This article can be cited before page numbers have been issued, to do this please use: D. Versace, J. Babinot, J. Malval, J. Lalevée, P. Mazeran, M. Condat, S. Abbad Andalloussi, I. Kang, F. Spillebout and S. Tomane, RSC Adv., 2016, DOI: 10.1039/C5RA25267A.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Development of photoactivable glycerol-based coatings containing quercetin for antibacterial applications


The development of new antibacterial coatings (against *Escherichia coli* and *Staphylococcus aureus*) using a natural dye, quercetin, according to a green chemistry process was investigated. Quercetin was used as both a photosensitizer and antibacterial agent. The synthesized material was developed according to a cationic photopolymerization process under light irradiation. The photosensitizing mechanism involving quercetin and an iodonium-based cationic photoinitiator was described for the first time according to steady state photolysis and fluorescence experiments. The resulting coatings showed excellent adhesion on stainless steel plate as demonstrated by nanoindentation and scratch tests, with a high thermal stability up to 375°C. Finally, a primary investigation was conducted to assess the antibacterial properties of the glycerol-derived coatings against *Escherichia coli* and *Staphylococcus aureus* under light illumination. Electron Paramagnetic Resonance spectroscopy confirmed the generation of reactive oxygen species, such as singlet oxygen, which is responsible for inhibiting bacteria proliferation.

1. Introduction

Contamination by microorganisms is of great concern in many fields such as health care products, medical devices, hospital surfaces/furniture, or surgery equipments. This problem is associated with an annual mortality of many thousands of people in the US and a constant increase of health-care costs. The lack of studies prompted us to develop new antibacterial coatings based on a natural dye, thus the originality of this article.

2. To solve the problem of bacteria resistance, the development of new antimicrobial systems in the biomedical industry for fighting infections has attracted considerable attention. The main strategies are based on the decrease of the capacity of bacteria to attach to a surface, the lethal contact, which induces the biochemical death of bacteria, the biocide leaching and finally the disinfection of surfaces using coatings that produce reactive oxygen species (ROS) upon light activation, also known as photodynamic inactivation (PDI). In this last method, the light excitation of a photosensitizer (PS) generates ROS which subsequently cause cell death. In this context, PSs do not have to penetrate the bacterium or even come in contact with the cell to be effective. Such antibacterial surfaces that work according to the PDI principle could help to reduce the transmission of multi-resistant microorganisms, which is of great importance in hospital hygiene, and can offer a constant prevention of microorganism adhesion and proliferation on any surface. Some investigations have already been performed essentially with porphyrins and phthalocyanine derivatives, hydroxyethyl Michler’s ketone, toluidine blue, eosin Y, methylene blue or rose bengal. However, the use of natural dyes instead of the aforementioned synthetic dyes is an interesting environmental challenge. To our knowledge, there is no investigation on the synthesis of antibacterial coatings using natural dyes which could act as a “bacteria killer” according to the PDI process. This lack of studies prompted us to develop new antibacterial coatings based on a natural dye. Thus, the originality of this...
study relies on two points: i) the use of a natural flavonoid dye, quercetin, which could be used as a reactive agent with dual functionality, i.e., an antibacterial promoter and a photosensitizer under light activation, and ii) the photochemical studies of (quercetin/iodonium salt) photoinitiating system (used for the initiation of the polymerization and the synthesis of the coatings) which have never been investigated yet.

Quercetin (3, 5, 7, 3’, 4’-pentahydroxyflavone) is one of the most abundant flavonol-type flavonoids found in many common fruits and vegetables (apple, grape, lemon, tomato, onion, etc.), beverages (tea, red wine), olive oil, and propolis from the bee hives. Quercetin has many health-promoting effects, including anti-inflammatory and anti-allergic effects, as well as the protection against cardiovascular health and cancer risk. In addition, it has been reported that quercetin enhances the antiviral activity owing to its strong antioxidant action and can be used as a skin antioxidant protector against UV radiation.

