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Review Article

Smart Biofilms and Integrating Biosensors for Real-Time Monitoring: A review

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Abstract

Biofilms are complex mixtures of organic and inorganic substances that are secreted by various organisms belonging to multiple groups, including bacteria, archaea, and protists. Biofilms have been known to humans since the discovery of the microscope, but later, in 1978, they were named. Initially, they were found to be beneficial for human use, including water purification and sanitation. In the modern era, biosensors have been combined with biosensors via multiple computer-aided technology techniques for real-time monitoring. Real-time monitoring involves monitoring various levels of drugs and physiological parameters on the basis of integrated systems in the body. Smart biofilms, especially biofilms made of bacteria, are common in medicine. Multiple biosensor-based biofilms have been formulated and used to detect cardiovascular, neural, gastrointestinal, and numerous other diseases. They work efficiently. However, there is a need to improve their sensitivity and specificity by improving sensor technology. In the future, these biosensors may become familiar with eradicating serious health issues in patients with deadly diseases.

Abbreviation
DNA: Deoxyribonucleic acid
AV Leeuwenhoek: Antonie Philips van Leeuwenhoek
UV: Ultraviolet
AMR: Antimicrobial Resistance
ECG: Electrocardiogram
WHO: World Health Organization
RNA: Ribonucleic acid

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1. Introduction

Multiple natural phenomena help humans with different nutritional, medicinal, and developmental opportunities [1, 2]. Likewise, many phenomena formed by microbes can assist humans in these developmental opportunities [3, 4]. Since the invention of the microscope, biologists have discovered multiple fashions of microbes that have greatly helped humans. Biofilm formation is a significant development [5–7]. Biofilm formation is a property of bacteria and other microbes that gained importance in the late 21^{st} century but was discovered in the early 17^{th} century [8]. After the discovery of the microscope in the 17^{th} century, AV Leeuwenhoek reported that certain microorganisms form film-like colonies while observing dental plaque samples obtained from teeth [9]. However, biofilms were named biofilms in 1978, inspired by the aggregating property of microorganisms as biofilms [10].

Biofilms are colonial forms of complex groups of microorganisms, including archaea, bacteria, and fungi or protists, in which the extracellular substances of these organisms exhibit adhesion (stickiness) properties [11, 12]. They are characterized by their ability to adhere to biotic (living) surfaces, i.e., the surfaces of different cells and tissues, as well as to abiotic (nonliving) surfaces [13, 14]. The group of microorganisms secretes some unique substances, leading to a defensive matrix of extracellular polymeric substances [15]. The defensive matrix comprises different substances, such as proteins, polysaccharides, and DNA [16]. This protective matrix plays a vital role in the prolonged survival of microorganisms and protection of organisms from the host immune response, disinfectants, UV radiation, antibiotics, and desiccation [17, 18].

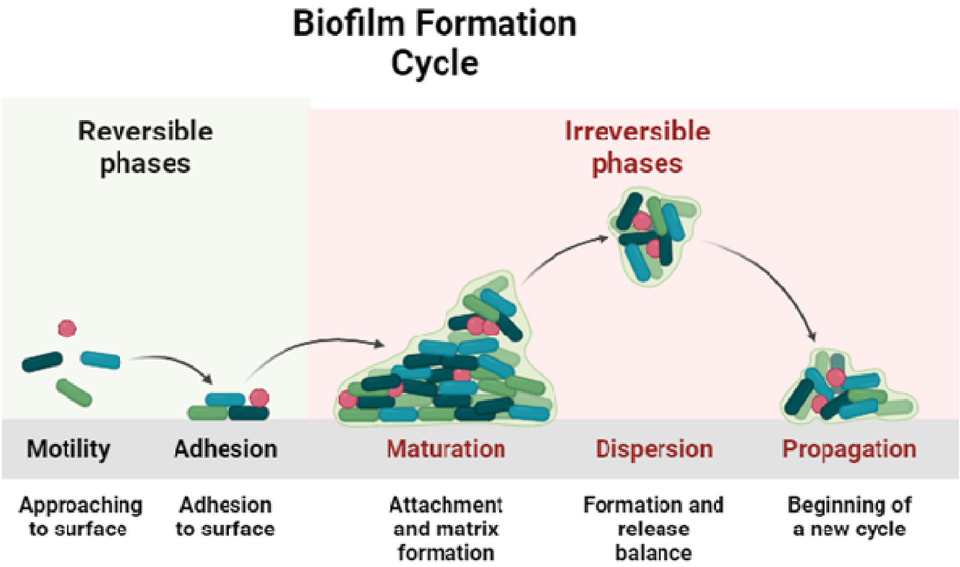

Figure 1: Biofilm formation cycle: stepwise path involved

Biofilm formation is a natural process for the survival of microbes. This process can also benefit humans [19]. Initially, the use of biofilms was limited to sanitation and wastewater treatment. They were subsequently designed for application in the medical industry [20, 21]. In the twenty-first century, the term "smart biofilm" was coined [22]. Smart biofilms are defined as those designed to have specific functions or properties, including the detection and sensation of particular ions and molecules, which help in real-time monitoring [23].

