Fluorescein pen: a novel tool with good ease of use for corneal fluorescein staining

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Research Article

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Abstract

Background

The purpose of this study was to validate a fluorescein pen for use in corneal staining and to compare this novel technique with two conventional tools.

Methods

An insulin pen was converted into a fluorescein pen for use in corneal staining. The fluorescein pen was provided to 15 ophthalmologists for evaluating the ease of use as compared with two other tools (standard fluorescein strips and the 1 mL syringe method). Each fluorescein pen was used multiple times and underwent microbiological cultivation weekly. If the microbial culture was negative, the pen was used until the fluorescein in the cartridge was used up. Cornea fluorescein staining with three tools was conducted on 16 patients. Images were taken under slit lamp cobalt blue light to assess image quality and consistency.

Results

The average application span for the fluorescein pen was 87.7 ± 17.0 days (n = 15). No microbial growth was detected in any sample during the application period. Ophthalmologists' assessments for ease of use via evaluation scores on a five-point Likert scale were 4.933 ± 0.26, 2.6 ± 0.83, and 2.2 ± 0.68 for the pen, strips, and syringe method, respectively. The fluorescein pen demonstrated greater ease of use than the other two tools ($p < 0.0001$). Image quality and consistency using the fluorescein pen were improved as compared with the other two tools.

Conclusions

We conclude that the fluorescein pen has several advantages over traditional staining methods, including greater ease of use, safety for multiple uses, and better yield in terms of improved corneal staining images.

Introduction

Fluorescein sodium has been widely applied within ophthalmology for the purpose of fundus angiography and ocular surface staining (since the 1880s)[1, 2]. When administered topically to the conjunctiva, fluorescein sodium disperses through the tear film and stains the damaged corneal epithelial cells[3]. These areas can be clearly highlighted with the use of a cobalt blue light filter under a slit lamp microscope. Ophthalmologists can assess the health of the ocular surface and the severity of ocular surface disease using fluorescein staining[4]. The techniques used for ocular surface evaluation are

constantly developing and numerous methods have emerged. The use of fluorescein sodium for ocular surface staining remains an invaluable examination in ocular surface health evaluations.

To facilitate more effective corneal fluorescence staining, we modified an insulin pen into a fluorescein pen, which can administer a precise concentration and quantity of fluorescein sodium solution into the conjunctiva and can be stored for a period of time for multiple uses. In this study, we introduced the application of a fluorescein pen and validated this pen tool against two conventional corneal staining methods. We conduct this study to validate that the fluorescein pen is superior for conducting ocular examinations as compared with the more traditional tools.

Methods

Patients

Sixteen patients aged 18–67 years (nine females, seven males) who visited the ophthalmology department at the Second Affiliated Hospital of Fujian Medical University between February 2021 and August 2021 were enrolled in this study. Corneal fluorescence staining was performed in accordance with the tenets of the Declaration of Helsinki. This study was approved by the Second Affiliated Hospital of Fujian Medical University Ethics Committee (ratification 2021 No. 43). All patients provided their written informed consent prior to participation.

Modifying and applying the fluorescein pen

The fluorescein pen was modified from a standard insulin pen (Novo Nordisk, Bagsværd, Denmark) containing 3 mL (300 units) of insulin fluid. The insulin liquid in the pen was extracted and rinsed with saline repeatedly until the insulin was completely removed. After removing all the saline solution in the pen, a 0.5% fluorescein solution constituted from a mix of fluorescein sodium and saline (5 mL:500 mg; Alcon Research LLC, Fort Worth, TX, USA) was injected into the cartridge, following which we attached a needle to form a pen injector (Fig. 1).

