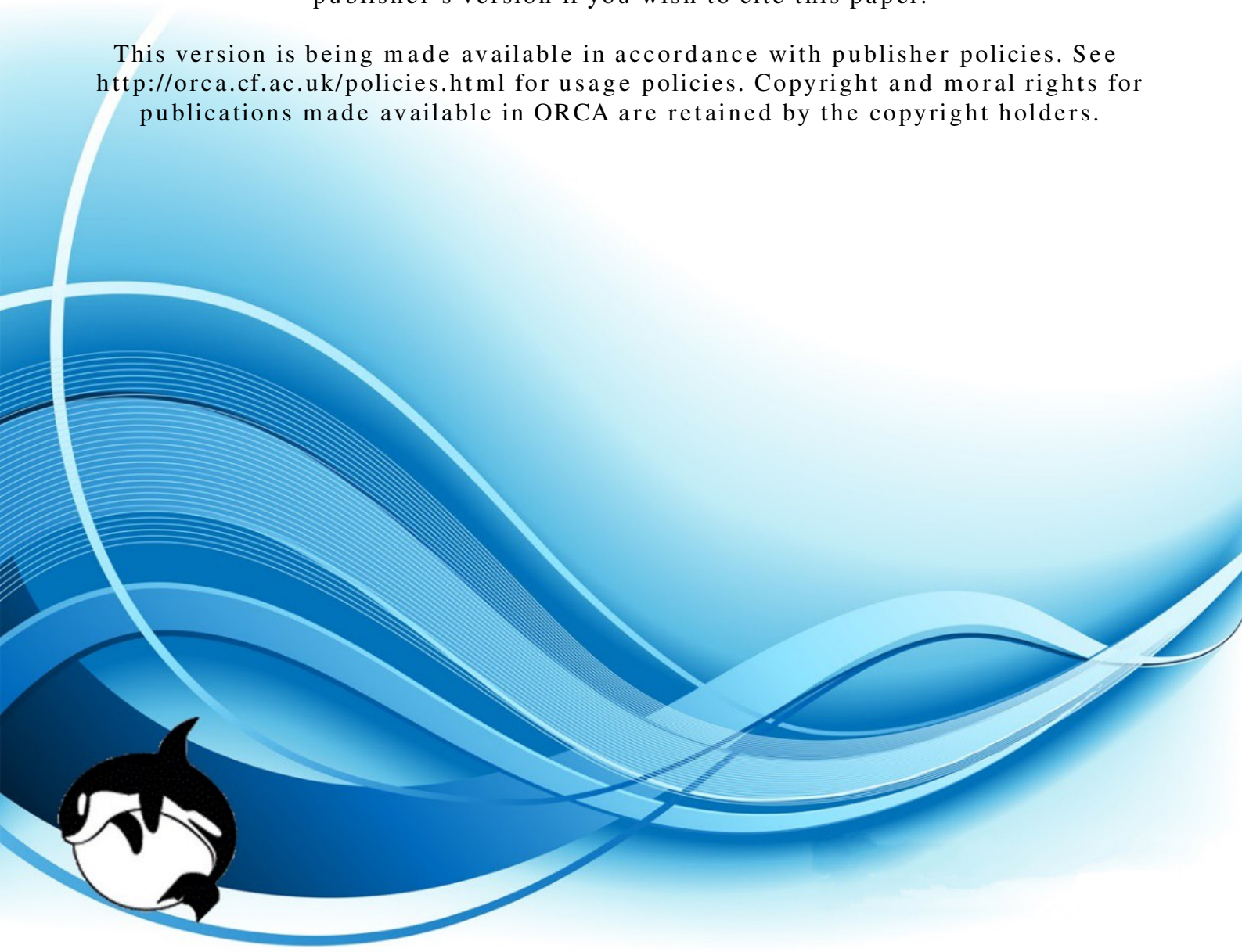

Mineralising agents to manage early carious lesions – part I: mode of action

Abstract

Dental caries remains a major global health challenge affecting millions of people worldwide, with both major health and financial implications. The minimum intervention oral healthcare (MIOC) delivery framework aims to improve caries management through early diagnosis and the use of remineralisation strategies in primary and secondary preventive approaches. The landmark discovery of fluoride in caries remineralisation resulted in an increase in research on such non-operative approaches. With an improved understanding of the biochemistry of caries and the demineralisation-remineralisation balance within dental hard tissues, researchers and clinicians currently seek new therapies to improve the non-operative management of early carious lesions. New remineralisation technologies have been introduced in recent years, with varying chemistries, modes of action and degrees of success.

This paper, the first of a two part series, will explore the chemistry and mode of action of currently available remineralisation technologies, outlining their clinical effectiveness and use in dental caries management.

Clinical relevance

A scientific understanding of ever-evolving remineralisation technologies is necessary to ensure clinicians are aware of the available options, their advantages and disadvantages, as well as to ensure these technologies are utilised correctly in different clinical scenarios.

Objectives

The reader should understand the chemistry and mechanism of action of currently available early carious lesion remineralisation therapies.

INTRODUCTION

Dental caries is one of the world's most prevalent non-communicable diseases, affecting adversely approximately 3.5 billion people, resulting in both a great health and financial burden[1,2]. Carious lesions start from dissolution of hydroxyapatite crystals at the tooth surface, at an atomic level, which occurs following the formation and stagnation of the dental plaque biofilm, if left undisturbed [3]. If no intervention occurs, the caries process will remain active and the lesion will progress which increases the complexity of its management[3]. The minimum intervention oral care (MIOC) philosophy applied to caries management has moved from invasive conventional operative management to more micro- / minimally invasive early interventions, with an increasing emphasis placed on early diagnosis and non-operative preventive strategies, among which, remineralisation plays a major part[4–7]. The early disruption of the caries process negates the requirement for extensive operative intervention later, leading to the preservation of more tooth structure and reducing the burden and complexity of future dental treatment[4].

