TITLE: Immediate effects of diamond burr debridement in patients with spontaneous chronic corneal epithelial defects, light and electron microscopic evaluation

AUTHORS: Charlotte Dawson, Carolina Naranjo, Belen Sanchez-Maldonado, Georgina V. Fricker, Rose N. Linn-Pearl, Natalia Escanilla, Christiane Kafarnik, David J. Gould, Rick F. Sanchez, Marian Matas-Riera

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IMMEDIATE EFFECTS OF DIAMOND BURR DEBRIDEMENT IN PATIENTS WITH SPONTANEOUS CHRONIC CORNEAL EPITHELIAL DEFECTS, LIGHT AND ELECTRON MICROSCOPIC EVALUATION

C DAWSON[1], C NARANJO[2,3], B SANCHEZ-MALDONADO[2], G FRICKER[4], R LINN-PEARL[4], N ESCANILLA[1], C KAFARNIK[4], DJ GOULD[4], RF SANCHEZ[1], M MATAS-RIERA[1]

[1] Ophthalmology Service, Royal Veterinary College, University of London.

[2] Departamento de Medicina y Cirugía Animal, Universidad Complutense de Madrid, Madrid, Spain

[3] Ocular Pathology Specialists, Madrid, Spain

[4]Davies Veterinary Specialists, Manor Farm Business Park, Higham Gobion, Herts

Running Title: Effect of diamond burr debridement on SCCEDs

Key Words: Diamond Burr, Cornea, Canine, SCCED, Ulcer, Superficial Keratectomy

Abstract:

Purpose: To evaluate immediate effects of diamond burr debridement (DBD) on the cornea of canine patients diagnosed with spontaneous chronic corneal epithelial defects (SCCEDs).

Animals studied: Eight client owned dogs with SCCEDs

Methods: Nine eyes from eight dogs with SCCEDs underwent superficial keratectomy (SK). The ulcerated area was divided into quadrants with a 300-micron restricted depth knife. Two of four quadrants underwent DBD for 40-60 seconds. A SK followed immediately. One burred and one non-burred section were fixed with formaldehyde 10% and underwent light microscopy (LM). The remaining quadrants from five eyes were fixed with gluteraldehyde 2.5% and underwent transmission electron microscopy (TEM). Masked pathologists evaluated the samples. A student's paired t-test was used to analyze the data.

Results: With LM all non-burred samples had a superficial stromal hyaline acellular zone (HAZ), seven of the burred samples had an intermittent HAZ and in two burred samples this zone was absent. The HAZ thickness of burred samples (1.062 ± 0.664µm) was significantly thinner than that of the non-burred samples (4.309 ± 1.348 µm) ($P<0.0001$). Transmission electron microscopy showed an absence of basement membrane and the presence of an amorphous, fine fibrillar material in the superficial stroma in non-burred samples. This material was intermittent or absent in burred samples.

Conclusion: DBD significantly reduces the superficial stromal HAZ in SCCEDs. A reduction of its thickness may be responsible for the healing rates reported with DBD.

Word count: 232

Introduction

Spontaneous chronic corneal epithelial defects (SCCEDs) are found in middle aged dogs of a wide variety of breeds.(1) Histopathologic evaluation of SCCEDs demonstrated the presence of a hyaline acellular zone (HAZ) within the superficial stroma of affected corneal samples.(1,2) This hyaline zone is theorized to interfere with the normal healing process of the cornea causing chronic corneal disease and ulceration.(2) Numerous treatment options have been reported in the literature including: simple debridement, punctate keratotomy, grid keratotomy, superficial keratectomy and diamond burr debridement. (3-4) A retrospective paper by Stanley *et al.* demonstrated faster healing, compared to simple debridements and keratotomies, when superficial keratectomy was performed in patients suffering from SCCED.(3) A recently described technique, diamond burr debridement, has been used in patients for the last few years and a study showed a 92% healing rate 10-15 days after its use.(4) A study by da Silva *et al* 2011 showed via light microscopy of normal post-mortem canine eyes, that the diamond burr would spare the corneal stroma and most of the basement membrane of the epithelium.(5) At present there is no data available on the corneal histopathologic and ultrastructural effects of those treatments performed in canine patients.

The aim of this study was to identify whether diamond burr debridement achieved removal of the hyaline acellular zone present in the superficial stroma in keratectomy samples diagnosed with naturally occurring SCCED, using light and transmission electron microscopy.

