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Spin coated chitin films for biosensors and its analysis are dependent on chitin-surface interactions

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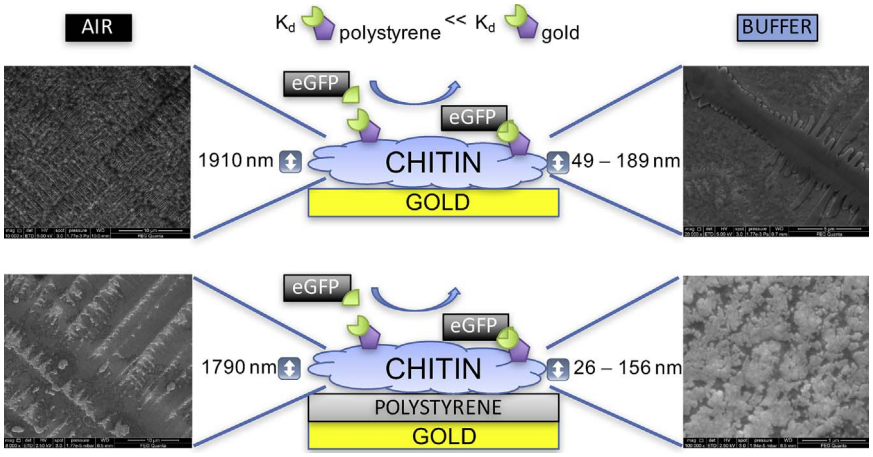
GRAPHICAL ABSTRACT



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ABSTRACT

Chitin, abundant in nature, is a renewable resource with many possible applications in bioengineering. Biosensors, capable of label-free and in-line evaluation, play an important role in the investigation of chitin synthesis, degradation and interaction with other materials. This work presents a comparative study of the usefulness of a chitin surface preparation, either on gold (Au) or on polystyrene (PS). In both cases the most common method to dissolve chitin was used, followed by a simple spin-coating procedure. Multi-parametric surface plasmon resonance (MP-SPR), modeling of the optical properties of the chitin layers, scanning electron microscopy, and contact angle goniometry were used to confirm: the thickness of the layers in air and buffer, the refractive indices of the chitin layers in air and buffer, the hydrophobicity, the binding properties of the chitin binding domain (CBD) of *Bacillus circulans*, and the split-intein capture process. Binding of the CBD differed between chitin on Au versus chitin on PS in terms of binding strength and binding specificity due to a less homogenous structured chitin-surface on Au in comparison to chitin on PS, despite a similar thickness of both chitin layers in air and after running buffer over the surfaces. The use of the simple method to reproduce chitin films on a thin polystyrene layer to study chitin as a biosensor and for chitin binding studies was obvious from the SPR studies and the binding studies of CBD as moiety of chitinases or as protein fusion partner. In conclusion,

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stable chitin layers for SPR studies can be made from chitin in a solution of dimethylacetamide (DMA) and lithium chloride (LiCl) followed by spin-coating if the gold surface is protected with PS.

1. Introduction

Chitin, along with its derivatives, is an increasingly popular biomaterial due to several useful properties, including biodegradability, low immunogenicity, non-toxicity, and biocompatibility [1–3]. Chitin is the main constituent of the exoskeletons of Arthropods, and is also found in fungal cell walls [3], cocoons of moths [4], diatoms, coralline algae, Molluscae, Protists, Polychaetes, within fish, [5] and marine and freshwater sponges [6,7], and it is the second most abundant biopolymer after cellulose [3]. Chitin easily be processed in to a number of derivatives, *e.g.* chitosan; these have shown promising applications in a broad range of areas, including food science, medicine, agriculture [8], and the biomedical field [9].

Chitin is an insoluble linear polymer of aminoglucopyrans with extended linear chains of ß-1,4-linked *N*-acetylglucosamine residues (Fig. 1), and three polymorphs of this polysaccharide are known [10,11]. It exists in three structural forms: α-, ß- and γ-chitin that have different mechanical properties depending on the arrangement of the polymer chains. The α-form represents an alternating antiparallel arrangement of polysaccharide chains (e.g. crustaceans [12]), the ß-form is a parallel chain arrangement (found e.g. in squid), and in the γ-form two parallel chains statistically alternate with an antiparallel chain (found e.g. in fungi) [14],13]. The α-chitin isomorph is most abundant and formed by: enzymatic polymerization [14], in-vitro biosynthesis [15,16], and recrystallization from solution [17]. Chitin is more than 50% acetylated, while chitosan is primarily deacetylated [11]. Shrimp chitin has been reported to be > 95% acetylated [18].

