SHINING THE BRIGHTEST LIGHT ON SENSITIVE TEETH.

Ever wondered the connection between sensitive teeth and Roman artifacts buried in the family villa of Julius Caesar? The answer is the size of eight soccer fields[1] and generates light that's more than a billion times brighter than the sun.[2] Welcome to synchrotron science, where remarkable imaging technology is shining a powerful light on sensitive teeth – and providing deeper insight into how desensitizing products work. Here's how.

> **I think it gives me the confidence that: if I understand it then I can, in good faith, prescribe it.**
>
> Dr. Liz Mitrani

In 2015, scientists used one of the biggest and brightest x-ray facilities in the world to uncover writing on ancient scrolls buried and carbonized by the Vesuvius eruption in 79 AD.[3] The Herculaneum scrolls, excavated in 1752 from a villa linked to Julius Caesar, were charred, fragile and impossible to read. Now – thanks to high-energy X-ray imaging in a gigantic machine called a synchrotron[4] – entire texts from the so-called 'invisible library'[5] are on the verge of being seen by a modern audience. The invisible is becoming visible.

Today, that same technology is being used to advance our understanding of sensitive teeth – with similarly illuminating results. Synchrotron light – one hundred billion times brighter than hospital X-rays[6] – is, for the first time, allowing scientists to look deep inside teeth to see how toothpaste formulations affect dentin occlusion over time. We believe that it is ground-breaking science. And it's driving a step change in toothpaste understanding.

THE PAIN POINT

Dentin Hypersensitivity (DH) is a global condition. As much as a third of the adult population suffers from it – but only around half of them actively address it.[7]

These numbers are all the more surprising when you consider something as simple as our choice of toothpaste can help relieve the pain.[8] Now, thanks to pioneering new research, we're beginning to see how in even greater detail.

Research into DH has been ongoing for over a century, with much focused on the hydrodynamic theory, which states that fluid moving through dentinal tubules is a primary cause of sensitivity.[9,10] Studies have explored how toothpaste formulations can occlude dentin tubules and block the pathways through the dentin. This can reduce fluid flow and prevents tooth nerves from firing.[8] But seeing is believing.

In recent years, conventional imaging techniques have helped us show the depth and durability of dentin occlusion on brushed teeth.[11] But, until now, we've been unable to visualize the impact our toothpaste formulations have on occlusion over a period of time in such spatial detail. It's a formidable task – after all, there can be thousands of tubules in a tooth. Monitoring the effect of a toothpaste on such a complex microstructure benefits from a whole new approach – and cutting-edge technology.

We've been working on Sensodyne Repair & Protect for about a decade now, and wanted to visualize the mode of action of a new, re-engineered formulation we've been developing for four years

Step forward GSK Consumer Healthcare, where our determination to advance the science of sensitivity continues to drive improvements in our Sensodyne formulations. That determination has led us to the European Synchrotron Radiation Facility (ESRF) in Grenoble, France – one of the biggest synchrotrons in the world – to push the boundaries of what's possible in understanding toothpaste technology.

Dr Christabel Fowler, Innovation Lead, Oral Health R&D, GSK Consumer Healthcare, explains why:

"If people can actually see and understand how the products work, it's easier for them to trust the science and in turn, believe it can help with their issues. So if we can better visualize how our toothpastes work, it's also easier for dental professionals to understand the science behind them and this helps patients choose a paste that's going to really help them get that relief from the pain of sensitivity."[2]

We know from our expert partners that understanding a toothpaste's mode of action can enhance the patient consultation. New York-based dentist, Dr Liz Mitrani, says: "So when I can tell them, listen "this is how it works" and they can visualize it [...] they think about it, and then they're more likely to be compliant."[12]

That's a big prize. And it's what motivated us to go to ESRF – to help dentists see that science in microscopic detail.

SYNCHROTRON SCIENCE

ESRF is a beacon for landmark science, with synchrotron light powering some of the world's most ground-breaking discoveries. For example, in Nobel Prize-winning work, scientists used ESRF to unravel the structure of the ribosome.[13] ESRF is also playing an important role in the development of new drugs and therapeutic strategies against COVID-19.[14]

In fact, the exceptional properties of synchrotron X-rays are helping unlock the secrets of everything from viruses and vital organs, to batteries and Herculaneum scrolls.[6,13] Now, in scientific research that's never been done before, ESRF is shining its light on teeth – allowing us to see what happens inside tubules in 3D, over time, following use with Sensodyne Repair & Protect.

HOW LONG IN THE TOOTH?

The science of DH has been advanced by conventional imaging techniques that have shown the effect of toothpaste on small samples of tubules. However, though techniques like Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) provide ultra-high resolution, they have a small field of view. Synchrotrons can examine much larger areas in 3D. In microscopic detail. And at high speed.