Material and Methods

This was a prospective multicentre study undertaken by the Royal Veterinary College, London (RVC), Davies Veterinary Specialists, Hertfordshire (DVS) and Universidad Complutense de Madrid (UCM). This study was approved by the RVC ethics committee (URN 2013 1234). The owners of patients diagnosed with SCCEDS (superficial corneal ulcers, without an underlying cause and that had loose epithelial edges and were present for 14 days or more) in both referral centers (RVC and DVS) were offered various treatment options available for SCCEDs. The treatments discussed included cotton bud debridement, grid keratectomy, diamond burr debridement and superficial keratectomy. Owners that elected treatment via superficial keratectomy were offered inclusion in this study. They were informed that DBD would be performed prior to the keratectomy and that the resected samples would be processed for further investigation. The only exclusion criterion was patients with corneal granulomatous changes over the diseased cornea.

The patients were anesthetized and a neuromuscular blocking agent was used. Anesthetic techniques were individualized to each case but in all neuromuscular blockade was monitored with peripheral nerve stimulator and acceleromyography. Prior to aseptic preparation of the surface of the eye, the estimated intraocular pressure was recorded by means of Tono-Pen Vet® (Reichert Inc., Depew, NY, USA). The ocular surface was prepared with povidone iodine solution diluted with saline 1:50 and the eyelids were prepared with a dilution of 1:10. The area around the ulcer was gently debrided with a sterile cotton bud to elevate and remove the loose epithelium unattached to the underlying stroma. The periphery of the area with exposed stroma was demarcated using a set depth knife (maximum depth 300μm) and half of its surface was debrided with the 3.5mm medium grit burr type (The Alger Company, Vista, Texas, USA). A circular or grid pattern was employed to ensure thorough and even coverage. Burring was continued slightly beyond the demarcated margin. The time of the burring was recorded with a minimum of 45 seconds and maximum of 60 seconds per eye. The other half

was left un-burred. The area was immediately resected by superficial keratectomy and the tissue obtained was perpendicularly divided resulting in two samples. Each eye therefore yielded four samples. One burred and one non-burred section were fixed with formaldehyde 10% and underwent light microscopy. A subset of samples were further divided for fixation with gluteraldehyde 2.5% and underwent transmission electron microscopy. In some cases, a bandage contact lens or temporary tarsorrhaphy was placed, depending on clinician preference as well as patient factors.

The samples fixed in formaldehyde were routinely processed for light microscopic evaluation. Four micron thick sections were obtained, which were routinely stained with hematoxylin and eosin (HE). Additional sections were stained with periodic acid-Schiff (PAS) and Masson's trichrome (MT) stains. Samples were assessed according to Bentley *et al.*, (2001). (2) The stromal features evaluated included: characterization and quantification of leukocytic infiltrate, if present (graded as absent, mild, moderate or severe); presence or absence of a superficial HAZ; keratocyte-spindle cell proliferation (graded as absent, mild, moderate or severe); and the presence or absence of vascularization. Epithelial features were not evaluated, since the epithelium had been debrided prior to keratectomy. Thickness of the HAZ was measured using the publicly accessible ImageJ software (ImageJ version 1.45s, NIH, Bethesda, MD, USA).

The samples kept in gluteraldehyde were kept refrigerated at 6-8^{o}C for 12-24 hours. Afterwards they were flushed in a sterile manner with sterile phosphate buffered saline (PBS) and submerged in the same fluid until they were processed. Samples were embedded in epoxy resin and semithin 1 micron sections were obtained and stained with toluidine blue. Approximately 70nm sections were obtained from the area where the HAZ had been observed. If no HAZ was present, sections were taken from the exposed superficial stroma. Samples were evaluated using a JEOL JEM 1010 electron microscope (JEOL Ltd. Japan).

The pathologists were masked to the type of treatment that each keratectomy sample received.

Statistical analysis of the averaged HAZ thickness between non-burred and burred fragments on light microscopy samples was performed using a Student's paired t-test after ensuring the data were normally distributed. The significance was set at $P<0.05$.

