display platform." *Archives of Microbiology*, **196**(7): 473-479. DOI: https://doi.org/10.1007/s00203-014-0989-8.

Hamzeh-Mivehroud, M., Alizadeh, A. A., Morris, M. B., Bret Church, W. and S. Dastmalchi, (2013). "Phage display as a technology delivering on the promise of peptide drug discovery." *Drug Discovery Today*, **18**(23): 1144-1157. DOI: https://doi.org/10.1016/j.drudis.2013.09.001.

Hashemi, H., Pouyanfard, S., Bandehpour, M., Noroozbabaei, Z., Kazemi, B., Saelens, X. and T. Mokhtari-Azad, (2012). "Immunization with M2e-displaying T7 bacteriophage nanoparticles protects against influenza A virus challenge." *PloS One*, **7**(9): e45765. DOI: https://doi.org/10.1371/journal.pone.0045765.

Hnasko, R. M. and L. H. Stanker, (2015). "Hybridoma technology." In: Hnasko, R. (ed.) *ELISA: Methods and Protocols*, New York, NY: Springer New York. pp. 15-28. DOI: https://doi.org/10.1007/978-1-4939-2742-5_2.

Hutchings, C. J. and A. K. Sato, (2024). "Phage display technology and its impact in the discovery of novel protein-based drugs." *Expert Opinion on Drug Discovery*, **19**(8): 887-915. DOI: https://doi.org/10.1080/17460441.2024.2367023.

Hyde-DeRuyscher, R., Paige, L. A., Christensen, D. J., Hyde-DeRuyscher, N., Lim, A., Fredericks, Z. L., Kranz, J., Gallant, P., Zhang, J., Rocklage, S. M., Fowlkes, D. M., Wendler, P. A. and P. T. Hamilton, (2000). "Detection of small-molecule enzyme inhibitors with peptides isolated from phage-displayed combinatorial peptide libraries." *Chemistry and Biology*, **7**(1): 17-25. DOI: https://doi.org/10.1016/s1074-5521(00)00062-4.

Jahandar-Lashaki, S., Farajnia, S., Faraji-Barhagh, A., Hosseini, Z., Bakhtiyari, N. and L. Rahbarnia, (2024). "Phage display as a medium for target therapy based drug discovery, review and update." *Molecular Biotechnology,* In press. DOI: https://doi.org/10.1007/s12033-024-01195-6.

Jaroszewicz, W., Morcinek-Orłowska, J., Pierzynowska, K., Gaffke, L. and G. Węgrzyn, (2022). "Phage display and other peptide display technologies." *FEMS Microbiology Reviews*, **46**(2): fuab052. DOI: https://doi.org/10.1093/femsre/fuab052.

Jo, S. J., Kwon, J., Kim, S. G. and Lee, S. J. (2023) "The biotechnological application of bacteriophages: what to do and where to go in the middle of the post-antibiotic era." *Microorganisms,* **11**(9): 2311. DOI: https://doi.org/10.3390/microorganisms11092311.

Kalt, D.A. (2019). "A retrospective analysis of corrected laboratory results in a large academic medical center." *American Journal of Clinical Pathology*, **152**(2): 200-206. DOI: https://doi.org/10.1093/ajcp/aqz036.

Köhler, G., Howe, S. C. and C. Milstein, (1976). "Fusion between immunoglobulin-secreting and nonsecreting myeloma cell lines." *European Journal of Immunology*, **6**(4): 292-295. DOI: https://doi.org/10.1002/eji.1830060411.

Köhler, G. and C. Milstein, (1975). "Continuous cultures of fused cells secreting antibody of predefined specificity." *Nature*, **256**(5517): 495-497. DOI: https://doi.org/10.1038/256495a0.

Ledsgaard, L., Kilstrup, M., Karatt-Vellatt, A., McCafferty, J. and A.H. Laustsen, (2018). "Basics of antibody phage display technology." *Toxins* (Basel), **10**(6): 236. DOI: https://doi.org/10.3390/toxins10060236.

