

METHODS

INVESTIGATION OF ADHESION OF A VITREOUS BODY TO HARDENED POLYMERS

V. A. Agafonov and S. I. Anisimov

UDC 617.741-089.28:615.462]-07:617.747-02:
615.462]-07

KEY WORDS: vitreous body; polymers; adhesion.

Alloplastic materials used in surgery must satisfy a number of requirements. They must not be toxic, must induce the minimal reaction on the part of the organism, and they must have the necessary physical properties [4]. Special requirements apply to materials intended for the manufacture of intraocular lenses (IOL), which are widely used in ophthalmology [5, 8]. These materials, after conversion into IOL, must be transparent and have good surface properties, and their reaction with the organism must not induce changes in them detrimental to these important properties of the IOL. The range of materials which can be used for making IOL is continually widening [4, 7, 9]. The need has therefore arisen for comparative evaluation of their compatibility with the eye tissues, and it is important to know the character of interaction between IOL and the high-molecular-weight components of these media and, in particular, with hyaluronic acid, whose concentration is highest in the vitreous body [3].

In this investigation the adhesive activity of various synthetic polymers in relation to the vitreous body (VB) of the eye was compared.

EXPERIMENTAL METHOD

VB from three rabbits weighing 2.5 kg (of the same age) were used. The rabbit VB is large enough for all the parallel tests planned in the investigation. In addition, the density of the gel of the rabbit VB is sufficiently homogeneous for investigations of this kind. The animals' eyeball was opened in the region of the flat part of the ciliary body, and 50- μ l samples of VB were withdrawn by means of graduated glass tubes with a bore of not less than 3.0 mm. The measured fragments of VB were placed on coverslips and distributed with a dissection needle within a circle with a diameter of 15 mm. Two parallel samples of VB from the same rabbit were tested on all polymers, after which the experiment was repeated using two other rabbits, and all measurements on the same polymers were considered to be parallel. Several synthetic polymers (of three types), whose physical properties satisfy the requirements for materials used to make IOL were used for the investigation: type I) polyorganosiloxane SKTN-F-MED (All-Union Research Institute of Synthetic Rubber); type II) "Vitur" polyurethane (All-Union Research Institute of Glass Plastics); type III) ST-1 polymethyl methacrylate (USSR). Before the experiments the coverslips were covered with these polymers during hardening. A groove was formed on the resulting materials within a circle 15mm in diameter in order to fix the edges of the VB sample inside the given circle. VB applied to glass was used as the control. Coverslips covered with polymer, with VB, and water were placed on different pans of damped analytical scales (VLA-200, USSR) and dried. Deviation of the pointer of the scales, i.e., the difference between the weight of VB and water ($M_{vb} - M_w$) was recorded every 5 min during drying with an accuracy of 0.1 mg.

The results were assessed with respect to the following special parameters [2]: 1) the drying index or ratio of the drying time of VB to the drying time of water (T_{vb}/T_w). This index characterizes the mean density of the gel throughout the volume of VB; 2) residual mass (M_{res}) — analogous to T_{vb}/T_w , but characterizes the density of the gel more accurately, for its value can be obtained before complete drying of VB, and is independent of the terminal stage of drying of VB; 3) the drying gradient $\left(\frac{M_{res}}{T_{vb}/T_w - 1} \right)$, which characterizes the unevenness of density of the gel; 4) the dry residue (M_{dry}), which shows how much dry substance is contained in the sample; 5) dispersion of the frame (M_{res}/M_{dry}), which shows the degree of dis-

Moscow Research Institute of Microsurgery of the Eye, Ministry of Health of RSFSR.
(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.)
Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 101, No. 3, pp. 370-372, March, 1986. Original article submitted March 11, 1985

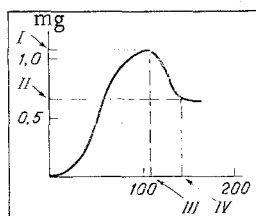


Fig. 1. Graphic recording of position of pointer of scales during drying of VB on polyurethane and water on glass, on different pans of the scales. Abscissa, drying time (in min); ordinate, difference in weight of objects on different scale pans (in mg). I) Residual mass, II) dry residue (M_{dry}), III) water drying time, IV) vitreous body drying time.

TABLE 1. Parameters of Drying of VB on Different Polymers ($M \pm m$)

Material	T_{vb}/T_w	M_{res}	$\frac{M_{res}}{T_{vb}/T_w - 1}$	M_{dry}	$\frac{M_{res}}{M_{dry}}$
Glass	1.28 ± 0.12	25.4 ± 2.5	18.2 ± 1.25	5.0 ± 0.1	5.1 ± 0.5
Polymethyl methacrylate ST-1	1.11 ± 0.04	16.6 ± 0.5	11.4 ± 0.32	5.7 ± 1.6	3.2 ± 0.1
"Vitur" polyurethane	1.28 ± 0.12	10.8 ± 0.5	6.9 ± 0.01	6.5 ± 0.7	1.8 ± 0.1
Siloxane SKTN-F-MED	1.16 ± 0.05	8.0 ± 0.4	5.6 ± 0.10	5.0 ± 0.9	1.6 ± 0.1

tribution of dry substance in the sample, maintaining the density of the gel (M_{res}). To obtain the values of these parameters, their mean values and error of the means were calculated.

EXPERIMENTAL RESULTS

After distribution of the samples of VB on the surface of the coverslips, covered with different polymers, some distinguishing features could be observed visually. In particular, when samples of VB were distributed by a needle on the surface of the siloxane, partial segregation of the frame of VB from its liquid part was observed. A similar phenomenon, but to a less marked degree, was observed when the VB were placed on polyurethane. The samples of VB appeared sufficiently homogeneous when placed on polymethyl methacrylate and glass.