Real-time monitoring collects information about events that occur continuously [24]. Multiple techniques are used to perform real-time monitoring, of which biosensors integrated with smart biofilms are the best candidates [25]. They are used for multiple purposes, i.e., continuous monitoring, early detection of changes, improved sensitivity and accuracy, and instant feedback [26, 27]. Combining bacterial biofilms with biosensors is the best available option for real-time monitoring [28]. Biofilms act as sensing elements that can be used in detection devices [29]. Multiple combinations of biofilms are attached to transduction devices Figure 2.

2. Integration of Smart Biofilms With Biosensors

Multiple problems occur in maintaining the health of an organism. Antimicrobial resistance (AMR) is one of the most important factors to focus on [30]. Most of the time, antibiotics do not help fight pathogens due to increased AMR against the given drugs (antibiotics), which is associated with improper dosage and delivery issues [31]. A major issue associated with a drug is accurate estimation of its dosage inside the body and its delivery. The drug given to the body must be known as to how much drug has been delivered and what the levels of the drug inside an organism are [32, 33]. To make drug delivery more potent and helpful, real-time monitoring of drugs and their mechanism of action inside the host body are needed [34, 35]. For that purpose, we formulate biofilms on the basis of biosensors. Biosensors can detect highly sensitive activity, which is beneficial for real-time monitoring [23]. Several techniques can be used to integrate smart biofilms with biosensors for real-time monitoring in various fields [25].

Biofilms can be integrated inside the body such that they can be immobilized on or with the surface of the biofilm via covalent bonding, encapsulation, and adsorption (attraction of the solid surface molecules with gases) [36–38]. Another method of biosensor integration is the functional grouping of living cells (proteins, microorganisms, or viable cells) with nonliving materials to form biohybrid products [39, 40]. Combining living cells with synthetic products helps create a biohybrid system of biosensors that can conjugate with biofilms [41, 42]. An integral process of biofilm integration involves the formation of biofilms by producing molecules with the help of genetically modified organisms, which makes biosensors capable of detecting them [43, 44]. The integration of biosensors based on biofilms through this process is also known as biofilm engineering [45]. Modification of the surface of biosensors can help integrate into biofilm surfaces [43]. Biosensors

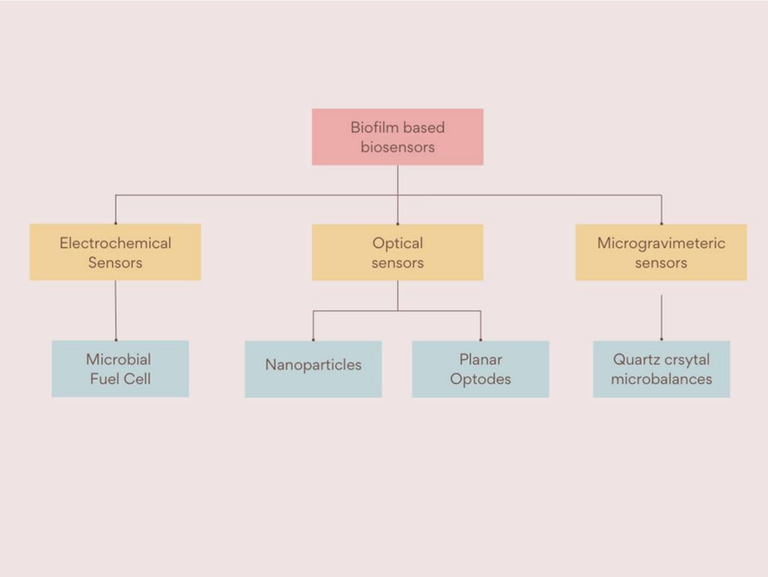

Figure 2: Various types of biosensors based on smart biofilms used in medicine

can be integrated with microfluidics (the use of devices that involve fluid channels that are smaller than 1 mm in size), in which the biofilm can grow quickly and can be detected by the biosensors [46, 47]. NPs can transport biosensors to biofilms and amplify signal detection [48, 49].