This investigation presents a simple route to efficiently synthesize an antibacterial coating derived from quercetin and glycerol monomer, using environmentally friendly method (photochemistry process), followed by a complete study of the (quercetin/iodonium salt) photoinitiating system to perform the synthesis of the quercetin-containing glycerol coatings. The first part of this study focuses on the photochemical properties of the photoinitiating system (quercetin/iodonium salt) using steady state fluorescence and electrochemistry experiments. In the second part, the thermal and mechanical properties of the quercetin derivated coatings were characterized by thermogravimetric analysis, nanoindentation and scratch tests. In a third part, the antibacterial properties of the coatings against *Escherichia coli* and *Staphylococcus aureus* were evaluated and correlated with the ability of the residual quercetin embedded in the film to photogenerate singlet oxygen.

### 2. Results and discussion

#### 2.1 Synthesis of the glycerol triglycidyl ether (GTE).

This monomer was synthesized using a two-step procedure. In the first step, glycerol was converted to triallyl glycerol by reacting it with allyl bromide and sodium hydride in dry DMF via a S$_2$2 reaction. After purification, the structure of the molecule was confirmed by $^1$H nuclear magnetic resonance (NMR) spectroscopy with the appearance of all signals at 5.91, 5.27 and 5.16 ppm, respectively (Figure 1A). The double bonds of triallyl glycerol were subsequently epoxidized using m-CPBA in CH$_2$Cl$_2$. The reaction was monitored by $^1$H NMR spectroscopy. Figure 1B shows the total disappearance of the ethylenic signals along with the appearance of signals related to epoxide moieties at 3.14, 2.78 and 2.59 ppm, respectively. The $^1$H NMR spectrum of glycerol triglycidyl ether appears more complex than that of triallyl glycerol. This is due to the non-stereospecificity of m-CPBA epoxidation, which leads to the formation of isomers.

#### 2.2 Photophysical fingerprint of quercetin

Figure 2 displays the normalized absorption and fluorescence spectra of quercetin in acetonitrile. The low energy side of the absorption spectrum is dominated by a distinctive band at 370 nm with moderate intensity ($e_{max} \sim 19000 \text{ M}^{-1} \text{ cm}^{-1}$).

![Normalized absorption and fluorescence spectra of quercetin in acetonitrile.](image)

The fluorescence spectrum shows a maximum located at 535 nm leading to a significant Stokes shift of approximately 8350 cm$^{-1}$. Such a large Stokes shift is a clear indication of a significant electronic change between ground and excited state. Moreover, quercetin is a very low emissive chromophore that exhibits a fluorescence quantum yield of less than $10^{-3}$ in acetonitrile. Similarly to the 3-hydroxyflavone and its 4’-substituted derivatives, the fluorescence of quercetin stems from the
radiative deactivations of two relaxed excited species which are in fast equilibrium at singlet excited state (Scheme 1).

Scheme 1. Schematic representation of the various radiative deactivation pathways of quercetin at $S_1$ state. (FC stands for: Frank-Condon state).

The first emitting species (blue emitting one) corresponds typically to an internal charge transfer (ESICT) from catechol to pyrone groups. Subsequent to this latter electronic relaxation, an internal proton transfer process occurs from the 3-hydroxyl function to the carbonyl oxygen atom, giving rise to the emission of an internal proton transfer (ESIPT) tautomer. Such a new fluorescent species exhibits a benzopyrylium-like configuration. It should be noted that the fluorescence spectra of ESICT and ESIPT are strongly overlapped in such manner that only one emission band is observed for quercetin. However, we will see hereafter that the presence of iodonium salts will lead to a specific quenching of the ESIPT species.

