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Reminova and EAER: Keeping Enamel Whole through Caries Remineralization

N.B. Pitts1,2 and J.P. Wright2

Abstract
This article aims to outline the early development of a King’s College London dental spinout company, Reminova, formed to commercialize a novel clinical method of caries remineralization: electrically accelerated and enhanced remineralization (EAER). This method is being developed to address the unmet clinical need identified by modern caries management strategies to keep enamel “whole” through remineralization of clinical caries as a form of nonoperative caries treatment for initial-stage and moderate lesions. A progressive movement within dentistry is shifting away from the restorative-only model, which, it is suggested, has failed. The high prevalence of initial-stage caries across populations provides a significant opportunity to prevent restorations and reduce repeat restorations over a patient’s lifetime. Reminova has set out to provide a method to repair lesions without drilling, filling, pain, or injections. The article outlines the rationale for and the chronological stages of the technology and company development. It then outlines corroborative evidence to show that EAER treatment can, in this preliminary in vitro investigation, remineralize clinically significant caries throughout the depth of the lesion as measured by Knoop microhardness and corroborated by scanning electron microscopy. Furthermore, the presented data show that EAER-treated enamel is harder than the healthy enamel measured nearby in each sample and is very similar in appearance to healthy enamel from the subjective interpretation made possible by scanning electron microscopy imagery. The data presented also show that this more “complete” remineralization to a high hardness level has been achieved with 2 remineralizing agents via in vitro human tooth samples. The broad clinical potential of this new treatment methodology seems to be very encouraging from these results. Reminova will strive to continue its mission, to ensure that, in the future, dental teams will not need to drill holes for the treatment of initial-stage and moderate caries lesions.

Keywords: dentistry, dental caries, dental white spots, secondary prevention, therapeutics, iontophoresis

Introduction
The first ICNARA (International Conference on Novel Anticaries and Remineralizing Agents), held in 2008, considered “what is known and what is the future” (Pitts and Wefel 2009), and although it is gratifying to see the progress made since then, advances at the clinical level are still frustratingly slow. The need for continuing collaboration for improving dentistry and health across a broad range of stakeholders, discussed and endorsed at the conference, is still required to make further progress. The stakeholders involved in driving progress include company-academic partnerships. In 2008, there was “broad agreement” that in the future “the aim of remineralization therapy is to facilitate caries control over a lifetime using evidence-based, clinically effective, multifactorial prevention to keep the caries process in balance” and that “over the coming years, the dental research community in this field should continue to apply new knowledge and methods from outside dentistry” (Pitts and Wefel 2009). Since that time, we have had the privilege to work with a range of stakeholders and with King’s College London to form a university spinout company to advance aspects of these aspirations. For transparency, it should be clear that the invitation to outline these developments in 2017 at ICNARA 3 was made to this new company, Reminova Ltd. (the authors relationships with the company are disclosed in the Acknowledgments). This article outlines the development of the company, which was formed to commercialize a novel method of caries remineralization—electrically accelerated and enhanced remineralization (EAER). This is being developed to address the unmet clinical need identified by modern caries management strategies to keep enamel “whole” through remineralization of clinical caries as a form of nonoperative caries treatment for initial-stage and moderate lesions. The article outlines the rationale and chronological stages of the technology development. It describes initial unpublished examples of the progress made to date with the development of EAER in the journey to ensure that, in the future, dental teams will not need to continue to drill holes for the treatment of initial-stage and moderate lesions.

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A supplemental appendix to this article is available online.

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How to Keep Enamel Whole: The Unmet Clinical Need Linked to Modern Caries Management

This section sets out the rationale for developing Reminova and its future products. Dental caries continues to be the most prevalent, costly, preventable, and silent global epidemic in humankind. Ninety-eight percent of the world’s population will have caries across the life course (Petersen 2008), while 35% of all ages combined (2.4 billion individuals globally) were estimated in the Global Burden of Disease Study to have untreated cavities in permanent teeth (Marcenes et al. 2013). Some $350 billion is spent annually treating caries in terms of treatment costs and the resultant losses to the global economy (Listl et al. 2015), and approximately 30% of people do not regularly visit the dentist (Oral Health Foundation 2017), mostly because traditional approach to caries prevention and management is not working for many patients or for society.

