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Lecithin Treatment to Prevent Protein Adsorption onto Contact Lenses

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Abstract—Two types of new never worn lenses made of hydroxyethyl methacrylate (HEMA) were treated with lecithin followed by in vitro immersion into individual protein solutions. The amount of protein absorbed onto untreated and treated lenses was compared using UV absorption spectroscopy. Basing on the results obtained a conclusion was drawn that lecithin prevented the protein adsorption onto contact lenses, and the lecithin concentration affected the amount of the sorbed protein.

Proteins are the main source of soft contact lenses contamination [1–3]. They are also the main reason of bacterial adhesion to contact lenses [4], and they are responsible for many problems associated with contact lenses, e.g., giant papillary conjuctivitis [5]. Proteins take part in damaging disposable contact lenses [6].

Our study was aimed at reducing to minimum the protein adsorption on contact lenses applying a biologically inert hydrophobic substance. Protein adsorption is always associated with lipid adsorption on different types of soft contact lenses [7]. Since the surface chemistry is possibly one of the main factors involved in the protein absorption [8], therefore we have modified the surface of some lenses with lecithin.

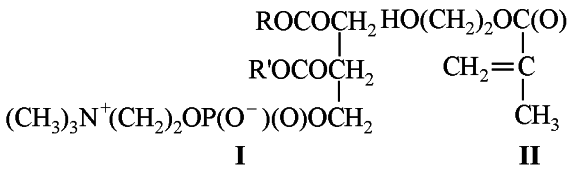
Once adsorbed, the protein is very difficult to remove, and many lens care solutions are only partly efficient in removing the adsorbed protein [9, 10]. The mechanism of protein adsorption to hydrogels was studied, and it was proved that it underwent some structural rearrangements in adsorption to different surfaces [11]. The biochemical aspect of lens spoiling was also well documented, and the fundamental mechanism of protein adsorption was demonstrated [12].

The protein adsorption onto contact lenses is just one of many adverse responses by body fluids to foreign materials. Another example is clodding of blood in the heart bypass surgery, and many efforts have been made to improve blood compatibility by using materials with chemically modified surface or with surface pretreated with plasma proteins, such as albumin, but the attempts have led to varying degree of success [13, 14].

Lipids, in particular phospholipids, are one of the major components of biological membranes. These molecules are amphiphilic: They contain both hydrophobic tails and hydrophilic head groups, and they are capable of self-assembling to form bilayers in an aqueous environment. We are currently using phosphatidyl choline (PC) to modify surfaces of some common biomaterials used in medicine and surgery, such as blood bags, catgut fibers, and contact lenses.

In this paper the results are reported on lecithin treatment of two types of contact lenses made of hydroxyethyl methacrylate [38 VH TINT^{TM}, 38% of hydroxyethyl methacrylate (HEMA), and SeeQuence^{TM} (polymacon), 38% of HEMA] in order to improve their resistance to protein adsorption.

Lecithin (PC) (**I**) is a natural phospholipid, it is amphiphilic and like the other phospholipids is capable of self-assembling to form bilayer in an aqueous environment. There is some structural similarity between hydroxyethyl methacrylate (II) and lecithin. Both materials have hydrophobic and hydrophilic domains and are therefore adsorbed on HEMA surfaces as a coating agent.



I **II**

R, R' are fatty acids rests.

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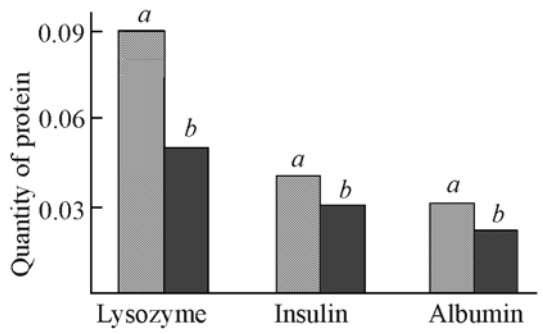

Fig. 1. Amount of different proteins adsorbed onto lenses of hydroxyethyl methacrylate before *a* and after *b* treatment with 5% lecithin solution in chloroform.

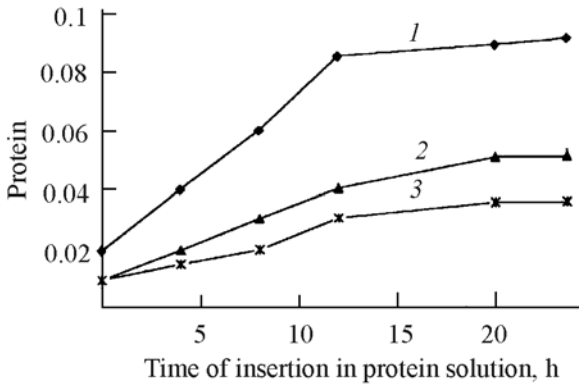

Fig. 2. Adsorption of lysozyme on lenses of hydroxyethyl methacrylate treated with 5% and 10% solutions of lecithin in chloroform. (*1*) untreated lenses; (*2*) lenses treated with 5% solution of lecithin; (*3*) lenses treated with 10% solution of lecithin.

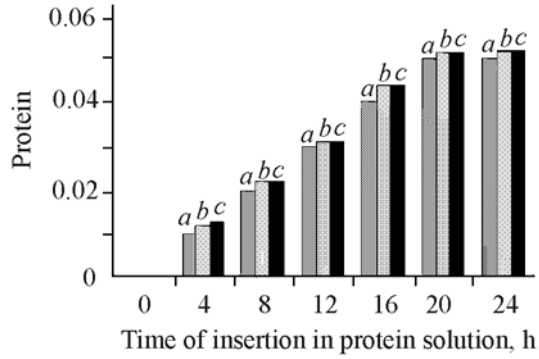

Fig. 3. The effect of duration of treatment with 5% lecithin solution in chloroform on the build up of lysozyme onto hydroxyethyl methacrylate lenses. (*a*) 10 min; (*b*) 1 h; (*c*) 24 h.

