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Corneal tolerance to micronised mineral pigments for keratopigmentation

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ABSTRACT

Purpose To study the tolerance and biocompatibility of micronised mineral pigments for corneal cosmetic pigmentation in an experimental animal model.

Methods Corneal intralamellar keratopigmentation was performed in 28 New Zealand white rabbits using micronised mineral pigments. Prophylactic actions using intraoperative antibiotic prophylaxis and gamma radiation of the pigment mixtures were performed to avoid infection. Animals were examined regularly by slit lamp to detect any sign of inflammation, pigment diffusion, colour changes or neovascularisation. Histopathological examination was performed to determine the level of pigment diffusion, the level of inflammation and the presence of neovascularisation.

Results No pigment diffusion or changes in colour, inflammation or neovascularisation were detected in the eyes treated. Histopathological examination corroborated clinical results regarding inflammation. Pigmented corneas showed a good cosmetic appearance without signs of ocular toxicity.

Conclusions Micronised mineral pigments could be a valid alternative treatment for cosmetic keratopigmentation. The intralamellar keratopigmentation technique presented good cosmetic appearance without adverse effects in the eyes treated.

INTRODUCTION

Corneal keratopigmentation (KTP) or corneal tattooing has been used to improve the cosmetic appearance of blind eyes with leukomas.[1–3] Galen (131–210 AD) is considered to be the first one to have used pigments for human cornea, using reduced copper sulfate to mask a corneal leukoma.[4–6] Thereafter, KTP gained limited popularity due to a variety of reasons. Various chemical products such as Indian ink, metallic powders, organic colours, animal uveal pigment, Chinese ink, gold and platinum chloride and even soot were used.[7–10] To obtain different shades, surgeons experimented with different combinations of such chemical products. The main problem which affected the outcome of previous KTP studies was the fading of the colours, which made results inconsistent with time.[6]

The therapeutic effect of corneal tattooing has also been evaluated in patients with symptomatic glare associated with iris loss, atrophy or trauma[4–6 11–13]; in cases of disabling light scattering[14] and photophobia in traumatic aniridia or iris coloboma[6 14–16]; and even in the treatment of incapacitating diplopia,[12 17] showing that this procedure is used in blind eyes but can also be used in sighted eyes for therapeutic reasons.[12 17]

Cosmetic contact lenses, enucleation or evisceration are the most frequently used methods to improve aesthetic appearance in cosmetically unacceptable, disabled eyes.[18–20] However, there is enough evidence of the excellent outcomes using KTP to achieve good cosmetic results and high patient satisfaction, avoiding extensive and mutilating reconstructive surgery.[1]

After reviewing the tolerance and biocompatibility of micronised mineral pigments for KTP,[1 12 13 16 17] the aim of our study is to investigate in an experimental animal model the potential of corneal tattooing using these pigments for cosmetic reasons. We also sought to demonstrate the safety, tolerance and medium-term durability of this procedure. The smaller particle size of micronised mineral pigments reduces the foreign body sensation and inflammatory response because of the imbedded pigment.

This work addresses the need of non-toxic and biocompatible pigments which may have fewer side effects. A variety of pigment colours to mimic different types of iris is also reported in this study. The poor practice and experience in corneal tattooing lies in the fact that reliable commercial products are unavailable. This is probably related to the lack of studies demonstrating the corneal biocompatibility of these pigments.

MATERIALS AND METHODS

Animal model

A total of 28 eyes of 14 New Zealand rabbits (average weight 3 kg) were included in this study. Intrastromal corneal tattooing was performed using a manual intralamellar keratopigmentation (MIK) technique and different colours of micronised mineral pigments, in aseptic and antiseptic conditions, under topical and general anaesthesia.

Pigments

For this study, micronised mineral pigments were used. The colours used to mimic the natural colour of the iris were blue, green, blue brown and greenish brown (figures 1 and 2) (Spanish Ministry of Health Registration No 280-PE and 281-PE).

