STAINING
What it is and why you need to know more

Experts define and explain solution-induced corneal staining and discuss its implications in your clinic.

Highlights from a roundtable held during the 2013 meeting of the American Academy of Optometry in Seattle

Sponsored by Alcon, a Novartis company
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David Kading, OD, FAAO, (moderator) owns two practices in Seattle. He writes, consults and lectures internationally on practice management, communications methods, ocular surface disease and specialty contact lenses.
DR. KADING: We’ve gathered three experts in the realm of contact lenses, corneal pathology and fluorescein to dig a bit deeper into what corneal staining is and what it means to our contact lens patients.

The idea of solution-induced corneal staining has changed over time. Today we have a shared idea of what corneal staining is under the slit lamp. At the same time, we’re continually evaluating how we need to change our practice habits in response to current research to enhance our patients’ contact lens-wearing experience.

Let’s begin our discussion with the person who in some ways started all of this, Gary Andrasko. He helped to publicize the idea of solution-induced corneal staining when he developed the Staining Grid in 2007.1 Dr. Andrasko, what prompted you to research staining, and where do we stand now with respect to solution-induced staining?

DR. ANDRASKO: Well, as early as 1994, one report2 showed that significant solution-induced corneal staining, or SICS, was found when a PHMB-based solution was used with a popular brand of contact lens. Although a few additional reports of SICS were published over the next few years, this phenomenon was largely ignored until silicone hydrogel lenses were introduced.

In 2002, Epstein3 published some very intriguing photos showing dense, diffuse superficial punctate keratitis when a popular PHMB-based solution was paired with PureVision contact lenses (Bausch + Lomb). The Epstein piece caught my attention and interest, and I began wondering what other lens-solution combinations might cause solution-induced staining. I began to design what eventually became known as the Staining Grid study,1 which ultimately quantified the biocompatibility of more than 100 lens and solution combinations. And we’re still learning about this phenomenon and the role of staining.

Causes of SICS

DR. KADING: Dr. Andrasko, you completed a very comprehensive work that has explained a great deal about biocompatibility. Could you tell us what you believe to be the potential causes of SICS?

DR. ANDRASKO: The mechanism is really very simple. As a contact lens is soaked overnight in a solution, the lens matrix absorbs some of the preservatives in that solution. This process is called preservative uptake. When the lens is placed on the eye, absorbed preservatives are released from the lens material into the tear film. If the concentration of released preservative is high enough, and if the preservative itself is toxic enough, the corneal epithelial cells will show staining. That is SICS.

DR. SINDT: It seems that the variable responsible for SICS is the concentration of the preservative and the length of time it’s in contact with the epithelial cells. When I read the literature and look at my patients’ corneas, it makes sense that these preservatives essentially work on the cell membranes as biocides. We want the beneficial effects of a biocide, but the preservative doesn’t discriminate between corneal epithelial cells and the pathogens that we want it to destroy.

DR. LAURENT: Dr. Laurent, could you comment on the thinking of some eye care physicians that fluorescein is simply sticking to the surface of the cell?

DR. LAURENT: That does not happen. Whoever came up with that idea didn’t look at stained corneal...
cells with a laboratory microscope. A study by Gorbet and colleagues in 2013 used confocal microscopy to show that cells stained with fluorescein have the dye in the cell cytoplasm, not adherent to the outside, as those who are promoting PATH, or preservative-associated transient hyperfluorescence, would have us believe.

A common thought used to be that these hyperfluorescent spots on the cornea represented a pooling of fluorescein in empty spaces. That's absolutely wrong, as evidenced by visualizing different concentrations of fluorescein solution. Even if you had a hole going the full 50-micron depth of the epithelium, you wouldn't see the level of contrast we see with hyperfluorescence.

**DR. KADING:** So can we agree on what it is we're seeing?

**DR. LAURENT:** We can agree that when we talk about staining, we're talking about hyperfluorescent cells that can be seen under the slit lamp. Those are the cells we're concerned about and the ones Dr. Andrasko is grading. We commonly think of that as superficial punctate keratitis, or SPK. Something is happening to the membranes of those cells. Whether the agent is mechanical or one of a variety of chemicals, the result is the same. Damaged cells take up stain, and we see hyperfluorescence from the concentration of fluorescein.

We can see the effects of epithelial cell membrane damage when the cornea is scraped, and then staining shows hyperfluorescent cells. Based on my experience with SICS and a review of the work on the subject over the last 10 to 15 years, we can presume that when hydrogen peroxide, PHMB, BAK, or some other preservative or disinfectant is on the eye, the hyperfluorescence we see is damage to the cell membrane.

**DR. ANDRASKO:** When we tested Polysquad and Aldox-based solutions, they caused minimal corneal staining with the lens material we used. Thus, staining can be minimized by recommending biocompatible contact lens-solution combinations.

**Patient Discomfort**

**DR. KADING:** It sounds like everybody agrees that when we see hyperfluorescence, something less than ideal is happening to the epithelial cells. How do patients experience the cell damage of SICS?

