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Vibrational spectroscopic study of disposable soft contact lenses. Correlations with the bacterial adhesion on their surface

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Raman and infrared spectra of two types of disposable soft contact lenses are presented and discussed in order to correlate the chemical structures of the polymers to bacterial adhesion on their surface. The results of the adhesion study show that Staphylococcus aureus adheres better to more hydrophilic lenses. The spectroscopic results point out that the different chemical structures of the polymers can explain not only the different water amounts in the lenses, but also play an important role on the adhesion capability of Staphylococcus aureus.

1. Introduction

The application of Raman and ATR/FTIR vibrational spectroscopies in the ophthalmological field is relatively recent. Since they are non-destructive and non-invasive techniques, they provide powerful tools for studying directly biomaterials at a molecular level [1].

Here we present the preliminary results of a vibrational spectroscopic characterization of two commercial disposable lens types. Moreover, the adhesiveness of Staphylococcus aureus was quantitatively evaluated in *vitro*, since this bacterium is one of the most frequent etiological agents of soft contact-lenses-associated infections.

It is well known that one of the main causes of soft contact lens spoilage is the formation of a protein layer, irreversibly adsorbed on the surface of the lenses. This layer can cause lens intolerance and favour bacterial adhesion. Normal cleaning treatments are not able to remove this protein layer: hence the necessity to frequently replace the contact lenses. Disposable soft contact lenses could represent a solution to avoid the problems related to lens spoilage: they must be replaced frequently and do not need regenerative treatments.

Some authors think that the infection complications associated with the use of disposable contact lenses occur far less frequently than those reported for traditional contact lenses [2–4]. According to other researchers, patients wearing the new lenses are equally exposed to a high risk of infection [5–9]. The investigators focused their attention on the detection of the characteristics which could favour organic material deposition, and therefore bacterial contamination, on the lenses [4, 5].

The aim of this research is to contribute to the study of factors which can affect bacterial adhesion taking into account also the chemical and structural characteristics of the polymers.

2. Materials and methods

2.1. Materials examined

The lenses examined are commercial Etafilcon A, consisting of crosslinked poly-2-hydroxyethylmethacrylate (PHEMA) copolymerized with methacrylic acid (MAA), and Polymacon, consisting of crosslinked PHEMA.

2.2. Spectroscopic and thermogravimetric analyses

A JASCO R500 spectrometer and a spectra Physics Ar^+ 488 nm laser source were used to record the Raman spectra. The lenses were examined directly in the respective buffered saline solutions according to an already described method [10].

The infrared spectra were collected by means of a JASCO FT/IR 5300 spectrophotometer, equipped with an ATR device. A ZnSe crystal and 45° incidence angle were used for the measurements.

Thermogravimetric (TG) analysis was performed in a Mettler TA 3000 system with a TG50 equipment.

2.3. Preparation of bacteria for the adhesion study

In order to evaluate bacterial adhesion [11–14], a strain of Staphylococcus aureus was used. It was

chosen because of its characteristic of the highest adhesiveness on polystyrene, in a group of 10 strains of Staphylococcus aureus isolated from surgical wounds following traditional methods.

To obtain the bacterial suspension to which each material had to be exposed the following procedure was adopted: an aliquot of the strain, preserved at − 20 °C, was transferred in trypticase soy broth (TSB) and incubated for 24 h at 37 °C in 5% CO_2 atmosphere. The seeding was then performed with a 2 µl loop on chocolate agar plate, followed by a 24 h incubation at 37 °C in 5% CO_2 atmosphere. After incubation, six isolated colonies were incubated in 8 ml TSB for 24 h at 37° in 5% CO_2 atmosphere. After incubation the suspension was washed three times at 3000 rpm for 10 min and finally resuspended in 2 µl saline solution.

2.4. Incubation of specimens with bacteria and turbidimetric evaluation

Into the culture wells already containing the lenses, 2 ml of TSB were added, then 20 µl of bacterial suspension. The specimens were then incubated for 24 h at 37 °C in 5% CO_2 atmosphere. After incubation the materials were washed three times with saline solution for 10 min each time and finally cultivated with TSB at 37 °C for exactly 24 h. The turbidimetric measure of the broth culture was then performed.

3. Results and discussion

Fig. 1 shows the ATR/FTIR spectra of Polymacon and Etafilcon A lenses after the subtraction of the spectra of the relative saline solutions. The band at 1556 cm^{-1} in the infrared spectrum of the Etafilcon A lens confirms the presence of a little amount of MAA copolymerized with HEMA. This band, which is absent in the infrared spectrum of the Polymacon lens, is due to the asymmetric stretching vibration of COO^- groups, which derive from the COOH groups of MAA ionized at pH 7.2.