2.3 Photosensitizing properties of quercetin. Steady state photolysis experiments in acetonitrile were first performed to demonstrate the photosensitizing role of quercetin in the presence of iodonium salts. Figure 3 displays the absorbance time profile at 370 nm of a solution of quercetin in the presence of iodonium salts. The kinetics relative to a reference solution of quercetin without a photoacid generator is also depicted to highlight the effects. In absence of iodonium salts, the absorption spectrum of quercetin remains constant upon irradiation whereas the presence of photoacid generator clearly induces the rapid photobleaching of quercetin. For instance, the intensity of the longest wavelength absorption band of quercetin collapses by 97 % after 17 min irradiation. Figure S2 shows the evolution of the UV-visible spectra of quercetin alone and quercetin/Iod.

A reaction between excited quercetin and iodonium salts is therefore demonstrated. Interestingly, such a reaction leads to the concomitant photogeneration of $H^+$ which can be detected using an acid indicator, such as Rhodamine B45, 46 (RhB). As shown in Figure 4A, the continuous irradiation of a solution of quercetin mixed with iodonium salts and RhB leads to the progressive growth of an absorption band in the 480-650 nm range corresponding to the acid form of RhB (RhBH$^+$). The photogeneration of $H^+$ is observed only when quercetin and iodonium salts are mixed together. This clearly confirms the photosensitizing properties of quercetin. Moreover, the amount of $H^+$ detected during the irradiation of a solution that was initially N$_2$-saturated is globally equivalent to that of a non-degassed solution, as illustrated in Figure 4B. It is clear that the presence of dissolved oxygen in acetonitrile ([O$_2$]$_{diss}$ ~ 10$^{-3}$ M47) does not have any inhibiting effects on the photoreaction between the excited quercetin and the iodonium derivative. This suggests that the photosensitizing process occurs mainly from the singlet excited states of quercetin.
This assumption is corroborated by the fluorescence quenching of quercetin in the presence of increasing amounts of iodonium salts (see Figure 5). The fluorescence spectrum clearly shows a decrease in intensity. It should be emphasized that this quenching mainly affects the ESIPT band which is located in the 530-680 nm range. As a consequence, after addition of iodonium salts, the relative contribution of ESIPT band to the total fluorescence spectrum drops drastically in such manner that the low intensive ESICT band can be detected in the 460-500 nm range. Noteworthy, the excitation spectrum collected from this latter emission band matches the absorption band of quercetin.

According to the strong electron-accepting characteristics of iodonium, it is proposed that the fluorescence quenching of quercetin can be mainly ascribed to an efficient photoinduced electron transfer (PeT) from the ESIPT state of quercetin to the photoacid generator. The free energy associated to this proposed mechanism can be estimated according to the Rehm–Weller equation:

$$\Delta G_{\text{PeT}} = E_{\text{ox}} - E_{\text{red}} - E_{\text{ESIPT}} + C$$

The following approximations were made: i) the Coulombic part of the stabilization energy (C) is negligible, ii) the energy of ESIPT state can be estimated by $E_{\text{ESIPT}} \approx \frac{1}{2} \hbar c (\nu_{\text{abs}} + \nu_{\text{fluo}})$ leading to a value of ca. 2.84 eV, and iii) $E_{\text{ox}}$ and $E_{\text{red}}$ correspond to the oxidation potential of the quercetin and the reduction potential of the diphenyliodonium salt ($E_{\text{red}} = -0.2$ V vs. SCE). The oxidation potential of quercetin in acetonitrile was measured by cyclic voltammetry. According to the cyclic voltammogram of the dye (see Figure S3), the first oxidation wave, which is irreversible, exhibits a half-wave potential of about 1.1 V vs. SCE. As a consequence, $\Delta G_{\text{PeT}}$ has an estimated value of ca. -1.54 eV in acetonitrile which is consistent with a largely exergonic PeT process.

Therefore, quercetin, as a natural dye, constitutes an interesting candidate that can promote acid photogeneration in the presence of iodonium salts. This photosensitizing process will be addressed to produce quercetin-derivative coatings by cationic photopolymerisation.

