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Blueron Enzigartges kapselfärbemittel mit herausragender Biokompatibilität

The invention relates to the use of an aqueous, physiologically compatible solution in which a biocompatible triphenylmethane dye is dissolved, for the production of a dyeing agent for colouring the

5 *Membrana limitans interna* to be removed from the eye in retinal or vitreous humor surgery, wherein the dye in addition to dead cells to distinguish living material also colours living cells.

Compositions for colouring epithelial cells by means of mitochondrial dyes, in particular for the diagnosis of

10 cancerous or precancerous epithelial tissue, are known from WO 02/07693 A. Aqueous, buffered solutions of malachite green and brilliant green can be used as dyes.

15 Compositions which contain *inter alia* 0.1 to 0.5 % of a triphenylmethane dye, these compositions being producible as solutions, are known from GB-A-2,026,015 for colouring cells.

A method for detecting and for determining leukaemic

20 cells by means of differential diagnosis, in which a triphenylmethane dye is used, is known from EP-A-0 037 905.

Furthermore, it is known from WO 99/58160 that trypan blue can be used as a dye. This compound known from

25 the class of diazo dyes is used in an aqueous solution to dye the anterior capsule for a cataract operation on the eye. By visualizing the anterior capsule, the surgeon identifies the contour of the capsulorhexis, thereby facilitating the phacoemulsification.

30 Trypan blue is a cytotoxic substance, as known, for example, from Solomon K.D. et al.: Protective effect of the anterior lens capsule during extracapsular cataract

extraction, OPHTHALMOLOGY, vol. 96, no. 5, May 1989
(1989-05), 591-597 and Veckener M. et al.: Ocular
toxicity study of trypan blue injected into the
vitreous cavity of rabbit eyes, Graefe's Arch Clin Ex
5 Ophthalmol (2002) 239: 698-704. When trypan blue is
used, a complete rinse-out, in particular of the eye
region in which the trypan blue has been used as a
dyeing agent, is therefore required immediately after
the cataract operation in order to avoid a situation
10 where it remains in the body or in the eye respectively
for a comparatively long time.

It is known from US-A-4,764,360 that a dye with a
molecular weight of at least 10,000 can be added to a
polymerizate of high molecular weight forming a
15 carrier.

In this way a situation is to be avoided where the dye
penetrates into the surrounding body tissue. The dye
is only to dye the carrier of high molecular weight.

Furthermore, it is known (E. Kutchera, "Vitalfärbung
20 der abgehobenen Netzhaut und ihre Defekte", Albrecht v.
Graefes Arch. klin. exp. Ophthal. 178, 72,87 (1969))
that the dye patent blue can be injected intravitreally
in order to make defects affecting the whole retina
visible in cases of retinal detachment. A 0.47% patent
25 blue hyaluronic acid solution was used for the
intravitreal injection. Visualization of retinal
detachment is extremely time-consuming and only ensues
a few days after the injection.

An object of the invention is to provide for the
30 production of a dyeing agent with an absence of
cytotoxicity that is suitable for making *Membrana
limitans interna* visible in the human or animal eye.

This object is achieved in accordance with the invention by means of the features of claim 1. The subclaims contain advantageous further developments of the invention.

5 In the case of the invention, as a result of the use of a biocompatible triphenylmethane dye without a carrier in a physiologically compatible aqueous solution of in particular sodium chloride that can be adjusted with a buffer to a pH of 6.8 to 7.8, in particular
10 approximately 7.4, a dyeing agent is provided to colour the membranes in the human and animal eye. The dyeing agent can be a non-polymer, water-soluble dye of low molecular weight. The dyeing agent applied in the case of the invention can be used to examine vitality, in
15 which case, however, in contrast with conventional vital dyes, with the biocompatible dye that is applied in the case of the invention in addition to the dead cells to distinguish from the living material the living cells can also be dyed.