Graphic recording of the position of the pointer of the scales during drying of VB and water on different pans yielded characteristic curves (Fig. 1). The results of analysis of these curves with respect to certain parameters are given in Table 1.

Table 1 shows that in a series of materials arranged in order of diminishing hydrophilicity of the surface from glass to siloxane [4], a tendency toward a decrease is observed in values of the residual mass, drying gradient, and dispersion of the frame. This is evidence of increasing adhesive activity in this series of substances relative to VB, for the decrease in residual mass (M_{res}) is linked with a decrease in density of the gel in the mass of VB, and a decrease in the ratio M_{res}/M_{dry} makes clear that the decrease in the previous parameter is connected with a decrease in dispersion of the frame of VB. The drying steepness index

$\left(\frac{M_{res}}{T_{vb}/T_w - 1} \right)$ is evidence more of a difference in the adhesive ability of the test materials relative to VB and of an increase in the change in gradient of the density of the frame of VB on the surface of materials in the given series. The parameters of drying and dry mass had no definite tendency to change when determined with the use of different polymers, i.e., they are inadequate for the solution of this particular problem.

When the mechanism of adhesion of VB, leading to collapse of the collagen frame, is discussed it will be recalled that in VB the collagen fibrils are covered with hyaluronic acid molecules [1]. Essentially adsorption of hyaluronic acid molecules takes place on the above-mentioned materials, and these precipitate the collagen fibrils bound with them. The result of this precipitation is destruction of VB, and this may be observed visually on the surface of siloxane and polyurethane, and it can be revealed quantitatively with the aid of the appropriate parameters during drying. On the less hydrophobic polymethyl methacrylate and the hydrophilic glass this adsorption was less marked. The degree of adsorption of hyaluronic acid was probably determined by the magnitude and density of the charges of the surface molecules of the materials tested, and also by hydrophobic interactions with the methyl groups of hyaluronic acid.

Investigation of the adsorption properties of materials *in vitro* can thus serve as a model with which to study adsorption processes taking place on the surface of IOL in the

living eye. Increased adsorption of hyaluronic acids may perhaps act as the trigger mechanism for subsequent adsorption of proteins on IOL, and in turn, this may induce an inflammatory reaction in the eye. Chronic inflammation in this case is an indication of incompatibility of the material with the surrounding media. Model investigations *in vitro* of this kind can provide speedy and reliable information on the adsorption properties of new materials for endoprostheses in ophthalmic surgery.

LITERATURE CITED

1. V. A. Agafonov, in: *Morphological Aspects of Ophthalmology* [in Russian], Moscow (1983), pp. 72-74.
2. V. A. Agafonov and S. I. Anisimov, *Byull. Eksp. Biol. Med.*, No. 12, 761 (1984).
3. A. Pirie and R. Van Heyningen, *Biochemistry of the Eye*, Springfield, Ill., 1956.
4. S. Manabu (editor), *Polymers of Medical importance* [Russian translation], Moscow (1981).
5. S. N. Fedorov, *Implantation of an Artificial Lens* [in Russian], Moscow (1977).
6. S. N. Fedorov et al., in: *Physiology and Pathology of Mechanisms of Adaptation of the Organ of Vision* [in Russian], Vol. 4, Vladivostok (1983), pp. 132-134.
7. R. B. Packard et al., *Br. J. Ophthalmol.*, 65, 585 (1981).
8. H. Ridley, *Trans. Ophthalm. Soc. UK*, 71, 617 (1951).
9. Zhou Kai-yi, *Chin. Med. J.*, 106, 175 (1983).

A METHOD OF PREPARATION OF LEUKOCYTES FOR ELECTRON MICROSCOPY

A. A. Pal'tsyn, N. V. Chervonskaya,
A. K. Badikova, and É. K. Uchaneishvili

UDC 616.155.3-076.4+[612.112+611-018.53]
-086.3

KEY WORDS: neutrophil, leukocyte, electron microscopy.

The usual method of precessing and embedding leukocytes for electron-microscopic investigation has certain disadvantages. These disadvantages are characteristic of any material composed of a cell suspension. In order to concentrate the cells in a volume small enough for ultramicrotomy, the suspension is usually centrifuged and all subsequent manipulations are carried out with the solid residue formed in the centrifuge tube. Under these conditions the cell pellet is in contact over the greater part of its surface with the glass of the tube, and only a small part of the surface remains exposed for penetration of the dehydrating and embedding media. A result of this state of affairs is poor dehydration and embedding. In turn, this leads to morphological disturbances in the material and makes the preparation of semi-thin and ultrathin sections much more difficult. In addition, if the cells composing the suspension are nonhomogeneous, centrifugation before fixation will disturb their uniform distribution in the suspension and will create zones consisting mainly or entirely of cells of only one type. Subsequent fixation consolidates this zonality of the residue (Fig. 1). Usually several pieces are cut out of the fixed and embedded residue. Under these conditions, because of the uneven distribution of the cells in the residue, the object of concern to the investigator may be absent not only in the section, but even in the whole fragment. Epoxide resins with low viscosity, suggested by Spurr [1], and BEEM capsules, which are in short supply, are necessary. However, even these of these resins and capsules does not dispense with the need for additional centrifugation of the material when introduced into the capsules. Only if this is done can the necessary density of distribution of the cells be achieved. Centrifugation of capsules is a tedious operation, and if it is not done exactly some resin may fall into the centrifuge vessels, polymerize in them, and make them unfit for further work. Specimens from each centrifuge tube must be extracted with a separate instrument to avoid accidental transfer of the crumbling material from one sample into another.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 101, No. 3, pp. 372-374, March, 1986. Original article submitted April 26, 1985