Corneal permeability for cement dust: prognosis for occupational safety

R.V. Kalmykov [a], D.V. Popova, [b] T.G. Kamenskikh,[a] E.A. Genina,[b] V.V. Tuchin,[b]
A.N. Bashkatov[b]
[a] Saratov State Medical University, 112 Bolshaya Kazachia str., Saratov, Saratov 410012, Russia
[b] Saratov National Research State University, 83 Astrakhanskaya str., Saratov, Saratov 410012,Russia

ABSTRACT

The high dust content in air of a working zone causes prevalence of pathologies of the anterior segment of the eye of workers of cement production. Therefore, studying of features of cement dust impact on structure of a cornea and development of ways of eye protection from this influence is relevant. In this work experimental studies were carried out with twenty eyes of ten rabbits. OCT-tomography was used to monitor the light attenuation coefficient of the cornea *in vitro* during the permeability of cement dust and/or keratoprotector (Systein Ultra). The permeability coefficients of the cornea for water, cement dust and keratoprotector were measured. A computer model allowing one to analyze the diffusion of these substances in the eye cornea was developed. It was shown that 1) the cement dust falling on the eye cornea caused pronounced dehydration of the tissue (thickness decreasing) and led to the increase of the attenuation coefficient, which could affect the deterioration of the eyesight of workers in the conditions of cement production; 2) the application of the keratoprotector to the eye cornea when exposed by cement dust, slowed significantly the dehydration process and did not cause the increase of the attenuation coefficient that characterized the stabilization of visual functions. At this, the keratoprotector itself did not cause dehydration and led to the decrease of the attenuation coefficient, which could allow it to be used for a long time in the order to protect the organ of vision from the negative effects of cement dust.

Key words: eye cornea, Systein Ultra, diffusion coefficient, permeability coefficient, OCT-tomography

1. INTRODUCTION

Working conditions of the workers of the cement industry are characterized by a combination of a number of adverse factors, in particular, of dust content[1]. The content of cement dust in air of a working zone exceeds maximum allowable concentration more than twice. That's why the workers, who work in the conditions of the raised dust content have prevailing dry eye syndrome. Therefore, studying of features of cement dust impact on the structure of a cornea and development of ways of eye protection from this influence is relevant. One of the most widely used methods of evaluation of cornea state and measurement of coefficient of its permeability is the OCT-tomography[2-7]. The used techniques are based on measurement of temporary dependence of change of optical properties of the tissue. As the values of refractive index of cement dust and corneal interstitial liquid differs significantly, penetration of this substance into the cornea will cause the change of its transparence for the probing radiation. The essential contribution to the OCT signal will be made also by the additional light scattering on the cement dust particles. The analysis of kinetics of this process allows us to estimate the rate of diffusion of cement dust in the cornea. The research goal is *in vitro* study of cornea permeability at the action of cement dust and keratoprotectoral medication.

2. MATERIALS AND METHODS

Experimental studies were carried out with twenty eyes of ten rabbits. Thickness and structure of rabbit and human cornea is similar that allows using the rabbit cornea for creation of experimental model. Samples of the rabbit cornea were received after enucleation of the eye immediately before the beginning of the research.

Aqueous solution of cement dust was prepared mixing cement with normal saline solution in concentration of 2 mg/mL. This concentration was chosen from the data on dust content in a working zone of cement production and rate of cement dust sedimentation on a surface[2]. In accordance with the technological features, the size of the main part of the dust particles (80-86%) doesn't exceed 2.5 microns. Refractive index of the particles is about 1.69. For exception of coarse cement particles from the solution, filter paper Whatman Grade 6, passing the particles with the size less than 3 microns was used.

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Keratoprotector Systein Ultra (Alcon, Spain), which showed good efficiency in treatment of a dry eye syndrome, was used. The solution was applied on the cornea surface by drop. The total amount of the applied solution was ~0.5 mL. Refractive index of the keratoprotector was 1.336 that allowed estimating an average value of diffraction of the dissolved substances as 1.412.

The measurements were performed using OCT system OCP930SR 022 (Thorlabs, USA) at the room temperature (~20°C). OCT-tomography was used to monitor the light attenuation coefficient of the cornea *in vitro* during the permeability of cement dust and/or keratoprotector. The permeability coefficients of the cornea for water, cement dust and keratoprotector were measured. Five experimental series were carried out:
1) Research of the cornea permeability for the cement dust particles (6 eyes);
2) Research of the cornea permeability for the keratoprotector (4 eyes);
3) Research of the impact of the keratoprotector on the cornea permeability for the cement dust particles (6 eyes);
4) Research of the impact of normal saline solution on structure and optical parameters of the cornea (2 eyes);
5) Research of the impact of cornea drying on its structure and optical parameters (2 eyes).