The use of chitin, and to a greater extent, chitosan films as constituents of biosensors have gained interest as the direct study of chitin is important in gaining insight into enzyme degradation pathways, interactions with natural composite materials (e.g., proteins and polysaccharides), and mechanisms behind biocompatibility and biofunctionality [19,20]. Chitin and chitosan are useful transducer surface modifiers and found many applications as a electrochemical (bio-)senor component, for example in Pt disk electrodes for choline-sensing, supercapacitors [21] for nucleic acid analysis (e.g. DNA microarrays), immunosensors (e.g. biomarker analysis), enzyme activity, and detection of trace elements (for comprehensive reviews please read Suginta et al. [11] and Kim et al. [22]). In addition, chitin can be easily modified via its hydroxyl and acetylamido groups for future biosensor advancements. Chitin has advantageous mechanical properties, such as a high mechanical stability (tensile strengths between 38–146 MPa have been reported) and a high thermal stability (e.g. shrimp shell chitin's thermal degradation is between 290–440 °C), though these properties are underused in industry [5].

In order to create chitin surfaces with representative properties similar to its natural structure, chitin needs to be dissolved first, and then deposited onto the sensor surface. One approach is the use of biomimetic methods, for example to use naturally occurring biocomposites. For instance, instead of spin-coating chitin on a silicon surface [23], silica-chitin based biocomposites, or other biocomposites, could be used and it is available from various sources and can even be obtained in-vitro [24–28]. A newly emerging area is the use of natural sources from environments where life is found under extreme heat or pressure, also referred to as extreme biometrics [5]. The solubility of chitin depends on the pH and ionic strength of the solvent used and is influenced by the percentage and distribution of acetylated (and deacetylated) moieties along the backbone of the polysaccharide [11]. It is high in crystallinity and does not dissolved in hot water [27]. The most common method to solubilize chitin is the use of dimethylacetamide (DMA) and lithium chloride (LiCl), already demonstrated at the beginning of the last century [29,30]. Recrystallizing chitin from a 5% LiCl in DMA solution by precipitation had an essentially crystalline form as the chitin prior to solution (be it the α- or the ß-form), though the α-form can be slightly disordered [23]. Alternative methods for the solubilization of chitin have been reported, such as using other polar solvents: LiCl/*N*-methyl-2-pyrolidone, chloride dihydrate/methanol [31], and hexofluoroisopropanol (HFIP) [32], or ionic liquids, such as 1-allyl-3-methylimidazolium bromide ([Amim]Br), [C_2mim][OAc], and 1-butyl-3-methylimidazolium acetate [31]. Thin chitin films are interesting for studies of chitinases which can convert chitin into cheap products such as *N*-acetylglucosamine for biomedical use [2]. Chitin films on silica surfaces by means of spin-coating were 50 nm thin, but rough [33]. Furthermore, a smooth, thin layer of amorphous chitin was obtained after the spin coating of trimethylsilyl chitin onto a silica or gold surface after which the films were regenerated to amorphous chitin [2].

One analytical method, which is highly compatible with thin layers is surface plasmon resonance (SPR). It is a highly sensitive, non-invasive, and label-free technique that is used extensively for surface interaction studies between polymers and proteins [34]. In addition, it has been used to evaluate a chitin-coated surface in the past using ionic liquids as a solvent [2]. However, no simple protocol for the preparation of thin chitin layers using DMA/LiCl as solvent on SPR gold sensors has been established yet.

In this contribution, we demonstrate that the precipitation of chitin, derived from shrimp shell, using the common DMA/LiCl solvation method onto a gold surface for subsequent SPR analysis poses a challenge due to the surface properties of gold, but can be protected by an additional coating of polystyrene prior to chitin coating. The composition, thickness, available binding surface, surface hydrophobicity, and ability to interact with a CBD of chitin were characterized with multi-parametric surface plasmon resonance (MP-SPR), scanning electron microscopy (SEM), and contact angle goniometer with two different SPR sensors: bare gold and a polystyrene coated gold. The binding constants of a chitin binding domain fused to a split intein and its split intein counterpart were determined for both systems, as well as the differences in behavior of chitin on gold versus polystyrene after spin coating.

2. Methodology

2.1. Materials

Unless specifically mentioned all commercial chemicals (dimethylacetamide (DMA), LiCl, hexofluoroisopropanol (HFIP), EDTA, DTT, NaN_3, triethanolamine hydrochloride (TEA), Tris, sodium chloride, ampicillin, Isopropyl β-D-1-thiogalactopyranoside (IPTG), magnesium

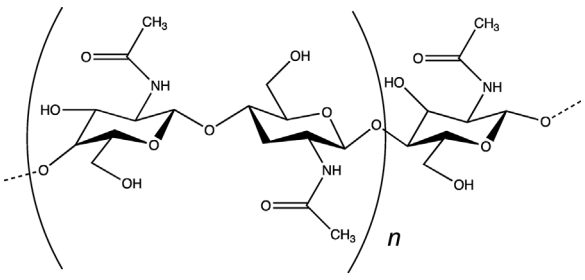

Fig. 1. Chemical structure of chitin. Chitin polymers derived from shrimp shell have a typical degree of polymerization of 493–605 [64].