TRANSMITTANCE AND SCATTERING DURING WOUND HEALING AFTER REFRACTIVE SURGERY.

S. Mar[*a], C. Martínez-García[b], J.T. Blanco[c], R.M. Torres[c], V. R. González[a], S. Nájera[a], G. Rodríguez[c] and J.M. Merayo[c].

[a]Departamento de Física Teórica, Atómica y Óptica, Facultad de Ciencias, Universidad de Valladolid, 47071 Valladolid, Spain.

[b]Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain

[c]Instituto Universitario de Oftalmobiología Aplicada, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain

ABSTRACT

Photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) are frequent techniques performed to correct ametropia. Both methods have been compared in their way of healing but there is not comparison about transmittance and light scattering during this process. Scattering in corneal wound healing is due to three parameters: cellular size and density, and the size of scar. Increase in the scattering angular width implies a decrease the contrast sensitivity. During wound healing keratocytes activation is induced and these cells become into fibroblasts and myofibroblasts. Hens were operated using PRK and LASIK techniques. Animals used in this experiment were euthanized, and immediately their corneas were removed and placed carefully into a cornea camera support. All optical measurements have been done with a scatterometer constructed in our laboratory. Scattering measurements are correlated with the transmittance, the smaller transmittance is the bigger scattering is. The aim of this work is to provide experimental data of the corneal transparency and scattering, in order to supply data that they allow generate a more complete model of the corneal transparency.

1. INTRODUCTION

Transparency is defined as the property of transmitting light without appreciable scattering so that structures lying beyond are seen clearly. Scattering is a phenomenon produced by light dispersion when light crashes with particles. There are different models to explain the corneal transparency and in particular the small quantity of scattering light produced by this tissue. All traditional theories to explain corneal transparency[1-7] have focused on light propagation in the stromal extracelular matrix.

From Benedek[4] it know that light is scattered by fluctuations in the index of refraction which wavelengths are larger than one-half of the wavelength of light in the medium. These fluctuations may be produced by the microstructural alterations. Then the corneal stroma contains irregular regions or lakes in which there is no collagen present at all. Recently Aghamohammadzadeh[8] by means of X-Ray scattering it has determined the preferred orientations of collagen in healthy corneas.

However there are important quantities of cell, like as keratocytes, and sometimes fibroblast or myofibroblast. It is reasonable to think about of its influence on the light transmitted. Møller-Pedersen[9] had studied these not clarified aspects. Many studies have tried to relate the form, density and size of the cells with the structure of the light scattered. Mourant[10] suggests that the cell itself is responsible for scattering at small angle, at slightly larger angle they data indicate that the nucleus is primarily responsible for scattering. Organelles, such as mitochondria and lysosomes, are likely responsible for scattering at larger angles.

All these models have been developed and proved in different types of tissues. However the cornea is a special tissue because of have an especially appropriate structure to transmit the light. This makes that even in a damage cornea, the transmission, absorption and scattering have behaviours that should be studied in a specifies way. In a general way we could affirm that the scattering in a damage cornea comes from the microstructural alterations in the stromal

[*] santiago@opt.uva.es; phone 34 983 423140; fax 34 983 423013

extracelular matrix, and the scattering by different cells like keratocytes, fibroblast or myofibroblast, even by some organelles of these cells. When the healing process has been completed the stromal extracelular matrix it recovers partial or totally his order and the cells alter its composition to acquire more similar refraction index to the means and to avoid the scattering.

In spite of all the works carried out it don't still have a totally reliable model that allows to explain the corneal transparency. The aim of this work is to provide experimental data about of the corneal transparency and scattering, in order to supply data that allow generate a more complete model of the corneal transparency.

In this work it is proof that a high correlation exists among the lost of corneal transparency and the scattering. However this last one doesn't allow to explain the lost of transparency; other phenomena like as the absorption or the reflection are necessary to understand the results. An increase in the scattering angular width implies a decrease in the contrast sensitivity, however the reflection, absorption or backscattering doesn’t change the contrast sensitivity. That mean that it is necessary a complete model of transparency of eye in order to know the visual performances.