The light attenuation coefficient μ_t, of tissue, representing the sum of absorption coefficient μ_a and scattering coefficient μ_s, can be determined from the slope of the A-scan of the OCT signal measured for the region of interest using the fitting parameters of the approximating curve calculated by means of the single-scattering model. The single-scattering model bases on the assumption that only singly scattered light keeps coherent properties and contributes into formation of OCT signal. The single-scattering model well describes low scattering tissues, in particular, eye cornea. OCT signal in this case is defined as[3]

$$R(z) \approx P_0 \alpha(z) \exp(-\mu_t z), \qquad (1)$$

where $R(z)$ is the OCT signal; P_0 is the optical power launched into the tissue; $\alpha(z)$ is the reflectivity of the tissue at the depth determined by the local refractive index and the local ability of tissue to reflect (to scatter) light back; z is the distance from a tissue surface to the area of reflection. According to the single-scattering model the reflected power is proportional to exp $(-\mu_t z)$, i.e. can be approximated by expression: $R(z) = A\exp(-\mu_t z) + B$, where A is the coefficient of proportionality equal to $P_0\alpha(z)$, and B is the background signal. In the assumption that the value $\alpha(z)$ remains a constant within some interval of values Δz, it is possible to define μ_t from the measurements of the reflectivity at various depths of z_1 and z_2 within this interval:

$$\mu_t = \frac{1}{\Delta z} \ln \frac{R(z_1)}{R(z_2)}, \qquad (2)$$

where $\Delta z = |z_1 - z_2|$. As for the cornea $\mu_a << \mu_s$[4], therefore $\mu_t \approx \mu_s$. In our experiments the interval Δz corresponds to the full thickness of the cornea determined from the analysis of OCT images.

The transport of cement dust and keratoprotector through the cornea can be analyzed within the model of free diffusion which is widely used in the research of diffusion of medicinal preparations in biological tissues[8,9]. Geometrically, the cornea sample can be presented as an infinite plane-parallel slab with finite thickness l, cm. The one-dimensional diffusion equation has the form:

$$\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2}, \qquad (3)$$

where $C(x, t)$ is the concentration of the preparation in the cornea; D is the diffusion coefficient, cm^2/sec; t is the diffusion time, sec; and x is the spatial coordinate, cm.

The boundary conditions for unilateral diffusion are:

$$C(0,t) = C_0 \quad \text{and} \quad \frac{\partial C(l,t)}{\partial x} = 0, \qquad (4)$$

where C_0 is the volume concentration of the solutions on the cornea surface (1.74×10^{-3} for the cement dust and 0.038 for Systein Ultra). The second boundary condition reflects difficulty of diffusion of the solutions through the corneal endothelium in the forward camera of eye.

The initial condition corresponds to the absence of the solution inside the cornea before the application:

$$C(x,0) = 0. \qquad (5)$$

The solution of Eq. (3) with the boundary Eq. (4) and initial Eq. (5) conditions is:

$$C(x,t) = C_0\left(1 - \sum_{i=0}^{\infty}\frac{4}{\pi(2i+1)}\sin\left(\frac{(2i+1)\pi x}{2l}\right)\exp\left(-\frac{(2i+1)^2 D\pi^2 t}{4l^2}\right)\right).$$

The volume-average concentration of the preparation in the cornea sample is:

$$C(t) = C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-(2i+1)^2 t\frac{\pi^2}{4}D\Big/l^2\right)\right). \qquad (6)$$

In a first-order approximation, the Eq. (6) is reduced to:

$$\mathrm{C}(t) \approx C_0\left(1 - \exp(-t/\tau)\right), \qquad \tau = \frac{4l^2}{\pi^2 D}, \qquad (7)$$

where τ is the characteristic time of diffusion, sec.

