Original article

CASE SERIES DESCRIBING THE USE OF LOW-TEMPERATURE VACUUM-DEHYDRATED AMNION (OMNIGEN®) FOR THE TREATMENT OF CORNEAL ULCERS IN CATS AND DOGS: 46 CASES (2016 – 2017)

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ABSTRACT

Amniotic membrane is widely used in the treatment of ocular surface disorders in human and veterinary patients. Preservation and storage of amnion has proven challenging, prompting the development of new preservation techniques. Omnigen®, a novel low-temperature vacuum-dehydrated amnion, is reported to possess enhanced structural properties and biochemical stability in vitro, but its clinical use in veterinary patients is not well-described. This study aims to document and describe the varied use of Omnigen for the surgical treatment of corneal ulceration in cats and dogs. A total of 45 patients (46 eyes) were recruited from the clinical record system of the Royal Veterinary College (London) between January 2016 and December 2017. Brachycephalic breeds were over-represented (37/45; 82.2%). Omnigen was used as a standalone graft in 5/46 (10.9%) eyes, as a supplementary graft in 29/46 (63.0%) eyes and as a patch in 12/46 (26.1%) eyes. Graft failure occurred in 10/46 eyes (21.7%). At final examination 43/46 eyes (93.5%) had healed and 31/33 eyes (93.9%) were visual. This study demonstrates the successful use of Omnigen for the surgical treatment of corneal ulceration in cats and dogs. Further studies are needed to clarify its properties and benefits in the clinical field.

Keywords: Amnion; Cornea; Omnigen; Ulcer; Vacuum-dehydrated

INTRODUCTION

Corneal ulceration is a common ocular condition encountered in cats and dogs. It is widely accepted that simple, uncomplicated ulcerative lesions often respond to medical management, while complicated ulcers are likely to require surgical intervention. This is particularly true in cases where the corneal defect is rapidly progressive, exhibits signs of keratomalacia or necrosis, invades deeper regions of the cornea, or perforates it entirely.

Surgical grafting may be helpful in providing tectonic support, vascular supply and locally available beneficial factors to the cornea. Many graft types and techniques have been described in the veterinary literature, including autologous conjunctival grafts[1-6], autologous corneoconjunctival transposition[7-9] and homologous/heterologous fresh and frozen corneal transplants[10-15]. In addition, the use of various synthetic, processed and unadulterated biological grafts and patches has been reported, including equine renal capsule[16], equine pericardium[17,18], bovine pericardium[19] and acellular submucosa derived from porcine small intestinal submucosa[20-28] and urinary bladder[21,29-32].

Amniotic membrane (AM) is one such grafting biomaterial which has garnered attention. Amniotic membrane is the avascular inner layer of the foetal placental membrane and comprises three layers: an epithelial monolayer, a thick basement membrane and an avascular stromal matrix. It is reported to promote epithelisation[33-43], and possesses anti-inflammatory[44-47], anti-fibroblastic[48-50], anti-angiogenic[44,51] and anti-microbial[52-55] properties. Additionally, it

exhibits immune-privilege (does not express HLA-A, B, or DR antigens) and hence is not rejected by the host immune system after transplantation[56-59].

The use of AM in the treatment of human ocular surface disease was first reported by de Rötth in 1940[60] who described the utilisation of AM in the repair of conjunctival defects in a small group of human patients. Although its use subsequently receded from the clinical field, AM enjoyed a resurgence in the 1990s. Since then, there has been increasing interest in the use of AM in the treatment of ocular surface disease in human and veterinary ophthalmology[49,61-86]. In addition to its use as a reconstructive grafting material, AM may also be used as a wound healing biological ‘bandage’ or ‘patch’. The decision as to whether AM is used as a graft or a patch in a specific case depends on the therapeutic aim. If the AM is being used with the aim of providing a substrate on which the host epithelium can grow and stromal cells repopulate, the AM is trimmed to the size of the defect and placed epithelium side facing up (stroma facing recipient cornea); during the healing process, the AM should be incorporated into the cornea. This is termed the ‘graft’ or ‘inlay’ technique. Alternatively, AM may be placed stromal side up (epithelium facing recipient cornea) as a removable ‘patch’ or ‘overlay’; this technique allows the epithelium to grow under the AM, which provides protection to the ocular surface, reduction of inflammation, reduction of pain and promotion of healing [46,77,80,82,83,87-90]. When used as a patch, the amnion is expected to slough from the ocular surface[91].