Corneal KTP technique

The animals were anesthetised with intramuscular injections of a 1:1 mixture of ketamine hydrochloride 20 mg/kg (Imalgene 1000; Merial, Lyon, France) and xylazine hydrochloride 4 mg/kg (Xilagesic 2%; Laboratorios Carlier, Barcelona, Spain). In addition, topical anaesthesia was applied to the cornea (tetracaine 0.1% and oxibuprocaine 0.4%, Colircusi; Alcon Cusi S.A., Barcelona, Spain). MIK, also called intralamellar corneal staining, was

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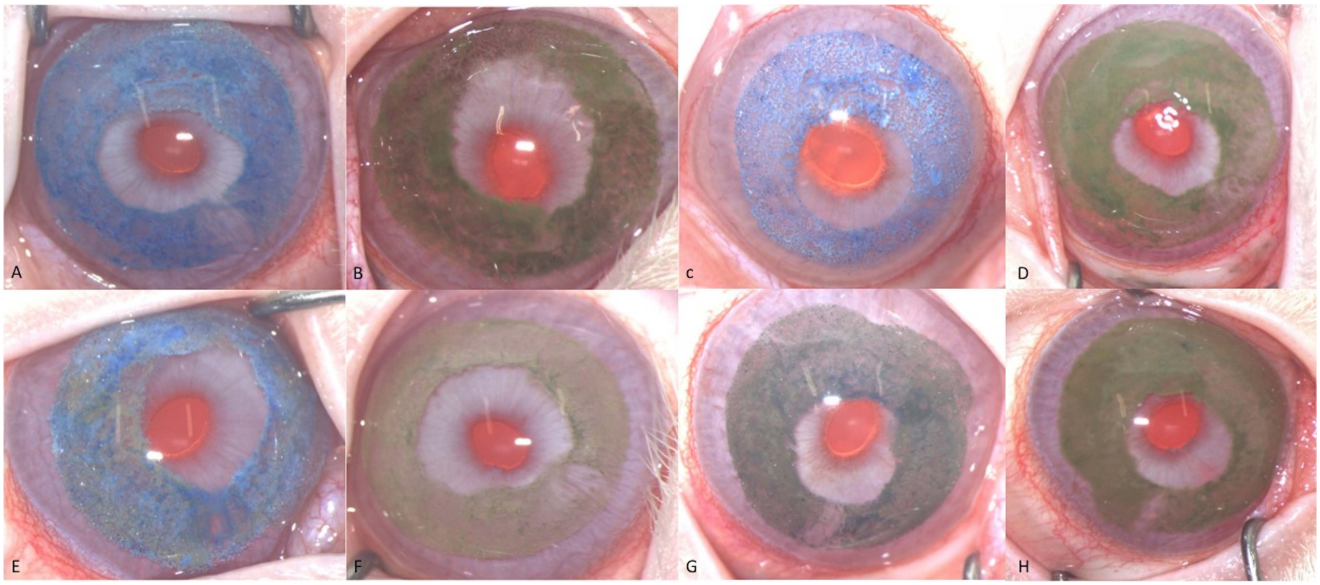

Figure 1 Results 2 months after keratopigmentation. Colours used: (A and C) blue; (B and D) green; (E and G) blue brown; (F and H) greenish brown.

performed by only one surgeon in 27 eyes (1 eye was used as control). The centre of the cornea was marked with a calliper and the pupil size determined by an RK optic zone marker (Katena, New York, USA). One free hand radial incision to mid-stromal depth was performed with a 45° knife from the limbus to the border of the marked pupil at 12 o'clock (Sharpoint, Surgical Specialties Corporation, Reading, Pennsylvania, USA). From the radial incision the cornea was dissected intralamellarly in the same plane with a microcrescent knife (Sharpoint, Surgical Specialties Corporation, Reading, Pennsylvania; USA) and then with a helical dissector (pigtail or spiral corneal dissector CPK, Epsilon, Irvine California, USA) 180° clockwise and 180° counterclockwise intrastromally and circumferentially along the route of the pupil margin (leaving the pupillary area intact).

The desired colour was injected inside the intrastromal tunnel in 26 eyes with a 30 gauge irrigation cannula (except in the control eye and in the pocket eye, where the tunnel was created but no pigment was injected). No corneal sutures were used. Antibiotic prophylaxis was topically applied using ciprofloxacin hydrochloride 3 mg/mL (Oftacilox, Alcon) and chloramphenicol ointment (Oftalmolosa Cusi, Alcon) twice a day for 7 days. Cyclopentolate hydrochloride 10 mg/mL (Colircusi cycloplegic, Alcon) was also applied topically twice a day for 3 days. Additional prophylactic actions were taken to avoid infection using intraoperative antibiotic and gamma radiation of the mixtures as preoperative sterilisation of the pigments.