**DR. ANDRASKO:** As practitioners, we're always concerned with patient comfort, and certainly, SICS has some implications for comfort.

In our study, we found that patients are able to tolerate mild amounts of SICS with little or no symptoms, but as staining becomes moderate to severe, lens-wearing comfort could be reduced. A study presented at AAO in 2012 by CCLR, School of Optometry and Vision Science, University of Waterloo, also found SICS to be associated with the specific symptoms of stinging and burning.

Patients with chronic SICS often drop out of contact lens wear because of persistent discomfort as well as the hyperemia that may accompany it. They don't realize that we may be able to alleviate their discomfort by simply recommending the most biocompatible contact lens-solution combinations.

**DR. KADING:** Dr. Sindt, you've been doing work in this area. What does corneal staining mean to your contact lens patients?

**DR. SINDT:** When people started talking about this idea of corneal staining about 14 years ago, I began looking at it for the first time. But it doesn't necessarily show up when you put a lens on the eye in your practice. It doesn't always happen the way we used to think about incompatibility with contact lens solutions, and this is something different.

Looking at immune cell migration, there's definitely a greater response with PHMB-based products than with non-PHMB-based MPS products. The hypothesis is that this chemical is driving an immune response. In other words, the tissue sees the chemical as foreign, so
dendritic cells migrate into the cornea either in direct response to the antigen, or perhaps, a greater inflammatory situation, such as inflammatory molecules, cytokines or something else in the cornea.

Having said that, a contact lens itself drives an immune response on the cornea. So just by placing the contact lens on the cornea, you double the number of resident immune cells compared to a non-contact lens wearer. But remember that there are twice as many immune cells in the cornea of PHMB users than of non-PHMB MPS users. What does this mean? We have to keep studying it to find out.

The Time Factor

**DR. KADING:** If SICS occurs over time, it may be difficult for clinicians to detect because we’re not seeing our patients in the right timeframe. Dr. Andrasko, was that rationale part of your study of SICS?

**DR. ANDRASKO:** In the Staining Grid study, we examined patients at three different time points. We performed a baseline exam before we put a lens on, to make sure there was no staining. Patients refrained from wearing their lenses on the day of the testing, so they came in with clear, clean corneas.

We then inserted lenses that had been soaked in the solution we were testing on that day. Patients wore those lenses for 2 hours, at which point we took the lenses out and examined them again. After that examination, the lenses went back on for another 2 hours, so we had baseline, 2-hour and 4-hour examinations.1

The rationale for the schedule was based on research by Garofalo and colleagues12 that showed solution-induced staining typically was present in the early part of the wearing period. It’s not the type of staining you see at the end of the day, but staining that shows up early because it’s related to toxicity from the preservative that’s released soon after insertion. You can see this type of staining within the first few hours of lens wear, and that’s when the discomfort also tends to appear. By consulting the Staining Grid, practitioners can get an idea if their patient’s individual lens-solution combination is one which has been shown to cause SICS.

**DR. KADING:** You’re alluding to the fact that we don’t always see our patients early in the wearing period or at the time of maximum staining.

**DR. SINDT:** Right. We see them whenever it’s convenient for them to come back into our practice. And some practitioners don’t see patients for follow-up visits after contact lens fittings at all.

But even a doctor who performs regular follow-up visits may not see the problem. Many practitioners don’t use staining during the follow up.

**DR. LAURENT:** There’s also some variability on the exam day that can affect staining. I tell patients to keep their lenses in for at least an hour before their exam, but they might have had them in for 30 minutes or 3 hours or 8 hours. So, I might see the state of the epithelium after a full day of wear, or I might see it after a fraction of the time.

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— Gary Andrasko, OD, MS

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**Understanding Cell Damage**

**DR. KADING:** What is meant when we say ‘taking up stain’? What’s wrong with those cells? Are they dead? Damaged?

**DR. LAURENT:** The epithelium is arranged with cells on the outermost surface that are dying cells and will be shed in a matter of hours. They don’t take up fluorescein. Below that is a layer of partially exposed cells that can become hyperfluorescent with staining. Under a microscope, I can see these hyperfluorescent cells overlapped by unstained dying cells.

**DR. KADING:** With regard to solution, why are those vital cells prone to take up stain?

**DR. LAURENT:** We put a solution on the lens that’s
meant to kill microorganisms, and then the lens in turn
soaks the front of the eye with this disinfectant. Thus, it
can be concluded that the solution damages the exposed
living cell membranes on the front of the eye.5-8

DR. KADING: So, the reason we don't see hyper-
fluorescence on the entire surface is because some of
the vital cells are covered by dying cells that don't take
up the solution and, as such, don't stain.