Similarly, liposomes are the best carriers of hydrophilic molecules because of their lipid bilayer structure. Nanoparticles based on liposomes have become excellent drug delivery vehicles [50]. Liposome-based nanoparticles can integrate biosensors with biofilms [51, 52].

The information mentioned above provides sufficient logic that the integration and delivery of biofilms are crucial for proper real-time monitoring on the basis of need [53, 54]. Optimizing for the appropriate integration route is necessary to properly function biofilm-based sensors inside the body for accurate real-time monitoring [55, 56]. Similarly, selecting delivery routes and vehicles is critical because of their role in the timely and accurate positioning of biosensors inside the body [57, 58].

3. Real-time Monitoring Via Biosensors in the Medical Field

Biosensor formation involves the encapsulation of biofilm-making microorganisms with sensors for real-time monitoring purposes [59]. The encapsulation of microorganisms has attracted researchers for the last decade [60, 61]. These encapsulated microorganisms effectively deliver biological substances, including hormones, probiotics, pain-killing agents, and cytostatic agents (for the treatment of cancer), to targeted body parts [62, 63]. Biohybrids can protect implanted biological substances against unwanted biological environments [64, 65]. Hence, the drug is delivered to the desired target of the body without any loss. To check the appropriate delivery system of the drug, we use biofilm-based biosensors that detect signals and provide real-time imaging of ongoing events in the body [66, 67]. There is a vast range of applications of biofilm-based biosensors in the medical field, some of which are as follows:

3.1. Biofilm-based Biosensors Used in Health Monitoring

Real-time health monitoring is essential for maintaining and screening the normal health of individuals and determining the accuracy of drug delivery procedures [68, 69]. Biosensors are used in many technologies for health monitoring purposes in the medical field [70, 71]. The biosensors used in electrocardiogram (ECG), pulse oximeter, and heart rate monitoring are discussed here. These medical fields are crucial because of the increasing risk of cardiovascular diseases worldwide [72–74]. According to WHO reports, it is estimated that over 17.9 million deaths occur because of heart failure or cardiovascular diseases around the globe [75]. Because of this tremendous risk of cardiovascular problems, smart real-time monitoring of all the events involved in heart failure problems is being conducted [76]. Below is a detailed overview of the biosensors and their use in ECG, heart rate monitoring, and pulse oximeters.

Biosensors Used in ECGs

Biosensors integrated with biofilms can be used for real-time monitoring of heart activity. ECG is the most commonly used technique for diagnosing and monitoring heart function status [77, 78]. Biosensors used in the monitoring of ECGs should have multiple properties, such as providing continuous electrical signals from the heart and providing insights into an individual's cardiovascular health [79]. The biosensors used in the electrocardiogram are of multiple types, i.e., optical, electrochemical, and mass types [79, 80]. Nanoparticle-based biosensors are also being used in ECGs, but their use is limited [81, 82]. Multiple biosensors, including the cardio chips, Savvy E.C.G., Neurosky BMD101, AD8232, etc., are being used [83, 84]. ECG biosensors can provide real-time monitoring, which helps detect the continuous activity of the heart [85]. This property aids in the quick detection of any abnormality or irregularity in heart activity [86]. The basic principle of the biosensors used for ECG is the configuration of the electrodes placed on the patient's chest to check the heart's activity [87]. These electrodes capture the electrical impulses (signals) generated with each heartbeat [88]. The signals captured from the electrical impulse transform into graphical ECGs [89]. These transformed graphical representations help us diagnose different heart conditions [90]. For the best graphical representations, we must provide the optimum frequency range for the electrodes, and high resolution is one of the important aspects [91]. The biosensors must precisely interpret the complex patterns of ECGs to obtain a graphical representation at a higher resolution [92]. Similarly, biofilm-based smart biosensors can monitor electrical pulses effectively [23]. Smart biosensors integrated with biofilms can be used to interpret ECG patterns precisely [93]. This will help us monitor whether the heart functions normally [94].