20 A triphenylmethane dye is used as a water-soluble dye of low molecular weight. The dye is used without a carrier. Examples of such suitable dyes are patent blue and brilliant blue R, with the latter being known from protein colouring in gel electrophoresis.

25 The patent blue is preferably a patent blue V ($C_{54}H_{62}CaN_4O_{14}S_4$, molecular weight: 1159.45) that is permitted as a food colour (food blue 3 = E 131).

A phosphate-, hydrogen-carbonate- or citrate-buffer whose pH value can be adjusted by means of sodium
30 hydroxide can be used as a buffer. The concentration of the biocompatible dye, for example patent blue V, in aqueous solution preferably amounts to 0.6 to 2.5 g/l, in particular approximately 1.2 g/l. Spontaneous

dyeing of the desired areas in the human or animal body is achieved.

The dyeing agent can be used to colour the lens capsule, in particular the lens anterior capsule in the case of a cataract operation. The colouring is effected before the capsulorhexis and phacoemulsification.

For the colouring, the aqueous humor is removed by suction through a corneal or scleral tunnel incision, and the anterior chamber is subsequently filled with a gas, in particular air. Approximately 0.3 ml dyeing-agent solution, for example patent blue V, is administered into the anterior chamber by means of a cannula. Colouring of the lens capsule limited by the pupillary margin of the iris results. After a few seconds, the anterior chamber is rinsed out with a sodium-chloride solution in order to wash out the dye that is not required.

Subsequently, in order to carry out the cataract operation in a conventional way a viscoelastic solution is introduced into the eye anterior chamber. On account of the blue discolouration of the anterior capsule the contour of the capsulorhexis stands out clearly and can be clearly distinguished from the grey tissue of the nucleus lentis.

Furthermore, the dyeing agent can be used to colour the membrana limitans interna or membranes that develop, for example, in consequence of PVR (proliferative vitreoretinopathy), in particular epiretinal membranes on the retina or on the rear surface of the vitreous humor limiting membrane, in particular in the case of retinal and vitreous humor surgery.

During removal, for example, of an epiretinal membrane from the retina, with the aid of a cannula inputting by way of the pars plana, the dye, for example patent blue V, in approximately 0.3 ml of the specified buffer solution is brought selectively to the membrane that is to be removed. The vitreous humor can be replaced beforehand completely or partly by means of a gas filling, as used in a conventional way in vitreous-humor or retinal, in particular macula, surgery. When colouring the epiretinal membrane, if applicable colouring of the adjacent retina tissue can be effected with a fainter degree of dyeing. When the membrane is removed from the retina tissue that lies underneath and is not dyed, a good contrast then ensues. After the colouring, excess dyeing-agent solution is rinsed out and the free space is filled by the gaseous vitreous-humor replacement identified. As a result of the dyeing it is possible to operate with an instrument that is not illuminated or is only faintly illuminated when cutting away the membrane. As a result, with sufficient contrast-awareness the light-toxicity is considerably reduced. In particular, when applied in connection with epiretinal membranes (epiretinal gliosis, "macular pucker", "surface wrinkling"), the use of the dyeing-agent solution constitutes a valuable aid when searching for and removing the membranes.

When in the case of macula foramen with increasing hole size removal of the membrana limitans interna is required, the dyeing of this membrane with the dyeing-agent solution proves to be an advantageous aid in the search for and removal of this membrane during vitreous-humor surgery.

Furthermore, it is possible to dye with the aqueous dyeing-agent solution a viscoelastic material, for example hyaluronic acid, which is used as an aid in

ophthalmological surgery. In particular, in the case of the cataract operation an improvement in the contrast of the viscoelastic aid in comparison with the intra-ocular tissue, in particular the eye iris and the fundus reflex, can be achieved as a result.

In comparison with the conventional trypan blue, which has teratogenic or mutagenic action (Cahen RL: Evaluation of the teratogenicity of drugs, Clin Pharmacol. Ther, 1964, 5, 480-514 and Product Information BLURHEX™, Dr. Agarwal's Pharma Ltd. Chennai (India)), the biocompatible solution in accordance with the invention, for example patent blue V or brilliant blue R, has no cytotoxicity.