### 2.4 Synthesis and characterization of the quercetin-derivative coatings

The photochemical reactivity of the photosensitive formulation containing glycerol triglycidyl ether (GTE)/Quercetin/Iodonium was evaluated by Real-Time Fourier Transform Infrared (RT-FTIR) spectroscopy by monitoring the decrease of the epoxy group at 910 cm$^{-1}$ upon irradiation (Figure 6A). The band associated with the epoxy group decreases concomitantly with the growth of a new absorbance at 1080-1100 cm$^{-1}$.
2.5 Thermogravimetric analyses. Thermal stability of the coatings was studied by thermogravimetric analysis (TGA). The results are displayed in Figure 8. The coating reached 50% weight loss at 375°C.

![Fig. 8. TGA thermogram of the photoinduced quercetin-derivative coating.](image)

2.6 Mechanical analyses of the coatings. The hardness of the glycerol-derived coatings was first determined relative to a standard set of pencil leads. Considering that the pencil hardness is always above 7H, it means that no macroscopic scratches were observed up to a hardness of 7H. To highlight more precisely the hardness of the coatings, further investigations, i.e. nanoindentation and scratch tests, were performed.

2.6.1 Nanoindentation. The loading and unloading curves during the nanoindentation tests were superimposed, showing that the coatings exhibit a viscoelastic behavior at room temperature, indicating that the polymer is in a rubber-like state at room temperature. The elastic modulus and the hardness, as measured by the Oliver and Pharr method, was approximately 189 ± 18 MPa (Mean ± Standard deviation) and 12 ± 1 MPa, respectively.

2.6.2 Scratch tests. Regarding the scratch tests, a comparison of the height profiles before and after scratch tests reveals the complete recovery of the material, confirming the rubber-like behavior (Figure 9). The lack of bulging on the height profile during and after scratching indicates the apparition of brittle fracture or delamination of the coating. Moreover, the optical images of the sample after scratching show no residual imprint, confirming that neither brittle fracture nor delamination occurs. Hence, there is a good adhesion to the substrate and the good resistance of the coatings to brittle fracture.

![Fig. 6. A) Photopolymerization kinetic of GTE under light activation and B) FTIR spectra of the coating during the 1200s of irradiation. Xe lamp, intensity= 70 mW/cm², film thickness = 100 μm.](image)
Fig. 9. Height profile before, during and after scratch. A complete recovery of the sample after scratch and the absence of bulge in the profile during scratch evidence the rubber like behavior of the coatings and its very good adhesion and resistance to brittle fracture.

2.7. Fluorescence of the coatings. After irradiation of the quercetin derived formulation (900s), unreactive quercetin molecules are left inside the coating, as observed by UV-vis spectrometry (Figure 10A, dot line) and its epifluorescence (Figure 10B). The remaining fluorescence of quercetin can be used to generate reactive oxygen species (ROS) able to eliminate bacteria, as it will be demonstrated in the following section.

Fig. 10. A) Evolution of the UV-vis spectra of the quercetin derived coating after 900s of irradiation. Solid line = before irradiation, dot line = after irradiation. Xe lamp, intensity = 70 mW/cm². Formulation was sandwiched under 2 glass plates. B) Epifluorescence of quercetin located into the polymer coating after 900s of irradiation. Inset = Fluorescence spectrum of the embedded quercetin into the polymer film after polymerization.

2.8. Antibacterial properties. Prior to investigate the antibacterial effect of the quercetin derivative-coatings, the ability of quercetin to inhibit bacterial proliferation in solution was investigated against Gram-Negative (E. coli) and Gram-Positive (S. aureus) bacteria after 2h and 6h, with and without light illumination. Initially, 6.5 x 10⁶ CFU/mL of each bacteria was introduced in solution at t = 0 h.