Pulse Oximeter And Heart Rate Monitoring Biosensors

Biosensors are used to measure the rate of oxygen saturation (the percentage of oxygen-saturated hemoglobin to total hemoglobin present in the blood) [95]. It is crucial in detecting cardiovascular problems [96]. Low oxygen levels in the blood can cause severe problems in the normal functioning of different body organs, including the heart [97]. This phenomenon leads to organ failure, and death occurs if the heart fails to perform its normal functions properly [98]. A pulse oximeter is a noninvasive electronic device used to measure an individual's oxygen saturation and heart rate [99]. Biosensors are pivotal in the formation of pulse oximeters [100]. MAX30102 is a biosensor mostly used to measure oxygen levels in the blood [101, 102]. MAX30102 can detect heartbeats per minute in a noninvasive and continuous manner [103]. This unique property of these biosensors is beneficial for real-time monitoring of cardiovascular systems [104]. The MAX30102 biosensor is widely known for its high sensitivity and maximum accuracy in measuring heartbeats and oxygen saturation levels in the blood [105]. These biosensors consume minimum power and provide the highest efficiency [106]. The basic working principle of these biosensors relies on the application of photoplethysmography (PPG) [107, 108]. PPG is an optical technique used to detect volumetric differences in the peripheral circulation of the blood [109]. Biosensors collaborate with pulse oximeters, which utilize PPG sensors that cast light on an individual's skin [110]. The light reflects the sensor with some variations because of changes in blood flow, which helps accurately detect heart rate and oxygen levels [111]. Likewise, smart biosensors integrated with biofilms can accurately observe oxygen saturation and heart rate [25, 112].

Table 1: Mechanisms of different biosensors, their applications, and challenges.

Sr no	Biosensor system	Applications	Mode of action	Highlights and challenges	Examples	References
1	Optical biosensors	Optical system-based biosensors are used to detect oxygen levels in blood, measure pH, and detect biomolecules, ions, and gases.	The sensing area is illuminated with the help of a light source, and the target analyte binds with the specific receptor. This binding results in a change in the optical signal, which is detected by the sensor.	They are very convenient, highly sensitive, more accurate, and low-invasive analyses. Some challenges to using optical biosensors are poor diffusion rate, low solubility, and sometimes cause toxicity.	Nanoparticles and Planar optodes.	[113]
2	Mechanical biosensors	They are widely used in medical studies, including disease monitoring, diagnostics, and toxicity studies. They can also detect changes in the viscosity and stiffness.	The central element (cantilever) used in the mechanical biosensors is very sensitive to biomolecules. The analyte binds with the receptor on the surface of the cantilever, resulting in a change of surface stress.	Low-invasive analysis, real-time monitoring, and in situ monitoring. calibration and validation are challenges when using such types of biosensors.	Interfacial tensiometry and rheometry	[114]
3	Electrochemical	These biosensors can detect the activities of enzymes and biotic (living) cells. They can also be used for diagnostic purposes.	The electrochemical reaction occurs when the analyte binds with the receptor present on the sensor surface. Change in the current because of the chemical reaction is detected and further processed for quantification.	They are susceptible and easy to use, but the real challenge to use them is their invasiveness, and they can easily compromise the structure of the biofilm.	Microelectrode probes	[115]
4	Immunoassay biosensors	Immunoassay biosensors detect bacterial activity in edibles (milk, cheese, water, etc.). They have shown a more significant effect on *Salmonella* spp.	The principle of immunoassay biosensors is antigen and antibody binding. This binding creates detectable signals, and then these signals are amplified with the help of various techniques like ELISA and then detected by biosensors.	They are very sensitive and provide quick, real-time responses. However, antibody specificity and cross-reactivity are significant issues in dealing with immunoassay biosensors.	Immunochroma tographic assay (ICA) biosensors, ELISA biosensors, and QCM.	[116]

Sr no	Biosensor system	Applications	Mode of action	Highlights and challenges	Examples	References
5	Gravimetric biosensors	They can detect the presence of bacteria in meat and water, specifically *E. coli.*	The target molecule binds with the analyte, which causes a change in the mass on the sensor's surface. The change in the mass results in the alteration of resonant frequency, which will be used to quantify the mass.	They are highly sensitive and selective, best for real-time monitoring, and have the potential for array-based detection. The challenge to using these biosensors is that they require precise control of humidity, temperature, and environmental factors	Quartz Crystal Microbalance (QCM) and Surface Acoustic Wave (SAW)	[117]
6	Metagenomic biosensors	These biosensors are helpful in the genetic analysis of different microbes which are present in each sample	DNA or RNA is extracted from the sample and amplified; after that, we hybridize the DNA to bind it to the specific genetic sequence. To detect the microbial community, we compare the results with a reference database.	These biosensors are very helpful in detecting microbial species but sometimes exhibit insufficient expression.	16S RNA biosensors and microbial community analysis biosensors	[118]