The optical model of a cornea represents the system of densely packed thin dielectric cylinders (collagenic fibers) located in parallel each other. The scattering coefficient of the corrnea sample can be estimated as[10]:

$$\mu_s(t) = N\sigma_s(t) = N\frac{\pi^2 a x(t)^3}{8}\left(m(t)^2 - 1\right)^2\left(1 + \frac{2}{\left(m(t)^2+1\right)^2}\right)\frac{(1-\varphi)^3}{1+\varphi}, \qquad (8)$$

where $N = \varphi\big/\left(\pi a^2\right)$ is the number of the scattering particles per unit area; σ_s is the scattering cross-section; $x = 2\pi a n_I(t)\big/\lambda$ is the size parameter; $m(t) = n_c\big/n_I(t)$ is the ratio of the refractive indices of the particle (n_s) and surrounding medium (n_I); $n_c = 1.416 \pm 0.004$ [11] is the refractive index of collagen fibers in the cornea, $\varphi \approx 0.2\pm0.06$[12] is the volume fraction of the tissue scatterers; a is the radius of the scattering particles; $n_I(t)$ is the refractive index of interstitial liquid in the moment t. Penetration of the keratoprotector into the cornea increased the refractive index of tissue interstitial liquid, i.e. $n_I(t) = n_{I0} + 0.079C_{sys}(t)$, where $n_{I0} = 1.357 \pm 0.01$ [11] is the refractive index of interstitial liquid in the initial moment, $C_{sys}(t)$ is the concentration of the dissolved keratoprotector determined from Eq. (7).

In case of the cement particles penetration into the cornea, the scattering coefficient is defined as:

$$\mu_s(t) = \mu_s^{cor}(t) + \mu_s^{cem}(t), \tag{9}$$

where $\mu_s^{cor}(t)$ is the eye cornea scattering coefficient changing due to the dehydration, determined by Eq. (8); $\mu_s^{cem}(t) = 0.75\frac{C_{cem}(t)}{\pi a^3}\sigma_s$ is the scattering coefficient on the cement particles penetrating into the cornea; $C_{cem}(t)$ is the concentration of cement dust determined by Eq. (7); a is the average radius of the particles; σ_s is the scattering cross-section determined by Mie's theory[13]. Diameter of collagen fibers in a stroma layer of the cornea (according to different data) is: 28±4 nm[10], or 31 nm[12] that allows to evaluate the average diameter of the scatterers as 30±2 nm.

Eqs. (8) and (9) define dependence of scattering coefficient on the concentration of cement dust or keratoprotector in the cornea, i.e. create the direct problem. The inverse problem in this case is the reconstruction of the diffusion coefficient on the basis of the temporal evolution of the scattering coefficient. This problem is solved by minimization of a target function:

$$f(D) = \sum_{i=1}^{N_t}(\mu_s(D,t_i) - \mu_s^*(t_i))^2, \tag{10}$$

where N_t is the sampling number; $\mu_s(D,t_i)$ is the value of the scattering coefficient calculated using Eq. (8) or (9) in the moment t; $\mu_s^*(t_i)$ is the experimentally measured value of the scattering coefficient in the moment t.

To minimize the target function, the Levenberg–Marquardt nonlinear least-squares-fitting algorithm described in detail in Ref. 11 was used. Iteration procedure repeated until experimental and calculated data were matched.

The coefficient of permeability is one of the major characteristics which, along with diffusion coefficient, is used for the analysis of transport of medicines through biological membranes (in this case the eye cornea). According to the first Fick's law, the number of the particles diffusing along an axis x in a unit of time through a single area perpendicular to this axis, is described by the equation:

$$J(t) = -D\frac{\partial C(x,t)}{\partial x}. \tag{11}$$

The solution of this equation can be obtained as:

$$C(t) = C_0\left(1 - \exp\left(-\frac{P}{l}t\right)\right), \tag{12}$$

where P is the coefficient of permeability of a membrane, cm/sec.

Comparing Eqs. (7) and (12) it can be obtained:

$$P = \frac{\pi^2 D}{4l}. \tag{13}$$

All obtained results were processed by the methods of variation statistics with calculation of an arithmetic average value (*M*) and root mean square deviation (*sd*). The method of confidence intervals was used to assess the significance of differences. Differences were considered significant if $p < 0.05$.