2. METHODOLOGY

The measurement device is a scatterometer, Fig. 1, working with three wavelengths constructed in the Optics Laboratory (University of Valladolid. Spain) to optimize some of its benefits. The light that comes from three lasers (Red He-Ne 632,8 nm, Green He-Ne 543,5 nm and infrared diode 830,0 nm) with the help of mirrors and beam splitters is lead towards the camera that contains the sample to measure. A set of polarizers controls the light intensity and defines the beam polarization plane. Always it has been employed p-polarization. A set of shutters allows selecting the measure wavelength.

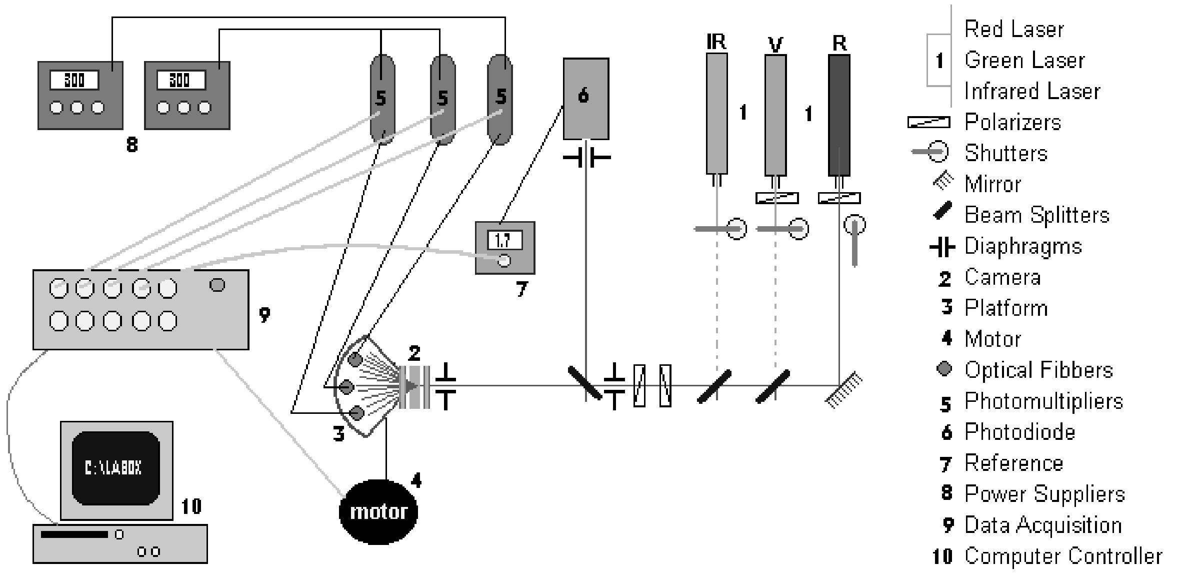

Figure 1: Experimental set-up.

Three optical fibbers collect the light spread in the camera and send to individual photomultiplied (5). The fibbers are mounted on a platform (3) that turns in a horizontal plane, driven by a stepping motor (4). The platform’s axis of rotation coincides with the impact point of the laser beam on the sample. The fibbers are placed 32.3 mm away from the axis of rotation and there is an angle of 30º between each two of them. With this arrangement, and supposing a symmetric scattering, rotating the platform 30º allows to obtain the whole angular distribution of scattered light. However, the main reason for having three detection channels is the strong dependence of the light intensity with the scattering angle. On this way, each channel is programmed with a different sensitivity depending on the measure angular interval. This procedure permits to obtain the angular distribution of scattered light in a range greater than six orders of magnitude.

There is also a fourth channel (6), which collects a part of the light emitted by the laser, before reaching the sample, in order to control the intensity variation of the laser beam.

The corneal support camera, made of stainless steel, has on both ends a Pyrex window, 26 mm in diameter These windows are 17 mm away from each other. A liquid maintenance medium, with constant temperature, flows continuously through the camera. This arrangement does not limit the external measure angle ranging from –85º to +85º, referred to the incident beam. The main limitation is caused by the limit angle of the second surface of the exit window. Depending on the refractive index of the liquid, the maximum scattering angle measured inside the camera is 54º. From now on, the scattering angles will be always referred to the inner part of the camera.

In order to place the cornea properly inside the camera, a device formed by two nylon or stainless steel sheets are used. The cornea is placed between the two sheets, like in a "sandwich". The sheets are shaped with the same cornea's curvature and have a hole that allows the light crossing.