When considering the prevalence of disease and the distribution of different stages and sizes of caries lesions at a population level, we are hampered by the historical World Health Organization convention, in traditional epidemiologic surveys, to score only established cavitated caries in dentine (Pitts 1994). However, the inclusion of initial-stage lesions in addition to moderate and advanced stages is becoming more common with the use of criteria such as the International Caries Detection and Assessment System (ICDAS; Ismail et al. 2007). Data on 15-y-old children from the 2013 National Child Dental Health Survey of England, Wales, and Northern Ireland underline the fact that the prevalence of initial-stage caries across populations is high (Pitts et al. 2015; Pitts et al. 2017), thereby providing a significant opportunity for secondary prevention. The percentage of children classified as having caries was 11% with inclusion of only ICDAS 5 and 6 lesions (equivalent to World Health Organization criteria), but when moderate dentine lesions (ICDAS 4) were included, this rose to 21% and with cavitated enamel lesions (ICDAS 3), to 25%. However, the percentage of children with caries dramatically increased to 52% when noncavitated enamel lesions (ICDAS 1 and 2) were also included (Pitts et al. 2017). This proportion would increase still further if radiographic information were also available (Agustsdottir et al. 2010).

There is an increasing international consensus on preservative caries management. This comes from a better understanding of the disease and the caries process, a holistic view of caries risk management, an improved focus on early detection of lesions, more unified caries classification systems that include the detection of lesions in enamel, the continuing promotion of “prevention” (primary and secondary), and a higher awareness of the limitations of restorations with the need to continue to replace them (Pitts and Zero 2016). The ICCMS—International Caries Classification and Management System (Pitts et al. 2014)—and its “4D” approach provides a framework that integrates determining patient-level risk assessment, detecting and assessing caries staging and activity, deciding a personalized care plan, and doing appropriate tooth-preserving and patient-level caries prevention and control (Pitts et al. 2017). In such systems, providing nonoperative care for initial- and moderate-stage clinical caries represents a significant opportunity to prevent restorations and repeat restorations and to improve patient experience and well-being. Therefore, there is now a sharper focus on more minimally interventive treatments for caries and on more patient-centered approaches to reducing anxiety and fear of the dentist. Dentistry needs new treatments that fit these approaches if it is to successfully move away from the restorative-only model, which fails economically and clinically and for patients (Pitts and Zero 2016).

However, despite the increasing consensus on addressing this need for caries prevention and control through nonsurgical care, in reality there has been a frustrating lack of progress since the 1970s. Remineralization has been well described in vivo (Koulourides 1968) and clinically with the concept of a caries balance (Pitts 1983). Lack of progress has been marked in 2 important areas. First is the lack of tools to produce effective remineralization (Silverstone 1972) deep into caries lesions to exploit caries being understood as a “dynamic disease process” (Featherstone 2008); Figure 1 summarizes the slow progress in using remineralization as a clinical tool and the aim for the EAER technology. Second, there has for decades been a lack of parallel developments with health systems worldwide to pay dental teams for delivering comprehensive caries detection, risk assessment, and prevention—as opposed to perverse and continuing incentives for restorative interventions (Pitts 2004; Pitts and Zero 2016). There are disturbing indications that a significant proportion of dentists and therapists still say that they would intervene invasively (restoratively) on carious lesions where evidence and clinical recommendations indicate that less invasive therapies should be used (Innes and Schwendicke 2017).

There are now some encouraging recent signs of progress. For instance, Reminova is developing a practice- and patient-friendly clinical remineralization system that will provide an alternative care choice to minimize the need for conventional fillings; also, broad discussions in a number of countries have revolved around the introduction of further diagnostic and treatment codes and the promotion of economic incentives in this area. A further stimulus for change in favor of caries prevention is the ratification of the UNEP Minamata Convention to phase down the use of amalgam and restorative treatment more generally (Pitts and Zero 2016).

