

Subconjunctival application of allogenic limbal cells in dogs with corneal disorders

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ABSTRACT

Limbal stem cells play a crucial role in maintaining the integrity of the corneal surface in a healthy condition or after injury through corneal renewal and repair. In pathologic conditions these cells could be replenished with cultivated allogenic limbal cells. In this investigation, limbal stem cells from small fragments of donor tissue were cultivated *in vitro* for treatment of corneal lesions in dogs. Fourteen dogs were divided into two groups of seven animals each: a group of dogs with corneal lesions treated using the classical veterinary ophthalmology approach, and a group of dogs with corneal lesions treated with the application of cultivated allogenic limbal cells. Regardless of the size and location of the corneal lesions, after 30 days in the group of dogs with corneal lesions treated with the application of cultivated allogenic limbal cells ophthalmological examination showed no signs of any eye disorder except corneal edema (14.29 %). In the group of dogs with corneal lesions cured using the classical veterinary ophthalmology approach, 30 days after the beginning of treatment ophthalmological examination showed 42.86 % mild/incisional blepharospasm, 85.71 % secretion, 100 % observable conjunctival congestion, 14.29 % eye liquor dimming, 85.71 % corneal edema and 85.71 % irritation. After application of cultivated allogenic limbal cells, corneal lesions were completely healed 15 days

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earlier than the lesions treated with conventional ophthalmological therapy. Allogenic limbal cells were capable of restoring corneal clarity with no development of ocular complications.

Key words: corneal disorders, allogenic limbal cells, dogs

Introduction

Stem cells at the sclerocorneal limbus maintain the integrity of the corneal surface in a healthy condition or after injury through corneal renewal and repair (SWIFT et al., 1996; HAAMANN et al., 1998; DUA and AZUARA-BLANCO, 2000). Studies have also demonstrated the presence of oligopotent cells in the central region of the cornea that appear to be important in the maintenance of intact corneal epithelium during homeostasis (MAJO et al., 2008). The authors showed that the corneal epithelium of a mouse can be serially transplanted, is self-maintained and contains oligopotent stem cells, with the capacity to generate goblet cells if provided with a conjunctival environment. Additionally, the existence and survival of a healthy sheet of corneal epithelial cells over a mean follow-up period of 60 months, in the presence of clinically apparent total limbal stem cell deficiency, suggests a limited role of limbal epithelial stem cell in physiologic homeostasis of the corneal epithelium (DUA et al., 2009).

Nevertheless, as a response to corneal injury when the regenerative/proliferative capacity of the oligopotent cells is reduced, activated stem cells from the sclerocorneal limbus moved to the surface and centripetally to become differentiated epithelial cells. This proliferation is limited and thus extensive epithelial destruction, results in corneal vascularization, with loss of corneal transparency, and can result in permanent visual impairment. In some cases there is a loss of stem cells or limbal deficiency, which consequently leads to conjunctivalization, chronic inflammation, vascularization, ulceration, calcification, melting and perforation of the cornea (DUA and AZUARA-BLANCO, 1999; DUA and AZUARA-BLANCO, 2000; DANIELS et al., 2001).

Deficiency of stem cells, caused by disease or injury, that usually leads to blindness, could be overcome by therapies that used cells and tissues cultivated *in vitro*. Despite numerous studies in this field, optimal protocols for *in vitro* cultivation of stem cells and their application to the patient are still undetermined (SHORTT et al., 2007; CHEN et al., 2007; SHY and CLEGG 2008). In recent clinical trials limbal stem cells have been used in different animal models and the beneficial effect of cultivated allogenic limbal cells has also been acknowledged (POPOVIĆ et al., 2009; VLAHOVIĆ et al., 2010; PIRKIĆ et al., 2010; NAM et al., 2013; SAMOILA et al., 2014; MORIYAMA et al., 2014).