3. RESULTS

OCT monitoring has shown significant decrease of cornea thickness under action of cement dust solution caused by the pronounced dehydrating properties of cement. Figure 1 demonstrates the relative change of the corneal samples thickness during exposition in the studied solutions and air. Values of thickness have been obtained from the analysis of OCT images of cornea samples, then averaged and normalized on the initial value. From the figure it is well seen that application of the solution with cement dust on the surface of the cornea leads to rather marked (approximately 10%) decreasing cornea thickness due to high hygroscopicity of cement dust. Drying of the cornea on air gives significantly lower level of dehydration (~4%). On the contrary, application of normal saline solution on the surface of the cornea leads to its swelling (thickness increases approximately by 6-8%). Preliminary application of the Systein Ultra considerably reduces cornea dehydration degree (decrease of thickness of the cornea is about 2%); the keratoprotector itself practically doesn't change the thickness of the cornea.

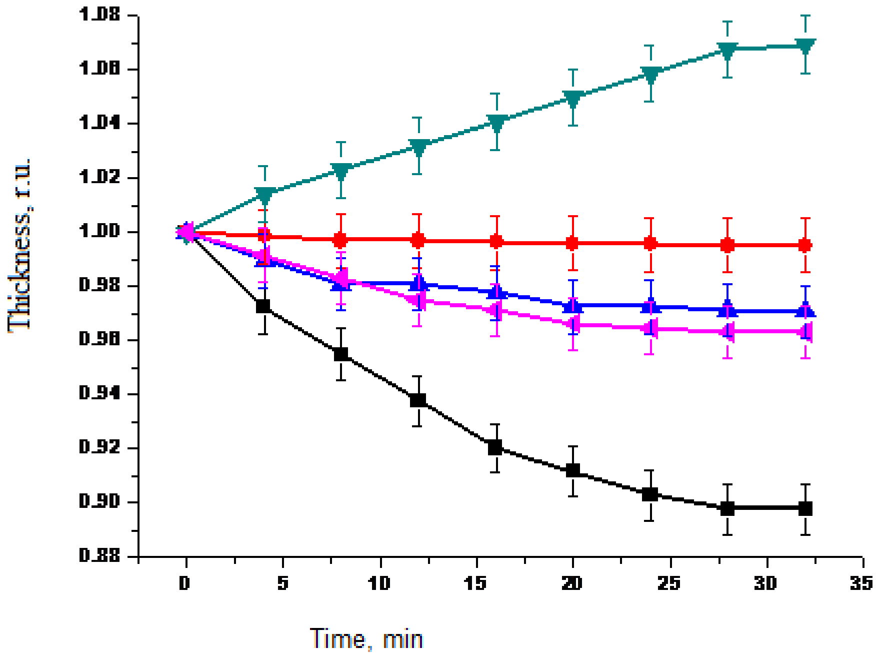

Figure 1. Relative change of thickness of the cornea *in vitro* at the exposition in cement dust solution (■), keratoprotector Systein Ultra (●), cement dust solution + keratoprotector (▲), normal saline solution (▼), and at the drying on air (◀). Symbols and bars correspond to the averaged experimental data with standard deviation.

In Figure 2 the kinetics of the change of the attenuation coefficient of the cornea measured at the impact of various solutions and at the drying on air and its approximation in the framework of the presented model is shown. Values of the attenuation coefficient were obtained from the analysis of OCT images of the studied corneas by means of Eq. (2), and then there were averaged and normalized on the initial value. It is well seen that the dehydration of the cornea leads to the increase of attenuation coefficient; it is especially pronounced when the cement solution has been used. In this case, the attenuation coefficient increases approximately in 2.3±0.12 folds. The cornea dehydration at the evaporation of water from a surface of a cornea causes the similar effect; the attenuation coefficient increasing is about 72±16%. In both cases the attenuation coefficient rises within the first 15 min and then the coefficient falls, however without reaching the initial value.