The whole system is computer (10) controlled: shutter opening, platform rotating, measure recording, etc. The measure of the whole angular distribution of scattered light lasts a few minutes that avoid the cornea damage.

In order to test the experimental apparatus, a measure was carried out using as scatter sample a suspension of polymeric microspheres (10% and 5%) in distilled water. The nominal diameter of the microspheres was 3.063±0.027 µm (Duke Scientific Corporation). The angular scattering distribution for each of the three lasers was measured. The good agreement between the measured and the calculated data answers for the reliability of the experimental arrangement. Obviously, it has been previously carried out the intensity calibration of lasers, fibbers, polarizators and detectors, the stability and fluctuation of lasers, the measure of shutters open and close times, platform's rotation angle, etc.

The measure protocol includes the recording of the angular distribution of light intensity with the camera filled with the maintenance liquid, without cornea. This "control" measurement is carried out every five or six measurements with cornea. From the point of view of calibration, these control measurements provide the angular resolution of the scatterometer. The light intensity becomes half maximum when the platform rotates 0.49º±0.01 for the red laser, 0.48º±0.01 for the green laser, and 0.47º±0.01 for the infrared laser; and it diminishes in a factor of 10 when the platform rotates 0.79º±0.01 for the red laser, 0.79º±0.02 for the green laser and 0.75º±0.01 for the infrared laser. Therefore, the angular resolution of the scatterometer is less than 1º.

With the experimental set-up previously described, it has been measured the light scattered by more than 100 corneas, corresponding to healthy and operated corneas. The corneas cover a wide interval of situations, from ones healthy with transmittances close to 100% until others with very severe lesions with almost absolute opacities.

The animals were cared for in accordance with the Guidelines for Use of Animals in Vision and Ophthalmic Research of the Association for Research in Vision and Ophthalmology (ARVO). The animals were euthanized, and the corneas were immediately removed and placed carefully into the cornea support. The experiment is optimized to reduce the time needed for "in vitro" measurements and thus to avoid loss of tissue transparency. The total measurement time for each cornea lasted ≈ 15 min. It has been proved that if the measurement time was four times this value, the tissue transparency was the same. Special care was taken to avoid the formation of small bubbles during injection of the maintenance liquid into the camera.

For each cornea, measurements were taken for 146 angular positions, in the range from –1.2º a +85º outside of cornea camera, corresponding to –0.9º y 48.6º inner of the camera. As it has been already said, every five or six corneas, a control measurement was taken, that is, a measurement without cornea.

3. RESULTS

In figure 2, it has been plotted the total transmitted energy integrated for all the angular positions (total transmittance) versus the transmittance in the same direction of the incident beam (zero transmittance) for each cornea. Excepting the corneas with transmittances less than 5%, there is a high lineal correlation between both transmittances, for all the wavelengths employed (R^2= 0.97 for the green laser, R^2= 0.96 for the red laser and R^2= 0.95 for the infrared diode). These results allow to conclude that both magnitudes have nearly the same information, whatever is the cornea's state.

Also it is observed that the line slope is very close to the unit (m=1.03 for the green laser, m=1.01 for the red laser and m=0.95 for the infrared diode). In contrast to sometimes it has been described, when a cornea loses transmittance in the incident direction is not completely due to the scattering, but to the absorption of the light by the tissue itself or to reflection and backscattering.

From Benedek[4] it know that light is scattered by the fluctuations in the refraction index. These fluctuations may be produced by the microstructural alterations then the corneal stroma contains irregular regions or lakes in which there is no collagen present at all. Benedek justifies the cornea opacity through the scattering produced by these fluctuations of the refraction index. If this it was the only cause of the opacity, the total transmitted energy integrated for all the angular positions should be higher, that is to say, the total transmittance should be bigger than the zero transmittance, and therefore the line slope should be bigger than the unit. This means that the absorption, reflection and backscattering should be play an outstanding paper. However it has not been demonstrated that the cornea damage have complex refraction index, that is to say that the coefficient of absorption is zero. With these alone hypotheses a strong reflection or backscattering would explain this result. As we will see later, the reduced angular interval of the scattering confirms this hypothesis.