Although the procurement of AM is relatively straightforward (usually collected via elective caesarean of human and veterinary patients), fresh AM is rarely used due to

its short shelf-life and, particularly in the human field, concerns about increased risk of disease transmission and post-operative complications[92]. Preservation of AM tissue and maintenance of its beneficial properties has proven challenging. Cryopreservation, lyophilisation (freeze-drying) and heat-drying are popular methods of AM preservation, but all three processes have been associated with inducing tissue fragility and/or loss of beneficial bioactive substances[92-98]. In addition, poorly standardised and validated preparation and storage methods have been known to result in variable and unpredictable end products[92]. This prompted the development of a novel and standardised method of AM preparation, which aims to preserve the regenerative properties of fresh amnion while overcoming the limitations associated with fresh amnion and conventional preservation techniques. This is achieved via a unique, patented low-temperature vacuum-evaporation dehydration process (Tereo®; Nu-Vision Biotherapies Ltd.), which is reported to produce a preserved AM tissue with enhanced structural properties and biochemical stability (Omnigen®; Nu-Vision Biotherapies Ltd.). Specifically, Omnigen exhibits more sustained release of EGF and TGF-ß1 in vitro and less visible structural damage when compared to cryopreserved AM; more akin to fresh AM. In addition, increased wound closure rates were observed in an immortalised human corneal epithelial cell co-cultures with Omnigen compared to cryopreserved and fresh AM, possibly attributable to the optimised retention of wound-healing factors (EGF, HGF, PDGF and TGF- ß) in Omnigen[92,99]. The thickness of rehydrated Omnigen (mean 69.78 µm) is comparable to fresh human amnion (mean 69.99 µm)[100].

The clinical use of Omnigen was briefly discussed at recent veterinary ophthalmology conferences[101,102], and is sparsely described in human and

veterinary literature[103,104]. The purpose of the present study was to describe the varied clinical use of Omnigen for the surgical treatment of corneal ulceration in a group of canine and feline veterinary patients.

MATERIALS & METHODS

Data Collection

This project was approved by the Royal Veterinary College Ethics & Welfare Committee on 13th February 2018 (Unique Reference Number SR2017-1296). A VetCompass™ clinical record search was performed to identify case records including the word 'amnion' within the data silo of the Royal Veterinary College (RVC, London, U.K.) between January 2016 and December 2017. During this period Omnigen was the only form of AM available to the surgeons at the RVC. Surgery was performed by one of five different surgeons; all were either board-certified ophthalmologists or ophthalmology residents in training. Data collected for each patient included species, breed, brachycephalic status, age, gender, eye, diagnosis, complicating factors, cytological/microbiological findings, concurrent ocular conditions, graft selection, AM orientation, surgeon, post-surgical treatment, post-surgical complications, anatomical outcome, epithelial healing time, follow-up, corneal opacification and visual outcome. Clinically apparent corneal malacia and corneal infiltration were recorded as either 'present' or 'not present' at the time of initial presentation. 'Omnigen utilisation' described whether Omnigen was used as a standalone material or in combination with another biomaterial, as well as how it was oriented with respect to the corneal surface: if Omnigen was sutured in place with the AM epithelium facing up (away from the corneal surface), this was termed a