Results

Nine eyes from 8 dogs diagnosed with SCCEDs were recruited, resulting in 9 burred and 9 non-burred corneal keratectomy samples. All non-burred samples had a superficial stromal HAZ that had a mean ± standard deviation (SD) thickness of 4.309 ± 1.348 µm. Seven of the burred samples had an intermittent HAZ with a mean (± SD) thickness of 1.062 ± 0.664µm and 2 did not show an obvious HAZ. There was a significant difference between the HAZ thickness of burred and non-burred corneal samples ($P< 0.0001$) (Fig. 1). The HAZ was PAS-positive (bright magenta staining) in all of the non-burred corneas, whereas the PAS was intermittently positive in 6 burred corneas and negative in the remaining 3. When present, the HAZ also stained strongly blue with MT, indicating the presence of collagen. All 9 non-burred corneal samples showed a strong blue staining of the HAZ with MT, whereas only 2 of the burred corneas showed strong blue staining. The remaining burred corneal samples showed either intermittent blue stain with MT (3 samples) or no staining (4 samples). Figures two and three show two examples of HE, PAS and MT stained samples. Inflammatory infiltrate was present in all samples and was neutrophilic in 16 of them and predominantly lympho-plasmacytic in 2 samples from the same patient. Four of the samples showed stromal vascularization. Keratocyte proliferation was seen in all of the corneal samples although the degree of proliferation varied between samples.

Transmission electron microscopy showed that no basement membrane was present in any of the samples examined (burred or non-burred). The superficial, exposed stroma of the non-burred samples showed disorganized to absent collagen fibrils that were admixed with an amorphous or fine fibrillar material; this area corresponded to the HAZ seen on light microscopy. This material was either not noted, or was noted intermittently in the burred samples (Fig. 4).

Intraocular pressures (IOP) estimated in patients under general anaesthesia were documented for all cases as between 8 and 12mmHg.

Discussion

Our understanding of the etiopathogenesis of SCCEDs has advanced considerably during recent decades;(2) however, the corneal effects of treating SCCEDs have not been thoroughly investigated. To the best of the authors' knowledge this is the first study that evaluates the microstructural and ultrastructural effects of corneal debridement with diamond burr in SCCEDs patients.

The results of this study show that DBD applied for between 45-60 seconds on a naturally occurring SCCED, after gentle epithelial debridement, can significantly reduce the thickness and partially or completely remove the HAZ. As the HAZ could be responsible for the poor attachment of the corneal epithelium in SCCEDs, it is possible that removal or a reduction of its thickness may be responsible for improving the healing rates reported with corneal DBD. (1,4)

There are many factors to take into account when evaluating DBD, such as contact-pressure variability with the probe and the cornea, the particle size of the diamond grit used and the intraocular pressure of the affected eye, all of which might have an effect on the variability

observed in the HAZ thickness reduction. The authors of the current study propose that a higher application of pressure during burring and/or a longer DBD time might increase the effectiveness of HAZ removal and therefore improve healing rates in SCCEDs however this specific area should be studied in the future to gain more information.

The epithelium was not assessed in this study as it was debrided prior to keratectomy. In the 2001 study by Bentley *et al*, the epithelium had specific characteristic features;(2) typically it demonstrated poor attachment and there was epithelial dysmaturation. However the HAZ was present in the non-burred samples and was consistent with the findings from previous studies where light and transmission electron microscopy were performed. (2)

There are some limitations to the study. Firstly, multiple surgeons were involved implying potential variabilities in ocular preparation, the cotton bud debridement and the diamond burr debridement technique, which could have affected the results. Secondly, the size of the SCCED area differed between eyes and therefore the relative time of DBD varied between each sample. Future studies are warranted, particularly focusing on the amount of time the burr is applied to the cornea. However, the chosen time of 45-60 seconds of debridement performed in a circular or crosshatch pattern led to a reduction of HAZ. The chosen minimum burr time of 45 seconds was based on both previous clinical experience and on the study by da Silva *et al* (2011) who found that with a burr time of 45 seconds more of the basement membrane was removed compared to the group where the burr time was 30 seconds.(5) Although, as already discussed, the amount of pressure of the operator cannot be standardized and will differ between individuals. The present study tried to consider estimated IOP as a measurable influencing factor. The IOP in anesthetised patients were spread over the low normal range of 8-12mmHg. Corneal biodynamics (corneal hysteresis, corneal resistance factor, corneal compensated IOP) influence measurement of IOP; the extent to which SCCED

will also influence the corneal biodynamics and IOP due to reflex uveitis is unknown.(6) However, this aspect should be considered in future studies with a larger study population and in studies using conscious DBD.