The directions for recommended usage for the fluorescence pen are as follows: (1) Remove the cap. (2) Attach the needle to the pen. (3) Prior to starting the sequence for a new pen, a priming dose of two units should be discarded. The priming dose should be repeated until no drops are observed at the top of the needle. (4) Dial a one-unit dose. (5) Gently push the plunger until a small drip appears at the needle tip. (6) The needle should be held close to the conjunctive sac and the drip should be delivered onto the ocular surface with a gentle touch (taking care that the needle does not touch the conjunctiva). (Fig. 2iii) (7) Expel and discard another drip. (8) Disinfect the needle and replace the cap back to its original state. (9) The application can be used for multiple administrations for a period of time. Please make sure to disinfect the needle prior to each use and to change to a new needle weekly. (10) The fluorescein inside each pen should undergo microbiological cultivation on a weekly basis. If the microbial culture is negative, the pen should be used until the fluorescein in the cartridge is used up. The 3 mL volume in the fluorescein pen is sufficient for approximately 300 uses.

Manipulation of fluorescein staining with standard fluorescein strips

Cornea fluorescein staining was performed using commercially available sterile fluorescein paper strips (Jinming New Technological Development Co. Ltd., Tianjin, China). Briefly, a drop of normal saline was instilled to the strip to wet the fluorescein end. The pen was then shaken to remove extra liquid (in order to minimise the volume of fluorescein fluid). Afterwards, the strip was gently touched with the inferior temporal bulbar conjunctiva and fluorescein was delivered into the conjunctival sac (Fig. 2i). The patients were then checked under a slit lamp.

Manipulation of fluorescein staining with 1 mL syringes

A 0.5% fluorescein solution (constituted from fluorescein sodium, 5 mL:500 mg; Alcon Research LLC, Fort Worth, RX, USA) was loaded into a 1 mL syringe. One drop of fluorescein solution from this syringe was instilled in the conjunctival sac (Fig. 2ii). The patients were then checked under a slit lamp.

Microbe culture and evaluation of fluorescein intensity

The transparency of the fluid inside the cartridge was evaluated each time prior to usage. Use of the fluorescein pen would be unsafe if the fluid becomes cloudy or if a decline in transparency is detected. Each pen was subjected to a weekly microbiological cultivation. Specifically, one unit of fluorescein solution was directly dripped onto agar plates and incubated at 37°C for 48 h. If the microbial culture was positive, the pen was discarded. If the microbial culture was negative, the fluorescein pen was used until the fluorescein solution in the cartridge was used up or until either a decline in transparency or the occurrence of a positive microbial culture.

In order to verify whether the fluorescein in the pen was quenched with time, leading to a poorer staining image, we compared differences in corneal staining and fluorescence intensity after blue light excitation for one pen at different time points after usage (days 0, 60 and 90).

Ophthalmologists’ assessments for ease-of-use and patient feedback

The fluorescein pen was provided to 15 ophthalmologists for evaluating its ease of use as compared with the other two tools (standard fluorescein strips and the 1 mL syringe method). Patients; evaluations of the corneal fluorescein staining methods were assessed via the following questionnaire item: “This tool makes corneal fluorescein staining easier.” Responses were measured on a five-point Likert scale ranging from 1 ("strongly disagree") to 5 ("strongly agree").

Patients’ evaluations

Patients’ evaluations of the fluorescein pen were graded on a five-point Likert scale: 1 (feel nothing), 2 (feel eye drops, no sting), 3 (feel eye drops, mild sting), 4 (feel eye drops, moderate sting), and 5 (severe

discomfort).

Image acquisition and evaluation

All patients (n = 16) underwent corneal fluorescence staining using the three aforementioned tools. For every patient, the three methods were each conducted on the same day with > 1 h intervals. After sodium fluorescein was dripped into the conjunctival sac, the patients were asked to blink three times. Cornea images were taken over the course of 30 s using a slit lamp (Huvitz Co. Ltd., Gunpo, South Korea) and a digital camera (Canon, Tokyo, Japan). This portion of the operation was performed by the first author under the same slit lamp (with brightness fixed). Images were analysed blindly with respect to method and were independently examined by two experts who evaluated the quality of each group of three photos and ranked the images in order from the highest to the lowest quality via score categories (1, 2, 3). At the end of the examination, patients were asked to evaluate their subjective experiences in using the three tools.

Statistical analysis

The statistical analyses were performed using GraphPad Prism 9(GraphPad Software, San Diego, CA). A p-value < 0.05 was deemed significant.