Where FIB-SEM studies can only analyze 100-200 tubules at a time, synchrotrons can scan thousands in a few minutes with a single scan.[11] This gives a more representative view of what's happening in a tooth. We can see how far occlusion has travelled, and how long it stays there over a period of time.

Similarly, most conventional techniques are destructive: scientists have to slice into samples to see inside, meaning every scan requires a different sample. Synchrotron imaging is non-destructive, enabling 'time lapse' studies that scan the same tubules, over and again, to show a toothpaste's effect on occlusion at different time points.[11]

3D

We've been working on Sensodyne Repair & Protect for about a decade now, and wanted to visualize the mode of action of a re-engineered formulation we've been developing for four years: Sensodyne Repair & Protect. The formulation contains stannous fluoride, which helps to build a reparative layer*† over and within dentin tubules.[15,16] The reparative*† layer formed by Sensodyne Repair & Protect is resistant to dietary acidic challenges and is maintained after repeated acidic challenge.[17]

Sensodyne Repair & Protect has a clinically proven ingredient for relief of – and lasting protection against – dentin hypersensitivity.*[18,19]

But we wanted to drill deeper and see in even greater detail how it worked on dentin over a period of time. According to Dr Kamel Madi, co-founder of

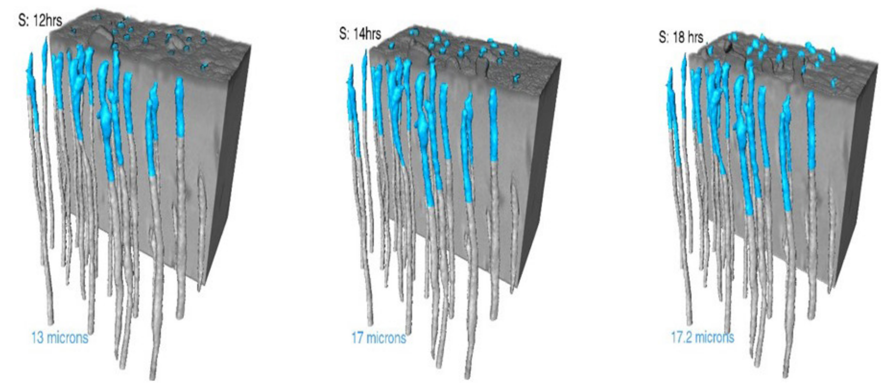

Figure 1. Visualization of average occlusion depth (um) in dentin specimens treated with Stannous Fluoride (S) based toothpaste formulation of Sensodyne Repair & Protect (containing 0.454% Stannous Fluoride).[20]

3Dmagination – who led the subsequent time lapse study at ESRF – a synchrotron was the only place to go:

"We can also detect if a tubule that was blocked at, for example, two hours after the treatment becomes unblocked after four hours. So, we call these the 'possibility of reopening'. And that is very useful because you can compare, for example, the old version versus the new version."[21]

The time lapse study, which Dr Madi says is 'like taking a 3D movie', used phase contrast tomography to visualize the complex features of dentin. "Once we achieved that, we ran a large scale campaign where the samples were treated with different formulations. Some of them were the new formulation of Repair & Protect. And then what we did was we used this technique call X ray tomography. [...] You play the sample in a sample stage and the sample is going to basically rotate around 360 degrees. After each micro rotation, say for example 0.1 degrees, you're going to collect a scan. [...] So this is the raw data that we collect. Once we have this raw data, we have super software that can turn this information- so all of these projections- into a 3D model."[21] (Figure 1)

THE INVISIBLE, VISIBLE

The study showed the new formulation gets deep inside*†‡ the microstructure of tubules and builds the repairing layer over the dentin surface for lasting sensitivity protection*. We turned, once again, to conventional imaging to validate the X-ray analysis. The same samples used at ESRF were taken to the Cavendish Microscopy Suite at Cambridge University for FIB-SEM imaging and analysis.

FIB-SEM provides higher resolution imaging that helps understand what's happening within the tubules. Dr Richard Langford, Head of the Cavendish Microscopy Suite, explains:

"So we use the focused ion beam system to make these very thin lamellas- these thin slices of the material- that we could put into another electron microscope called transmission electron microscope, where we could do high resolution and mapping and imaging of the cross sections- i.e of these thin lamellas, thin slices- which enabled us to look at the chemical and structural composition of the material that's occluding the tubules."[11]

"We could see that the new formulation resulted in the occlusion of the tubules. We could certainly identify that the new formulation resulted in more fluorine being incorporated into the formed material."[11]

BIG SCIENCE, FOR TINY MOMENTS

Dr Mitrani thinks the scientific advances coming out of the studies can only improve oral health. "I think it's very exciting to learn more of the science, and get down to the nitty gritty in the data and understanding a little bit more of the mechanism, on a very microscopic level, because that's our foundation. And once we understand the foundation and all the information, we can build on that, understand more, and then be more confident prescribers of the product."[12]

SENSODYNE

Longer term, the synchrotron study could drive step change in toothpaste engineering. Dr Madi says: "We have a tool now to really investigate, deeply in detail, different formulations, comparing the between each other, looking inside and not just at the surface, and this is in a non destructive way. So I would say it's going to revolutionize the way we look at tubule occlusion."[21]

The science of sensitivity will continue to advance, but the story so far is exciting for patients. Because it took four years – and the brightest light imaginable – to test it in the lab and show how deep Sensodyne Repair & Protect goes. But it only takes a split second – and a sip of ice cold lemonade – to prove that it works in real life.