‘graft’, if it was sutured in place with the AM epithelium facing down (in contact with the corneal surface) this was termed a ‘patch’. In cases where Omnigen was used as a standalone graft, 1-4 layers were used. In cases where the ‘Swiss Roll’ method was used, this was performed as described by Chan et al.[66] i.e. by rolling the AM and suturing the rolled tissue into the defect. In eyes where Omnigen was used in combination with another type of graft, it was either used as a supplementary top layer graft (epithelium up) or as a ‘patch’ (epithelium down) on top of the other graft. The other graft material was either porcine urinary bladder submucosa (ACell Vet™; ACell, Inc. Columbia, MD, USA) or frozen donor cornea. Regardless of utilisation technique, Omnigen was always fixated with 8/0-9-0 absorbable suture. The surgeons decided whether to use Omnigen as a patch or graft, depending on whether it was meant to integrate into the cornea for tectonic support and the provision of beneficial factors, or as a protective layer providing improved comfort and healing of the underling graft. Only complications thought to be related to the initial surgery were noted. Complications were divided into ‘minor graft complications’ and ‘graft failures’; ‘minor graft complications’ included any changes that did not require further medical or surgical intervention. ‘Graft failures’ included any deterioration that required additional intensive medical management or surgical intervention. Anatomical outcome was categorised as follows: eyes that ‘healed’, eyes that ‘healed after intensive medical or surgical intervention’, and eyes that were ‘enucleated’. ‘Epithelial healing time’, recorded in days, was determined by means of negative fluorescein uptake. ‘Follow-up time’ (time from presentation until final examination) was also recorded in days. Corneal neovascularisation was denoted as ‘present’ or ‘absent’ at any point during the follow-up period. The presence or absence of two features of corneal opacification were noted as per final examination

records: ‘corneal pigmentation’ and ‘corneal fibrosis’. ‘Visual status’ was determined at the final post-operative examination and was characterized by the presence or absence of a menace response, as recorded in contemporaneous clinical notes.

Data and statistical analysis

Percentages and medians were used to summarise categorical and continuous variables. Age and epithelial healing time were assessed as continuous variables and species, breed, brachycephalic status, gender, eye, diagnosis, complicating factors, concurrent ocular conditions, graft selection, AM orientation, post-surgical complications, anatomical outcome, corneal opacification and visual outcome were assessed as nominal variables. Some nominal variables were analysed by Fischer’s exact test. Variables with $P<0.05$ were considered significant. Statistical analyses were performed using IBM SPSS software (IBM SPSS Statistics for Macintosh, Version 24.0).

RESULTS

Animals

A total of 46 eyes (from 45 animals) were included in the study. This comprised 5/45 (11.1%) cats and 40/45 (88.9%%) dogs. Brachycephalic breeds were over-represented (37/45; 82.2%). The most common breed was Pug (14/45; 31.3%), followed by French Bulldog (8/45; 17.8%), Shih Tzu (8/45; 17.8%), domestic shorthair cat (4/45; 8.9%), followed by one each (1/45; 2.2%) of the listed breeds: Chihuahua, British Bulldog, Cavalier King Charles Spaniel, domestic long hair cat, Jack Russell Terrier, Lhasa Apso, Miniature Schnauzer, Pekingese, Pomeranian and

Yorkshire Terrier. The sample population included 4/45 entire females (8.9%), 9/45 (20.0%) spayed females, 9/45 (20.0%) entire males and 23/45 (51.1%) neutered males. Median age was 4.7 years (interquartile range 6.0 years, range 0.9 years – 13.2 years).