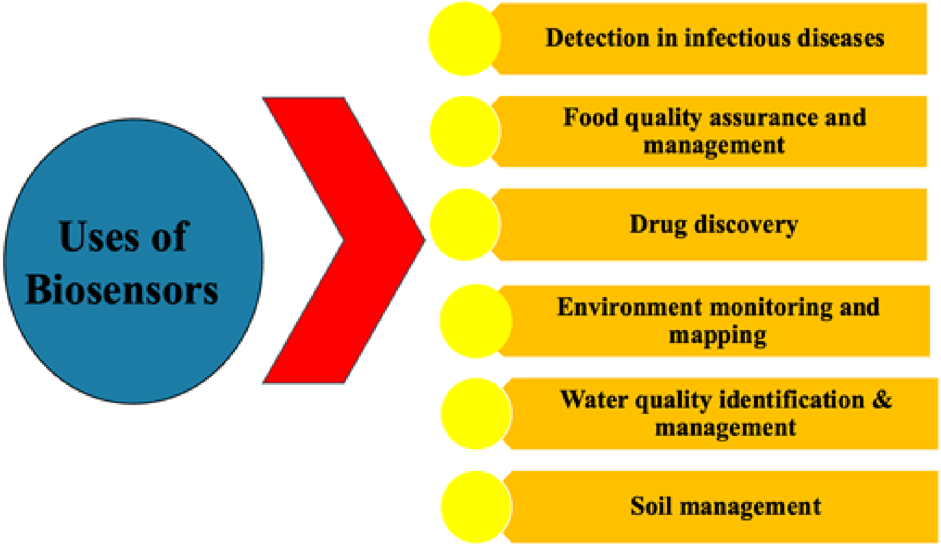

Figure 3: The uses of biosensors are shown in this flowchart

4. Role of Biofilms In The Food Industry

Biofilms are microbial communities generated by one or more species within an extracellular matrix. This matrix's composition varies on the basis of the food production environment and the species involved. Both bacteria and fungi are capable of forming biofilms. The presence of multiple bacterial species within a biofilm enhances its ability to attach to surfaces, yielding significant ecological benefits, even in species lacking specialized fimbriae. Notably, mixed biofilms are relatively resilient to disinfectants such as quaternary ammonium compounds and other biocides [119].

Primarily composed of polysaccharides such as cellulose, proteins, and foreign DNA, the extracellular matrix adheres to hard surfaces, such as food industry equipment; transport vehicles; storage areas; and soil and biological structures such as vegetables, meat, bones, and fruits. This matrix is crucial for biofilm persistence in the food sector, as it creates nutrient and oxygen gradients, contains nutritional enzymes, facilitates cell communication, and protects cells from harmful chemicals. Biofilm formation on various surfaces, including raw milk tanks, pipelines, butter centrifuges, cheese tanks, pasteurizers, and packing tools, can lead to pathogenicity, metal surface corrosion, and changes in organoleptic properties. This issue is particularly significant in the dairy industry, where different processes and structures provide substrates for biofilm formation across various temperatures and colonizing species [120].

Biofilms may include psychrotrophic *Pseudomonas* spp. and thermophilic *Geobacillus stearothermophilus*. Pathogenic species such as *Aeromonas hydrophila*, L. *monocytogenes*, S. *enterica*, and *Vibrio* spp. can form biofilms on fresh fish products, posing substantial health and economic risks. Biofilm-forming bacteria can exhibit chromosomal differences in critical genes, resulting in distinct biofilms in various environments. The eradication of biofilms in the food sector presents a challenge due to the complexity of the affected habitats and the diversity of bacterial species involved [121].

Limitations

Smart biofilms are a modern method for developing biosensors for real-time monitoring, and they are effective in providing data from body systems [25]. However, some issues exist when using smart biosensors integrated with biofilms. The formation of suitable biofilms is a complex process, as biofilms are very fragile and unstable [122]. They can be disrupted at any time when they are integrated with smart biosensors [23]. Some biofilms cannot provide accurate real-time monitoring of different body systems of an individual [123]. They can interfere with other microorganisms that are not targeted, but some biosensors are not very sensitive or selective [27, 124, 125]. Another drawback of biofilm-based smart biosensors is the high maintenance cost, as they require maintenance and regeneration regularly [56, 126]. The use of biofilm-based biosensors is also limited because of the invasive nature of some biofilms in body systems [127, 128].

5. Conclusion

In conclusion, integrating biosensors within smart biofilms significantly advances real-time monitoring technologies. These innovative systems provide unparalleled insights into the dynamic processes of biofilms, providing critical data for diverse applications, from environmental monitoring to medical diagnostics. The synergy between biofilm robustness and biosensor precision allows for continuous and accurate detection of biochemical changes, enhancing our capacity to respond swiftly to microbial activities. Future developments in this field are anticipated to refine sensor sensitivity, improve data integration, and broaden the range of detectable parameters, thereby advancing biofilm research and practical applications. Adopting these smart biofilm technologies will undoubtedly pave the way for more efficient and responsive solutions across health, industry, and environmental management.

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