Volume 66, Issue 3, 2022
Journal of Scientific Research
of
The Banaras Hindu University

Applications of Hydrophobins in Medical Biotechnology

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Abstract: **Class I and class II Hydrophobins are small, low molecular weight cysteine-rich protein produced by filamentous fungi, of approximately 100 amino acids, whose size ranges between 5-20 kDa. Research over the last 10 years gave an improved insight into the applications of hydrophobins in the medical and pharmaceutical fields. The study of hydrophobins, self-assembly between hydrophobic and hydrophilic interfaces led to a better understanding of physical properties and their applications. Different coatings which use anti-fouling agents like hydrophobins are used for fabricating medical devices to provide bacteriocidal and antiplatelet activity for instance by fusion of SC3-NO-releasing medical-grade polymer; formation of antibacterial surfaces. Hydrophobins have been used for making Nano suspensions of water-insoluble drugs to increase their efficiency/effectiveness and for better oral administration. In addition to it, Hydrophobins are used for immobilization of molecules and cells, Detection of antigen-antibody interaction. This review highlights the study of a significant wide range of applications by various methods, also showcasing the need for developing new product strategies to improve the low yields for their broader use. Hydrophobins being a promising protein gives widespread opportunities for their application in medical and therapeutics.**

Index Terms: **Antifouling coating, Antimicrobial surfaces, Drug carrier, Hydrophobin, Self-assembly.**

I. INTRODUCTION

Hydrophobins are small, low molecular weight cysteine-rich proteins. As these proteins contain hydrophobic amino acids, that's where the name 'hydrophobin' originates. These proteins get produced from cells and are secreted into liquid media or remain at the surface of mycelia (Stübner, et al., 2006). This results in several proposals, where hydrophobins can be operated in various important scientific and technological applications.

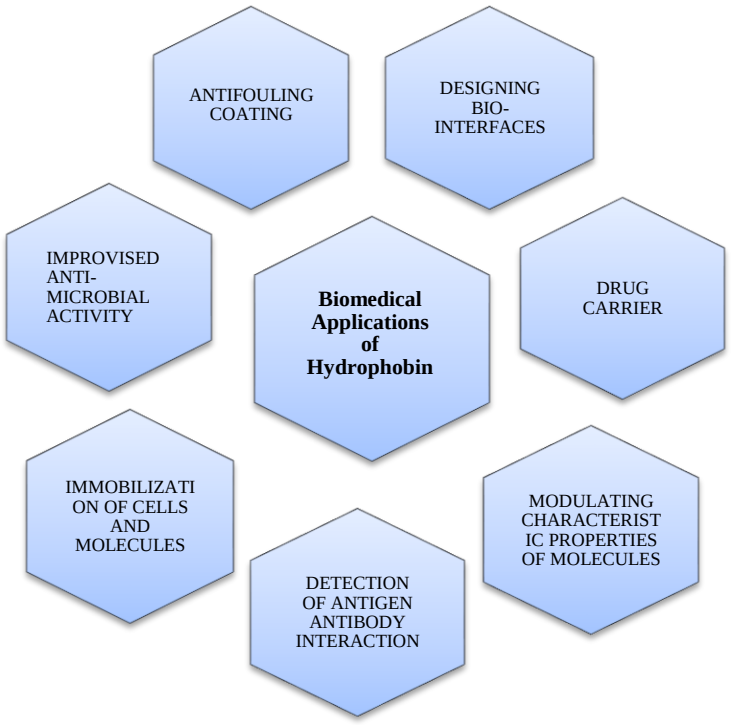

Fig. 1. Applications of Hydrophobins

Hydrophobin attracts an interesting application in the field of medicine and pharmaceutical formulations "Fig. 1" as they cause an increase in the stabilization of drugs. Hydrophobins enhance the biocompatibility of medical implants by increasing their chances of validation. Isolation of hydrophobins from new sources would increase the chances of novel industrial applications of the protein. An attempt was made to isolate and purify hydrophobin from strain *P. islandicum* isolated from malted barley waste (Kulkarni, et al., 2020). To increase the yield and production of hydrophobin different modes of fermentation, such as submerged liquid and solid state fermentation were carried out (Kulkarni, et at., 2019). However, the low yield of hydrophobin restricts its industrial applications. Several strategies and bioprocesses can be implied to overcome this issue (Kulkarni, et al., 2017). Exclusive commercialization of hydrophobin is crucial for understanding its various aspects.