Ophthalmic examination and diagnostics

Out of 46 total eyes, 22/46 (47.8%) were right eyes and 24/47 (52.2%) were left eyes. Forty-four out of forty-five (97.8%) patients were affected unilaterally and 1/45 (2.2%) was affected bilaterally. The most common diagnosis was deep stromal ulceration (23/46 eyes; 50.0%), followed by corneal perforation (14/46 eyes; 30.4%), descemetocoele (5/46 eyes; 10.9%), mid-stromal ulceration (2/46 eyes; 4.3%), corneal sequestrum (1/46 eyes; 2.2%) and corneal laceration (1/46 eyes; 2.2%). Corneal malacia was detected in 15/46 (32.6%) eyes and corneal infiltration was present in 26/46 (56.5%) eyes. Evidence of microorganisms was found in corneal samples from 19/46 (41.3%) cases, by cytology, microbiology and/or histopathology. Of those 19 cases, cytological findings were reported for 18/19 (94.7%) eyes; 6/18 (33.3%) exhibited cocci or diplococci, 6/18 (33.3%) exhibited rods, 4/18 (22.2%) exhibited a mixed bacterial population and in the remaining 2/18 (11.1%) samples no microorganisms were found. Microbiological diagnostics were reported for 8/19 (42.1%) cases and included the identification of *Pseudomonas aeruginosa* (2/8; 25%), *Pasteurella spp*. (1/8; 12.5%), *Streptococcus canis* (1/8; 12.5%), *Staphylococcus pseudointermedius* (1/8; 12.5%) and *Escherichia coli* (1/8; 12.5%); there was no growth from 4/8 (50%) samples. Sensitivity testing was performed for all positive microbiology samples and topical antibacterial treatment was adjusted accordingly. Corneal histopathology, performed in 1/19 (5.3%) cases, revealed the

presence of fungal elements (species unidentified). ‘Entropion and euryblepharon’ was the most common concurrent ocular finding (13/46; 28.3%), followed by ‘keratoconjunctivitis sicca (KCS)’ (5/46; 10.9%), ‘entropion’ (5/46; 10.9%), ‘euryblepharon’ (2/46; 4.3%), ‘entropion and ectopic cilia’ (1/46; 2.2%), ‘entropion and distichia’ (1/46; 2.2%), ‘eyelid mass and retinal degeneration’ (1/46; 2.2%), ‘ipsilateral cataract’ (1/46; 2.2%), ‘ipsilateral facial nerve paralysis and KCS’ (1/46; 2.2%) and ‘euryblepharon and KCS’ (1/46; 2.2%). There were no concurrent ophthalmic conditions in 14/46 (30.4%) cases.

Omnigen utilisation

Cases were allocated to one of three different groups according to how Omnigen was utilised: Omnigen was used as a standalone graft (epithelium facing up) in 5/46 (10.9%) cases, as a supplementary graft in combination with another biomaterial (epithelium side facing up) in 29/46 (63.0%) cases and as a ‘patch’ (epithelium facing down) either on its own or over another graft in 12/46 (26.1%) cases. In those cases where Omnigen was used as an adjunctive graft or as a patch over a graft, the additional graft materials comprised porcine urinary bladder submucosa (ACell Vet) in 33/46 (71.7%) cases and frozen donor cornea in 7/46 (15.2%) cases. Omnigen patches were not removed but were left to spontaneously slough from the ocular surface. The date of sloughing was recorded in 2/12 cases as 1 and 3 days, respectively, following surgery. In 3/12 cases the presence of the patch was described at 2, 2 and 33 days, respectively, following surgery. In the remaining 7/12 cases, neither date of sloughing nor post-operative presence of the patch was recorded.

234 Post-surgical treatment

235 Post-surgical treatment, which varied according to clinician and patient factors,
236 included the use of topical chloramphenicol 0.5% w/v (generic form), topical
237 ofloxacin (Exocin®, Allergan, Dublin, Ireland), topical ciprofloxacin (Ciloxan®,
238 Novartis, Surrey, U.K.), topical atropine (Minims atropine sulphate 1%®, Bausch &
239 Lomb, Surrey, U.K.), topical serum (prepared in-house from canine blood donors),
240 topical carmellose sodium (Celluvisc® 1% w/v, Allergan, Westport, Ireland), topical
241 ciclosporin (Optimmune® 2mg/g ointment, MSD Animal Health, Milton Keynes,
242 U.K.), systemic meloxicam (Metacam® 1.5mg/ml, Boehringer Ingelheim Animal
243 Health Ltd., Bracknell, U.K.), systemic carprofen 4mg/kg once daily (Rimadyl®,
244 Pfizer, Tadworth, Surrey), systemic firocoxib (Previcox®, Boehringer Ingelheim
245 Animal Health Ltd., Bracknell, U.K.), systemic cefalexin (Rilexine®, Virbac, Bury St
246 Edmunds, U.K.) and systemic doxycycline (Ronaxan®, Boehringer Ingelheim Animal
247 Health Ltd., Bracknell, U.K.).