DR. LAURENT: Probably. These dying surface cells
do not take up stain and they do cover the cells below
them. If those cells below are completely covered,
I think they're shielded from chemical insult and also
from fluorescein. However, if a cell is only partially
covered by a dying surface cell and is also partially
exposed to the surface, I think it can be compromised by
a chemical agent and become hyperfluorescent. With
the lab microscope, I've seen a hyperfluorescent cell
partially covered by a dying cell and the entire cell was
visible because the dying cell was mostly transparent. In
the case of exposure to a chemical such as BAK or
PHMB, I'm not sure that every exposed cell that isn't
already dying will be damaged and become hyperfluo-
rescent. There may be some cells that are simply more
resistant to injury than others and they may not suffer
membrane damage or become hyperfluorescent.

We did the original paper on hyperfluorescent cells
in 1995,6 and found that certain cells have the optimum
concentration of fluorescein in the cell cytoplasm to
hyperfluoresce. But we didn't know why some cells took
up enough stain to make them hyperfluorescent and
others did not. In 2011, a group in Australia did some
really fabulous work to determine the process cells
undergo that makes them hyperfluoresce.8

DR. ANDRASKO: The Australian authors found
that the hyperfluorescent cells are neither healthy cells
nor dead cells, but rather the cells that are injured. Is
that correct?

DR. LAURENT: Yes. Healthy cells do take up stain.
If you put fluorescein on a perfectly healthy cornea,
you'll see fluorescein in the anterior chamber of the
eye because it permeates those cells.5 But they won't be
hyperfluorescent. You won't see them as bright spots
under the slit lamp.5

DR. KADING: Without corneal staining, can you
see SICS-damaged, apoptotic cells?

DR. LAURENT: We've all seen corneas that have a
granular appearance on the slit lamp, and then we see a
concentration of stain where those granules were, but I
haven't looked at this with a laboratory microscope.

DR. ANDRASKO: While conducting the Staining
Grid study, I sometimes scanned a white light across
the cornea before I instilled the fluorescein, and I saw
this grayish granular group of spots. The pattern of
moderate to severe fluorescein staining was exactly
where those grayish areas were located.

Cell Shedding

DR. KADING: So we've got dying cells that don't take
up stain, healthy cells that take up stain but don't
appear hyperfluorescent, and SICS. Is toxicity affecting
the epithelial cell life cycle?

DR. LAURENT: As you know, every hour we lose
hundreds of cells, with some coming off with each blink
just like we shed skin cells. In our lab, we refer to these
shedding cells as terminally differentiated. They've got
polygonal shapes and they average 30-some microns
across, with some as large as 70 microns. Mostly, these
cells come off in what looks like complete cells.

Some other cells are undergoing a different mecha-
nism of cell death called programmed cell death, or
apoptosis. Instead of becoming terminally differentiated,
they shrink and give off blebs. The Australian group
found that cells that hyperfluoresce are in the early stages
of apoptosis.7 Based on work I've done in clinic looking
at cultured cells with hydrogen peroxide, BAK, or
PHMB, I see that all of the hyperfluorescent cells are
circular. They’ve got blebs, and they’re undergoing apoptosis. It’s striking physical evidence that the Australians findings are correct.

**DR. ANDRASKO:** When Gorbet and colleagues evaluated the effect of SICS on corneal epithelial cells, they found a statistically significant increase in epithelial cell shedding, defined as “removal of cells no longer useful,” after SICS. By using a series of dyes, the researchers found that the cells shed due to SICS were often still alive, indicating they were probably healthy, viable cells prior to SICS. This study adds to the growing evidence that SICS represents true corneal staining likely caused by a toxic response of the corneal epithelial cell to preservatives found in different multipurpose solutions.

**DR. LAURENT:** The transient nature of SICS has been well known for a while, but there hasn’t been a good explanation for it. The work by Gorbet and colleagues would indicate that cell shedding is the explanation. It appears that SICS is visible evidence of premature cell death that results in these cells being shed from the corneal surface. Like most research, this study makes us realize that there are additional questions to answer before we have a complete picture of corneal fluorescein staining.

**DR. SINDT:** I perform a good deal of confocal work, so I’m looking at all the layers of the epithelial cells with the ideas we’ve discussed in mind. Cells at the surface don’t pick up stain, while the second layer hyperfluoresces due to increased apoptosis. So, does SICS increase sloughing of that vital layer off the cornea, not just the dying surface layer? Does SICS increase turnover of the cornea?

**DR. LAURENT:** Yes, I would say that it probably does increase turnover.

**DR. ANDRASKO:** And it’s not just SICS that causes apoptosis, staining and turnover. When we see corneal staining, no matter what the cause, the effect is the same.

**DR. LAURENT:** We’ve evaluated chemical versus mechanical epithelial cell damage, and there is no difference in the staining. A scratch causes a bright line of stain, and if you look at it under a laboratory microscope, you’ll see the cells that line the injury are taking up stain. Their membranes have been disrupted, and they’re hyperfluorescing. Just like a physical injury, the wrong lens-solution combination causes cell damage that can be revealed by hyperfluorescence. The damaged cells will die and be shed prematurely.

**DR. KADING:** In conclusion, we need to consider SICS as a reality. But it’s important to note that SICS can be minimized simply by selecting a biocompatible solution-lens combination.

**References**