Results

Clinical assessment

In all 16 sets of images, both experts agreed that corneal images using the fluorescein pen were of the best quality. For two sets of images, the two experts had different assessments of the quality of the images taken using the 1 mL syringe and the fluorescein strip methods (Table 1). Figure 2 shows a representative example of corneal staining using three tools. Compared with the other two methods, the corneal staining with fluorescein pen resulted in less tear overflow and a clearer display of corneal lesions (Fig. 2).

Table 1
Comparisons of image quality following three blinks after administering each of the three fluorescein tools.

	Sodium luciferin pen	1 mL syringe	Fluorescein strips
1	16 (16)	0 (0)	0 (0)
2	0 (0)	5 (6)	11 (10)
3	0 (0)	11 (10)	5 (6)
* the values indicate counts			

The ophthalmologists' assessments for ease-of-use scores (on a five-point Likert scale) were 4.933 ± 0.26, 2.6 ± 0.83 and 2.2 ± 0.68 for the pen, strip, and syringe methods, respectively. All 15 ophthalmologists agreed that the fluorescein pen had a greater ease of use as compared with the other two tools ($p < 0.0001$). There was no statistically significant difference between the fluorescein strip and the 1 mL syringe methods ($p = 0.227$) (Fig. 3i). In terms of patient evaluations of the methodologies, the fluorescein pen caused less discomfort as compared with each of the other two tools (all $p < 0.0001$) (Fig. 3ii).

Microbial culture, stability of sodium fluorescein and the application span for the pen

The average application span for the fluorescein pen was 87.7 ± 17.0 days (n = 15). The liquid in the cartridge remained crystal clear during the application period for each of the pens. Additionally, 182 culture cycles were performed and no microbial growth was detected in any of the samples during the application period. No side effects were detected during the application phase.

The fluorescein in the pen tool was dripped in the culture dish on days 0, 60, and 90. The colour and the fluorescein intensity remained unchanged under white light and blue light illumination and the effect on corneal staining remained unchanged (Fig. 4).

Discussion

There are several accepted methods for delivering fluorescein sodium solution onto the ocular surface when performing fluorescein staining.[5] In the current study, we compared standard fluorescein strips and a 1 mL syringe method with an innovative newly developed fluorescein pen. The standard fluorescein strip method cannot administer exact concentrations and quantities. In contrast, the 1 mL syringe method can fix concentrations but it is difficult to accurately control the infusion amount. Additionally, the above two methods are either single-use or their viable single-use period is short. These shortcomings may reduce the use of corneal fluorescence staining in clinical practice, resulting in missed diagnoses or misdiagnoses. To the best of our knowledge, we have conducted the first study evaluating the use of a fluorescein pen, a novel methodology newly developed by our research group for use in ophthalmic evaluations.

In the current study, images were taken immediately following three blinks after the fluorescein was dripped into the conjunctival sac. This difference is likely one reason that the image quality in the fluorescein pen group was best as compared with the other two tools. Because the amount of fluorescein dripped into the conjunctiva sac (controlled by the pen plunger) can be subtle enough to not cause changes in the volume of the tear film, the fluorescein immediately forms an evenly distributed thin green film, allowing the fine corneal lesion to be easily detected. Both the fluorescein strip and 1 mL syringe methods may lead to overdosing. However, our data indicate that prolonged observation under slit lamps as well as rinsing fluorescein from the ocular surface within the latter two tools can lead to an equally

good images as compared with the pen tool, though necessitating more time and the performance of a more complex procedure to derive results of similar quality (data not shown).

The reported concentrations of fluorescein used in ocular surface staining differ, ranging from 0.1–1%[5–7]. We applied a concentration of 0.5% in our study, because this was the optimal concentration identified in our pilot study and because we use this concentration for corneal staining in our practice (data not shown). A higher concentration might lead to a darker background, with an image that is undiscernible immediately after staining; a prolonged observation period is needed under this scenario.