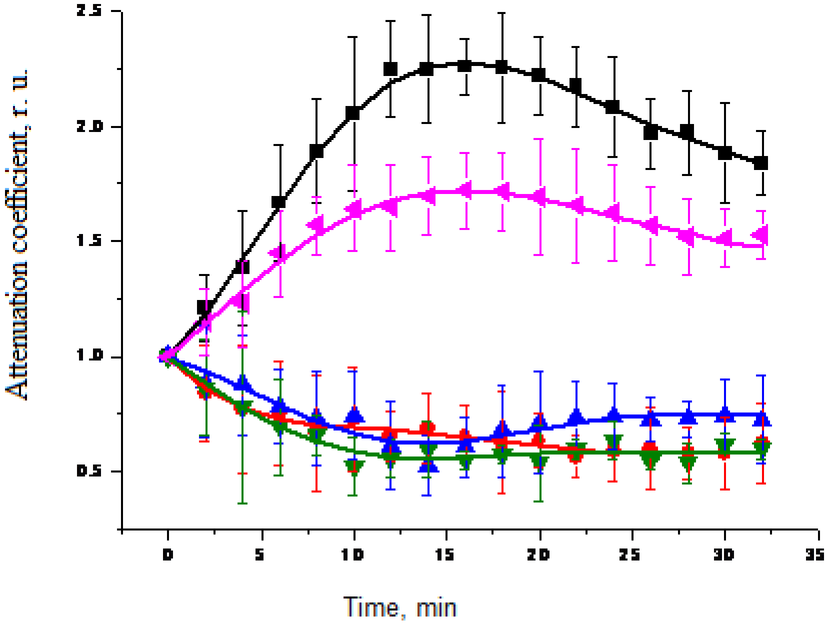

Figure 2. Relative change of the attenuation coefficient of the cornea *in vitro* at the exposition in cement dust solution (■),keratoprotector Systein Ultra (●), cement dust solution + keratoprotector (▲), normal saline solution (▼), and at the drying on air (◀).Symbols and bars correspond to the averaged experimental data with standard deviation. Solid lines correspond to the approximation in the framework of the presented model.

Impact of both normal saline solution and keratoprotector on optical properties of the cornea is shown from the decrease of the value of the attenuation coefficient (approximately by 40%). At the preliminary application of the Systein Ultra on the cornea surface with subsequent application of the cement dust solution, the absence of dehydration has been observed.

4. DISCUSSION

The use of the presented model allows one to explain experimentally observed dependences and to evaluate the rate constants characterizing process of penetration of cement dust and keratoprotector into eye cornea (Table 1).

Table 1. The rate constants characterizing process of penetration of cement dust and keratoprotector into eye cornea.

Preparation	Characteristic time, min	D, cm^2/sec	P, cm/sec
The filtered cement dust	9.84±0.48	$(1.26\pm0.33)\times10^{-6}$	$(7.41\pm1.93)\times10^{-5}$
Keratoprotector Systein Ultra	6.62±0.17	$(2.19\pm0.39)\times10^{-6}$	$(1.18\pm0.21)\times10^{-4}$
Saline	4.31±0.79	$(2.67\pm0.49)\times10^{-6}$	$(1.61\pm0.31)\times10^{-4}$
Drying on air	7.81±1.61	-	-

The increase of the attenuation coefficient is caused by the increase of density of packing of fibers due to the dehydration. The so-called "interferential member" $\varphi\frac{(1-\varphi)^3}{1+\varphi}$, the term in Eq. (8), is responsible for that. The additional light scattering on particles of the cement dust takes place. This conclusion is confirmed also by comparison of times characterizing rate of the process. It is seen that process of the dehydration goes faster at the use of the cement dust solution than at the evaporation. The impact of the cement dust particles is also manifested in the additional increase of the attenuation coefficient value leading to the cornea turbidity growing, that is confirmed by clinical trials and complaints of patients on the deterioration of vision[15].

Preliminary application of the keratoprotector on the cornea decreases significantly this effect, partially preventing both the cornea dehydration, and penetration of particles of cement dust into the tissue. As it is well seen from figure 2, at the initial stage (10-15 min) the attenuation coefficient decreases that is caused by action of the keratoprotector, and then in process of penetration of the dust particles into the cornea, its increase is observed in about 30-40 min.

The decrease of light scattering in the cornea under action of saline is connected with tissue swelling that leads to the decrease of volume fraction of fibers and, respectively, to the decrease of the attenuation coefficient (Eq. (8)).

5. CONCLUSION

OCT-tomography was used to monitor the attenuation coefficient of the cornea *in vitro* during the penetration of cement dust and/or keratoprotector. It was shown that 1) the cement dust falling on the eye cornea caused pronounced dehydration of the tissue (thickness decreasing) and led to the increase of the attenuation coefficient, which could affect the deterioration of the eyesight of workers in the conditions of cement production; 2) the application of the keratoprotector to the eye cornea when exposed by cement dust, slowed significantly the dehydration process and did not cause the increase of the attenuation coefficient that characterized the stabilization of visual functions. At this, the keratoprotector itself did not cause dehydration and led to the decrease of the attenuation coefficient, which could allow it to be used for a long time in the order to protect the organ of vision from the negative effects of cement dust.

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