First, a reference study referring to the light irradiation against the two bacteria strains was performed (Figure 11 and Figure S5). The results show that the irradiation does not have any influence on the proliferation of E. coli and S. aureus alone. The two strains proliferate in the same way with or without irradiation. A second reference study was performed in the presence of DMSO, because quercetin is insoluble in water. According to these results, DMSO does not affect the bacterial activity because the decrease in CFU is not significant regardless of the stains used with or without light illumination (Figure 11 and Figure S5).

Fig. 11. Influence of the incubation time (0, 2h, 6h) and the irradiation on the growth of A) E. coli and B) S. aureus. [Quercetin] = 500 mM.
The last interesting results are the effect of light irradiation on the proliferation of bacteria. The antibacterial effect of quercetin was then evaluated in DMSO, under light activation. The results show that irradiated quercetin only leads to an antibacterial effect with S. aureus. Indeed, the development of gram-negative bacteria (E. coli) is neither inhibited by the illumination nor affected by the presence of quercetin in the dark. On the contrary, the irradiated quercetin solutions allow the total death of S. aureus after 2h of incubation. The antibacterial effect against S. aureus remains efficient even after 6h. The variation in quercetin killing efficacy at different exposure durations was statistically significant (p value < 0.05 on comparing percentage survival). In the absence of irradiation, more than half of bacteria are always alive after 6h of incubation with S. aureus.

Some studies on the inactivation of bacteria demonstrate that the photosensitizers do not need to penetrate the cell membrane or even to come into contact to be effective. Indeed, if sufficient quantities of singlet oxygen can be generated near the outer membrane of the bacteria, it will lead to its damage. However, only gram-positive bacteria (S. aureus) are affected by this oxygen singlet process. Indeed, the lipopolysaccharide (LPS) coatings of the cell wall of the gram-negative bacteria offers some protection from the toxic effects of exogenous agents. In addition to possibly forming a structural barrier to penetration, this outer membrane may form a chemical trap for singlet oxygen; it is composed of unsaturated fatty acids and proteins, which are compounds known to react chemically with singlet oxygen.

As a result, some strains that fail to produce a large portion of the LPS displayed greater sensitivity to exogenous singlet oxygen than the strains with this capability. Most gram-positive bacteria (S. aureus) lack this protective structure analogous to the gram negative LPS and the outer membrane in which it is anchored. This can explain the 99% inhibition of the proliferation of S. aureus after 2h of incubation. ROS and particularly singlet oxygen probably diffuse readily through the relatively open structure of the peptidoglycan layer of the S. aureus cell wall to react with the vital target.

In this study, Electron Paramagnetic Resonance (EPR) spectroscopy was used to monitor the ROS generation ability of quercetin upon photo-illumination (Figure 12). Amount of 2,2,6,6-tetramethylpiperidine (TEMPO), a diamagnetic molecule, was used to capture oxygen radical species by yielding a paramagnetic product, the nitroxide radical TEMPO. As shown in Figure 12, the EPR spectral signal of three lines of equal intensity, which is attributed to the TEMPO nitroxide radical, was observed when an oxygen-saturated solution of quercetin was irradiated in the presence of TEMPO at room temperature. During illumination of quercetin solution, the intensity of the EPR signal increases gradually, indicating the formation of reactive oxygen species like singlet oxygen.

According to these results, it is likely that quercetin, which was introduced and immobilized in the coatings, could generate reactive oxygen species like singlet oxygen to inhibit the proliferation of bacteria on stainless steel substrates. Mechanistic interpretations, have already demonstrated that the resulting reactive species, such as singlet oxygen could be generated by these immobilized photosensitizers on the material surface, followed by its diffusion, resulting in the damage of the bacteria envelope. Interestingly, the diffusion of quercetin through the synthesized coatings was not observed until 6h of incubation. The ability of the quercetin derivative-coatings (deposited on stainless steel substrates) to inhibit the bacteria adhesion/proliferation was investigated with and without the light activation (Figure 13). A quantitative and well-tried method in biological studies for quantifying the total biofilm population was used, as reported by many antibacterial investigations.