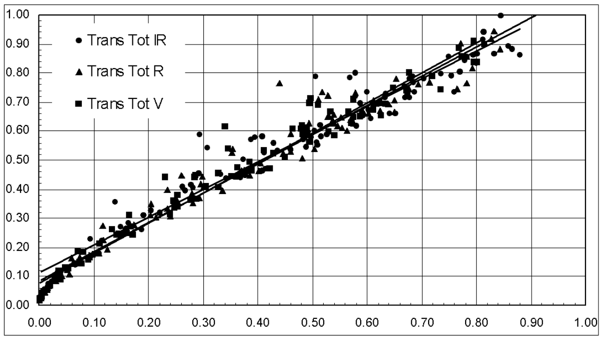

Figure 2: Total transmittance versus zero transmittance for each cornea and wavelength.

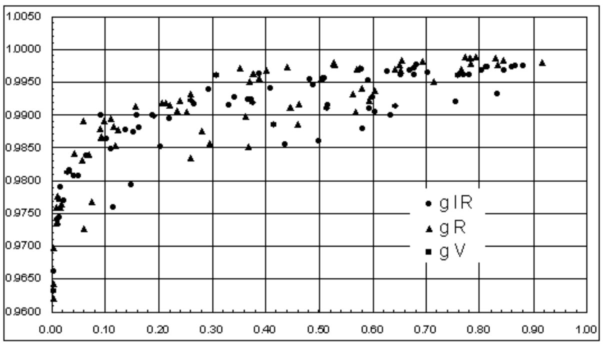

Figure 3: Mean cosine of scattering angle (g) versus zero transmittance.

This result is compatible with other experimental data. Jester[11] has measured the reflection and backscattering and he has found a remarkable increase in those regions damaged, with a high density of keratocytes. This behaviour is interpreted as the lack of protein ALDH3 in the keratocytes and the consequent reduction of the homogeneity in the refractive index of corneas. On the other hand the theory of Mie predict a backscattering very small. If both ideas are true, we would have to conclude that most of the light sent backwards must to the reflection. If the absorption is small these hypotheses locates to us in a very delicate position, since the lack of homogeneity in the refractive index would be responsible for a very important reflection, about 70%, and the forward scattering and backscattering, small.

At sight of the experimental data it seems more reasonable to admit than most of the energy that does not reach the retina is as a result of two reason: the absorption and the reflection. The absorption must be due to the breakage of the structure in the stromal extracelular matrix. The reflection is maybe due to an increase in the separation of the fibbers of collagen that would generate destructive interference towards ahead and constructive interference backwards, just the opposite to the normal behaviours of these fibbers.

This effect is more accusing in low that in high wavelengths and would be compatible with an interferencial phenomenon like as we have indicated. Despite as much the absorption as the reflection or backscattering they would not have to affect of significant form the quality of the retinal image. As it will be seen ahead, the lost of transmittance is associate to a greater angular dispersion in scattering. In any case, this phenomena has a minor importance from the point of view of energy

Assuming single scattering, it is possible to calculate the mean cosine of scattering angle, g, from the angular measurement I(θ) via:

$$g = \frac{\int_{4\pi} \cos\theta \times I(\theta) \times d\omega}{\int_{4\pi} I(\theta) \times d\omega}$$

The correlation among the mean cosine of scattering angle (g) and the transmittance is shown in the figure 3. As can be appreciated, except for corneas with very severe lesions (transmittance <5%), the value of g is very close to the unit (g > 0.98). This value is reasonable in corneas healthy, since the scattering is very small to be able to obtain a clear image in the retina. However in most of the tissues[12] g is usually between 0.60 and 0.90. This behaviour of g indicates that corneas, even the very seriously damaged ones, present a very small scattering, which is compatible with its function of forming image. In order to loses this properties it is necessary that the lesion is extraordinarily severe (transmittance < 5%).

The shape of the central part of scattering curve is related to the kind of cells that form the corneal tissue, and thus is different from each cornea, depending on the course of healing. On the other hand, the transmittance depends on the organized structure of the tissue. Therefore, the scattering curve and the transmittance should not be necessary related. Obviously, as greater the corneal wound is, there are a larger number of cells which contribute to the scattering, and more disorganized the tissue is. For this reason, there is always a correlation between the scattering widths and the transmittance, but this correlation can not be high because it depends on the particular state of the cornea healing. In the case of the scattering curve wings, they are not related to the particular state of each cornea, and then the correlation with the transmittance is better.