In veterinary medicine, the applications of limbal stem cells in corneal surgery are rare, but their use in veterinary ophthalmology could certainly be applied to pets and high-quality animals. In the present study, our objective was to isolate and cultivate *in vitro* canine corneal stem cells for reparation of corneal lesions, after application of allogenic limbal cells.

Materials and methods

Dogs. Fourteen dogs of different breeds, clinical patients of the Surgery, Orthopedics and Ophthalmology Clinic of the Veterinary Faculty, University of Zagreb, were used in this experiment. The animals were randomly divided into two groups, regardless of breed, sex and age, each comprising 7 dogs: a group of dogs with corneal lesions treated by conventional veterinary ophthalmological access (Tobramycin drops in combination with Cyclopentolate and Atropine eye drops) and a group of dogs with corneal lesions treated with subconjunctival application of allogenic limbal cells (Tables 1 and 2). All dogs were treated with the consent of the owner and released for home care. Experimental and animal management procedures were conducted in accordance with the Directive for the Protection of Vertebrate Animals used for Experimental and other Purposes (86/609/EEC).

Table 1. Dogs treated with conventional veterinary ophthalmological therapy

Breed	Age/Gender	Corneal pathology
French Bulldog	7 years, male	Ulcus corneae chron OD*. Keratitis pannosa OD
German boxer	9 years, male	Ulcus corneae chron OS**. Keratitis pannosa OS
Pekingese	4 years, male	Perforatio bulbi susp.. Keratitis pannosa OD
West Highland White Terrier	9 years, female	Ulcus corneae OS. Keratitis pannosa OS
French Bulldog	1.5 years, male	Ulcus corneae chron OS. Keratitis pannosa OS
Shih-tzu	1.5 years, male	Contusio bulbi OS. Ulcus corneae chron OS
Shih tzu	7.5 years, male	Ulcus corneae OD. Keratitis pannosa OD

*OD - oculus dexter; **OS - oculus sinister

Table 2. Dogs treated with the application of cell allotransplantate

Breed	Age/Gender	Corneal pathology
Mops	1.5 years, male	Ulcus corneae. Keratitis pannosa OS
Alaskan Malamute	7 years, male	Perforatio corneae OS. Keratitis pannosa OS
Pekingese	6 months, male	Ulcus corneae OS. Keratitis pannosa OS
Pekingese	4 years, male	Ulcus et granulatio corneae OS. Keratitis pannosa et pigmentosa OS
Mops	4 years, female	Descemetocele OS. Keratitis pannosa OS
Shih-tzu	7 years, male	Descemetocele OS. Keratitis et Keratomalacia OS
Shih-tzu	7 years, male	Descemetocele OS. Keratitis pannosa OS

*OD - oculus dexter; **OS - oculus sinister

Biological material. Samples of healthy eyeballs were taken from 10 euthanized dogs and stored at 4 °C for 24 hours in transport medium: DMEM with 4.5 g/L glucose, 1 % ABAM solution (penicillin-streptomycin-antimycotic) and 5 % foetal bovine serum (Invitrogen-Gipco). Limbal specimens (5mm²) were obtained from the cadaveric eyes using penetrating keratotomy.

Cell cultivation in explant culture. Limbal tissue fragments (1-2mm²) were placed on cultivation plates with 6 wells containing 3 mL of medium without cell growing serum (Serum Free Media SFM, Invitrogen-Gibco). The fragments were incubated at 37 °C and 5 % CO₂ atmosphere. The medium was changed every three days under sterile conditions.