In order to demonstrate the absence of cytotoxicity, mouse cells L 929 and ARPE 19 cells were treated with the dyeing agent patent blue V in accordance with the invention with different concentrations, over 68 to 72 hours, in the incubator. The vitality of the cells and derived cytotoxicity are determined quantitatively by determining the protein content of the treated cell cultures in comparison with untreated control cultures. Using a standard method, the protein content is ascertained colorimetrically.

It is thereby shown that cytotoxicity is not present to a significant level, corresponding to a growth inhibition of more than 30%.

The invention proves to be particularly advantageous when carrying out cataract operations with dense cataracts and/or heavily pigmented fundi, in which the fundus reflex is only weakly formed or is missing. With the aid of the dyeing agent, a good contrast between the coloured anterior capsule and the underlying tissue is achieved

Exemplary embodiments of the dyeing agent in various buffer solutions are specified in the following.

Example 1

Patent blue V with a concentration of 1.2 g/l in a
5 phosphate-buffer solution.

200 ml solution contain:

0.240 g patent blue V

0.380 g disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \times 2 \text{ H}_2\text{O}$)

0.060 g sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \times 2 \text{ H}_2\text{O}$)

10 1.640 g sodium chloride (NaCl)

sodium hydroxide for the pH-adjustment.

Example 2

Patent blue V with a concentration of 1.2 g/l in a hydrogen-carbonate-buffer solution.

15 200 ml solution contain:

0.240 g patent blue V

0.420 g sodium hydrogen carbonate (NaHCO_3)

1.640 g sodium chloride (NaCl)

sodium hydroxide for the pH-adjustment

20

Example 3

Patent blue V with a concentration of 1.2 g/l in a citrate-buffer solution.

200 ml solution contain:

25 0.240 g patent blue V

0.216 g trisodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \times 2 \text{ H}_2\text{O}$)

1.640 g sodium chloride (NaCl)

sodium hydroxide for the pH-adjustment

Identical exemplary embodiments in accordance with
30 Example 1, 2 and 3 can also be produced with brilliant blue R with a concentration of 1.2 g/l.

In the case of the buffer solutions, the pH value is preferably adjusted by means of sodium hydroxide. It is, however, also possible to adjust the solution itself to the desired pH value (neutral, slightly
5 acidic, slightly alkaline) within the preferred range of 6.8 to 7.8. The concentration of patent blue is adjusted to preferably 0.6 to 2.5 g/l, in particular approximately 1.2 g/l, by means of a corresponding amount of patent blue V.

Patentkrav

1. Anvendelse af en vandig fysiologisk kompatibel opløsning, i hvilken et biokompatibelt triphenylmethanfarvestof er opløst, til fremstilling af et farvemiddel til farvning af *Membrana limitans interna*, der skal fjernes fra øjet ved nethinde- eller glaslegemekirurgi, hvorved farvemidlet foruden døde celler til adskillelse af levende materiale også farver levende celler.
2. Anvendelse ifølge krav 1, hvorved farvestoffet opløses i en neutral eller svag sur eller svag alkalisk buffer.
3. Anvendelse ifølge krav 1, hvorved farvestoffet opløses i en buffer med en pH-værdi på 6,8 til 7,8.
4. Anvendelse ifølge et af kravene 1 til 3, hvorved der anvendes en phosphat-, hydrogencarbonat- eller citrat-buffer.
5. Anvendelse ifølge et af kravene 1 til 4, hvorved koncentrationen af farvestoffet i bufferopløsningen udgør 0,3 til 2,5 g/l, især ca. 1,2 g/liter.
6. Anvendelse ifølge et af kravene 1 til 5, hvorved der som farvestof anvendes patentblå V.
7. Anvendelse ifølge et af kravene 1 til 6, hvorved der som farvestof anvendes brillantblå R.