An ease-of-use evaluation by 15 ophthalmologists illustrated the superiority of the fluorescein pen as compared with the other two tools. This result is likely due to the portable pen style, which can be kept in the physician's pocket for ready administration. In addition, the fluorescein pen is safe for multiple uses, and can be used for as long as three months as observed in our study.

The patients' subjective assessments of the three methods illustrated that the pen tool causes less discomfort as compared with the other two tools. It is important that staining methods induce little irritation and prevent reflex tearing, as this dilutes the solution and causes epiphora. Good compliance and cooperation from patients are vital to achieving stable and repeatable results within ocular surface check-ups.

Although fluorescein quenching is a common phenomenon, prolonged usage of the pen (up to a maximum of four months) did not result in colour fading or in debilitating the staining effect. Prolonged and multiple usage is the major advantage of the pen tool and chemical stability is a precondition. No side effects were detected during the application. In contrast, the latter two tools (as well as other tools not evaluated in the current study) are single-use or their viable single-use period is short.

The generation of a fluorescein pen was inspired by the fact that an insulin pen can be used multiple times (i.e., for prolonged use) and that the insulin solution can only be discharged without suction, thus preventing microbial contamination.[8] In addition to the substantial strengths of this study, including its novelty and innovation, limitations of this investigation include the fact that the needle attached to the pen is sharp and carries the potential risk of puncturing the eyeball upon incautious performance. However, we note that we did not cause any damage to the eyeball in this study. Innovating the device with a silicone needle in order to avoid damaging the eye as well as with a slimmer cartridge (i.e., with one dial for delivering a suitable quantitative volume of fluorescein) would greatly enhance the advantages of the fluorescein staining procedure in the future.

Conclusions

In summary, in this innovative report of a novel ophthalmic methodology, we demonstrated that fluorescein pens are easy to use and safe. The fluorescein pen evaluated in this study substantially improved the image quality in corneal staining compared with more traditional tools. Our results guide

future research directions, medical guidelines, and clinical decision-making with respect to ophthalmic evaluations, imaging methods, and diagnostics.

Declarations

Funding

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Corneal fluorescence staining was performed in accordance with the tenets of the Declaration of Helsinki. This study was approved by the Second Affiliated Hospital of Fujian Medical University Ethics Committee (ratification 2021 No. 43). All patients provided their written informed consent prior to participation.

Consent for publication

Not applicable.

Competing interests

The authors have no actual or potential conflicts of interest to declare.

Author's contributions

YJ and YG wrote the main manuscript text; JW, LZ and MH prepared figures 1-4. All authors reviewed the manuscript.