248

249 Epithelial healing time and follow-up time

250 Median epithelial healing time was 19.0 days (interquartile range 17 days, range 8 –
251 67 days). Median follow-up time was 84 days (interquartile range 256 days, range 2
252 – 1046 days).

253

254 Complications, outcome and corneal opacification

255 Complications, outcome and corneal opacification according to Omnigen utilisation
256 and respective outcome variables are summarised in Table 1. Thirty-one out of
257 thirty-three (93.9%) eyes were visual at the final examination; this figure excludes
258 three eyes which were enucleated and 10 cases for which the visual status was not

recorded. The remaining 2/46 cases that were not visual were blind due to extensive corneal neovascularisation (1/46; 2.2%) and retinal degeneration (1/46; 2.2%), Successful anatomical healing (including eyes that required additional intervention) occurred in 43/46 eyes (93.5%). Graft failure lead to enucleation in 3/46 (6.5%) cases, due to persistent malacia in 2/46 (4.3%) cases where Omnigen was used as a supplementary graft and corneal perforation under the graft in 1/46 (2.2%) case where Omnigen was used as a standalone graft. Eyes that were enucleated (3/46; 6.5%) were excluded from the corneal neovascularisation, pigmentation, fibrosis and visual outcome data. Minor graft complications (10/46; 21.7%) included partial graft retraction (4/46; 8.7%), corneal degeneration adjacent to the graft (2/46; 4.3%), anterior synechiae (2/46; 4.3%), secondary glaucoma due to extensive anterior synechiae (1/46; 2.2%) and development of an inclusion cyst within the graft site (1/46;2.2%)). Graft failures (10/46; 21.7%) were attributable to complete graft dehiscence (3/46; 6.5%), progressive malacia (3/46; 6.5%), perforation under the graft (3/46; 6.5%) and persistent aqueous leakage (1/46; 2.2%). Fisher's exact test was performed on some categorical variables; results are summarised in Table 2. Significant associations were found between brachycephalic status and corneal pigmentation and between complications and anatomical outcome; brachycephalic dogs were more likely to develop corneal pigmentation ($P=0.002$) and eyes with graft failures were more likely to be enucleated ($P<0.001$).

DISCUSSION

Amniotic membrane is used in humans and in veterinary species for the treatment of a wide range of ocular surface disorders, including the treatment of ocular burns,

284 severe chemical and thermal eye injuries, refractory neurotrophic ulcers, deep
285 corneal ulcers, partial limbal stem cell deficiency, persistent epithelial defect,
286 pterygium, canine dermoids, canine complicated ulcers, equine keratomalacia,
287 equine immune-mediated keratitis and feline sequestrum
288 [49,60,62,63,67,73,75,76,78,79,105]. In this study, a novel low-temperature vacuum-
289 dehydrated AM, Omnigen, was applied in the primary surgical repair of 46 eyes from
290 45 cats and dogs with corneal ulceration.
291
292 The varied utilisation of Omnigen in this study demonstrated satisfactory overall
293 anatomical and visual outcome (93.5% and 93.9% respectively). The present study
294 demonstrates the use of Omnigen in several different ways; as a standalone graft, as
295 a supplementary graft and as a patch. In those cases where Omnigen was used as a
296 standalone graft, the anatomical and visual outcomes were 80% and 100%
297 respectively; these results are comparable to previous studies on the use of fresh
298 and cryopreserved AM for treatment of corneal defects in veterinary species, which
299 demonstrated satisfactory to good overall outcome (71.4% - 100%) [63-
300 65,72,79,84,85,105,106] The clinical use of Omnigen in combination with corneal
301 cross-linking was briefly described in a recent veterinary study[104]; this study
302 reported good anatomical and visual outcomes of 84.6% (11/13) and 76.9% (10/13)
303 cases, respectively. In the present study, the subset of cases where Omnigen was
304 used as a standalone graft was very small (5/46) which is a limitation. For those
305 cases where Omnigen was used as a supplementary graft with either ACell Vet or
306 frozen donor cornea, the anatomical and visual outcomes were 93.1% and 90.5%,
307 respectively. Similar results were reported for the use of ACell Vet alone (73.3% –
308 100% good outcome)[29-32] and frozen donor cornea alone (95% - 100% good

