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Antiviral effects of polyphenols: Development of bio-based cleaning wipes and filters

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Polyphenol molecules play multiple essential roles in plant physiology such as defences against plant-pathogens and micro-organisms. The present study reports a chemical modification of the surface of non-woven cellulosic fibre filters (Kimwipes®) by fixing polyphenol in order to confer them antiviral properties. The grafting of the non-woven fibres by the antiviral entity was performed using laccase. T4D bacteriophage virus of *Escherichia coli B* was used as virus model. Catechin polyphenol was tested as antiviral entity. Proteomic experiments were performed to quantify the potential protein target of catechin on viruses. When the modified filter was in contact with the viral suspension a large improvement in the reduction of the viral concentration was observed (5-log after 1 h). Thus, we propose that this material could be used as virucidal wipes for the virus elimination from contaminated surfaces. Virus filtration experiments were performed by spraying an aerial suspension of T4D bacteriophage virus through the designed filter. The best virus capture factor f (ratio of upstream to downstream virus contents) was obtained when using 2 functionalized filters ($f = 2.9 \times 10^3$). When these 2 layers were placed inside a commercial medical mask in place of its cellulose layer (Kolmi M24001 mask) ($f = 3.5 \times 10^4$), the f ratio then reached 2.6×10^5 for 2 h of filtration. Based on these results, this novel bio-based antiviral mask represents a significant improvement over conventional medical masks.

1. Introduction

The removal of pathogenic viruses from the environment represents nowadays a major public health concern. The recent viral epidemics observed in the world (Ebola, HIV, A (H5N1) avian influenza, SARS) highlight that there is a real need for protection against emerging viral diseases. In most cases, conventional protection methods such as facial medical masks are not effective because the pore sizes of the filter material are often too large to allow virus removal (Derrick et al., 2006). Only expensive face-piece respirators (FFP2 or FFP3 type by European standards or N95 type by US standards) currently provide effective protection against airborne viruses (Derrick et al., 2006). Antiviral compounds addition to the surface of a low-cost filter, in order to built affordable and easy-to-produce antiviral filters, constitutes a valuable alternative. Surface cleaning represents also an essential disinfection procedure for virus elimination from contaminated surfaces. For instance, the fomites represent potential sources of viruses transmission inside or outside hospital (Brady et al., 1990). The cleaning by modified wipes containing antiviral compounds is therefore of major interest.

Polyphenols are secondary metabolites produced by all higher plants. They play multiple essential roles in plant physiology such as defences against pathogen plants and animals, and as response to the various abiotic stress conditions (Daglia, 2012).

Numerous healthy properties of polyphenol on human organisms (mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, antibacterial, antifungal and antiviral agents) have been already highlighted. It has been stated since a long time by Japanese tobacco growers that a spray containing tea extracts is effective against the tobacco mosaic virus (Hara, 2001). The antiviral properties are correlated to the presence of catechin and theaflavin molecules that bind to the virus nucleic acids. Catechin, flavan-3-ol, is a polyphenol molecule belonging to the flavonoids family. Its antiviral activities have been described extensively in the literature. Thus, Song et al. (2005) highlighted the remarkable activity of catechin extracted from green tea on influenza viruses A/H1N1, A/H3N2 and B virus. The compounds

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trigger hemagglutination and neuraminidase inhibition activities, and lead to the suppression (when used at high concentration) of the viral RNA synthesis and the alteration of viral membranes physical properties. Catechins from green tea are also able to inhibit in vitro growth of influenza cells by acidification of lysosomes and endosomes (Imanishi et al., 2002). Catechin derivatives such as epigallocatechin gallate, show also activities against HIV, on the virus replication (Fassina et al., 2002) and on the reverse transcriptase (Nakane and Ono, 1990). In addition, they are effective against flu virus by binding to the hemagglutinin (Nakayama et al., 1993).

Only few studies describe the polyphenol compounds addition into wipes or masks in order to introduce antimicrobial properties. US patents 5888527 (Kazuo and Yoshikazu, 1999) and 5747053 (Nashimoto et al., 1998) describe the preparation of antiviral masks impregnated by tea polyphenolic antioxidants (catechin and theaflavin) that inactivate viruses (by inhibiting the viral replication and by deteriorating the physical properties of the virus membranes).

The aim of the present work was to modify commercial cellulose non-woven layers (Kimberly-Clark®) with catechin polyphenol to provide the antiviral property. The catechin grafting on the cellulose material was performed using laccase as enzymatic coupling reagent. The experiments on viruses were performed on suspensions of T4D bacteriophage of *Escherichia coli*. The modified layers were tested as wipes and as filters. The wipes were tested on the suspensions. The filtration experiments were performed by spraying the aerial suspension through the filters, using a sterilized experimental set-up developed in the laboratory. The most effective filters were placed inside medical masks and tested against airborne viruses.

