

Morphological analysis of the corneal endothelium in eyes of dogs using specular microscopy¹

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ABSTRACT.- Pigatto J.A.T., Cerva C., Freire C.D., Abib F.C., Bellini L.P., Barros P.S.M. & Laus J.L. 2008. **Morphological analysis of the corneal endothelium in eyes of dogs using specular microscopy.** *Pesquisa Veterinária Brasileira* 28(9):427-430. Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: pigatto@ufrgs.br

Both healthy eyes of 10 six-year-old male and female mongrel dogs were studied. With a contact specular microscope the corneal endothelium was examined. Endothelial cells were analyzed in the central and peripheral cornea. Morphological analysis with regard to polymegathism and pleomorphism was performed. Three images of each region with at least 100 cells were obtained. The analysis showed that polygonal cells formed a mosaic-like pattern uniform in size and shape. The predominant number of cells was hexagonal. The polymegathism index was 0.22. The study demonstrates that the morphology of the normal corneal endothelial cells of dogs is similar to that found in the human cornea.

INDEX TERMS: Dogs, corneal endothelium, morphology, specular microscopy, *Canis familiaris*.

RESUMO.- [Análise morfológica com microscopia especular do endotélio corneal em olhos de cães.] Foram estudados 20 olhos de 10 cães sem raça definida, machos e fêmeas com 6 anos de idade. A morfologia das células do endotélio da córnea foi analisada utilizando-se microscópio especular de contato. Foram estudadas as regiões central e periférica da córnea. De cada região da córnea foram realizadas três micrografias. De cada mi-

crografia foram analisadas no mínimo 100 células endoteliais. Foram obtidos os valores do polimegatismo e pleomorfismo. O endotélio corneano de cães caracterizou-se por uma monocamada de células poligonais uniformes em tamanho e forma. A forma predominante das células endoteliais foi hexagonal. O índice de polimegatismo foi 0.22. Este estudo demonstrou que a morfologia das células do endotélio da córnea de cães é semelhante à observada em humanos.

TERMOS DE INDEXAÇÃO: Cães, endotélio corneano, morfologia, microscopia especular, *Canis familiaris*.

INTRODUCTION

The corneal endothelium is a single layer of polygonal cells on the inner surface of the cornea. The endothelial integrity and metabolic activity are essential for continuous maintenance of corneal dehydration and transparency (Waring et al. 1982). Ageing results in a gradual reduction in endothelial cell density and an increase in cell area variability in those species that exhibit minimal mitotic potential (Jackson et al. 1995). Furthermore, morphological changes of endothelium associated with cell loss occur in many conditions including corneal dystrophies, keratoconus, glaucoma, uveitis, blunt ocular trauma, cataract ex-

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traction, and penetrating keratoplasty (Olsen 1980, Tuft & Coster 1990).

The specular microscopy has become a standard technique to determine endothelial cell counting and cell morphology (Laing et al. 1979). Among the useful parameters to determine endothelial cell status are the endothelial cell density and cell morphology.

The morphological parameters that can be quantified are principally the polymegathism and the pleomorphism. The polymegathism is an index that provides a quantitative measurement of variation in cell size and the pleomorphism is the variation of cell shape such as percentage of hexagonal cells (Laing et al. 1979). Despite the relative widespread availability of instruments to the assessment of the endothelial mosaic, there are few detailed reports on endothelial morphology of vertebrates, particularly mammals (Peiffer et al. 1981). The objective of this study was to assess the morphology of the corneal endothelial cells in normal eyes of dogs using specular microscopy.

MATERIALS AND METHODS

Twenty normal eyes from 10 six-year-old male and female mongrel dogs, with 15 kg weight, were studied. All procedures were performed in compliance with the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic and vision research. The dogs were euthanized for reasons unrelated to this study. Eyes that showed evidence of ocular disease were excluded. Eyes were enucleated immediately after euthanasia of the dogs and transported to the laboratory in moist chamber containing physiologic saline. Studies of these corneas were initiated within 1 h after enucleation.

Eyes were mounted on an eyeball holder and examined using a contact specular microscope (Bio-Optics, model LSM-2100 C, Inc. Arlington, MA, EUA) with software for corneal endothelium analysis. Specular microscopy was performed on all eyes in orders to determine the conditions of the corneal endothelium and those with evidence of alteration were excluded. All analyses were carried out by the same investigator (FCA.). Three micrographs of the corneal endothelium were taken from the central and peripheral region of each cornea. Peripheral readings were taken in the superior regions of the cornea 2 to 3mm from the limbus.