visual outcome, 86% good anatomical outcome)[11,107]; however, these studies do not provide a perfect comparison as they describe the sole use of these biomaterials without supplementary Omnigen or other AM.

The prevalence of post-surgical complications in the present study was reasonably high, with a total of 10/46 (21.7%) eyes experiencing minor complications and 10/46 (21.7%) eyes exhibiting complete graft failure. Interestingly, corneal degeneration adjacent to the graft was reported a minor complication in 2/46 (4.3%) eyes; in both cases this had the clinical appearance of corneal lipid and/or calcium deposition. Corneal calcification following AM graft has been reported in 12.8% of cases in the human literature[108], but has not previously been described in veterinary patients. A common risk factor for corneal calcification in the cited study included the post-operative use of phosphate containing eyedrops; however, these were not prescribed for the two cases in the present study. Graft failures occurred predominantly due to complete graft dehiscence, progressive malacia and perforations under the graft. The highest graft failure rate occurred in the group where Omnigen was utilised as a supplementary graft and slightly lower in the groups where Omnigen was used as a standalone graft (20.0%) or as a patch (8.3%). The reasons for the high complication rate within this sample population are difficult to elucidate, due in part to the combination of graft materials used and the relatively small sample size. The authors feel that an important factor to consider is the complicated nature of the corneal defects included in this study. According to the surgeons involved, Omnigen was typically selected for use in ‘high-risk’ or ‘catastrophic’ ulcers; this would have led to an inevitable population bias.

The occurrence of corneal neovascularisation was high (100%) which was somewhat unexpected given the purported anti-angiogenic and anti-inflammatory effects of amnion. This could perhaps be attributable to a degree of graft rejection, as has been speculated upon in previous studies on the use of xenografts in veterinary species[64,72]. If so, this could have implications for our understanding of immunogenicity of amniotic xenotransplants in the cornea[59]. Alternatively, it may be that the initial neovascularisation is a transient response; longer-term studies would be helpful in determining if this is the case.

Corneal opacification is a parameter of interest when considering the use of AM, due to previous reports of anti-fibroblastic and anti-inflammatory properties [44,48]. In the present study, the overall occurrence of corneal pigmentation and fibrosis at final examination was reasonably high, at 55.8% and 100% respectively. Specifically, in the group where Omnigen was used as a standalone graft, the pigmentation rate was lower (20%) although the fibrosis rate was still high (100%). This is comparable to a recent study by Plummer et al [85], which revealed some degree of 'corneal scarring' in 100% of equine patients that underwent amnion transplantation for ocular surface reconstruction. Assessment of corneal opacification was limited in the present study, due to lack of an objective scale of opacification in the contemporaneous clinical notes, and the variation in follow-up time. Here, again, longer-term studies would be helpful in determining how the use of Omnigen affects the final corneal opacification outcome.

Statistical analysis in general was limited in the present study, due to the nature of the study. As a retrospective study with a relatively small sample size, much of the

data was not suitable for extensive analysis. Statistically significant findings were limited to the following: brachycephalic dogs were more likely to develop corneal pigmentation and those eyes with graft failures were more likely to be enucleated. The propensity of brachycephalics to corneal pigmentation post-surgery is a generally accepted anecdotal finding, and the correlation between graft failure and enucleation makes biological sense; these findings are therefore easy to accept.