Clinical evaluation

The follow-up period after the surgery was 3 months. The eyes were examined under a slit lamp 1 week and 1–3 months after the surgery by an independent observer. At each examination time point the degree of inflammation was quantified using an anterior segment inflammation scale, measuring conjunctival injection (0–3), corneal haze (0–3), corneal neovascularisation

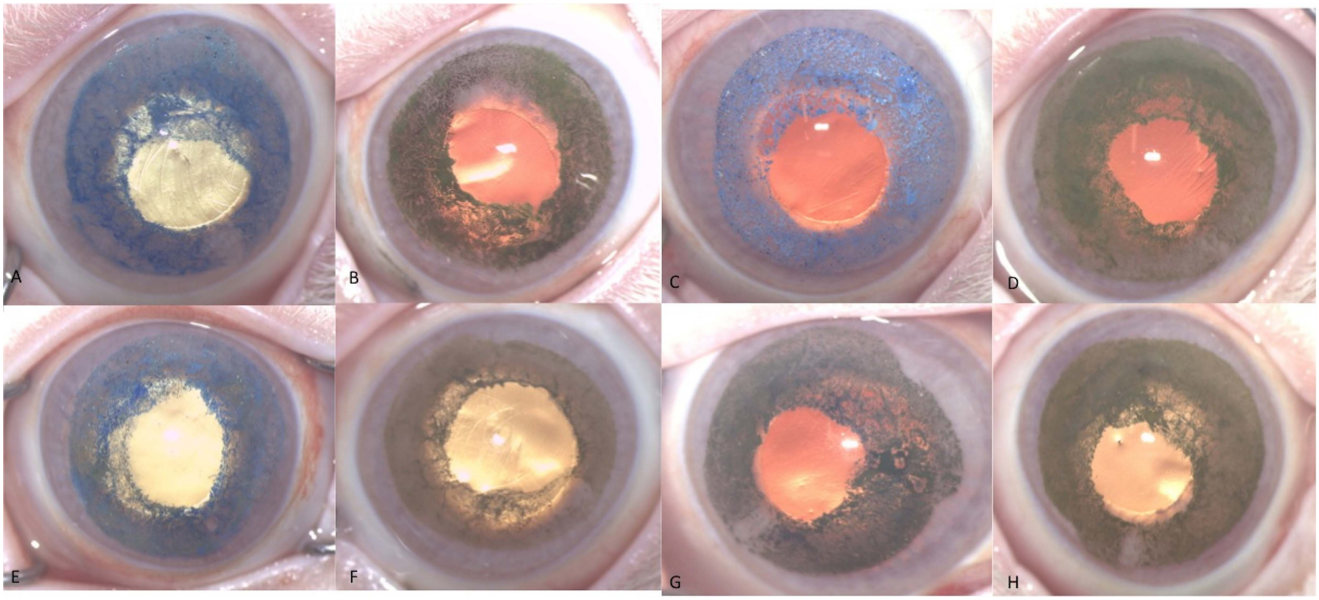

Figure 2 Results 3 months after keratopigmentation. Colours used: (A and C) blue; (B and D) green; (E and G) blue brown; (F and H) greenish brown.

Table 1 Anterior segment inflammation scale

Clinical examination		Histological study	
Conjunctival injection	0–3	Inflammation	0–3
Corneal haze	0–3	Neovascularisation	0–3
Neovascularisation	0–3	Pigment diffusion	Yes/No
Epithelial defect	Yes/No		
Pigment diffusion	Yes/No		
Changes in colour	Yes/No		

(0–3) and epithelial defect (in mm). Other parameters documented included the presence of oedema, pigment diffusion or changes in the colour and/or texture of the pigmentation (table 1).

Histological analysis

The eyes were enucleated and placed in Davidson liquid for 48 h and then placed in 70% ethanol until tissue processing. They were embedded in paraffin and sectioned in microslides with a microtome. Sections were stained with haematoxylin eosin to determine the presence and distribution of inflammatory cells (lymphocytes, macrophages and neutrophils) surrounding the pigment. Any inflammatory infiltrate was described as acute or chronic on the basis of its cellular composition. The presence of neovascularisation was also documented if present.

RESULTS

Clinical evaluation

Twenty-eight eyes were treated. One week after the procedure, 7 eyes had a minimal degree of conjunctival injection (grade 1: in 6 eyes, grade 2: in 1 eye), 5 eyes had an epithelial defect coinciding with the radial incision area, 3 eyes had corneal haze (grade 1). The eyes were also examined to detect the presence of neovascularisation from the limbus toward the centre of the cornea and no cases with such characteristics were found during the clinical follow-up.

The health of these 28 eyes was satisfactory during the first and second month (figure 1). The favourable postoperative status was also observed in the last review 3 months after surgery (figure 2). By that time, all corneas were clear, even in those in which no pigment was applied or pocket eyes. The pigmented corneas showed a good cosmetic appearance without changes in colour or dispersion of the pigments (figures 1–4).