Cell cultivation in secondary culture. After the primary cell culture covered 80 % of the plate bottom, it was treated with 1 mL of 0.25 % trypsin for 2-3 minutes and consequently the cells were elevated from the cultivation plate bottom. The cell suspension obtained was centrifuged at 1100 rpm for 4 min. Cell sediment was resuspended in 1 mL SFM medium and grown in cultivation plates with 6 wells for adherent cell cultures in 3 mL medium without cell growing serum (Serum Free Media, SFM, Invitrogen-Gibco). When the cells in the secondary culture covered 80 % of the plate bottom, they were treated with 1 mL of 0.25 % trypsin for 2-3 minutes, which caused elevation of the confluent limbal transplant from the bottom of the cultivation plate. The cells were centrifuged at 1100 rpm for 4 min. and the sediment was resuspended in 1 mL SFM medium. The number of cells in the suspension was measured in a Neubauer cell counting chamber, using trypan blue (Sigma), and 1×10⁶ cells in 1 mL SFM medium were prepared for clinical application by injection. The morphological characteristics of those samples were analyzed using an electronic microscope XL30 ESEM, Philips, Netherlands (the Ivan Vučetić Forensic Science Centre, Zagreb, Croatia). The phenotype of cells was determined by routine the immunocytochemical method using a fluorescent dye conjugate monoclonal mouse antibody against the p63 cornea of the dog (antibodies-online.come GMbb, Germany).

Clinical applications of cultivated allogenic limbal cells. 1×10⁶ cells in 1 mL SFM medium were administered subconjunctivally in the area of the affected eye under local anesthesia (1 % tetracaine hydrochloride drops three times in about 5 minutes, Alcon). Treated dogs were under local antibiotics (Tobramycin drops, Alcon) 3 days before and seven days after this procedure. Also, 2 days before and 5 days after application of the allogenic limbal cells, local corticosteroids were administered three times a day to the affected eye (Dexametason drops, Alcon).

Ophthalmic clinical examination. Anterior ocular segment was examined on days 0, 10, 30, and 45 of the experiment, using focal illumination with a magnifying glass. Comparisons were made of: blepharospasm, vascular congestion, secretion, eye liquor dimming, corneal edema, adhesions and irritation. All parameters were graded numerically from 0-3 using a scale of subjective assessment (NELMS, 1994) and results were statistically processed.

Statistical analysis. Quantitative data (based on 0-3 scale) were expressed as percentages and compared by the chi-square test. A probability of $P < 0.05$ was considered statistically significant.

Results

Limbal cells from all samples showed the morphological characteristics of canine corneal epithelium and most of the cells were densely grouped in a single layer. In explant the culture of canine limbal cells reached 80 % of confluence 7 days after ingrowing, while the cells in secondary cultures reached 80 % confluency two days earlier (Fig. 1). Immunocytochemical analysis of cells within the secondary culture 5 days after ingrowing, using fluorescein dye conjugated mouse monoclonal antibody against p63 cornea of the dog, showed that the cells are positive to p63. Using an electronic microscope, it was visible that cells within the secondary cultures were of equal size, approximately $14.5 \mu\text{m} \times 13.4 \mu\text{m}$, five days after ingrowing.

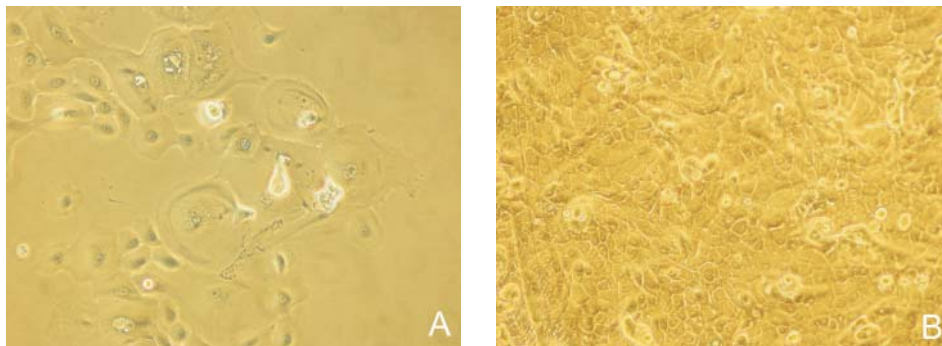


Fig. 1. In explant culture of canine limbal stem cells cultivated in SFM medium 7 days after ingrowing, $\times 40$ (A) and the secondary culture of canine limbal cells cultivated in SFM medium 5 days after ingrowing, $\times 20$ (B).