Big science, for the small, special moments. **Because life's too short for sensitivity.**

8

The surface area of ESRF can fit about 8 soccer fields[1]

844

Electrons travel round a circular tunnel that has a circumference of 844m[6]

44

There are 44 beamlines[6]

9000 scientists from around the world visit ESRF every year to conduct experiments[6]

6

6 ESRF users have won Nobel Prizes[22]

100

The synchrotron at ESRF generates light **100 billion** times brighter than medical X-rays[6]

* with twice daily brushing

† Sensodyne Repair & Protect is intended for relief of occasional dentin hypersensitivity that occurs when sensitive teeth are exposed to hot or cold substances. It contains stannous fluoride, a well-established, effective tooth desensitizer with remineralization properties. There is general scientific consensus that occluding exposed dentin effectively relieves the occasional pain of dentin hypersensitivity.

‡ as shown in an *in vitro* study

Source: ESRF (https://www.esrf.eu)

SENSODYNE

REFERENCES:

1. Mobilio, S et al (2015) Synchrotron Radiation, Springer-Verlag Berlin Heidelberg, Ch.1, p7.https://www.springer.com/gp/book/9783642553141
2. Dr. Christabel Fowler, interview July 28, 2020.
3. Mocella, V., Brun, E., Ferrero, C. et al. (2015) Revealing letters in rolled Herculaneum papyri by X-ray phase-contrast imaging. Nat Commun 6:5895.
4. David, N. (2019), Ancient scrolls charred by Vesuvius could be read once again, Guardian News & Media Ltd. Sept 9, 2020. https://www.theguardian. com/ science/2019/oct/03/ancient-scrolls-charred-by-vesuvius-could-be-read-once-again
5. Diamond Light Source (2019), 2,00 year-old Herculaneum Scrolls from Institut de France being studied using UK's Synchrotron, Diamon Light Source. Oct, 3, 2019. https://www.diamond.ac.uk/Home/News/LatestNews/2019/03-10-2019.html
6. ESRF (2020) What's ESRF?, https://www.esrf.fr/home/education/what-is-the-esrf.html (Last accessed June 2021).
7. Addy, M. (2002), Dentine hypersensitivity: New perspectives on an old problem. International Dental Journal, 52: 367-375. doi:10.1002/j.1875- 595X.2002.tb00936.x
8. JADA vol 134, December 2003 . Sensitive teeth causes and treatments
9. D. G. Gillam, Management of dentin hypersensitivity, Current Oral Health Reports, vol. 2, no. 2, pp. 87–94, 2015.
10. M. Brännström, "A hydrodynamic mechanism in the trans- mission of pain producing stimuli through the dentine," in Sensory Mechanisms in Dentine, D. J. Anderson, Ed., pp. 73–79, Pergamon Press, Oxford, UK, 1963.
11. Richard Langford, interview, July 29, 2020.
12. Dr. Elizabeth Mitrani, interview, 13 August 2020.
13. Ramakrishnan, V., Alsari, M. (2019), The Ribosome Under Synchrotron Light, Scientific Protocols.
14. ESRF (2020) World X-ray science facilities are contributing to overcoming COVID-19, ESRF, Sept 9, 2020, https://www.esrf.eu/home/news/general/content-news/general/world-x-ray-science-facilities-are-contributing-to-overcoming-covid-19.html
15. Earl JS and Langford RM. Am J Dent 2013; 26 (Spec Issue): 19A-24A
16. Burnett G. Am J Dent 2013; 26 (Spec Issue): 15A-18A
17. A proof of technology study investigation dentin tubule occlusion of toothpastes in a controlled in situ model. 2009
18. Parkinson C et al. Am J Dent 2013; 26 (Spec Issue): 25A-31A
19. Parkinson C et al; "Confirmation of efficacy in providing relief from the pain of dentin hypersensitivity of an anhydrous dentifrice containing 0.454% with or without stannous fluoride in an 8-week randomized clinical trial"; American J Dent; 2015; 28 (4): 190-196
20. *In vitro* report number G7322/014; Occlusion depth measurements and visualisation of Sensodyne Repair & Protect with Deep Repair using Synchrotron X-ray Tomography and Focused Ion-Beam Scanning Electron Microscopy; 2020
21. Kamel Madi, interview, July 31, 2020.
22. ESRF history ESRF (2020), https://www.esrf.fr/home/about/History.html (Last accessed June 2021).