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Figures

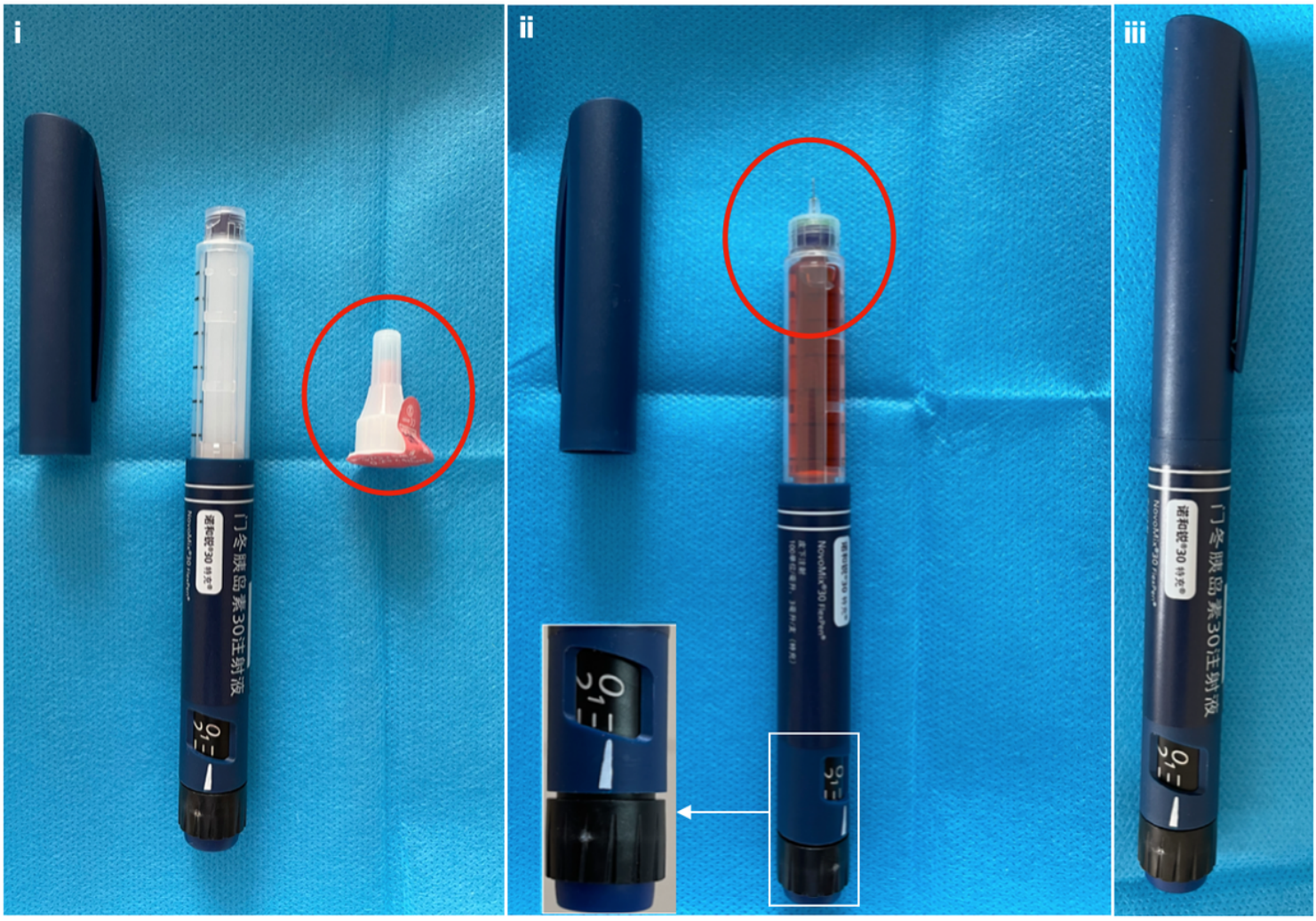

Figure 1

The insulin pen adapted for transformation to a fluorescein pen consists of pen fills, a pen cap, and a needle. (i) After the insulin is completely replaced by 0.5% fluorescein, the fluid in the cartridge changes to a clear red colour. This image depicts the attachment of the needle to the cartridge (red circle). (ii) A dose of one unit is dialled prior to usage (white rectangle). (iii) the fluorescein pen has the shape and size of a standard pen, fits well into a pocket, and is easy to carry and store.