![Figure 12](image-url)
Conclusions

This study examined the synthesis of antibacterial coatings derived from a natural dye (quercetin) and a glycerol triglycidyl ether monomer. The ability of quercetin to be used both as a photosensitizer of iodonium salt and as an efficient antibacterial agent (even incorporated into a coating) against S. aureus was demonstrated for the first time. For this purpose, the photochemical behavior of quercetin in the presence of iodonium salts was described by steady state and fluorescence experiments, demonstrating i) the efficiency of this system to be used as a photoinitiating couple and ii) its ability to generate H⁺ photoacid for the cationic photopolymerization. The resulting coatings synthesized under light illumination showed very good adherence properties to the stainless steel substrates and a high thermal stability up to 375°C.

The synthesized coatings containing quercetin led, under light activation, to an inhibition of S. aureus proliferation of 99% after 2h and 6h of incubation. This new coating could be used successfully to avoid bacteria proliferation and be deposited onto disposable paramedical devices, such as clamp or scalpsels, which can be used one-time for a few hours.

3. Experimental

Materials. Glycerol (≥ 99%), sodium hydride (97%), 3-chloroperbenzoic acid (≤ 77%, m-CPBA), quercetin (≥ 95%, Qr), 2,2,6,6-tetramethyl-1-piperidinyloxyl (98%, TEMPO), 2,2,6,6-tetramethylpiperidine (≥ 99%, TEMP), rhodamine B (≥ 95%, RhB) and anhydrous DMF were purchased from Sigma-Aldrich. Iodonium 4-(2-methylpropyl)phenyl-hexafluorophosphate (Iod) was purchased from Badische Anilin und Soda Fabrik (BASF). Allyl bromide (99%) was acquired from Alfa Aesar. Petroleum ether, ethyl acetate (analytical grade) and dichloromethane (synthesis grade) were obtained from Carlo Erba. All chemicals were used as received. Table 2 lists the chemical structure of the compounds used in this study.
Table 2. Chemical structure of the compounds used in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Function</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>Monomer precursor</td>
<td><img src="image" alt="Structure of Glycerol" /></td>
</tr>
<tr>
<td>Quercetin</td>
<td>Photosensitizer</td>
<td><img src="image" alt="Structure of Quercetin" /></td>
</tr>
<tr>
<td>Iodonium 4-(2-methylpropyl)phenyl-hexafluorophosphate (Iod)</td>
<td>Cationic photoinitiator</td>
<td><img src="image" alt="Structure of Iodonium 4-(2-methylpropyl)phenyl-hexafluorophosphate" /></td>
</tr>
<tr>
<td>2,2,6,6-Tetramethylpiperidine (TEMP)</td>
<td>Singlet oxygen scavenger</td>
<td><img src="image" alt="Structure of 2,2,6,6-Tetramethylpiperidine (TEMP)" /></td>
</tr>
<tr>
<td>2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)</td>
<td>Determination of ( ^1 \text{O}_2 )</td>
<td><img src="image" alt="Structure of 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)" /></td>
</tr>
<tr>
<td>Rhodamine B (RhB)</td>
<td>Acid indicator</td>
<td><img src="image" alt="Structure of Rhodamine B (RhB)" /></td>
</tr>
</tbody>
</table>

**Synthesis of triallyl glycerol.** Triallyl glycerol was synthesized using a procedure reported elsewhere. Glycerol (5 g, 54.3 mmol) and NaH (6.84 g, 285 mmol) were dissolved in 50 mL of anhydrous DMF at 0°C. After 10 min, allyl bromide (17 mL, 285 mmol) was added and the solution was stirred for 3h at room temperature. The mixture was then poured into water and extracted with CH₂Cl₂. The organic phase was concentrated and the residue was purified on a silica gel column (petroleum ether-EtOAc 1:1.5 as eluent). The evaporation of the solvent and subsequent drying under high vacuum led to 8.272 g (yield = 72%) of triallyl glycerol. The product structure was confirmed by \(^1\)H NMR. \(^1\)H NMR (CDCl₃) \( \delta \) (ppm) 5.91 (m, 3H), 5.27 (m, 3H), 5.16 (m, 3H), 4.15 (dt, J = 5.7, 1.4 Hz, 2H), 4 (dt, J = 5.6, 1.4 Hz, 4H), 3.69 (m, 1H), 3.54 (m, 4H).