Histopathological study

The corneal epithelium was intact, with no hyperplasia or atrophy in the 28 eyes; normal thickness was maintained (between 3 and 5 cells). Bowman's membrane was not examined because rabbits do not have this structure despite the great similarity with the human eye. Keratocytes around the pigment were larger than elsewhere with an eosinophilic cytoplasm containing small thin pigmented granules. The core was discretely bigger with increased chromasia and small or non-identifiable nucleolus. There was no fibrosis in the corneal stroma and collagen fibres maintained their wavy shape. Type I collagen was predominant followed by type III.

Stromal extracellular matrix remained intact. The presence of macrophages was not found and a defined, continuous and homogeneous layer of pigments was found in the mid stroma of all eyes, with no signs of pigment diffusion. The cohesiveness of the pigment was demonstrated, although in some areas it formed denser clusters which did not affect the final cosmetic appearance. The edges of the pigmented areas were clearly defined, without dispersion. Peripheral corneal nerves were normal. There were no histological changes in the endothelial layer or Descemet's membrane in tattooed or control eyes (figures 3–5).

DISCUSSION

Corneal tattooing is a useful procedure that can be used for cosmetic and therapeutic indications in selected groups of patients.[21] Even though its use has been described since ancient

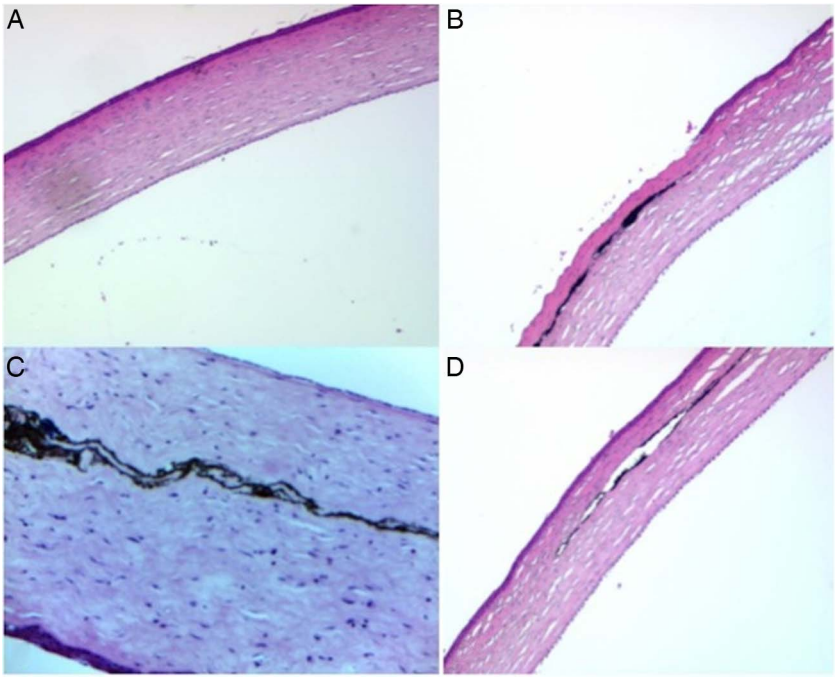

Figure 3 (A) Rabbit 1, left eye: corneal stroma without histological alterations in pocket eye without pigment. (B) Rabbit 6, right eye (green). (C) Rabbit 7, left eye (blue-brown). (D) Rabbit 9, left eye (greenish brown). The absence of inflammation and neovascularisation is evidenced in all images.

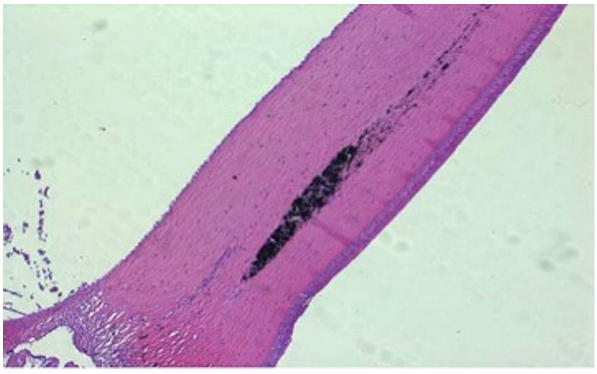

Figure 4 Pigment remains in the stroma 3 months after surgery and no pigment dispersion is observed.

times, it is seldom practiced today. Current practice generally uses indiscriminate pigments and poorly systematised surgical techniques. With adequately studied pigments from the perspective of histopathological tolerance, immune reactivity and local toxicity, we believe that this technique may offer important applications from therapeutic and cosmetic perspectives. For these reasons corneal KTP may play a significant role in future ophthalmic surgical practice.