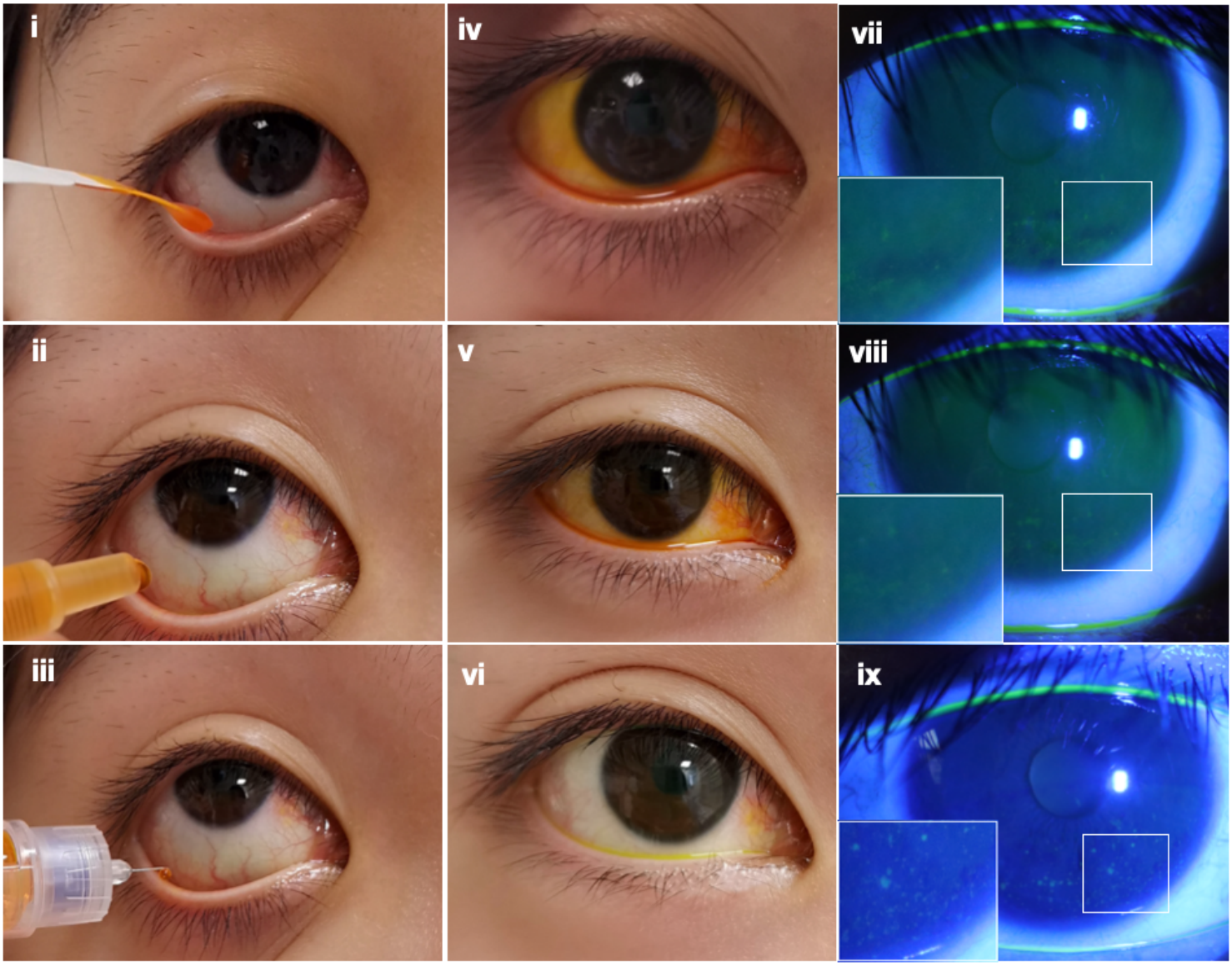

Figure 2

Depiction of the procedure for corneal staining and accompanying image differences for the same patient using three staining methods. Imaging following staining with the fluorescein pen(i) and observation of the ocular surface just after three blinks(iv). Imaging following staining with a standard fluorescein strip (ii) and observation of the ocular surface just after three blinks (v). Imaging following staining with 1 mL syringe (iii) and observation of the ocular surface just after three blinks (vi). Corneal lesions can be discerned well using the fluorescein pen method immediately following three blinks after staining (vii). Images are vague when using fluorescein strips (viii) and the 1 mL syringe method (ix) due

to excessive dye. The white boxes show that the fine punctate corneal lesions are discerned well after using the fluorescein pen but are obscure after using strips and a 1 mL syringe, though a prolonged checking time or rinsing with saline can lead to similar stain effects (data not shown).