Not only in Europe and the United States but globally, there is increasing evidence indicating a parallel desire between the dental profession and patients for preventive care. The profession also seeks more emphasis on preservative philosophy in dental education. In dental education, a modern core cariology curriculum was developed in 2011 through consensus across Europe by the Association for Dental Education in Europe and the European Organisation for Caries Research (Pitts et al. 2011; Schulte et al. 2011). Since then this core curriculum, with some local modifications, has been implemented widely: in Europe, across Colombia (Martignon et al. 2014), and most recently, across the United States (Fontana et al. 2016), as well as in further countries. International collaboration in implementing modern education in the future is exemplified by the King’s College London / Association for Dental Education in...
Europe / American Dental Education Association meetings held on May 7 to 9, 2017 (Association for Dental Education in Europe et al. 2017).

With a focus on initial and moderate caries lesions, Reminova has targeted an idealized treatment objective, defined as one that

- Preserves all healthy tissue
- Repairs the full depth of the caries lesion
- Involves no pain and uses no drills or injections
- Retains (or restores) the mechanical strength of the enamel structure
- Has similar or better acid resistance than natural enamel
- Is a fast and efficient treatment for patients and dentists
- Evidences the successful treatment for reimbursement purposes
- Has a positive aesthetic and health appeal to patients

With this in mind, the founders of Reminova set out to invent such a solution, a method to repair lesions without drilling, filling, pain, or injections.

**Initial Technology and Company Development for EAER**

At the outset of the technical brainstorming aimed at identifying a tentative solution, the founders—with full recognition of the role played by Reminova cofounder Chris Longbottom—considered the following:

*Natural caries remineralization*—to learn from the natural demin-remin mechanism: could we re-create it or borrow from it?

*Reviews of the literature*—we sought to understand what did not work and why, to learn from history rather than ignore it.

*A multidisciplinary approach*—to bring learnings from other sciences and industries. Typically, some of the technologies are not familiar to dentists or dental researchers.

This brainstorming and exploratory work led us to some surprising and counterintuitive conclusions (to the established teachings of dentistry) that were protected by way of patent applications and that we termed *EAER* for “electrically accelerated and enhanced remineralization.” Three Scottish universities (Dundee, St. Andrews, and Abertay) collaborated on this, bringing together dentistry, chemistry, and soil science expertise, the last surprisingly relevant to dental analysis. We tested several approaches before focusing on implementing EAER by adapting the use of iontophoresis, a widely used and accepted method for transdermal and ocular drug delivery (Sarraf and Lee 1994; Prausnitz et al. 2004), to the caries remineralization challenge.

Since the 1960s, particularly in Eastern Europe, iontophoresis has been applied to treat dentine hypersensitivity (Gangarosa and Park 1978) and to attempt to drive greater quantities of fluoride onto the surface of enamel for caries prevention. Simone et al. (1995) reported such a study but with no apparent benefit. Following the publication of our original patent application in many regions of the world, more recent work utilizing iontophoresis and fluoride has produced additional promising results, including an acceleration effect in relation to the remineralization of artificial lesions (Ivanoff et al. 2013) and an enhancement effect (Ivanoff et al. 2011). In EAER, we focus on conditioning of the untreated lesion as an essential step: cleaning extraneous material and lipids (Shellis et al. 2002) from the bulk of the subsurface lesion.

Figure 2 shows the essential steps in using EAER to restore caries lesions to the equivalent of healthy enamel. EAER works by using iontophoresis to apply a small electrical field from a custom-made dental device to drive mineral molecules from a reservoir into the deepest parts of caries lesions, which have been cleaned and conditioned. This creates an environment with a surfeit of suitable minerals collecting within the
subsurface lesion, in an environment that encourages remineralization to occur and that matures to give desirable hardness and mineral density (which we continue to investigate).