KTP has proven to be effective in the cosmetic correction of cosmetically disfiguring corneal scars.[1 21] It has also been demonstrated to be a good technique to correct optical problems such as glare, photophobia and monocular diplopia due to iris defects, iridectomies or traumatic iris loss.[11–13 15 21]

The intrastromal surgical technique maintains the integrity of the basement membrane. Damage to this membrane, whether mechanical, chemical or traumatic, is cited as a cause of recurrent corneal epithelial erosions.[22] If the corneal epithelium is removed leaving an intact basement membrane, the epithelium regenerates within 48 h and strong adhesion to the underlying stroma is achieved in 7 days. However, if the basement membrane is also removed, then epithelial adhesion requires up to 8 weeks.[22] In all treated eyes, we observed no complications associated with the surgical technique and this is probably because we did not affect the basal membrane. In addition, the corneal architecture was not affected. The intrastromal tunnelling technique is advantageous because the epithelium was not affected and because the pigment was retained in the tunnel, avoiding dispersion and reducing its inflammatory potential, having no contact with the ocular surface, and providing good results for both cosmetic and therapeutic use.

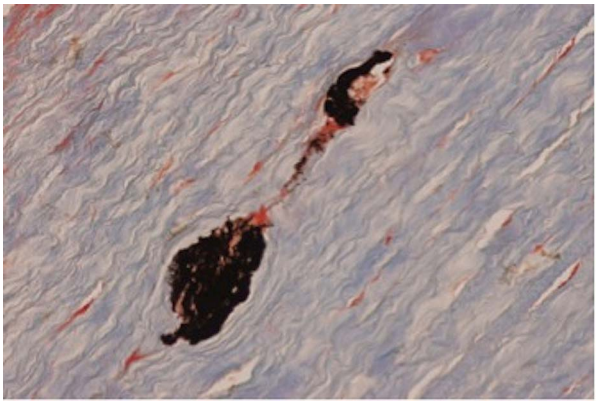

Figure 5 Masson trichrome staining. The pigment remains in the stroma, without dispersion. Activated keratocytes around the pigmented area and some neuronal cells are recognised.

Some authors have suggested femtosecond-assisted keratopigmentation to create the lamellar pockets and facilitate the technique in human eyes, achieving predictable configuration and distribution of the pigments in the stroma.[11 13]

Possible complications of KTP are underpigmentation, overpigmentation, discolouration, pigment migration, accidental perforation, healing problems and uveitis.[2] These complications were not observed in our study. We believe that pigment components have no toxic potentials because there were no local signs of toxicity. Intraoperative antibiotic prophylaxis and gamma radiation of the pigment mixtures were used as preoperative sterilisation methods to prevent infections and increase the safety of the surgical technique.

Micronised mineral pigments present an additional advantage over other natural pigments because their particle size is reduced by micronising procedures. The small particle size diminishes the chances of developing a foreign body reaction against the pigment introduced into the corneal stroma.

Another major advantage of the mineral micronised pigments is the wide range of colours available. In this study we have used several colours (figures 1 and 2) and no inflammatory complications were observed with any of them. This is very useful in KTP because the main goal is to accurately mimic the natural colour of the patient's eye to obtain the best possible cosmetic result. The mixing of different pigments to obtain a matching colour can make KTP a laborious and time-consuming procedure. Micronised mineral pigments can be mixed and prepared in advance in monodose vials adequately sterilised and ready to use during the surgery.

However, even simple colours such as black are obtained by combining several iron oxides and other additives and stabilisers. Their effect in the eye must be assayed to guarantee the safety of each pigment before they are applied to the human eye.

The safety of the technique, tolerance and biocompatibility of the micronised mineral pigments have been demonstrated in this study clinically and histopathologically. Although the intrastromal KTP technique has shown good results in this study, one limitation is that all corneal scars may not be amenable to lamellar pigmentation and might require different or more superficial techniques to cover the affected area.

CONCLUSION

No signs of toxicity were found in corneas coloured using the micronised mineral pigments selected for this investigation, which suggests that they could be a viable alternative for use in KTP for cosmetic and therapeutic reasons.

Contributors JLA: conception, design, analysis and interpretation of data, critical revision of manuscript, final approval given. MAA: design, animal experimentation (surgeon), acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision, final approval given. AER: micronised mineral pigments preparation, animal experimentation, final approval given.

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Competing interests None.

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