The corneal endothelial cell analysis was done with a variable frame analysis method. A minimum of 100 well defined endothelial cells obtained from each frame was analyzed. The polymegathism and pleomorphism were obtained from both eyes. Statistical data analysis was conducted using the Tukey test. Values of $P < 0.01$ were considered significant.

RESULTS

Reproducible images were obtained from all eyes. Specular microscopy demonstrated that the dog corneal endothelium was continuous and without detectable abnormalities in all eyes examined. Normal dog corneal endothelium was characterized by a monolayer of polygonal cells of uniform size and shape (Fig.1 and 2). Regarding the pleomorphism of the endothelium, the majority of cells were six-sided (68%), five- (17%), seven-

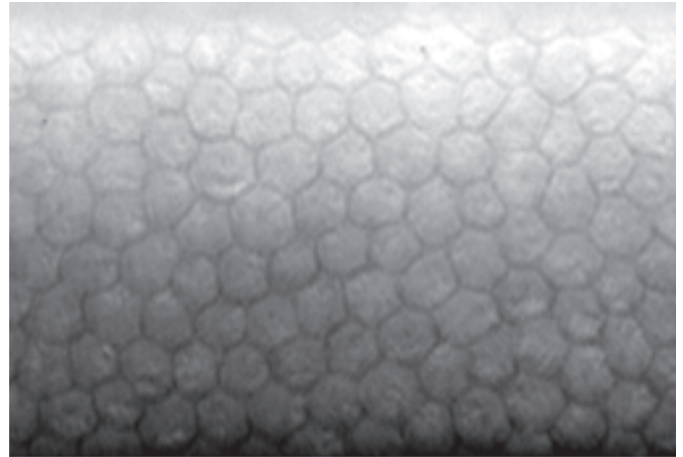


Fig.1. Specular micrograph of the normal corneal endothelium of the right eye of a mongrel dog with 6 years of age. The endothelium has a regular polygonal appearance with 68% hexagonal cells and polymegathism index of 0.22.

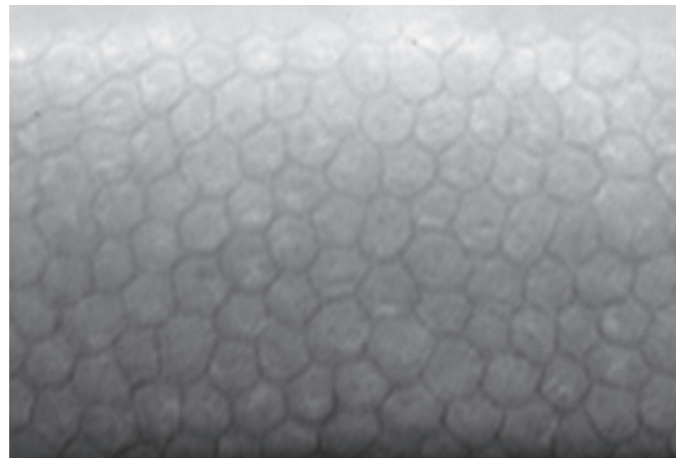


Fig.2. Specular micrograph of the normal corneal endothelium of the left eye of the same dog shown in Figure 1. The endothelium has a regular polygonal appearance with 69% hexagonal cells and polymegathism index of 0.22.

(11%), four- (2%) and eight-sided (2%) constituting the remaining corneal endothelium. The polymegathism index was 0.22. The parameters evaluated did not differ significantly between the right and the left eye from the same dog. The comparison between central and peripheral cornea did not exhibit statistically differences within the parameters evaluated.

DISCUSSION AND CONCLUSION

The endothelium is a monolayer of polygonal cells which maintains corneal transparency by keeping the stroma in a state of relative dehydration (Waring et al. 1982). Endothelial alterations are considered important parameters of surgical trauma and are essential in estimating the safety of surgical techniques (Tuft & Coster 1990).

Currently, scanning electron microscopy and specular microscopy are used and accepted methods to evaluate the corneal endothelium (Yee et al. 1987, Abib & Barreto, 2001, Andrew et al. 2002). Changes in endothelial cell dimensions can occur as a result of processing cornea for scanning electron microscopy (Schutten & Van Horn 1980). In spite of this limitation, the scanning electron microscopy has been widely used to compare the endothelial ultrastructure of vertebrates, and to evaluate the effects of medications, chemicals or surgical procedures on the endothelium (Collin & Collin 1998, Pigatto et al. 2004, Pigatto et al. 2005).