In Fig. 2 the Raman spectra of the samples between 4000 and 2500 cm^{-1} are reported. The different hydrophilicity of the two types of lenses is evident by comparing the relative intensities (I_W/I_p) of the water band at 3420 (I_W) and the band at 2945 cm^{-1} due to the polymer (I_p). The I_W/I_p values are 0.21 and 0.46 for Polymacon and Etafilcon A lenses, respectively. This result is confirmed by TG measurements which give for the two types of lenses 39.4 and 58.5% water content, respectively.

The Raman spectra of the two lenses between 1800 and 1300 cm^{-1} (Fig. 3) do not show any band at about 1640 cm^{-1}. This region of the spectrum is characteristic for the evaluation of residual monomers

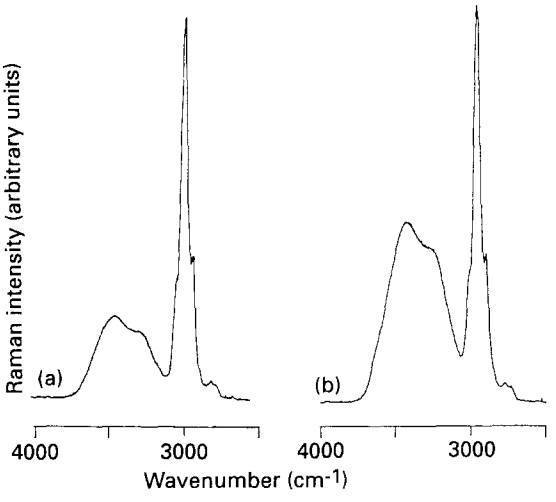

Figure 2 Raman spectra of (a) Polymacon and (b) Etafilcon A lenses between 4000 and 2500 cm^{-1}.

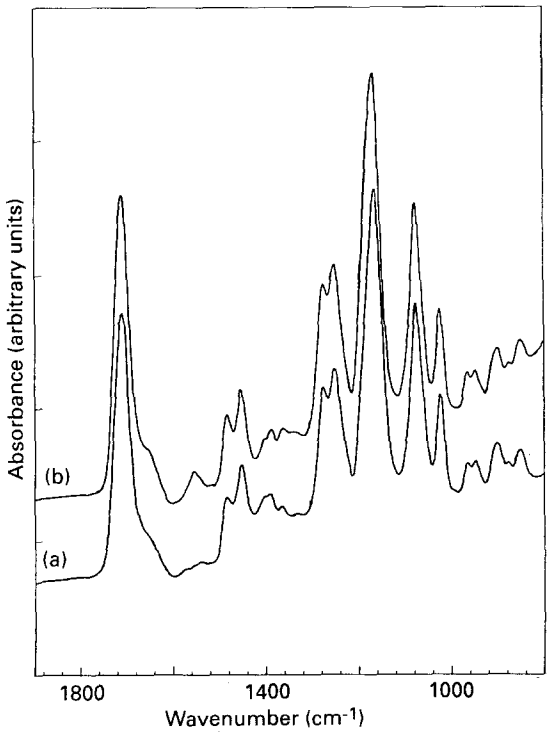

Figure 1 ATR/FTIR spectra of (a) Polymacon and (b) Etafilcon A lenses after subtraction of the spectra of the relative saline solutions.

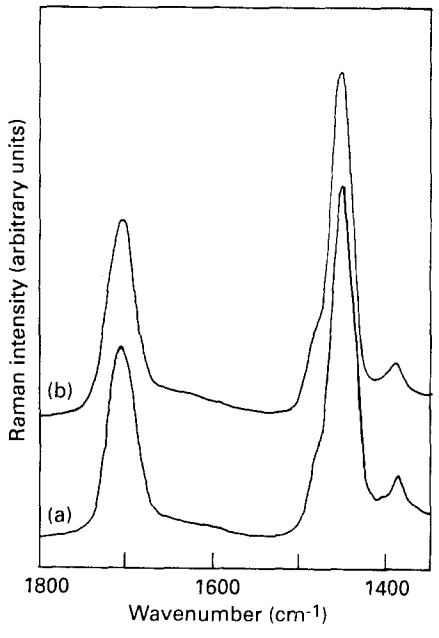

Figure 3 Raman spectra of (a) Polymacon and (b) Etafilcon A lenses between 1800 and 1300 cm^{-1}.

which are responsible for the chemical toxicity when they are present in polymeric biomaterials [15]. In fact, the Raman band at about 1640 cm^{-1} is attributed to the stretching vibration of the C = C bonds which are present in the monomers. Our previous studies on hydrophilic soft contact lenses [16, 17] showed that Raman spectroscopy is a suitable, non-destructive tool for monitoring the chemical purity of the materials during the different stages of preparation and purification. The lack of the band at 1640 cm^{-1} in the Raman spectra shows the good chemical biocompatibility of the two types of lenses.