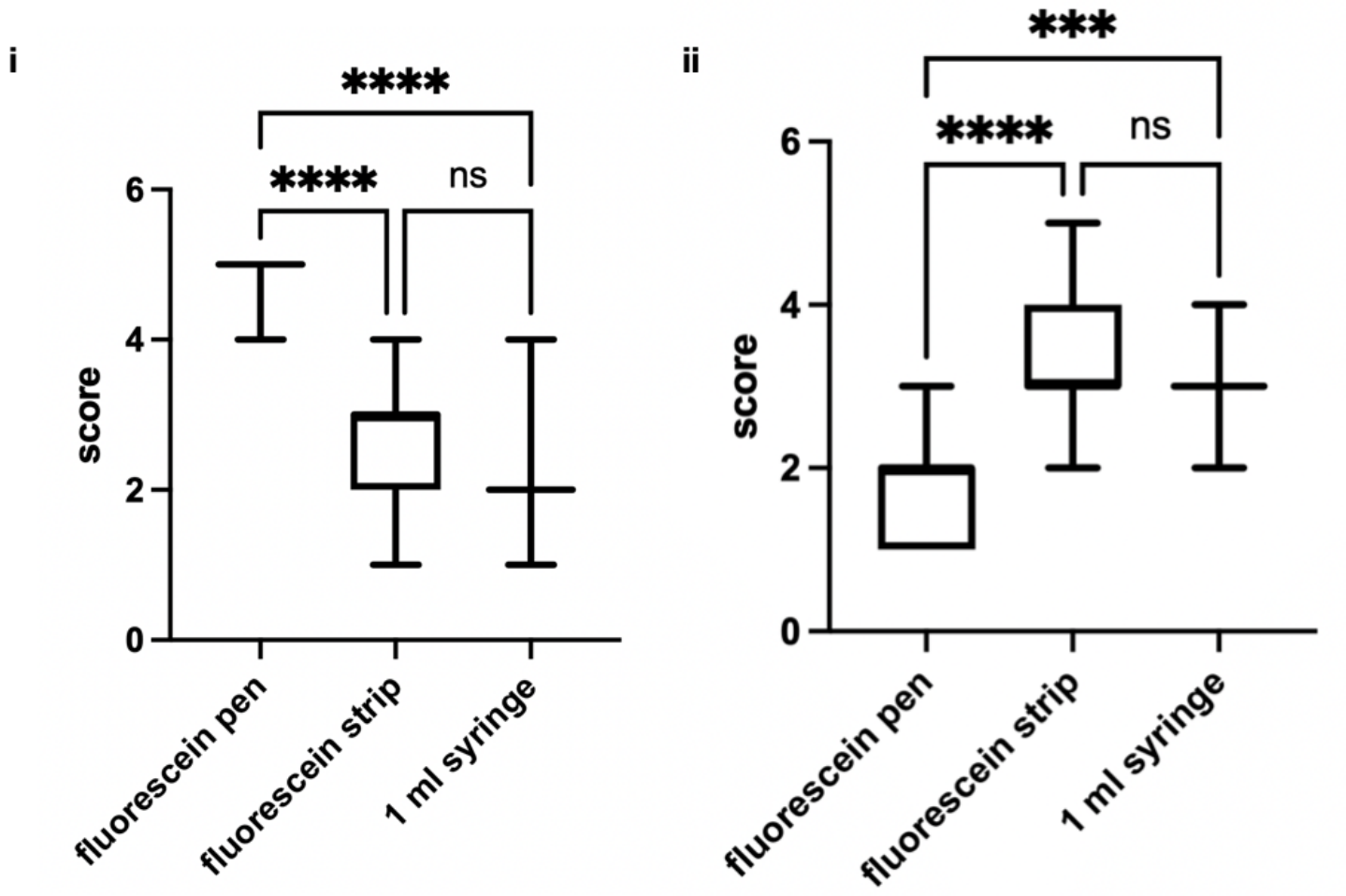

Figure 3

Ophthalmologist and patient assessments. (i) Comparison of ophthalmologists' assessments for ease of use scores with respect to the three tools. (ii) Comparing patients' evaluations of the three tools. *p<0.05, ***p<0.001, ****p<0.0001 ***p<0. 001; ns, no statistically significant differences.