Specular microscopy has become a standard technique to determine endothelial cell density and cell morphology *in vivo* and *ex vivo* without artifacts or damage of the cornea (Pigatto et al. 2006). Rapid quantitative analysis regard the state of the corneal endothelium has been cited as another advantage of this method (Laing et al. 1979). Specular microscopy may be either a contact microscope, in which the front of the objective lens touches the cornea, or a non contact specular microscope, in which there is an air space between the front of objective lens and the cornea. In our study contact specular microscope allows observation of the corneal endothelium in whole eye. Thus only healthy corneas were studied. Furthermore, the applanation drastically reduces the light reflected of the air-tear interface and dampens eye movement, facilitating the acquisition of images with higher resolution than non-contact devices provide.

Enucleated eyes were used in this study. The utilization of eyes within 1 h after enucleation allowed the maintenance of endothelial ultrastructure. Specular microscopy demonstrated that the endothelium was continuous and without detectable abnormalities in all eyes examined. The methods used to analysis of specular images are fixed frame and variable frame analysis (Laing et al. 1979). The variable frame method used in our study eliminates the problem of counting fractional cells along the boundary and provided a more accurate determination of parameters evaluated than the fixed frame analysis.

The specular microscope has been used to quantify endothelial cell size in humans, dogs, rabbits, horses and other animal species (Stapleton & Peiffer 1979, Peiffer et al. 1981, Gwin et al. 1982, Morita et al. 1994, Morita 1995, Abib & Barreto 2001, Andrew et al. 2001, 2002, Pigatto et al. 2006).

In the most vertebrates the shape of the normal corneal endothelial cells was typically a mixture of hexagonal and pentagonal cells (Yee et al. 1987, Collin & Collin, 1998). The results observed in this study show that normal dog corneal endothelium consisted of a continuous monolayer with mainly hexagonal cells, but also pentagonal and heptagonal variability. This study showed that, despite, minor interspecies differences, the apical morphological characteristics of corneal endothelial cells of dogs were similar to those found in man and rabbit, in that some 65-80% of corneal endothelial cells were hexagonal (Jackson

et al. 1995, Morita 1995). Other studies on endothelial cells of lower species including bullfrog, trout, gecko and goldfish demonstrated that these species exhibit more variability (Yee et al. 1987).

The size and regularity of the shape of endothelial cells are influenced by their ability to regenerate. Our results showed endothelial cells with minimal variation in size and shape, probably because all animals were of the same age, and only healthy corneas were studied. In other species, endothelial morphologic features and cell densities are dependent on age, with a decrease in endothelial cell density and corresponding increases in cell size and shape variation with ageing (Gwin et al. 1982, Matsuda et al. 1985). Furthermore, reduction in the percentage of 6-sided cells have been reported after penetrating keratoplasty and cataract extraction (Tuft & Coster 1990).

The index of polymegathism observed in this study was similar than those described in human. Our analysis demonstrated that the parameters evaluated did not differ significantly between the eyes from the same dog. Such findings are in agreement with previous studies that show no differences between the right and the left eye from the same animal (Tuft & Coster 1990, Morita 1995, Andrew et al. 2001, Pigatto et al. 2004). In addition, we observed that the comparison between central and peripheral cornea did not exhibit significant differences in the endothelial cells variables determined. Similar results have been observed in healthy corneas of man and other vertebrates (Blackwell et al. 1977, Gwin et al. 1982, Andrew et al. 2001).

Microscopy of the corneal endothelium can be used to evaluate the functional reserve of the cornea and to provide better understanding the wound healing process following intraocular surgery (Mishima 1981, Tuft & Coster 1990). Previous reports have demonstrated that the use of specular microscope to determine endothelial cell density is a practical way to evaluate corneal endothelium for eyes that have been harvested for tissue banks or corneal transplantation (Andrew et al. 2001). Specular microscopy can thus help to improve the quality of the tissue used in penetrating keratoplasty and reduce the number of grafts that fail. Furthermore, knowledge of the endothelial cell morphology before surgical procedures involving opening of the anterior chamber is useful for better defining the risk of endothelial decompensation. This study demonstrates that the morphology of the normal corneal endothelial cells of dogs is similar to that found in the human cornea.

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