Modification of hydrophobicity could open new applications. A pandect resulting in the existing probabilities for applications might help to understand their environmental behavior stimulating the improvement in techniques for isolation, purification, and other uses. Various attachments can be manipulated by modifying the surface hydrophobicity. The self-assembling and the nontoxic nature allow hydrophobins to induce several products which include emulsions and personal care, separation technologies, and biosensors. Immunogenic reactions are not hindered by hydrophobin utilization in medical applications. Therefore, hydrophobin functionalized materials can replace the traditional methods thereby improving the applications of biomaterial. This review foregrounds the scope of medical applications of the unique isolated hydrophobins.

II. APPLICATIONS

A. Antifouling Coating

In the medical device design, the vital need for antifouling coating which prevents fouling by micro molecules, which can lead to device-related complications, thrombosis, infection, to avoid this, SC3 hydrophobin is coated on polymer which is a Nitrogen oxide (NO) releasing Medical-grade Polymer to form an antifouling layer which works with Nitrogen oxide's bactericidal and SC3-NO's antiplatelet activity "Table I" . When the experimental viable count was checked for *Staphylococcus aureus* on hydrophobin SC3-NO, It was seen to be lesser than the control sample, also reduction in adherence of platelet was seen on SC3-NO surface (Devinr, et al., 2019). This coating also improves the biocompatibility of medical implants and devices (Damodaran & Murthy, 2016). The first stage in the foreign body response (FBR) starts with the triggering of surface fouling when a layer of exopolymeric substrate (EPS) is formed by nonspecific absorption of proteins onto the foreign surface. This surface of the EPS layer provides adhesion receptors for attachment for inflammatory (Anderson, et al., 2008) bacterial cells (Stewart & Franklin, 2008) in devices that are in contact with platelet, blood cells to attach to a foreign surface (Gorbet & Sefton, 2004). Adhesion of these types of cells on the surface of the device can further result in its failure as a result of foreign body response (FBR) (Wilson & Gifford, 2005).

In blood-contact devices, thrombus formation may result in various complications like local tissue necrosis, surgical complications, lethal cardiovascular complication (Silverstein, et al., 2008). These acute complications of exopolymeric substrate formation are the motivation for the research of versatile "all-in-one" materials which can combine both anti-microbial and antifouling action, which can not only prevent protein absorption and platelet activity but can also effectively kills bacteria.

In this application, the effect of SC3 hydrophobin on the wettability of SNAP which is S-nitroso-N-acetylpenicillamine, polydimethylsiloxane, and CarboSil® 2080A is verified (Scholtmeijer, et al., 2009; Zykwinska, et al., 2014). SC3 hydrophobin was coated on the surface using a simple physical methodology and thereby providing an alternative for the antifouling coating and forbidding the drawbacks of Nitrogen Oxide releasing medical device coating.

B. Designing Bio interfaces

The extensive use of medical devices has led to challenges for devices in the healthcare system, as patients are in direct contact with the materials, facilitating the colonization of microorganisms.

Many devices are made of hydrophobic materials making them susceptible to bacterial contamination. Therefore, to improve and inhibit the growth of these species, an improved structure and design are required. To this end, the expansion of Pediocin PA1 improves the antibacterial activity of poly-caprolactone electrospun [PCL], which is a combination of PA1 and HGFI. PCL is a biodegradable polymer with multiple hydrophobic properties that work for biomedical applications such as surgical materials, repair of internal tissue damage, and drug delivery systems. Previously, several strategies have been implemented to increase the antibacterial activity of PCL materials. Studies indicate that an effective and safe therapeutic agent for preventing device-associated infections is an important requirement (Jang, et al., 2015; Tran, et al., 2014). Bacteriocins are cationic, ribosomal, membrane-permeable antibacterial peptides (AMPs) produced by bacteria (Castillo, et al., 2013). However, Pediocin PA1 bacteriocin is a peptide produced from *Pediococcus acidilactici*, consisting of 44 amino acids that exhibit strong activity against bacterial species and also inhibit other Gram-positive bacteria (NietoLozano, et al. ., 2006). The HGFI that can be used from *Grifola frondosa* "Table I" is an 8 kDa type I hydrophobin (Yu, et al., 2008). The design goal of antimicrobial interfaces that can be achieved using anti-mobilization PA1 on PCL using self-assembled HGFI will improve the antibacterial ability of PCL fibers by a simple, inexpensive method and safe. This fusion protein improves the bacterial resistance of the PCL membrane covering PCL fibers to the recombinant pH protein.