Lu, R.-M., Hwang, Y.-C., Liu, I. J., Lee, C.-C., Tsai, H.-Z., Li, H.-J. and H.-C. Wu, (2020). "Development of therapeutic antibodies for the treatment of diseases." *Journal of Biomedical Science*, **27**(1): 1. DOI: https://doi.org/10.1186/s12929-019-0592-z.

Merkuleva, Y.A., Shcherbakov, D.N. and A.A. Ilyichev, (2022). "Methods to produce monoclonal antibodies for the prevention and treatment of viral infections." *Russian Journal of Bioorganic Chemistry*, **48**(2): 256-272. DOI: https://doi.org/10.1134/S1068162022020169.

Mirick, G., Bradt, B., Denardo, S. and G. Denardo, (2004). "A review of human anti-globulin antibody (HAGA, HAMA, HACA, HAHA) responses to monoclonal antibodies. Not four letter words." *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, **48**(4): 251. PMID: 15640788.

Mitra, S. and P.C. Tomar, (2021). "Hybridoma technology; advancements, clinical significance, and future aspects." *Journal of Genetic Engineering and Biotechnology*, **19**(1): 159. DOI: https://doi.org/10.1186/s43141-021-00264-6.

Moreira, G., Fühner, V. and M. Hust, (2018). "Epitope mapping by phage display." In: Hust, M. and T. Lim, (Eds), *Phage Display. Methods in Molecular Biology*, Vol 1701. Humana Press, New York, NY, pp. 497-518. DOI: https://doi.org/10.1007/978-1-4939-7447-4_28.

Mustafa, M. I. and A. Mohammed, (2024). "Developing recombinant antibodies by phage display technology to neutralize viral infectious diseases." *SLAS Discovery*, **29**(3): 100140. DOI: https://doi.org/10.1016/j.slasd.2024.01.001.

Nagano, K. and Y. Tsutsumi, (2021). "Phage display technology as a powerful platform for antibody drug discovery." *Viruses*, **13**(2): 178. DOI: https://doi.org/10.3390/v13020178.

Nicastro, J., Sheldon, K. and R.A. Slavcev, (2014). "Bacteriophage lambda display systems: developments and applications." *Applied Microbiology and Biotechnology*, **98**(7): 2853-2866. DOI: https://doi.org/10.1007/s00253-014-5521-1.

Omidfar, K. and M. Daneshpour, (2015). "Advances in phage display technology for drug discovery." *Expert Opinion on Drug Discovery*, **10**(6): 651-669. DOI: https://doi.org/10.1517/17460441.2015.1037738.

Parmley, S. F. and G. P. Smith, (1989). "Filamentous fusion phage cloning vectors for the study of epitopes and design of vaccines." In Atassi, M. Z., (Ed.) *Immunobiology of Proteins and Peptides V: Vaccines Mechanisms, Design, and Applications*, Boston, MA: Springer US. pp. 215-218. DOI: https://doi.org/10.1007/978-1-4757-2046-4_21.

Parray, H. A., Shukla, S., Samal, S., Shrivastava, T., Ahmed, S., Sharma, C. and R. Kumar, (2020). "Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives." *International Immunopharmacology*, **85**: 106639. DOI: https://doi.org/10.1016/j.intimp.2020.106639.

Pierzynowska, K., Morcinek-Orłowska, J., Gaffke, L., Jaroszewicz, W., Skowron, P. M. and G. Węgrzyn, (2024). "Applications of the phage display technology in molecular biology, biotechnology and medicine." *Critical Reviews in Microbiology*, **50**(4): 450-490. DOI: https://doi.org/10.1080/1040841X.2023.2219741.

Pouyanfard, S., Bamdad, T., Hashemi, H., Bandehpour, M. and B. Kazemi, (2012). "Induction of protective anti-CTL epitope responses against HER-2-positive breast cancer based on multivalent T7 phage nanoparticles." *PloS One*, **7**(11): e49539. DOI: https://doi.org/10.1371/journal.pone.0049539.

Rahbarnia, L., Farajnia, S., Babaei, H., Majidi, J., Veisi, K., Ahmadzadeh, V. and B. Akbari, (2017). "Evolution of phage display technology: from discovery to application." *Journal of Drug Targeting*, **25**(3): 216-224. DOI: https://doi.org/10.1080/1061186X.2016.1258570.