Limitations of the present study were as expected in an observational retrospective of study small sample size, including the limited ability to measure key findings e.g. visual status (limited to the relatively crude assessment of absent or present menace response), epithelial healing time (result of fluorescein testing was not always noted in the clinical record), corneal opacification (limited to presence/absence of corneal pigmentation and fibrosis, where an objective corneal clarity score or other grading scheme would have been more helpful), time until sloughing of the patch (not recorded for most cases), significant biases (selection of 'high-risk' cases for Omnigen use), corneal ulcer size (not recorded in clinical notes) different surgeons and surgical techniques, disparity in post-operative treatments, and variation in follow-up time.

CONCLUSIONS

This study describes the successful use of Omnigen for the treatment of corneal ulceration in 46 eyes from 45 cats and dogs, using a variety of methods, resulting in satisfactory anatomical and visual outcomes in the majority of cases. Future clinical research comprising prospective studies designed to reduce the limitations

encountered here would undoubtedly provide more accurate information on the clinical use and potential benefits of Omnigen, particularly its use as a standalone graft and in comparison to other forms of amnion.

CONFLICT OF INTEREST

The authors declare that they have no financial or personal relationships that could inappropriately influence or bias the content of the paper.

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TABLES

Omnigen® utilisation	**Total eyes**	**Complications**			**Anatomical outcome**			**Corneal neovascularisation**	**Corneal opacification at final examination**		**Visual at final exam**
		Total complications	**Graft minor complication**	**Graft failure**	**Healed without intervention**	**Healed with intervention**	**Enucleated**		**Pigmentation**	**Fibrosis**	
Omnigen® standalone graft	5/46 (10.9%)	2/5 (40.0%)	1/5 (20.0%)	1/5 (20.0%)	4/5 (80.0%)	0/5 (0.0%)	1/5 (20.0%)	4/4 (100.0%) 1/5 enucleated	1/4 (25.0%) 1/5 enucleated	4/4 (100.0%) 1/5 enucleated	2/2 (100%) 1/5 enucleated 2/5 not recorded
Omnigen® supplementary graft Combined with ACell® or frozen cornea	29/46 (63.0%)	14/29 (48.3%)	6/29 (20.7%)	8/29 (27.6%)	21/29 (72.4%)	6/29 (20.7%)	2/29 (6.9%)	26/26 (100.0%) 2/29 enucleated 1/29 not recorded	15/27 (55.6%) 2/29 enucleated	27/27 (100%) 2/29 enucleated	19/21 (90.5%) 2/29 enucleated 6/29 not recorded
Omnigen® patch	12/46 (26.1%)	4/12 (33.3%)	3/12 (25.0%)	1/12 (8.3%)	11/12 (91.7%)	1/12 (8.3%)	0/7 (0.0%)	11/11 (100.0%) 1/12 not recorded	8/12 (66.7%)	12/12 (100.0%)	10/10 (100.0%) 2/10 not recorded
TOTAL	46/46 (100%)	20/46 (43.5%)	10/46 (21.7%)	10/46 (21.7%)	36/46 (78.3%)	7/46 (15.2%)	3/46 (6.5%)	41/41 (100.0%) 3/46 enucleated 2/46 not recorded	24/43 (55.8%) 3/46 enucleated	43/43 (100.0%) 3/46 enucleated	31/33 (93.9%) 3/46 enucleated 10/46 not recorded

Table 1. Anatomical outcome, complications, corneal opacification and visual outcome according to Omnigen® utilisation

		Complications	Corneal pigmentation	Anatomical outcome	Visual outcome
Predictor variables	Brachycephaly	0.825	**0.002**	0.179	0.549
	Gender	0.238	0.126	1.000	0.818
	Eye affected (right vs. left)	0.798	0.760	0.800	1.000
	Diagnosis	0.503	0.122	0.648	0.374
	Corneal malacia	0.889	0.743	0.170	1.000
	Corneal infiltration	0.449	0.547	0.486	0.136
	Omnigen® utilisation	0.803	0.370	0.711	0.152
	Complications	-	0.532	**<0.001**	0.641
	Corneal pigmentation	0.532	-	0.517	1.000

Table 2. Results of Fischer's exact test expressed as P-values; values in bold are statistically significant (P<0.05).

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