Human saliva is naturally saturated with calcium and phosphate ions necessary for remineralisation. This is an important part of the natural homeostatic mechanism maintaining the demineralisation-remineralisation balance that occurs on exposed dental hard tissue surfaces. The clinical efficacy of saliva on its own to maintain this demineralisation-remineralisation balance can be affected by many factors including saliva quality and quantity (xerostomia), the latter commonly being affected by patient polypharmacy; these factors affect adversely patients' susceptibility to caries. As a result, adjunctive remineralisation products, protocols and strategies are required to supplement this process[8,9].

The purpose of this two-part review is to provide a comprehensive review of the mineralisation technologies available and to discuss comparatively the clinical efficacy of these strategies in dental caries management.

CHEMISTRY OF REMINERALISATION - MODE OF ACTION

The natural formation of enamel (amelogenesis) is a complex biomineralisation process involving cellular activity[10]. Proteins self-organise to regulate the crystallisation of hydroxyapatite in an ordered manner, i.e., the extracellular protein matrix continuously forms when enamel crystals grow in length and the enamel thickens, followed by the transition and maturation stage when the protein matrix degrades as crystals grow in width and is eventually removed upon the completion of enamel formation. Therefore, mature enamel is acellular and once demineralised, any repair cannot occur through autonomous biomineralisation, but through extrinsic remineralisation processes. Remineralisation (perhaps better termed mineral deposition) can be described as the relatively disordered deposition of apatitic minerals onto / within the demineralised tissue. Efforts in investigating these remineralisation processes have resulted in several commercialised agents available for clinical use. Depending on the mechanism, these can be categorised into the following groups[8,11]:

1. Fluoride-based systems
2. Non-fluoride-based systems
3. Biomimetic systems.

Fluoride-based systems

Fluoride is often considered the “gold standard” in enamel remineralisation. The mechanisms by which fluoride facilitates enamel mineralisation have been well documented. In an aqueous environment saturated with mineral ions and with a pH > 5.5, fluoride will absorb into the

demineralised enamel acting as nucleation sites and attracting calcium ions due to its electronegative ionic charge[12]. It is suggested that a set of reactions including ion-exchange, chemisorption and hydrolysation can occur when fluoride reacts with hydroxyapatite (HA), which eventually results in transformation of HA to fluorapatite (FA) or fluoridated hydroxyapatite (FHA)[13]. FA and FHA have lower solubility in oral fluids at the critical pH for carious lesion formation (pH 5.5) thus increasing its resistance to demineralisation[14].