2. Materials and methods

2.1. Wipes and filters

Non-woven cellulose materials were used as support for polyphenol grafting. They were purchased from Kimberly-Clark® (Kimwipes® Lite KWL; density: 29 g cm^{-2}; pores size: 25 μm; thickness: 113 μm).

2.2. Reagents

Catechin (>98.0%) was used as polyphenol without further purification (Sigma–Aldrich, France) (Fig. 1). Laccase from *Trametes versicolor* (concentration > 20 Units mg^{-1}), sodium tartrate and Tween 80 were also purchased by Sigma–Aldrich, France.

2.3. Grafting

Catechin grafting on cellulose was performed using laccase. This enzyme allows mild oxidation of phenol molecules and polysaccharides by the mean of copper ions fixed on the active sites of the enzyme (Riva, 2006) (Fig. 2).

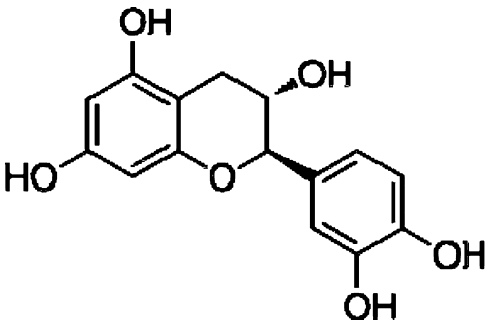

Fig. 1. Formulae of catechin.

Grafting treatments were carried out in glass Petri dish (5 cm of diameter) by immerging cellulose samples (5 cm of diameter, 50 mg) in sodium tartrate buffer (4 cm^3, 50 mM, pH 4) supplemented with Tween 80 at 0.05% (w/v), laccase (>80 Units) and catechin (3.5% w/v for activities measurements and 1% w/v for filtration experiments). Samples were incubated at 50 °C at 30 rpm during 4 h in the dark. Control cellulose samples were treated under similar conditions without polyphenol and laccase enzyme. Then, the cellulose wipes were washed extensively in distilled water for 2 h with shaking (30 rpm) and finally air-dried over night at room temperature (Fillat et al., 2012).

2.4. Characterization of the grafting

The efficiency of the grafting reaction was checked by FTIR measurements using a Nicolet Avatar 360 FTIR (Thermo Scientific, Courtaboeuf, France). For each measurement, series of 128 scans were collected with a 4 cm^{-1} resolution over the 650–4000 cm^{-1} range at room temperature. The spectra of the samples were compared before and after grafting treatment.

The grafting efficiency was also assessed by measurement of the changes of colors of the samples after treatment using the CR-200 Minolta Chromameter (Colombes, France). The samples color was described according to the CIE $L^*a^*b^*$ three dimensional model. L^*, a^* and b^* are the coordinates in a cylindrical color space of the perceived color, where L^* is the lightness (black and white), a^* the red-green and b^* the yellow-blue sensations. The chroma (C^*) was also used as optical parameter. It represents the perpendicular distance from the lightness axis (Fillat et al., 2012):

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

The efficiency of the grafting was also measured by contact angle measurements. This method is usually used to characterize surface modifications after treatment (Hossain et al., 2009). It was used to highlight the changes in the hydrophily of the cellulose support after grafting. The measurements were performed with ultra-pure water (milliQ Water-System, resistivity 18 MΩ cm^{-1}) and glycerol 99% (Sigma–Aldrich, France) as test liquids at room temperature and relative humidity. Contact angles (θ) were measured using

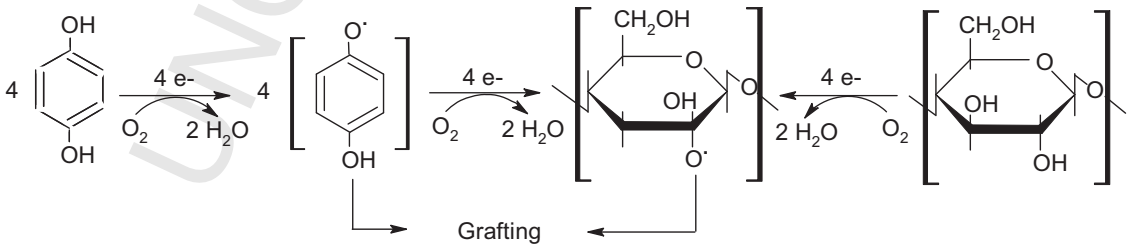

Fig. 2. Mechanism of action of laccase enzyme on polyphenol and cellulose.

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