**Synthesis of glycerol triglycidyl ether (GTE).** Glycerol (5 g, 23.6 mmol) was dissolved in 50 mL of CH₂Cl₂. m-CPBA (24.42 g, 142 mmol), dissolved in 75 mL of CH₂Cl₂, was added dropwise over a one hour and the solution was stirred overnight at room temperature. After filtering the precipitate, the solution was concentrated to approximately 50 mL and placed at -20°C. The precipitate was then filtered and the filtrate was evaporated 3 times until no more precipitate appeared. Finally, the solution was passed through a silica gel pad, evaporated and dried under vacuum, yielding 4.93 g of glycerol triglycidyl ether (yield = 80%). The total conversion of double bonds to epoxide was confirmed by \(^1\)H NMR spectroscopy. \(^1\)H NMR (CDCl₃) \( \delta \) (ppm) 3.90 (dt, J = 11.7, 2.7 Hz, 1H), 3.78 (dd, J = 11.6, 2.6 Hz, 2H), 3.71 (m, 1H), 3.59 (m, 5H), 3.39 (m, 2H), 3.14 (m, 3H), 2.78 (t, J = 4.6 Hz, 3H), 2.59 (m, 3H).

**Photopolymerization procedure.** For the cationic photopolymerization, 8 mg of Iod (4 wt % with respect to the epoxy monomer), 5 mg of Quercetin (2.5 wt % with respect to the epoxy monomer) were dissolved into epoxy monomer formulation (GTE, 200 mg) containing 100 μL of acetone. The kinetics of photopolymerization were followed by real time Fourier transform infrared spectroscopy (RT-FTIR) using a Thermo-Nicolet 6700 instrument. The liquid samples were applied to a BaF₂ chips by means of calibrated wire-wound applicator. The thickness of the UV-curable film was evaluated at 100 μm. The RT-FTIR analyses were carried out under air conditions. Samples were irradiated at room temperature, by means of a Lightningcure LCB-03 lamp from Hamamatsu, equipped with a xenon lamp (200 W) coupled with a flexible light guide. The end of the guide was placed at a distance of 4 cm. The maximum UV light intensity at the sample position was found to be 70 mW/cm². The photopolymerization was monitored by the disappearance of the epoxy function of the GTE monomer at 910 cm⁻¹. The decreases of epoxy function at 910 cm⁻¹ and the increase of polyether at 1080-1100 cm⁻¹ demonstrated the efficiency of the photopolymerization. Conversion rate was calculated with the followed equation (Eq 1):

\[
\text{Epoxy conversion (\%)} = \frac{1}{2} \left( 1 - \frac{A_t}{A_0} \right)
\]

A₀ represents the area at t = 0s and Aₜ represents the area at time t.

**Synthesis of the glycerol-derivative coatings.** Prior to the deposition of formulation on stainless steel plate, the latter was intensively cleaned with ethanol and toluene. The photosensitized formulation containing GTE, Iod and Quercetin (100 μL) were then deposited and spin-coated (2000 rpm during 3s) on the dried stainless steel plates. Both sides of stainless steel plate were irradiated at a distance of 4 cm at room temperature using a Lightningcure LCB-03 lamp from Hamamatsu, equipped with a xenon lamp (200 W) coupled with a flexible light guide. The irradiation time was fixed to 900 s per side with an intensity of 70 mW/cm².