In our ICNARA 3 presentation, we detailed the commercial development path of our company (www.reminova.com), including 1) the rationale for our decision to raise investment directly by crowdfunding from a global base of passionate believers in preservative dentistry, rather than by conventional venture capital, and 2) the worldwide press coverage that we received at the time of company launch to exemplify the tremendous appeal that preservative dentistry has for the general public.

Reminova has its headquarters in Scotland and a base in London at King’s College London. The company at the time of presentation at ICNARA 3 had 17 granted patents. The company is working to address our customers’ needs for a reparative treatment for early and moderate enamel caries as a first target. We are seeking to offer a quick and painless way to repair and strengthen caries-affected tooth enamel.

**Development of EAER So That Dental Teams Will Not Need to Continue to Drill Holes—Preliminary In Vitro Results**

From our very first exploratory brainstorming work, we continually challenged ourselves with the question “How will this translate into a practical dental treatment?” This made us focus on developing a method that utilized the tools and chemicals typically available to dentists in their practices already. In our method, the tooth sample would have normal physiologic hydration levels and then be dried with compressed air for a few seconds, similar to the start of many dental procedures. Conditioning of the lesion area involves a series of chemical applications and washes with water, which are the subject of our continued patent applications.

The remineralizing agents that we have used during development have been applied to lesions as pastes or more fluid liquid formulations in such a way as to create a reservoir of mineral material near to or on the lesion surface. The iontophoretic electric field is then created by the placement of suitable metal electrodes into or indeed near to the remineralizing agent reservoir and an opposing polarity electrode placed to accelerate the agents into the enamel lesion, as the electrically shortest path to the opposing electrode. When a battery or electrical power supply is connected, the remineralizing agents are electrically accelerated toward the opposite polarity electrode and away from the electrode of similar polarity. The direction of movement is dependent on the charging potential of the remineralizing agents or particles. A neutral charged agent may still be moved by being within an electrolyte medium, much as a wooden log moves in the current of a river. This arrangement has the effect of driving remineralizing agents as far as they can go into the physical structure of the enamel lesion.

To provide maximum experimental flexibility during our earliest brainstorming, we used noninvasive micro-computerized tomography imaging on our (human) teeth samples so that we could compare before-and-after treatment mCT images of lesions and infer changes in mineral density levels and reductions in demineralized volumes within a lesion. Early signs of success included demonstrating a natural caries lesion “shrinking” after EAER treatment by using mCT imaging; the lesion continued to shrink, in depth and volume, after further EAER treatment, as shown in Figure 3.

We developed this method to give more convincing evidence by using a sample tooth that had 2 “similar” caries lesions on approximal surfaces. Lesion 1 was treated with EAER and a remineralizing agent, while lesion 2 was treated without EAER but with the same treatment and remineralizing agent but no iontophoresis as a control (mimicking natural diffusion only). Significant changes in mineral levels were calculated from the before-and-after mCT image analyses in the EAER-treated lesion 1 (before, 76%; after, 92%), but there was no change in mineral levels in the control diffusion-only lesion 2 (before, 83%; after, 83%).

Our next goal was to understand more clearly where our remineralizing agents travel to during EAER. A simple theory of iontophoresis suggests that charged particles (also, paradoxically, uncharged particles) will progress as far as possible toward the opposing polarity electrode until the electric field reduces. For our application, we were keen to understand 1) how deeply remineralizing agents were being driven into the lesion and if they were contained in some way and 2) how...
effective the preconditioning is in providing a pathway through the porous network of the surface zone layer and the deeper lesion. We chose to use gold nanoparticles instead of a remineralizing agent for this study, as gold is easier to detect and visualize within enamel, as it is not naturally present in enamel and therefore much simpler to confirm what we had added and where it traveled to within our test lesions.

After EAER treatment, we fractured open the lesions to minimize any posttreatment artifact from polishing the sample, to examine where the gold was found. Figure 4 shows a histologic view of an EAER-treated and then fractured lesion (with purple gold nanoparticles visible deep in the lesion), as well as x-ray diffraction (XRD) scans and scanning electron microscopy (SEM) images. Gold was found almost everywhere in the lesions; it was not found in healthy enamel.