Fluoride delivers significant remineralisation potential at a low concentration (sub-ppm level)[13,15,16], but with a significant positive dose-response, with increased mineral gain with an increasing fluoride concentration[17]. However, a recent in-vitro study noted that the fluoride dose-response may also be dependent on the severity of the lesion, i.e. the earlier the lesion, the better the dose-response, with possible reasons being that in more demineralised lesions the developed surface zone blocks the influx of mineral ions due to hypermineralisation and/or the lack of soluble mineral ions leaves more acid-resistant minerals in the lesion[18].

The clinical mode of action of fluoride in remineralisation / mineral deposition is more sophisticated as biological factors may affect its efficacy. Fluoride can diffuse into the natural plaque biofilm which may act as a reservoir to maintain fluoride concentration in the oral fluids close to the tooth surface[19]. When the biofilm is immature, in-vitro studies have revealed that fluoride failed to promote remineralisation significantly, implying that elevated levels of fluoride in plaque and saliva exert a significant impact[20,21]. The levels of fluoride in plaque is associated with many factors including plaque disruption rate and pH[22]. In addition, calcium fluoride (CaF_2) may form alongside FA and FHA and be deposited onto the enamel surface. CaF_2 is readily soluble in acidic pH when it dissolves and releases both calcium and fluoride into oral fluids, therefore acting as an alternative reservoir of calcium[23]. However, high-dose fluoride leads to excessive formation of CaF_2, which may in turn clog the lesion surface, resulting in reduced remineralisation capacity[24].

Non-fluoride-based systems

Non-fluoridated mineralisation agents can be further subcategorised into pH modifiers, and calcium-phosphate systems[25] (see Table 1).

pH modifiers

Generally, pH modifiers promote remineralisation by affecting the pH of the microenvironment. An example of this is xylitol, a non-fermentable, non-acid-producing sugar substitute. Xylitol can help maintain the pH of dental biofilm above 5.5 and meanwhile increase the flow rate of the stimulated saliva which has a higher concentration of calcium and phosphate, thereby creating a suitable environment for remineralisation[26]. Likewise, arginine is an amino-acid which has also been found to boost remineralisation through raising pH in the oral environment due to the production of ammonia after the amino acid is deaminated by arginine deaminase, found within saliva[27]. However, high-quality clinical evidence of pH-modifying remineralisation agents is scarce and sometimes contradicting. Therefore these agents show potential but more clinical trials are required before more robust conclusions can be drawn[28,29].

Calcium-phosphate systems

Several compounds can be classified as calcium-phosphate remineralisation agents, including casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), bioactive glasses, calcium silicate, functional tricalcium phosphate (f-TCP), nano-hydroxyapatite (n-HA) and amorphous calcium phosphate (ACP).

Though the specific mode of action of these agents differs, their chemistry is broadly similar. They all release calcium phosphate ions into the oral fluid and maintain concentrations that are supersaturated with respect to hydroxyapatite (HA), therefore resulting in mineral gain at the surface of carious lesions. For example, the amino acid sequence "-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-" from CPP is found to stabilise calcium and phosphate by formation of amorphous phosphate nano-clusters, approximately 2nm in size[30]. These nano-complexes prevent crystal growth to the critical size needed

for nucleation and phase transformation and hence act as a mineral ion supplier for later remineralisation.