Table 3. Ophthalmological parameters in dogs with corneal disorder after classical veterinary ophthalmology treatment or application of limbal cell allograft at day 0

Parameter	Treatment	Unobservable	Mild/ incisional	Moderate/ incisional + dorsal cornea	Severe/ diffuse	P value
Blepharospasm	classical veterinary ophthalmology treatment	/	/	5 71.43 %	2 28.57 %	1.000
	application of limbal cell allograft	/	/	5 71.43 %	2 28.57 %	
Secretion	classical veterinary ophthalmology treatment	/	/	1 14.29 %	6 85.71 %	0.008
	application of limbal cell allograft	/	/	6 85.71 %	1 14.29 %	
Conjunctival congestion	classical veterinary ophthalmology treatment	/	/	/	7 100.00 %	/
	application of limbal cell allograft	/	/	/	7 100.00 %	
Eye liquor dimming	classical veterinary ophthalmology treatment	5 71.43 %	1 14.29 %	/	1 14.29 %	n/a
	application of limbal cell allograft	6 85.71 %	1 14.29 %	/	0 0.00 %	
Corneal edema	classical veterinary ophthalmology treatment	/	/	1 14.29 %	6 85.71 %	1.000
	application of limbal cell allograft	/	/	1 14.29 %	6 85.71 %	
Irritation	classical veterinary ophthalmology treatment	/	/	1 14.29 %	6 85.71 %	1.000
	application of limbal cell allograft	/	/	1 14.29 %	6 85.71 %	

P<0.05 - statistical significant difference between two treatments; n/a - chi-square test cannot be performed because number in one or more categories is zero

Table 4. Ophthalmological parameters in dogs with corneal disorder after classical veterinary ophthalmology treatment or application of limbal cell allograft at day 10

Parameter	Treatment	Unobservable	Mild/ incisional	Moderate/ incisional + dorsal cornea	Severe/ diffuse	P value
Blepharospasm	classical veterinary ophthalmology treatment	0 0.00 %	4 57.14 %	3 42.86 %	/	n/a
	application of limbal cell allograft	5 71.43 %	2 28.57 %	0 0.00 %	/	
Secretion	classical veterinary ophthalmology treatment	0 0.00 %	1 14.29 %	6 85.71 %	/	n/a
	application of limbal cell allograft	5 71.43 %	2 28.57 %	0 0.00 %	/	
Conjunctival congestion	classical veterinary ophthalmology treatment	/	0 0.00 %	7 100.0 %	/	n/a
	application of limbal cell allograft	/	6 85.71 %	1 14.29 %	/	
Eye liquor dimming	classical veterinary ophthalmology treatment	6 85.71 %	/	1 14.29 %	/	n/a
	application of limbal cell allograft	7 100.00 %	/	0 0.00 %	/	
Corneal edema	classical veterinary ophthalmology treatment	0 0.00 %	1 14.29 %	6 85.71 %	/	n/a
	application of limbal cell allograft	1 14.29 %	3 42.86 %	3 42.86 %	/	
Irritation	classical veterinary ophthalmology treatment	0 0.00 %	1 14.29 %	6 85.71 %	/	n/a
	application of limbal cell allograft	1 14.29 %	6 85.71 %	0 0.00 %	/	

P<0.05 - statistical significant difference between two treatments; n/a - chi-square test cannot be performed because number in one or more categories is zero

Table 5. Ophthalmological parameters in dogs with corneal disorder after classical veterinary ophthalmology treatment or application of limbal cell allograft at day 30