4. CONCLUSION

The lost one of transmittance of each cornea is not related to an increase of the light scattered, but by absorption of the tissue or reflection, more likely due to the lack of structure of the damaged tissue. In any case the scattering concentrate the light on very small angles, if we compare it with the scattering in another type of tissues. This behaviour would be reasonable in corneas healthy or with small lesions, but it remains in corneas with very severe lesions. This fact allows that corneas with very high opacities, they still conserve a certain capacity to form images in the retina. It haven't data to affirm that in the damage corneas the absorption is high, so an important part of the incident light in damage corneas is reflected or back scattered. These results allow to conclude that in situations with the luminance of the scene is almost constant, the quality of image is acceptable. Only when exist very intense blinding points, the sensibility to the contrast it will be seriously affected and with it the vision performances. Everything seems to indicate that the lost of transmittance of a damage cornea it is more related with the reflection or the back scattering that with the scattering. The bigger is the scattering the smaller transmittance is.

ACKNOWLEDGMENTS

The authors thank S. González for his collaboration in the experimental arrangement. The Spanish Dirección General de Investigación Científica y Técnica (Ministerio de Educación y Ciencia), the Fondo de Investigación Sanitaria (Instituto Carlos III) and the Consejería de Educación y Cultura (Junta de Castilla y León), for their financial support under contracts No. BFM2002-01002, PI 01/0270 and VA004/04 respectively. The Alcon-Cusi Laboratories, for the donation and maintenance of the excimer laser. The Novosalud (Dr. R. Gutierrez), for the donation of the mycrokeratome.

REFERENCES

1. D.M. Maurice,. "The structure and transparency of the cornea", J. Physiol. Lond. **136**, p. 263, 1957.
2. J.N.Goldman and G.B. Benedek, "The relationship between morphology and transparency in the non swelling corneal stroma of the shark", Invest. Ophthalmol. **6**, p. 574, 1967.
3. R.W. Hart and R.A. Farrell, "Light scattering in the cornea", J. Opt. Soc. Am. **59**, p. 766, 1969.
4. G. B. Benedek, "Theory of Transparency of the Eye", Applied Optics **10-3**, p. 459, 1971.
5. R.A. Farrell, R.L. McCally and P.E. Tatham, "Wave-length dependencies of light scattering in normal and cold swollen rabbit corneas and their structural implications", J. Physiol. Lond. **233**, p. 589, 1973.
6. H. Liu, B. Beauvoit, M. Kimura and B. Chance, "Dependence of Tissue Optical Properties On Solute-Induced Changes In Refractive Index and Osmolarity", J. of Biomedical Optics **1-2**, p. 200, 1996.
7. J. R. Mourant, M. Canpolat, C. Brocker, O. Esponda-Ramos, T. M. Johnson, A. Matanock, K. Stetter, J. P. Freyer, "Light Scattering from Cells: the Contribution of the Nucleus and the Effects of Proliferative Status", J. of Biomedical Optics **5-2**, p. 131, 2000.
8. H. Aghamohammadzadeh, R. H. Newton and K. M. Meek, "X-Ray Scattering Used to Map the Preferred Collagen Orientation In the Human Cornea and Limbus", Structure **12**, p. 249, 2004.
9. T. Moller-Pedersen, "Keratocyte Reflectivity and Corneal Haze", Experimental Eye Research **78**, p. 553, 2004.
10. J. R. Mourant, J. P. Freyer, A. H. Hielscher, A. A. Elck, D. Shen and T. M. Johnson, "Mechanisms of Lights Scattering from Biological Cells Relevant to Noninvasive Optical-Tissue Diagnostics", Applied Optics **37-16**, p. 3586, 1998
11. J.V. Jester; T. Moller-Pedersen, J. Huang, C.M. Sax, Wm.T. Kays, H.D. Cavangh, W.M. Petroll and J. Piatigorsky. "The cellular basis of corneal transparency: evidence for 'corneal crytallins'". J. of Cell Science **112**, p. 613, 1999.
12. I. A. Vitkin, J. Woolsey, B. C. Wilson and R. Rox Anderson, "Optical and Thermal Characterization of Natural (Sepia Officinales) Melanin", Photochemistry and Photobiology **59-4**, p. 455, 1994.