The PCL membrane formed by PH (PA1 – HGFI) can prevent biofilm formation. PH-coated PCL can inhibit the growth of S. aureus ATCC6538 was demonstrated with a PH-coated PCL membrane when minimal bacterial cell mass was found. Bacterial surface coverage was reduced on PCL due to PH and thus an inhibitory effect on biofilm could be observed. This result is understandable since biofilm formation is involved in bacterial growth and adsorption. Furthermore, the increase in the active aggregates occurred more readily with increasing pediocin concentration at low temperatures (Bhunia, et al., 1988).

HGFI properties, such as diversity and synthesis, can affect the activity of PA1 in solution, resulting in a lower PH activity than PA1 in solution. PH fusion protein has much stronger antibacterial activity than PA1 although PH activity is less in solution. This is one of the steps to antibacterial surfaces. Studies have shown that hydrophobin has low immunogenicity (Aimanianda, et al., 2009) and is, therefore, a safe and functional biomaterial that can fulfill the needs of medical devices. Pediocin PA1 is one of the major members of bacteria. It can be replaced with Immobilize others to create antibacterial bio-masks. This fusion protein offers an economical and versatile technique for antimicrobial surface modification.

C. Development of Antimicrobial Surfaces using a Hydrophobin Chimeric Protein

In this context, hydrophobins, special category proteins are coated on surfaces to make them repel or kill the other microbes to reduce microbial infections. Hydrophobins can be used to prevent biofilm formation which can be an ecological way to reduce the spread of diseases (Artini, et al., 2017; Wang, et al., 2016). Though most of the biofilms are reduced, the cell viability of some of the strains of *Staphylococcus epidermis* was not reduced also was not effective against *staphylococcus aureus* biofilm. So to magnify the development of antimicrobial surface, a chimeric protein with a combination of several other potentials of Vmh2 hydrophobin with antimicrobial LL37 peptide found in humans "Table I". The combination of Vmh2 with the human antimicrobial peptide LL37 broadens the activity of the protein, adding the properties such as biocidal activity.

D. Formulation of Water Insoluble Drug

The safest, most convenient, and inexpensive way of drug delivery is oral administration. In this application, crystal nano suspension, polymeric nano-carriers, formulations based on lipid are described to enhance bioavailability (Rawat, et al., 2006; Rabinow, 2004). With such approaches, small particles are formulated. These Small particles consist of an out-sized surface-to-volume ratio. In which the bioavailability, as well as desolation rate, is increased (Rabinow, 2004). When the particle size is furthermore decreased to sub-micron levels, then there is the uptake of entire drug particles within the gastrointestinal tract (Rabinow, 2004). An encapsulated drug can be protected by polymeric nano-carriers from the environment. Where, the limitations are loading capacity, aggregation, and toxicity issues. On the other hand, a formulation based on lipid is hampered by instability. Whereas, nanosuspensions are colloidal of drug particles, and can be stabled by using hydrophobins, and prepared by milling technique or precipitation. Finally, stability against the degradation of the solid-state drug ends up increasing. Here, they analyzed that the suspension of the small drug particles is synthesized using hydrophobins surfactant property. The formulation of the drug increment of SC3 results in increased homogeneity and reduction of particle size. Experimentally it is seen that involve uptake of nifedipine and CyA resulted that this approach ending uprising the bioavailability of the drugs. Additionally, remarkable pharmacokinetic property is seen when CyA drug is formulated with SC3 hydrophobin. The use of SC3 hydrophobin resulted in reducing the peak concentration of the drug for a longer duration in the blood. Thus, the hydrophobin functionalized drugs, challenge existing methods, especially the formulation only contains non-toxic hydrophobin protein as an excipient, and also the method is generic. Likelihood small scale experiments when combined with simple preparations allow the hydrophobin implementation for shielding the drug contender during the process of high-scalability (Haas, et al., 2009).