Parameter	Treatment	Unobservable	Mild/ incisional	Moderate/ incisional + dorsal cornea	Severe/ diffuse	P value
Blepharospasm	classical veterinary ophthalmology treatment	4 57.14 %	3 42.86 %	/	/	n/a
	application of limbal cell allograft	7 100.00 %	0 0.00 %	/	/	
Secretion	classical veterinary ophthalmology treatment	1 14.29 %	6 85.71 %	/	/	n/a
	application of limbal cell allograft	7 100.00 %	0 0.00 %	/	/	
Conjunctival congestion	classical veterinary ophthalmology treatment	0 0.00 %	7 100.00 %	/	/	n/a
	application of limbal cell allograft	7 100.00 %	0 0.00 %	/	/	
Eye liquor dimming	classical veterinary ophthalmology treatment	6 85.71 %	1 14.29 %	/	/	n/a
	application of limbal cell allograft	7 100.00 %	0 0.00 %	/	/	
Corneal edema	classical veterinary ophthalmology treatment	1 14.29 %	6 85.71 %	/	/	0.008
	application of limbal cell allograft	6 85.71 %	1 14.29 %	/	/	
Irritation	classical veterinary ophthalmology treatment	1 14.29 %	6 85.71 %	/	/	n/a
	application of limbal cell allograft	7 100.00 %	0 0.00 %	/	/	

P<0.05 - statistical significant difference between two treatments, n/a - chi-square test cannot be performed because number in one or more categories is zero



Fig. 2. Clinical examination of the anterior segment of the eye (Shih-tzu; 7 years old male): before treatment (A), 10 days (B) and 30 days (C) after application of cultivated limbal cell allograft.



Fig. 3. Clinical examination of the anterior segment of the eye (Mops; 4 years old female): before treatment (A), 10 days (B) and 30 days (C) after application of cultivated limbal cell allograft.

Regardless of the size and location of the corneal lesions, after 30 days in the group of dogs with corneal lesions treated with the application of cultivated allogenic limbal cells, ophthalmological examination showed no signs of any eye disorder except corneal edema (14.29 %). In the group of dogs with corneal lesions treated by the classical veterinary ophthalmology approach, 30 days after the beginning of treatment ophthalmological examination showed 42.86 % mild/incisional blepharospasm, 85.71 % secretion, 100 % observable conjunctival congestion, 14.29 % eye liquor dimming, 85.71 % corneal edema and 85.71 % irritation. While at the beginning of treatment statistical data expressed as percentages and compared by the chi-square test, shows no difference between conventional and stem cell treatment except in the secretion, probably due to the small sample size (Table 3), 10 days into treatment significant differences were already occurring (Table 4). At day 30, general improvement could be seen in both types of therapy, but stem cell therapy still clearly showed better results (Table 5). While the small sample size prevents any meaningful statistics, the chi-squared test was performed where applicable, showing a very significant difference in corneal edema. After application of cultivated allogenic limbal cells, corneal lesions were completely healed 15 days earlier than the lesions treated with conventional ophthalmological therapy. Subconjunctival

application of limbal cells ensures fully regeneration of corneal lesions after 30 days, regardless of the size and extent of the defect (Figs 2 and 3).

Discussion

Limbus is a thin border between the sclera and cornea that has special characteristics which distinguish it from the peripheral and central cornea. Although the central region of the cornea possesses oligopotent cells with certain regenerative/proliferative capacity, as a response to corneal injury, maintenance and regeneration of the corneal epithelium is provided by proliferation and differentiation of stem cells located at the limbus, and thus, severe damage of the limbus leads to stem cell deficiency, which ultimately results in conjunctivalization, vascularization, and chronic inflammation (ESPANA et al., 2004; MAJO et al., 2008; PAUKLIN et al., 2009).