Conclusions:

Diamond burr debridement significantly decreased the thickness and partially removed the HAZ when applied for 45-60 seconds. As removal of this HAZ is considered a mainstay of treatment of SCCED lesions, this technique may be considered, when applied for this time interval, as a less invasive alternative to superficial keratectomy.

Acknowledgements:

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Figure 1. Graph showing the significant decrease in HAZ thickness between the non-burred and burred samples (P < 0.0001). The burred and non-burred samples of each sample are joined with a line.

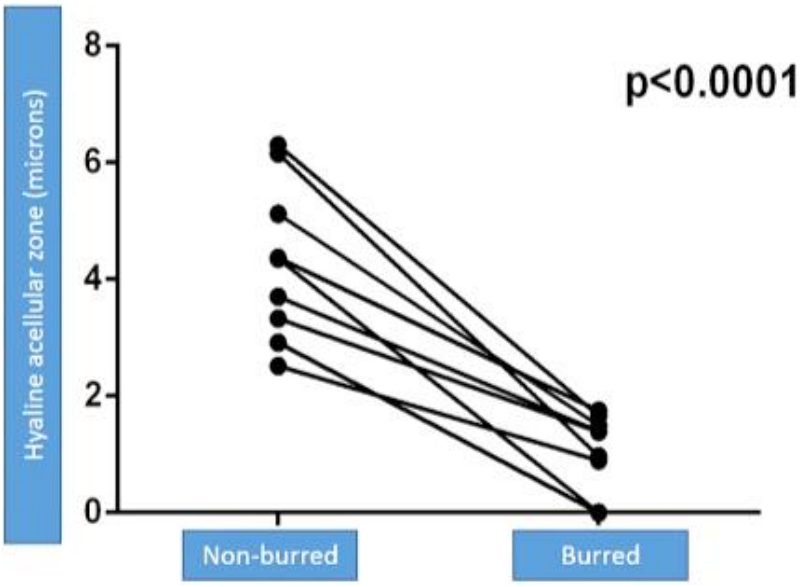

Figure 2. Case no.7 demonstrating the light microscopic findings of the (A) non-burred and (B) burred samples of the same sample in HE, MT and PAS. The burred samples show a decreased HAZ (black arrow). All pictures shown at 20× and the bar is 50 μm.