E. Drug Carrier to Improve Serum Stability

Glucagon-like peptide-1 (GLP-1) has multiple physiological properties which make sure that it is a promising drug candidate against type 2 diabetes (diabetes that's characterized by high blood glucose, insulin resistance, and lack of insulin). But there is one restriction for GLP-1, where quick degradation by the enzyme dipeptidyl peptidase-IV (DPP-IV) and renal clearing function shortens the in vivo half-life. Poor serum stability of GLP-1 restricts the clinical utility even though the focus is on the studies that extend the serum stability. To avoid protease degradation and to stabilize GLP-1 hydrophobin protein is used as a drug carrier by forming a cavity and encapsulating. Experimentally by glucose tolerance test, it is elucidated that the clearance activity was retained for almost 72 hours against blood glucose, which suggests that the administered drug candidate may be utilized for 2-3 days. In addition, an ideal pH condition for self-assembly can be achieved by mutagenesis of hydrophobin. This implies that hydrophobin will be a strong tool for drug carrier or drug-release compounds. There is a possibility of a reduction in future interest in hydrophobin indicating the novel pharmaceutical applications of hydrophobin (Zhao et al., 2016).

Clinically the efficacy period of GLP-1, hydrophobin/GLP-1, and hydrophobin E24K/GLP-1 complex is measured in Wistar rats after administration of each drug it is seen that the GLP-1 alone shows poor glucose tolerance than those of with hydrophobin and hydrophobin E24K complexes. These complexes show a 2-3 times higher efficacy period than GLP-1 alone. This results in the reduction in the frequency of administration to one injection of the drug every 2-3 days. Further, it suggests that a dose of 250 μg/kg

body weight gives sufficient efficacy (Woodroofe, et al., 2004). It is also seen that the hydrophobin/GLP-1 is ideally stable at pH 7 and hydrophobin E24K/GLP-1 is ideally stable at pH 3.

F. Enhanced Antitumor Efficiency of Curcumin-loaded Poly D, L-lactic-co-glycolic acid (PLGA) Nanoparticles Coated with Unique Fungal Hydrophobin

Biologically derived proteins or peptides were the materials used for the construction of Nanocarriers by delivering small molecular drugs or other therapeutics as they are useful due to their properties like biocompatibility and biodegradability (Yu, et al., 2017, Shitara, et al., 2017). Studies have shown that hydrophobins have great potential in Nano technological coatings, cosmetic emulsions, food, and biomedical applications (Valo, et al. 2011).

Curcumin is extracted from the rhizome of turmeric. It is a yellow polyphenol, also known as diferuloylmethane or Curcuma long. It is useful in biological and pharmacological activities which include antimicrobial, antioxidant, anti-inflammatory, and anti-carcinogenic activities. Curcumin has been established in preclinical models, effectively from solid tumors (Bisht et al., 2010). Due to their hydrophobicity and poor oral bioavailability, different-nano carriers which also include polymer nanoparticles, nanocrystals, self-assemblies, lipid nanoparticles, nano emulsions, and protein-based drug delivery systems, were proposed to improve the stability, solubility, and bioavailability as well as target delivery (Gupta, et al., 2009; Kim, et al., 2010; Yallaou, et al., 2015; Niu, et al., 2020).

The cells treated with HPB PLGA NPS had stronger cellular uptake than those treated with free curcumin and PLGA NPS, while bare PLGA NPS showed no, or less cellular uptake compared with raw curcumin. The results could be attributed to improved uptake of nanoparticles due to the surface modification to PLGA nanoparticles with hydrophobin and thus enhanced killing of tumor cells.