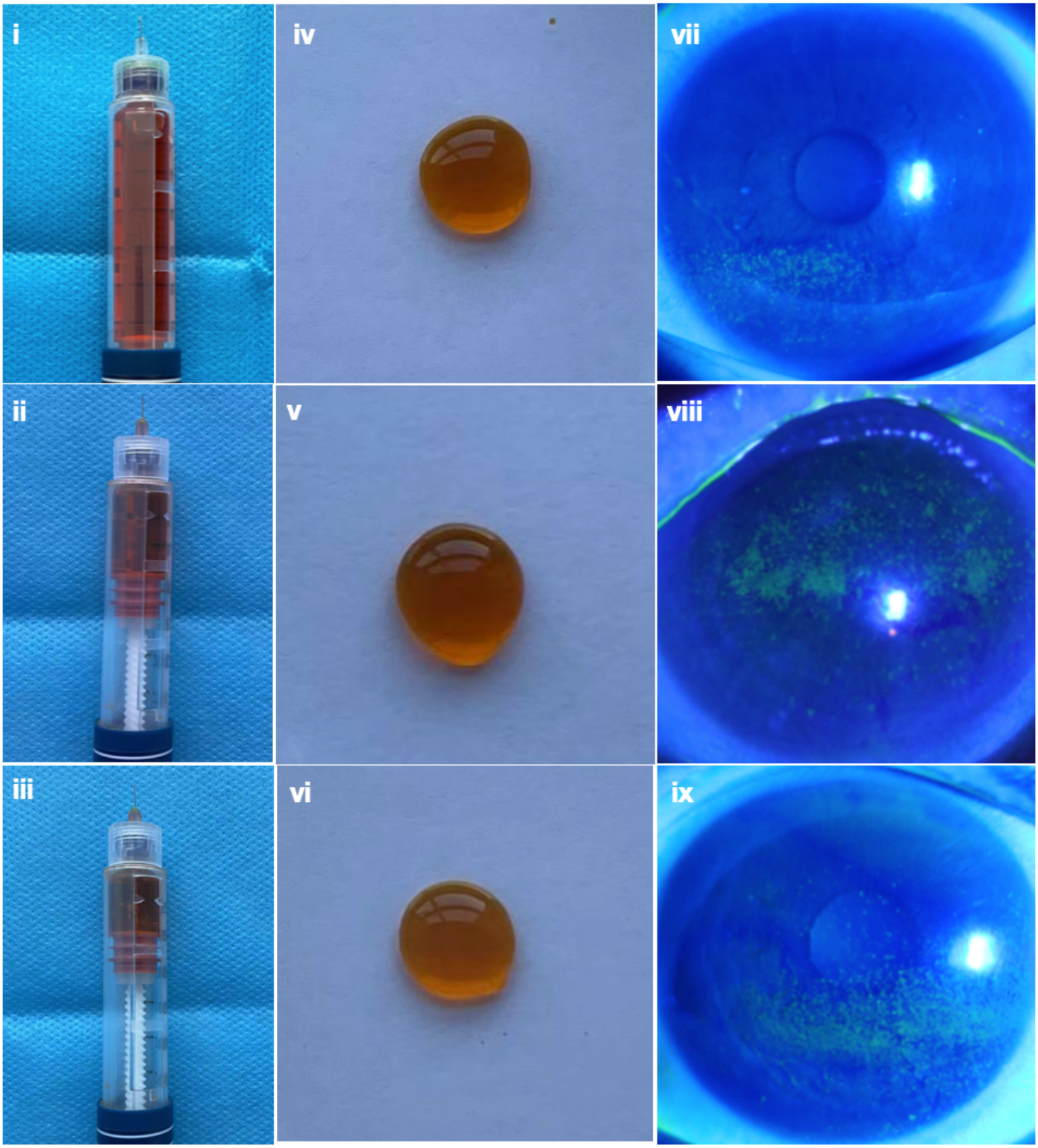

Figure 4

Differences in the fluorescein solution and fluorescence intensity at different time points.

This image depicts the same fluorescein pen at day 0, day 60 and day 90 after usage (i, ii, iii), photographed under white light illumination following administering fluorescein solution via the fluorescein pen at day 0, day 60 and day 90 (iv, v, vi). Additional images depict corneal fluorescein

staining at day 0, day 60 and day 90 (vii, viii, ix). All images were taken with the same camera in the same environment; 100 uL was poured into Petri dishes for microbial culture.