**UV-Visible absorption and fluorescence measurements.** The absorption measurements were carried out using a Perkin-Elmer Lambda 2 spectrometer. Steady-state fluorescence spectra were collected from a FluoroMax-4 spectrophotometer. All emission spectra were spectrally corrected.

**Fluorescence microscopy.** An inverted microscope IX73 from Olympus equipped with a 75W Xe Lamp housing was used. The excitation and emission light is filtered by a fluorescence mirror unit (U-FUN from Olympus) associating a band pass filter centered at 365 nm (BP360-370), a dichroic mirror (DM410) and a long pass filter (BA420IF).
measurement begins with the lowest pencil and continues up the scale to determine the maximum hardness able to scratch the surface of the glycerol-derived coatings (Method: ASTM D3363-74, 2000). The surface hardness is determined by scratching the leads across the coating at a controlled angle of 45°, the value given are the lowest grade of pencil that could induce a scratch on the coating surface. The pencil hardness was measured using a No. 553 pencil hardness tester (Yasuda Seiki Seisakusho LTD.). Pencils were supplied by Staedtler Mars Lumograph 100 (Germany).

Nanoindentation and scratch tests. Nanoindentation and scratch tests were carried out on the coatings deposited on a stainless steel substrate using a Nano Indenter G200 (Agilent Technologies) with a Berkovich tip (Micro Star Technologies). Twenty Nanoindentation tests per sample were performed. Samples were loaded and unloaded at constant strain rate (0.05 s⁻¹) using the Continuous Stiffness Measurement (CSM) method until an indentation depth of 1 µm was reached. The unloading stage was performed after a hold load plateau of 300 s to exhibit the viscous behavior. Twenty scratch tests were performed; face forward, with an increasing load from 0.1 to 100 mN for a scratching distance of 500 µm. The distance between two scratches was fixed to 500 µm.

Thermogravimetric analyses (TGA). 10 mg of the glycerol-derivative films were introduced into aluminum pans and analyzed using a Setaram Setsys Evolution 16 thermobalance by heating the samples at a rate of 15 °C/min from 20 to 800 °C under argon atmosphere.

Antibacterial properties of the coatings. The initial adhesion assays were performed using two strains of bacteria, namely E. coli ATCC25922 and S. aureus ATCC6538 on the quercetin derivative-coatings. Before the in vitro antibacterial tests, the bacterial strains were grown aerobically overnight in Luria–Bertani broth at 37 °C with stirring. Overnight cultures of E. coli and S. aureus grown in Luria–Bertani broth were diluted to an optical density (OD 600 nm) of 0.05 in sterile LB broth. At this point, the stainless steel supports and stainless steel substrates with coatings (1.5 cm x 1.5 cm) were immersed in the culture. The corresponding vials were placed on a slantwise rotating wheel to avoid the sedimentation of bacteria, and incubated for 2h and 6h at room temperature under illumination or not. The volume of DMSO corresponds to 3% v/v of the total bacteria solution. The process is the same as described in the previous paragraph. The suspensions were pooled and diluted serially. A 100 µL volume of the viable bacteria solution was introduced onto the surface of a Plat Count agar plate.

Finally, the number of viable bacteria was determined by a counting of the CFUs, after overnight statically incubation of the agar plates at 37°C. Each experiment was done four times.

Statistical analysis. All values corresponding to the anti-adherence property of E. coli and S. aureus are expressed as mean ± standard deviation. Statistical analysis was performed using Student’s t-test to calculate the significance level of the data. Differences were considered statistically significant at P < 0.05. Ten samples per group were evaluated.

Acknowledgements

We would like to thank CNRS institute and University of Paris-Est Creteil (UPEC) for financial support, Léon Preira for the cutting process of stainless steel substrates and Séna Hamadi for the TGA analysis.

References

Synthesis of antibacterial coatings derived from glycerol and quercetin for the inhibition of bacteria proliferation.