G. Modulation of Fluorescent Dye

Today, fluorescence imaging is turning out to be the most powerful and interesting technique for obtaining efficient pathways for specific targets and processes in living organisms (Xia, et al., 2013; Stennett, et al., 2013). Fluorescent dyes play a crucial role in fluorescence-based analysis (Yin, et al., 2016). However, in the techniques such as bio-imaging and bio-labeling, several issues such as low water solubility, deficient membrane permeability are faced by these dyes (Liu, et al., 2013; Qin, et al., 2012). To overcome these drawbacks efforts have been made by appending hydrophilic groups to the core of dyes which include, sulfonate, pyridinium, glycol, and carboxylate, thereby increasing their aqueous solubility (Zhang, et al., 2015). However, the modification in the existing skeleton led to various new problems such as functional interference of biomolecules due to the changed properties (Wysocki, et al., 2011). Thus, these modified molecules have acquired a functional limitation for certain methods such as amyloid labeling.

For resolving these issues, herein, Boron-dipyrromethene (BODIPY) can be used as a model fluorescent dye. BODIPY has exceptional abilities it is the most popular dye in fluorescence imaging (Zheng, et al., 2008).

BODIPY derivative is characterized by 1 H NMR is beneficial in the NIR window (650–900nm) and can be utilized for investigating the different aspects of hydrophobin HFBI and BODIPY derivative. Hydrophobin BODIPY derivative can be functionalized by employing hydrophobin HFBI "Table I" to overcome the insolubility problem of BODIPY dye. Hydrophobin 1 is an amphiphilic protein, having about 18% hydrophobic area of its total surface area (Kallio, et al., 2007). The hydrophobic patch interacts and binds with different hydrophobic materials (Liu, et al., 2012). It is observed that the fluorescence spectrum of BODIPY increases when the HFBI concentration is increased. This indicates that the protein concentration is the key for dye dispersion and hence the HFBI protein provides a supreme solvent for BODIPY. Extensive shreds of evidence support the nature of hydrophobic coating as nontoxic on different interfaces (Aimanianda, et al., 2009). As the BODIPY surface is covered with HFBI film, the cell interaction with HFBI/BODIPY comes out to be non-toxic. Thus, in the future, HFBI-functionalized BODIPY can be implemented in the living cell system for bio-imaging. Evaluating the water solubility, cytotoxicity, and the membrane permeable ability of this HFBI/BODIPY complex, the results signify an enhanced dispersion and membrane-permeable properties of BODIPY by dispersing in the HFBI solution of 200 μg/mL and efficient permeability through the cell plasma membrane and nuclear membrane.

After sonication small holes are detached from huge BODIPY aggregates forming spherical HFBI/BODIPY complexes. The BODIPY dye, in general, due to its high lipophilic property tends to accumulate in the subcellular membranes. Since there is a widespread of HFBI functionalized BODIPY through the cytoplasm, there is an ease in the image formation of specific biomolecules and cell organelles by binding the target group to the HFBI protein. Hence fusing HFBI with BODIPY, we could image not only the targeted biological molecules/drugs but also the nuclei of the molecules thoroughly in the future.

H. Hydrophobin for Detection of Antigenic Antibody Interface

The fields like medical and healthcare diagnostic, biomolecular interaction detection, environmental monitoring have greatly captivated the use of optical biosensors (Fan, et al., 2008). Optical fiber sensors operate on different sensing mechanisms (Daems, et al., 2016). Due to their advantageous features such as affordable fabrications, convenient shaping, less consumption of samples in real-time, etc (Huang, et al., 2015). Optical sensors have been used as immune sensors as well for the detection of antigen antibodies (Liu, et al., 2017; Lu, et al., 2017). The assessment of physical parameters such as temperature, humidity, refractive index, strain

Table 1. Applications of Hydrophobin Proteins in Medical Biotechnology

Hydrophobin	**Organism**	**Applications**	**Reference**
SC3	*Schizophyllum commune*	Antifouling Coating	(Wösten, & Scholtmeijer, 2015) (Devine, & Murthy, 2019)
		Formulation of water insoluble drugs for oral administration	(Haas, et al., 2010)
HGFI	Mushroom *Grifola Frondosa*	Designing Biointerfaces (Antibacterial interfaces)	(Wang, et al., 2016)
		Detection of Antibody	(Duan, et al., 2020)
Vmh2	*Pleurotus ostreatus* PC15	Development of Antimicrobial Surfaces using a Hydrophobin Chimeric	(Sorrentino, et al., 2020)
		Immobilization of Antibodies	(Stanzione, et al., 2021)
HFBI	*Trichoderma reesei*	Formulation of a Florescent Dye	(Wang, k., et al., 2016)
		Enhanced Antitumor Efficiency of Curcumin-loaded PLGA Nanoparticles Coated with Unique Fungal Hydrophobin	(Sun, et al., 2020)
		Immobilization of enzymes by using Hydrophobins adsorbed on the substrate.	(Wösten &Scholtmeijer, 2015)