The absence of any gold traveling into healthy enamel during EAER in these preliminary results was a further positive development: specifically, we infer that healthy enamel may present a physical barrier to the progression of remineralizing agents, enabling lesion-specific treatments with no or minimal impact on surrounding enamel; however, we recognize this as an area that will need further research and investigation.

Having satisfied ourselves, for this early stage of investigation and development, that our EAER developments could deliver materials to the deepest part of enamel caries lesions, we have undertaken preliminary in vitro studies on natural caries lesions in human teeth and analyzed these by cross-sectional microhardness at various depths into the lesions and extending deep into the enamel layer (600 to 1300 µm).

In this small sample of teeth and microhardness data presented at ICNARA3, the hardness values for healthy (untreated) enamel and untreated lesions conform broadly with the previously established typical Knoop hardness trends reported in the literature (Feagin et al. 1971). Almost all of the hardness measurements taken inside the EAER-treated lesions were found to be slightly harder than those of healthy enamel measured in their same tooth samples. The full depth of the lesion is apparently remineralized to a level that makes it “harder” than healthy enamel.

Figure 5 shows a graph of the average microhardness values measured in 6 teeth included in this preliminary in vitro study and discussed at the conference. In line with the exploratory nature of this preliminary work, these teeth were not treated with the exact same treatment conditions; indeed, a different remineralizing agent (tooth mousse) was used in 1 sample, and a nonremineralizing gold nanoparticle is included for reference in another. The striking information is the high averaged microhardness measurement in all treated lesions, with the exception of the nonremineralizing gold sample. By averaging the microhardness values measured within the lesion at all depths, we aim to communicate that the lesion is “hard” and substantially remineralized. In 5 of the 6 samples shown on Figure 5, we were able to locate and measure the microhardness of untreated lesions present at another site on each tooth sample. The average values of the untreated lesions are in line with expected values from the literature and further confirm that there is nothing exceptional or “out of the ordinary” about the tooth samples selected for the study; in fact, they may even give some insight into the possible starting condition of the treated lesions, as the treated and untreated lesions (of each tooth sample) would have been exposed to the same long-term oral environmental conditions.
In perhaps the most compelling example of our studies presented at ICNARA 3, Figure 6 shows microhardness data taken from a tooth with 2 discrete ICDAS 2 lesions that were in close proximity to each other on the same approximal surface and that had shared a very similar oral environment. Only 1 lesion was treated with EAER. Microhardness profiles showed low values into the untreated lesion, typical values for the healthy enamel measured surrounding but near to these lesions, and harder values for the EAER-treated lesion extending 1300 µm below the enamel surface.

Figure 7 shows typical SEM imagery from these sample examinations carried out by the Department of Chemistry at the University of Edinburgh that corroborate the enamel microhardness results. In the untreated lesions, the enamel prism structure is evident: rods are broken and demineralized. However, in the EAER (electrically accelerated and enhanced remineralization)–treated lesions, the SEM appearance is very similar to healthy enamel, with no degraded prisms or broken rods visible.

This corroborated evidence from our small preliminary studies suggests 1) that the in vitro EAER treatment can remineralize clinically significant caries throughout the depth of the lesion, 2) that the EAER-treated enamel can be harder than healthy enamel but almost completely indistinguishable from it (via SEM), and 3) that it also shows success with different remineralizing materials and parameters based on the EAER process.

Reminova is continuing to build a more complete analytic data file for these preliminary in vitro study samples utilizing human teeth and ICDAS 2 lesions, as well as for other studies that we are pursuing at this time. There is a long road ahead: our company’s development activities continue to focus on treatment optimization, and our protocol development is progressing in parallel with our regulatory work, which we hope will support our move into in vivo studies in 2018. Notwithstanding, our continued commercial activities are moving toward the launch of our first commercial product and having all the supporting infrastructure in place to be able to bring this exciting development to dentists and patients around the world.

**Author Contributions**

N.B. Pitts, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J.P. Wright, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. Both all authors gave final approval and agree to be accountable for all aspects of the work.

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References