The chemical structure of phosphatidyl choline where are present hydrophilic and hydrophobic domains suggests that the compound forms a weak bond with the surface of hydroxyethyl methacrylate due to hydrophobic interactions.

The thickness of PC film depends on the concentration of lecithin solution used for coating. Since the phosphatidyl choline film is insoluble, it cannot be removed with water, artificial tears or by some lens care solutions, but some surfactant cleaners can wash out the adsorbed lipids based on PC. Efficiency of some cleaning systems, as well as the one formulated in our laboratory, was studied in another set of research work.

Untreated lenses and those treated with lecithin were inserted in separate 4% solutions of albumine and lysozyme for 24 h. The quantity of protein in each solution was measured at 280 nm before and after insertion of lens. The difference in the amount of protein in solution was due to the adsorption on the contact lenses. Figure 1 illustrates the amount of different proteins adsorbed onto lenses before and after treatment with 5% lecithin solution in chloroform.

It is evident that lysozyme is absorbed stronger than albumin and insulin, and the treatment procedure has significantly reduced the quantity of absorbed protein.

The concentration of lecithin solution affected the thickness of the PC film and therefore also the efficiency of the treatment procedure.

On Fig. 2 is demonstrated a comparison between lysozyme built up on SeeQuens^{TM} lenses treated with 5% and 10% lecithin solutions. It is evident from Fig. 2 that the 10% solution is more effective in reducing lysozyme adsorption than the 5% solution which proves that a thicker film has been formed in the former case. However the duration of treatment with lecithin does not affect the film thickness (Fig. 3).

An important conclusion follows from the above data that lecithin treatment can significantly reduce the protein adsorption, and the treatment efficiency is the higher the thicker is the lecithin film.

Comparison of isoelectric points of the three proteins (see table) shows that lysozyme possesses a net positive charge, while insulin is almost uncharged, and albumin is charged negatively at neutral pH. It can be suggested from these data and the amount of each protein adsorbed on similar lenses

that the charge more than the size of the protein determines the adsorption onto the charged surface of the lenses produced of polymer from hydroxyethyl methacrylate contaminated with methacrylic acid (MMA).

We formerly studied in detail factors influencing lens damage by proteins. It is very important that the poly(hydroxyethyl methacrylate) constituting these lenses contains trace amount of MMA units generating a negative charge on their surface which favors better adsorption of positively charged protein compared to neutral and negatively charged species. This was reported in our previous work [16]. We have also shown that other impurities, such as *N*-vinylpyrrolidone, in the HEMA contact lenses facilitate absorption of charged proteins [17].

Treatment of contact lenses with natural phospholipids (such as lecithin) can significantly reduce the protein adsorption onto their surfaces. A simple process "dip, drip, and dry" using the chloroform solution of lecithin provides a very efficient film on the lenses which is not removed by wearing, handling, washing and storage of lenses. However the cleaners containing surfactants should be used with care and a repeated lecithin treatment is recommended after application of the surfactant cleaner. An important advantage of our method consists in the possibility to easily teach the user of lenses to treat them with lecithin solution before use and after cleaning them with surfactant cleaner.

EXPERIMENTAL

The protein concentration was measured on UV spectrophotometer (Utroscop^{R} 3000, Pharmacia Biotech).

The study was carried out on contact lenses 38 VH TINT^{TM}, 38% of hydroxyethyl methacrylate (HEMA), and SeeQuence^{TM} (polymacon), 38% of HEMA].

Lecithin (phosphatidyl choline, PC) from eggs (Merck, Art. 533) was used as supplied without purification, 5 and 10% solutions in chloroform were prepared.

Albumin (Merck) was used as 4% solution in phosphate buffer (pH 7).

Lysozyme (Merck, Art. 588) from hen egg white was used as 4% solution in phosphate buffer (pH 7). Standard solutions of the protein were prepared by dissolving a weighed portion in distilled water.

Isoelectric points and molecular weights of proteins used in the *in vitro* studies of lens damage [15]

Protein type (source)	M(kDa)	pH_i
Serum albumin (bovine)	66.5	4.9
Insulin (bovine)	5.9	6.8
Lysozyme (hen eggs)	14.3	11.0

Bovine insulin (Novo Nordisk, Denmark) was purchased in a drugstore.

Procedure of lecithin treatment of contact lenses. The lenses at room temperature were immersed for 24 h into separate 4% solutions of albumin and lysozyme placed into a rotating device; it was expected that within this period the maximum protein adsorption would be attained [16]. Four separate lenses prior to protein absorption test were treated with 5 and 10% lecithin solutions in chloroform. Further procedure was similar to the above described one. The PC surface treatment was carried out as a simple "dip, drip, and dry" process. As a result due to the physical adsorption the lens surface became coated with a lipid film. When the lenses were kept in the PC solution for 5 or 10 h, the coating efficiency was no better than at the simplest procedure, therefore the latter was used in all experiments.

The protein adsorption on both types of HEMA lenses was similar, and thus we used a single type in further studies.

The protein concentration was measured spectrophotometrically on λ 280 nm before and after lens immersion; the calibration curve was obtained using standard protein solutions. The amount of protein adsorbed on lenses was calculated from these data.

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