In animals with stem cell dysfunction, limbal allotransplantation is increasingly being used for treatment of severe ocular surface diseases (HENRY et al., 2004). YANG et al. (2008) demonstrated that epidermal adult stem cells can also be induced to differentiate into corneal epithelial cell types *in vivo*. They managed to successfully reconstruct damaged cornea in goats with total limbal stem cell deficiency by using tissue-engineered cell sheets composed of adult epidermal stem cells. SAMOILĂ et al. (2014) isolated and cultivated rabbit corneal stem cells *in vitro*, into an epithelial tissue with preserved proliferative state to allow transplantation onto the cornea. Indeed, recent progress in this field is encouraging and offers an opportunity to develop cell- and tissue-based therapies for corneal lesions (PINNAMANENI and FUNDERBURGH, 2012). In contrast to human medicine, in clinical veterinary ophthalmology it is easier to apply cellular rather than tissue limbal grafts. Also, the use of allografts is more common, although the cornea contains no blood vessels and is immunologically extremely privileged.

A variety of surgical techniques have been described for corneal epithelial reconstruction, but cultivated limbal stem cell transplantation seems to be the most promising. Treating corneal lesions with *ex vivo* expanded limbal cells is a widely accepted technique (RAMAESH and DHILLON, 2003; SHORTT et al., 2009). In the present study, limbal stem cells cultivated on amniotic membrane or contact lenses are often in use for transferring cells into the affected eye (SANGWAN, 2012; GORE et al., 2014). The RHEINWALD and GREEN (1975) method for *in vitro* cell cultivation on a nutritive layer of γ -rays radiated or mytomicin-C treated 3T3 cells is still the commonly used method, but it can cause the problem of infection transmittance from mouse fibroblasts, along with the possibility of the development of unwanted immune responses. In order to avoid these problems, the nutritive layer of 3T3 cells could be replaced by a nutritive layer of human epithelial amniotic membrane cells, which seems to withdraw cells with less differentiation but with more stem cells (FIGUEIRA et al., 2007). PIRKIĆ et al. (2010)

concluded that the method of choice can be an *in vitro* explant culture in SFM medium. The authors have also shown that cells isolated by explant culture are phenotypically identical to those obtained by the conventional method, which has also been confirmed by other authors (PRIYA et al., 2012). An additional advantage of using this method instead of the 3T3 cell method, would be that the cells are not additionally treated with enzymes, which can have a negative influence on cell cycle establishment and maintenance. Moreover, an explant culture is a gentle and simple technique that may be particularly useful for obtaining cell cultures in cases of limited starting material. SAMOILĂ et al. (2013) showed the possibility of *in vitro* expansion of corneal stem cells, starting from a few cells, to obtain enough tissue, as needed for transplantation into the injured eye. OUYANG et al. (2014) pointed to a new strategy for treating corneal surface diseases. They found that the transcription factors p63 (tumor protein 63) and PAX6 (paired box protein PAX6) act together to specify limbal stem cells, and WNT7A controls corneal epithelium differentiation through PAX6. After transduction of PAX6 in skin epithelial stem cells and upon transplantation onto the eyes in a rabbit corneal injury model, these reprogrammed cells are able to replenish corneal epithelia cells and repair the damaged corneal surface. These findings suggest the central role of the WNT7A - PAX6 axis in corneal epithelial cell fate determination.

The outcome of stem cell transplantation can be adversely affected by several risk factors, such as: ocular surface inflammation, hemorrhage, progressive vascularization and glaucoma. There is a risk of infection because the cornea has no blood vessels, so it heals relatively slowly, and during that time it might become infected by various microorganisms. In some cases, more than one limbal biopsy was necessary in some animals because of the failure of outgrowth after the first biopsy. Moreover, the explant can remain on the peripheral cornea and later form a small granuloma. There is also the possibility of rejection of the transplanted tissue. Treatment complications after autologous limbal stem cells *ex vivo* expansion and cultivation may be partial stem cell deficiency in the used eye. TSUBOTA et al. (1999) performed transplantations of corneal epithelial stem cells from cadaveric eyes into the eyes of patients with severe ocular-surface disorders and limbal dysfunction. Complications of the first transplantation included persistent defects in the corneal epithelium, ocular hypertension, and rejection of the corneal graft.