The results of the experiments performed for the quantitative evaluation of bacterial adhesiveness on the lenses, expressed in nefelometric turbidity units (NTU), are reported in Fig. 4.

The turbidimetric measure of the broths, obtained as described earlier, indicated the number of bacteria still adherent to the examined material after *in vitro* contact and the following washings. In this way bacterial adhesiveness was indirectly measured. Each experiment was considered valid only if the broth culture of the last washing of the examined material resulted negative.

The results show that Staphylococcus aureus adheres less to Polymacon than to Etafilcon A lens.

Food and Drug Administration divides soft contact lenses into the following classes: (I) non ionic low hydrophilic lenses; (II) non ionic high hydrophilic lenses; (III) ionic low hydrophilic lenses; (IV) ionic high hydrophilic lenses. In compliance with the above classification, some authors have examined the effects of water content on the bacterial adhesion to polymers, obtaining conflicting results [18, 19].

In previous studies we examined the effect of water content on bacterial adhesion to type I and type II lenses and we found that the number of adhered bacteria was higher to polymers with lower water contents [12]. Our study suggested that bacterial adhesion is "mediated" by the surface water molecules, which are linked by hydrogen bonds of different strength, depending on the hydrophilic surface groups, and a model for this was subsequently proposed [10].

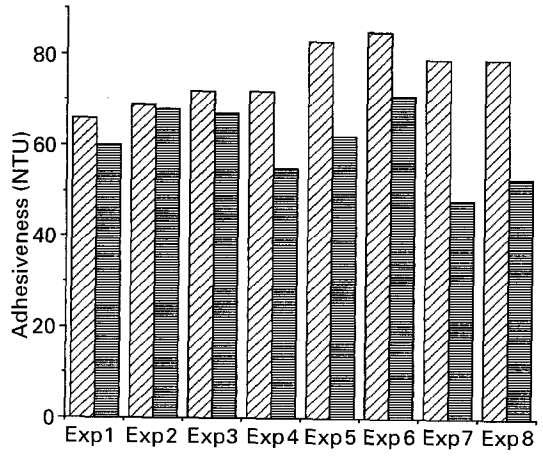

Figure 4 Adhesiveness values obtained for the single experiments after 24 h contact of disposable contact lenses with Staphylococcus aureus. (▨ Etafilcon A; ▤ Polymacon).

In this work, type I and type IV lenses, with opposite characteristics for hydrophilicity and ionicity, were examined. The vibrational spectra of the lenses point out some interesting differences in the structure and in the properties of the two materials.

The considerable difference in water contents pointed out by Raman spectra and TG measurements can be reasonably explained if the introduction of the anionic comonomer (MAA) in the PHEMA lattice of Etafilcon A is taken into account. At pH 7.2 (pH of the buffered saline solution) the COOH groups are ionized. The resulting COO^- groups undergo repulsive forces thus causing an enlargement of the polymer network; moreover, the water molecules can interact by a stronger H-bond with these ionic groups which are absent in the structure of Polymacon material.

According to the results of our *in vitro* experiments it can be seen that bacterial adhesion occurs after 24 h on both types of lenses. The amount of such adhesion is remarkably higher on the more hydrophilic lenses. This fact is apparently in contrast with our previous measurements on non-ionic hydrophilic lenses [12], but it can be explained if one considers the presence of the new type of electron donor group in Etafilcon A, the COO^- group, which can interact more strongly not only with the absorbed water molecules but also with the polar groups present in the proteic component of bacteria as shown by our previous infrared measurements [11, 14].

In conclusion, the prevention of infections in contact lens users could therefore be improved by the complete explanation of the mechanisms of bacterial adhesion to the lenses and the role played by proteins in mediating bacterial adhesion phenomena. Only in this way will it be possible to project lenses with characteristics able to effectively inhibit protein deposition and bacterial adhesion.

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