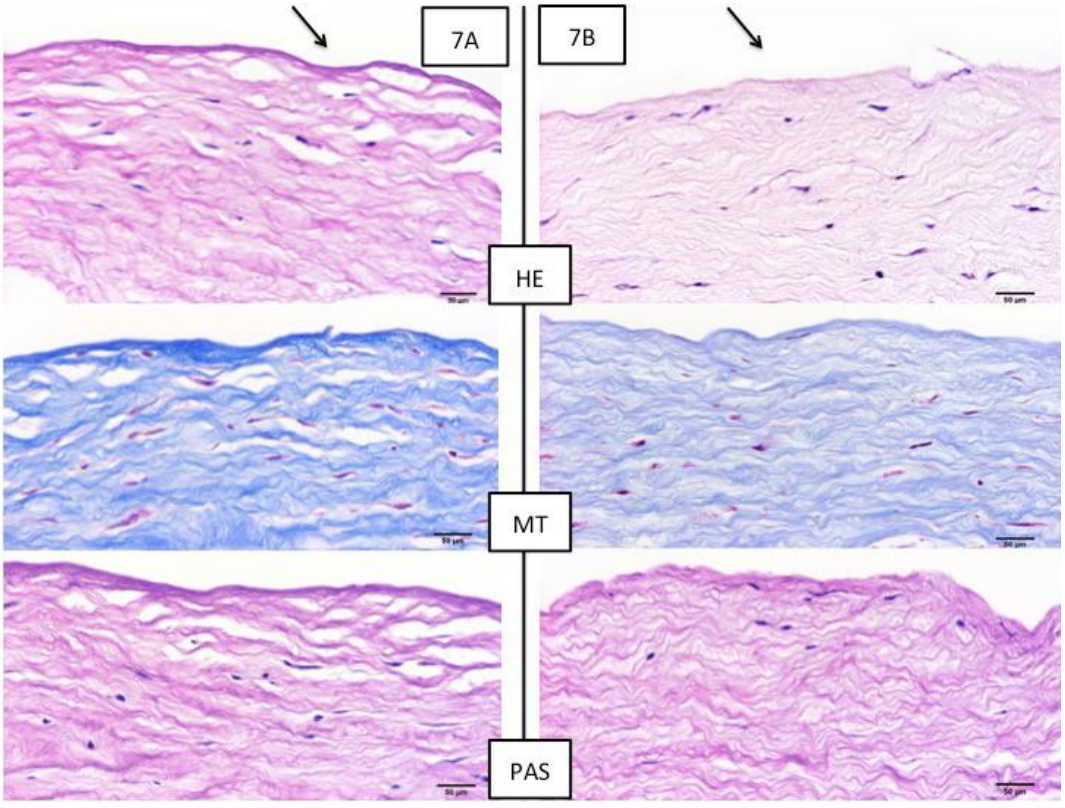

Figure 3. Case no. 9 demonstrating the light microscopic findings of the (A) non-burred and (B) burred samples of the same sample in HE, MT and PAS. The burred samples show an absent HAZ (black arrow). All pictures shown at 20× and the bar is 50 μm.

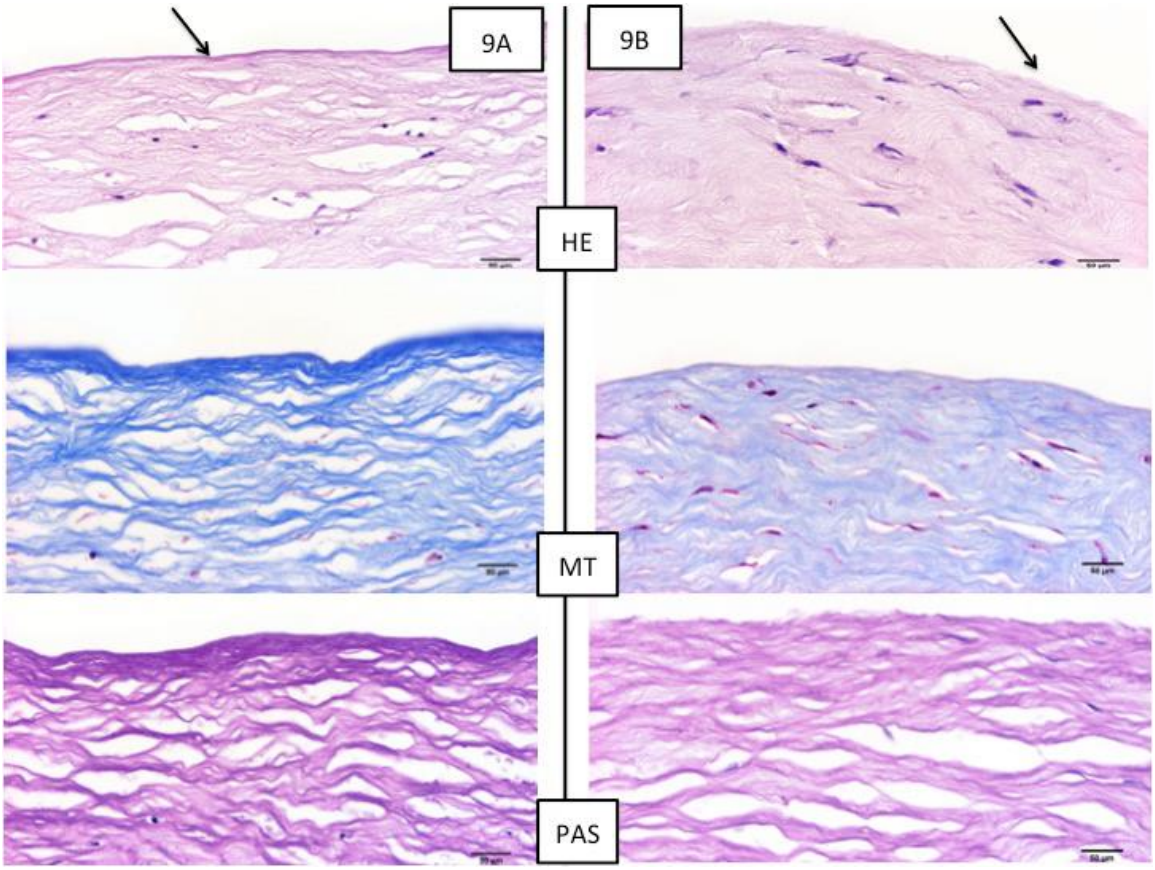

Figure 4. Transmission electron microscopy showing (A) non-burred and (B) burred sections of the case no. 5. A portion of the amorphous material remaining to the right of the image in B) (black arrow). The bar is 0.5 μm.