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CASE STUDY

MEASURING TOOTH DEMINERALISATION AND REMINERALISATION IN VITRO

In vitro oral care product testing

A key aspect of fluoride toothpastes is their ability to remineralise acid-damaged enamel. To substantiate claims and provide evidence that some products are more effective than others, in vitro studies can be used to quantify the amount of remineralisation of human or bovine enamel in the laboratory.

Demineralisation & remineralisation

The process of demineralisation and remineralisation has been studied extensively, in particular to support the development of oral care products such as toothpastes which have potential for repair of acid-damaged tooth enamel. Conventionally, fluoride has been formulated into a wide variety of dentifrices.

During the remineralisation process, it can become incorporated into the enamel crystalline matrix in the form of fluorapatite. Fluorapatite is harder than hydroxyapatite and is itself more resistant to further rounds of demineralisation.

More recently, products containing nano-hydroxyapatite (n-HAP), a biocompatible and bioactive material, have been considered as an alternative to fluoride and laboratory studies have also demonstrated the potential for this new material to repair dental enamel.

$36.98Bn
Forecasted Global Market

REF: Global Industry Analysts, Inc, Market Research report, April 2018

The toothpaste market was valued at $26.09 billion in 2018, and projected to reach $36.98 billion by 2024 with whitening, senstivity and reminearlisation toothpastes playing a key role in the forcasted growth.

To substantiate claims and provide evidence of efficacy versus fluoride, carefully designed in vitro studies can be used to evaluate the effects of different n-HAP dentifrices on the remineralisation of enamel.

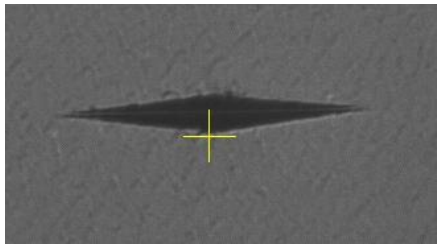
In vitro measurement

Given that the hardness of enamel is governed by the extent of demineralisation / remineralisation of the structure, we can effectively measure the condition of tooth enamel by measuring its surface microhardness. Enamel that has had mineral ions extracted from the hydroxyapatite matrix is effectively softer than the parent material.

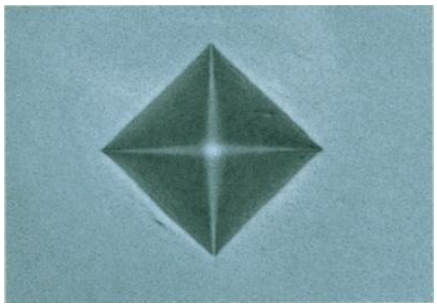
To measure surface microhardness, we place an indent into the surface of the enamel using a diamond tip indenter. The dimensions of the indent are related to how hard or soft it is; softer enamel allows to diamond to penetrate more deeply leading to larger indents.

In hard enamel we see a small indent, perhaps 40-50 microns in length dependent on the force applied and the duration of the indenting process. Typical measurement parameters for a process of this type would be a loading of 50g for 10 seconds. If the same enamel block is then exposed to acid for several minutes, the size of subsequent indents can increase significantly.

There are several different types of indenter available for these studies. In Figure 1, you can see an example of a Knoop indent on the left and a Vickers indent on the right. Either works well for tooth enamel though there is a slight preference for Knoop when the samples are perfectly flat.



a) Knoop indent



b) Vickers Indent

Figure 1 Indents into the surface of the enamel using a diamond tip indenter

MEASURING TOOTH DEMINERALISATION AND REMINERALISATION *IN VITRO*

Modeling study

Using bovine or human enamel blocks we can model the demineralisation / remineralisation process in the Laboratory using a pH-cycling process involving several rounds of acid exposure and then repair. The blocks are typically around 5mm square, 1-2mm thick, polished to a 3 micron finish (Figure 2).

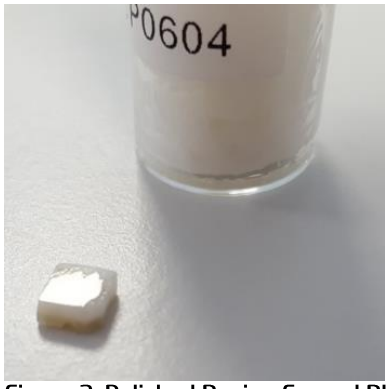


Figure 2: Polished Bovine Enamel Block

At least 5 indents are placed in the surface of the enamel and the average length is measured. In this particular example sound enamel has a mean indent length of 45 microns. This is then followed by immersion in grapefruit juice for approximately 30 minutes, after which the mean indent length increases to around 58 microns.

Then, after a single exposure to toothpaste and saliva slurry, in this case either a fluoride paste (product A) or a non-fluoride paste (product B), the enamel is re-hardened and following measurement we see a corresponding decrease in indent length (Figure 3).

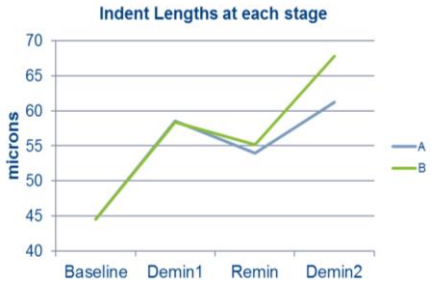


Figure 3: Indent Lengths at each stage where A=Fluoride Paste, B=non-Fluoride paste

Conclusion

In this case study, a small but significant benefit for remineralisation by the fluoride product over the non-fluoride product is measured and this difference is magnified when the blocks are subjected to a second acid challenge or demineralisation. The fluoride-repaired enamel is much more resistant second time round leading to a 15 micron difference in mean indent length.

Supporting your product development and claim substantiation

The design of such in vitro remin-demin studies can vary enormously and can be engineered to mimic several rounds of acid exposure and repair. Such studies along with clinical in situ variations can lead to compelling enamel repair and re-hardening, stronger teeth and enamel protection claims.

Such tests are normally used for demonstrating the relative efficacy of fluoride-containing salts such as NaF and SMFP but can also be used to assess the effect of novel delivery systems or the effect of different formulation ingredients on the ability of fluoride to repair enamel.

They can also be used to evaluate the ability of novel hydroxyapatite actives to reharden enamel. A well-designed in vitro test can often yield the required data much more quickly and at a lower cost in comparison to clinical studies and can deliver robust data to help you meet development milestones and substantiate claims.

Total Quality Assurance

Our Laboratory team can deliver Total Quality Assurance for our customers and have been a trusted partner for those manufacturing oral care products for many years. Intertek is a leading service provider in the oral care sector offering in vitro method development , bespoke models and rigorous screening of oral care products, supporting safety, product development, regulatory compliance and advertising claim support.

Meet Our Expert: Peter Hall

Peter is the Laboratory Director at Intertek Clinical Research Services. He has 30 years of industry experience in Colloid, Formulation, Materials, Analytical and Clinical Sciences. With experience of R&D Oral Care Project Management and expertise in oral Instrumentation and experimental design, he currently heads up Intertek's team of scientists delivering a variety of in vitro methodologies for product evaluation and claim support, including enamel remineralisation, stain prevention/removal and chemical whitening.

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