Sadraeian, M., Maleki, R., Moraghebi, M. and A. Bahrami, (2024). "Phage display technology in biomarker identification with emphasis on non-cancerous diseases." *Molecules*, **29**(13): 3002. DOI: https://doi.org/10.3390/molecules29133002.

Sharma, P., Joshi, R. V., Pritchard, R., Xu, K. and M.A. Eicher, (2023). "Therapeutic antibodies in medicine." *Molecules*, **28**(18): 6438. DOI: https://doi.org/10.3390/molecules28186438.

Singh, R., Chandley, P. and S. Rohatgi, (2023). "Recent advances in the development of monoclonal antibodies and next-generation antibodies." *ImmunoHorizons*, **7**(12): 886-897. DOI: https://doi.org/10.4049/immunohorizons.2300102.

Singh, S., Tank, N. K., Dwiwedi, P., Charan, J., Kaur, R., Sidhu, P. and V. K. Chugh, (2018). "Monoclonal antibodies: a review." *Current Clinical Pharmacology*, **13**(2): 85-99. DOI: https://doi.org/10.2174/1574884712666170809124728.

Sioud, M. (2019). "Phage display libraries: from binders to targeted drug delivery and human therapeutics." *Molecular Biotechnology*, **61**(4): 286-303. DOI: https://doi.org/10.1007/s12033-019-00156-8.

Smith, G. P. (1985). "Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface." *Science*, **228**(4705): 1315-1317. DOI: https://doi.org/10.1126/science.4001944.

Song, B.P.C., Ch'ng, A.C.W. and T.S. Lim, (2024). "Review of phage display: A jack-of-all-trades and master of most biomolecule display." *International Journal of Biological Macromolecules*, **256**: 128455. DOI: https://doi.org/10.1016/j.ijbiomac.2023.128455.

Suzuki, M., Kato, C. and A. Kato, (2015). "Therapeutic antibodies: their mechanisms of action and the pathological findings they induce in toxicity studies." *Journal of Toxicologic Pathology*, **28**(3): 133-139. DOI: https://doi.org/10.1293/tox.2015-0031.

Tikunova, N. and V. Morozova, (2009). "Phage display on the base of filamentous bacteriophages: application for recombinant antibodies selection." *Acta Naturae*, **1**(3): 20-28. PMID: 22649612.

Veggiani, G., Giabbai, B., Semrau, M. S., Medagli, B., Riccio, V., Bajc, G., Storici, P. and de A. Marco, (2020). "Comparative analysis of fusion tags used to functionalize recombinant antibodies." *Protein Expression and Purification*, **166:** 105505. DOI: https://doi.org/10.1016/j.pep.2019.105505.

Wang, Z., Wang, G., Lu, H., Li, H., Tang, M. and A. Tong, (2022). "Development of therapeutic antibodies for the treatment of diseases." *Molecular Biomedicine*, **3**(1): 35. DOI: https://doi.org/10.1186/s12929-019-0592-z.

Wu, C.-H., Liu, I. J., Lu, R.-M. and H.-C. Wu, (2016). "Advancement and applications of peptide phage display technology in biomedical science." *Journal of Biomedical Science*, **23**(1): 8. DOI: https://doi.org/10.1186/s12929-016-0223-x.

Wu, J., Tu, C., Yu, X., Zhang, M., Zhang, N., Zhao, M., Nie, W. and Z. Ren, (2007). "Bacteriophage T4 nanoparticle capsid surface SOC and HOC bipartite display with enhanced classical swine fever virus immunogenicity: A powerful immunological approach." *Journal of Virological Methods*, **139**(1): 50-60. DOI: https://doi.org/10.1016/j.jviromet.2006.09.017.

Zhang, X., Das, S., Dunbar, S. and Y.-W. Tang, (2020). "Chapter Two - Molecular and non-molecular approaches to etiologic diagnosis of gastroenteritis." In: Makowski, G.S. (Ed.) *Advances in Clinical Chemistry*, Vol. 99, Elsevier. pp. 49-85. DOI: https://doi.org/10.1016/bs.acc.2020.02.007.