can be operated by S-tapered interferometers (Tian, et al., 2018; Li, et al., 2018). The factors like fabrication, structural ease give a potential benefit for S-tapered fibers for determining antigen-antibody interactions. Before biomolecular detection, the sensor fibers must be functionalized for immobilizing examined molecules on the fiber surface. The HGFI Nano layer self-assembles on a fiber surface that offers a platform to manage biocompatible binding which gives amphiphilicity, optical and biochemical properties. This is an extended antigen detection scheme, for other biomolecules owing to its high amount of integrating property, good specificity, real-time detection, and simple method. HGFI with concentration of 300g/ml forms a film

on the fiber surface which is self-assembled amphipathic that smoothly adsorbs antibody with a rapid response, fine repeatability, and no side effects.

HGFI modified-S-tapered fiber-based label-free immunosensor can work for the antigen-antibody detection procedure. By using the S-tapered fiber, real-time results could be observed for modification technique for surfaces and antigen identification. Experiment indicates that the fabricated S-tapered fiber functionalized with HGFI shows a detection limit of less than 1 g/ml for antigens.

The venerable capability of HGFI Nanofilm to bond antibodies with glass surfaces gives a distinguished way to implement a modified biocompatible interface. This reveals that antigens by interacting with HGFI, without chemical complexities and difficult methodologies, could make a stable immobilization on the desired surface.

I. Immobilization of Molecules and Cells

Hydrophobin functionalization methods for surface modification have proven to be a non-covalent alternative in this field because the other methods are intrinsically unstable (Sapsford, et al., 2004). The alteration of properties is related to the immobilization of the molecule as one of its components and can be achieved by adsorbing hydrophobins on the substrate (Qin, et al., 2007; Zhang et al., 2011). To elucidate them, here are some implications and strategies focusing on the function of hydrophobin. Self-assembled Hydrophobin II provides hydrophilicity of the bioactive surface proteins of mica and polydimethylsiloxane (PDMS), providing immobilization absorb the required molecule, thereby immobilizing the enzyme.

Thus, meeting the need for biosensors (Corvis, et al., 2005; Zhao, et al., 2007; Zhao et al., 2009). Furthermore, the human endothelial call for biocompatible surface binding was successful through immobilization using HFBI-coated media (Zhang et al., 2010). Studies have shown that HFBI assembly can be a versatile and suitable method for immobilization of biomolecules in different substrates with the hope that potential applications in microscopic networks, biosensors, immunoassays, and many more.

III. CONCLUSION

Hydrophobins are viable for many different applications. Hydrophobins, are cautious in the medical field due to their non-toxic, non-cytotoxic and non-immunogenic nature. The special properties of hydrophobins make many applications in science and technology possible. In addition, its unique properties contribute to improved foam stability. Hydrophobin coating appears to be an intermediate step in attaching cells and molecules to hydrophobic surfaces, as in biosensors. Nowadays, an in-depth study of drug formation using hydrophobins with regard to their surface characterization has become an interesting topic. Hydrophobins that self-assemble on hydrophobic/hydrophilic surfaces have absorbed medical and engineering applications by altering their biophysical properties (wetting. biocompatibility, and bioavailability). Genetic engineering allows proteins or peptides to cross-link and fuse with hydrophobins, thereby providing a common concept for surface modifications. Therefore, studying the binding sites at the molecular level is essential for the development of industrial applications based on foams and surface coatings. It is necessary to expand the production and isolation of hydrophobin protein. Currently, the low production of hydrophobins limits its widespread use, precluding its ability to meet sufficiently in small-scale applications such as biomedical devices and biosensors.

IV. ACKNOWLEDGEMENT

We would like to thank, the Department of Biotechnology, Sinhgad College of Engineering, for providing laboratories and research facilities.

V. DECLARATION

We declare that there are no conflicts of interest associated with this manuscript.

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