In our investigation, small fragments of limbal tissue from the cadaveric eyes were successfully used for *in vitro* cultivation of allogenic limbal cells on SFM medium, and application into the affected eyes. Cells within the secondary culture, at the moment of their preparation for clinical use, had the characteristics of epithelial limbal cells, e.g. they had positive p63. PELLEGRINI et al. (2001) concluded that within the cornea, nuclear p63 is expressed by the basal cells of the limbal epithelium, but not by transient amplifying cells covering the corneal surface. Moreover, the corneal cells failed to be subcultured,

whereas the limbal cells could be subcultured with increasing cell size (MORITA et al., 2014). However, the same authors detected p63 in both limbal and corneal cells, and it decreased gradually in the limbal cells with the cell passages. In our research, limbal cells in the primary and secondary culture achieved sufficient confluence 5-7 days after ingrowing. These data are in concordance with previous investigations of canine and porcine limbal cells *in vitro* cultivation (VLAHOVIĆ et al., 2010, PIRKIĆ et al., 2010). BRUNELLI et al. (2007) demonstrated the efficiency of limbal autograft transplantation in dogs with damaged cornea. In our current research, postoperative assessment of ophthalmological parameters in dogs with corneal lesions showed that after application of cultivated allogenic limbal cells, corneal lesions were completely healed 15 days earlier than the lesions treated with conventional ophthalmological therapy.

The present study has shown that subconjunctivally applied allogenic limbal cells were capable of restoring corneal clarity independently of the size and extent of the defect and with no development of ocular complications. Although further investigation is needed for optimizing therapy protocols, the current results encourage the continued use and refinement of this procedure as a treatment for corneal lesions. Application of cultivated allogenic limbal cells has potential as novel approach in veterinary ophthalmology that could be applied to pets and high-quality animals.

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SAŽETAK

Limbalne matične stanice igraju ključnu ulogu u održavanju integriteta površine rožnice u zdravom stanju ili nakon ozljede, kroz obnavljanje i popravak rožnice. U patološkim stanjima te se stanice mogu nadomjestiti s uzgojenim alogeničnim limbalnim stanicama. U ovom istraživanju limbalne matične stanice iz malih fragmenata tkiva donora uzgojene su *in vitro* radi liječenja ozljeda rožnice u pasa. Četrnaest pasa bilo je podijeljeno u dvije skupine od sedam životinja: skupina pasa s ozljedama rožnice liječenim klasičnim veterinarskim oftalmološkim pristupom i skupina pasa s ozljedama rožnice liječenim primjenom *in vitro* uzgojnih alogeničnih limbalnih stanica. Neovisno o veličini i smještaju lezije rožnice, deset dana nakon primjene stanica, oftalmološki pregled je pokazao 80% manje izražene simptome blefarospazma, 85% smanjenu sekreciju, 43% manje uočljivu konjunktivalnu kongestiju, 31% manji edem rožnice i 55% smanjenu iritaciju u usporedbi s psima liječenim klasičnim oftalmološkim pristupom, bez znakova zamućenja očne vodice ili priraslica. Nakon primjene uzgojeni alogeničnih limbalnih stanica, lezije rožnice u potpunosti su bile izliječene 15 dana ranije nego lezije liječene konvencionalnim oftalmološkim načinom. Supkonjunktivalna primjena limbalnih stanica omogućuje potpunu regeneraciju ozljeda rožnice nakon 30 dana bez obzira na veličinu i opseg defekta. Alogenične limbalne stanice sposobne su obnoviti prozirnost rožnice bez razvoja komplikacija. Iako su postignuti rezultati bili ohrabrujući, potrebna su daljnja istraživanja za optimizaciju protokola liječenja.

Ključne riječi: poremećaji rožnice, alogenične limbalne stanice, psi
