Photopolymerization as a promising method to sense biorecognition events

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This work addresses the main topics related to the photopolymerization process to develop and to monitor the analytical signal derived from biorecognition assays. We review basic aspects of photopolymerization, together with the nature and the reactivity of the chemicals involved. Focusing on DNA and protein determination and medical applications, we envisage as relevant photopolymers that generate biocompatible hydrogels.

We also review the major characterization techniques applied to ascertain the composition of the products formed and to monitor the progress of the reaction. In this sense, we present optical microscopy, fluorescence imaging, ATR-FTIR spectroscopy and profilometry measures.

Finally, we discuss analytical examples and applications of protein-to-protein, protein-to-antigen and DNA-hybridization photopolymerization. We demonstrate implementation of CD/DVD player technology to the detection and quantitation of such biointeractions, highlighting the advantages of this powerful methodology in biosensing.

Keywords: Amplification; ATR-FTIR spectroscopy; Biosensing; Biorecognition assay; Characterization; Fluorescence imaging; Initiator; Molecular recognition; Photopolymerization; Protein determination

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1. Introduction

1.1. Methods for biointeractions detection

Detection of biointeractions is a broad interdisciplinary research field that connects analytical, biological and surface chemistry to medical, agricultural, electronic, environmental or agricultural sciences. A specific biointeraction is assumed to take place with high affinity between the appropriate receptor and the target substance [1] (*e.g.*, between proteins or DNA strands). The goal is to develop a biorecognition system that generates a specific signal with a very low analyte concentration present in the sample.

The main detection strategies when monitoring biointeraction events are referred to as labeled and label-free. Label-free methods include those that do not require the presence of any tag to generate a readable signal. The most outstanding approach has been based on the analysis of the interaction between the evanescent field and biomolecules {*e.g.*, surface-plasmon resonance (SPR) technologies [2]}. Other label-free techniques (not based on evanescent field measures) include ellipsometry, atomic force microscopy or quartz-crystal microbalance, but their use is limited. More recently, approaches [e.g., biophotonic sensing cells (BICELLS)] were reported to be a promising technology for the quantification of biointeractions [3]. However, the full use of these new techniques is still undergoing development.

Regarding methods using labels, the molecule marked with the tag is commonly referred to as a tracer, and it has one or more labels pending. When these labels receive the appropriate stimulus, a final readable signal (colorimetric, fluorescent, electrochemical, etc.) is generated. With light-excitable labels, a high quantum yield is desirable to ensure an intense signal, while the addition of an appropriate reacting substrate is needed with catalytic or enzyme labels. Fig. 1 exemplifies the steps followed to obtain a readable signal from a labeled biointeraction assay.

The development of diagnostic tools that target human diseases through the analysis of pathogens or genetic biomarkers

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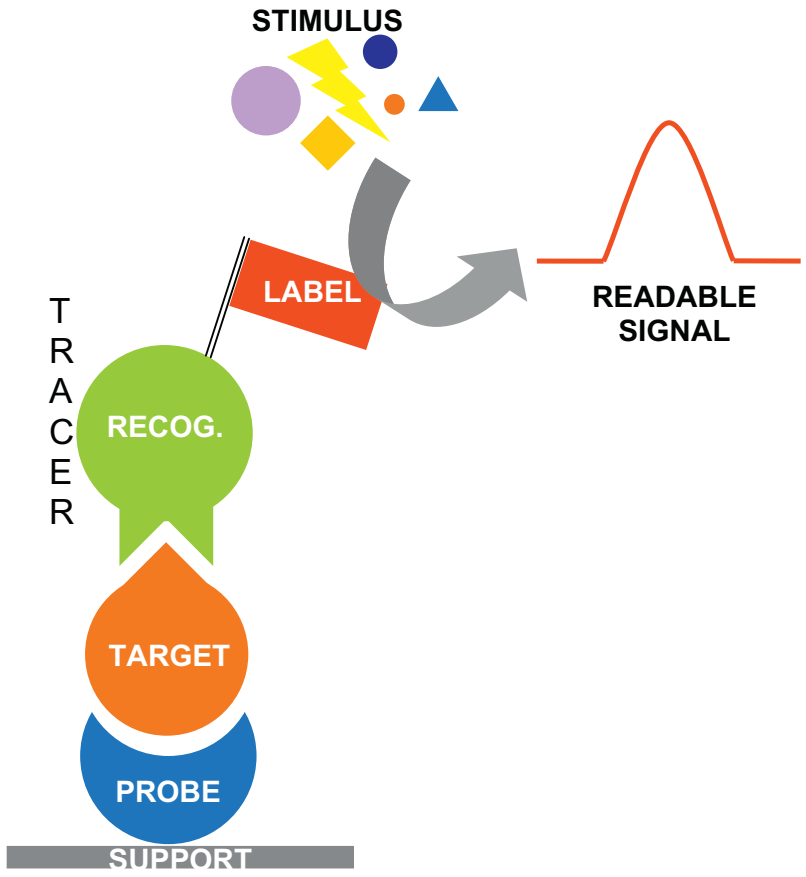

Figure 1. Labeled biorecognition format.

has been the motivation behind much of the effort made in this area. DNA diagnostic possibilities have been around since the early 1970s, but it was not until the 1980s that use of the polymerase chain reaction (PCR) spread in routine laboratories [4]. Later in the 1990s, the development of DNA chips became one of the most relevant gene technologies [5]. When determining specific gene sequences [6], hybridization with a target or labeled probe is the most common method employed.

When interest is in proteins, catalyzed methods employing enzymes are mostly used [e.g., alkaline phosphatase or horseradish peroxidase (HRP)] to develop colored products when appropriate substrates are added [7]. As enzymes are required to produce the amplification effect from a single (or a few) initial interaction(s), such approaches are referred to as enzymatic amplifications. Nevertheless, non-enzyme-mediated amplification can also be achieved with tracers bearing nanoparticles (gold or silver in nature) [8], fluorophores [9] or radioactive moieties [10]. Certain limitations prevent the widespread use of this type of amplification, mainly due to analyzers being expensive and non-portable, or to the intrinsic instability of reactants.

Another labeled amplification strategy, which has not been studied so much, is based on formation of polymer or gel deposits. It is a rapid procedure capable of offering the visual discrimination of the presence of labeled molecules in the sample. Several approaches have been provided to determine targets by polymer deposition, and some consider chemistries to generate radical initiators [11], while others explore how to accentuate polymer growth by either using macroinitiator substances [12] or forming self-assembled monolayers of labeled molecules [13]. Light-induced polymerization has become a relevant field of research, and its applications involve different practical uses. First reports on the photopolymerization topic were published in the early 1900s. Since then, interest in this area has grown, and more than 8000 reports have been published since the year 2000 [14].

Signal amplification by light-mediated polymerization has attracted attention since reported results first appeared [15]. So-called non-enzymatic signal amplification (NESA) [16], or photopolymerization reaction (PMR), allows the formation of polymeric deposits exclusively in the regions where the reactive mixture (incorporating one or more types of monomer) comes into contact with light-sensitive labels.

In general, photopolymerization amplification offers potential advantages if compared to enzymatic