When introduced into an aqueous environment, calcium sodium phosphosilicate bioglasses (bioactive glasses) can release calcium and phosphate as well as increase the pH through surface ion exchange reactions[31,32]. These result in deposition of hydroxycarbonate apatite (HCA)-coated glass particles onto carious enamel surfaces [33]. The glass particles can bind to enamel surfaces and continually release mineral ions to facilitate mineralisation[34].

Likewise, calcium silicates have a similar mode of action to bioglasses in remineralisation, by releasing calcium via ion exchange from surface reactions with body fluids[35]. Recent in-vitro studies have indicated that calcium silicate can precipitate HA and help repair demineralised enamel[36].

ACP, n-HA and f-TCP are similar histologically to enamel apatite[8]. ACP is an unstable, transitional apatitic phase which, after formation, can release calcium and phosphate ions for remineralisation before transformation to a thermodynamically stable phase such as HA[37]. n-HA are nanosized hydroxyapatite particles that exhibit significantly increased surface area. The precise mechanism that n-HA enhances remineralisation is unclear; some studies suggest that the large surface area allows n-HA particles to directly bind to enamel surface defects like a filler and attract calcium and phosphate ions to grow[38,39]. Others claim n-HA's function as a calcium phosphate reservoir[40]. With respect to f-TCP, originally designed to boost fluoride remineralisation efficacy, its functionalisation by organic compounds allows a protective barrier to prevent calcium from reacting prematurely with fluoride, hence providing bioavailable calcium when interacting with saliva[41].

Biomimetic systems

Biomimetic approaches have gained increasing interest in recent years. Self-assembling peptides are an analogue to enamel matrix proteins which could guide oriented apatite growth. P11-4 peptides, for example, are tailored for subsurface remineralisation. Some studies indicate that P11-4 can diffuse into the carious lesion body, self-assemble into 3D hierarchical structures under highly ionic

concentration to provide nucleation sites for mineral ions, therefore favouring remineralisation[42,43]. Although laboratory studies have found conflicting results regarding its efficacy in remineralising incipient carious lesions[44,45], recent clinical trials showed that P11-4 induced significantly greater remineralisation than other agents including f-TCP and fluoride[46,47]. This material has been successfully launched as Curodont™ by Credentis for dental professionals.

HISTOLOGICAL CHARACTERISTICS FOR MINERALISATION

Incipient carious lesions (white spot lesions (WSLs)) present clinically as a white, chalky and roughened enamel surface due to the light scattering that occurs due to the difference in refractive index between the air/water in the porosities within the lesion and mineral, which intensifies with air-drying. This poses an aesthetic and functional problem[48]. A histological diagram is shown in Figure 1. A recent in-vitro pH-cycling study monitored the colour change in artificial WSLs using a spectroradiometer and attributed the greatest reversal of the white appearance observed in 5000 ppm fluoride group to remineralisation, which was evidenced by scanning electron microscopy (SEM) and electron microprobe analysis[49]. However, despite this remineralisation, the white spot remained clinically evident after fluoride application. Similarly, CPP-ACP was found to show a significantly greater colour reversal compared to artificial saliva in-vitro[50]. Further examinations suggested that such colour change was associated with mineral deposition filling the microporosities at the lesion surface. However, another in-situ study compared the aesthetic characteristics of artificial WSLs remineralised by 5000 ppm fluoride, bioglass and CPP-ACP and found no significant difference amongst all materials tested[51].

Carious lesions are unique because histologically they consist of a well mineralised surface zone in which the porosity is 1-2%, overlying the body of the lesion which accounts for 25-50% porosity[52] (see Figure 1). Remineralisation will affect these histological zones and consequently induce a series of changes.

The remineralisation capabilities in the surface zone have been evidenced by studies employing various remineralisation agents. For example, in an in-vitro study, Milly et al. demonstrated that bioglass, either pure or modified with polyacrylic acids, could precipitate apatitic crystals and assist the growth of the existing enamel prisms, therefore filling the superficial interprismatic spaces[53]. Some reports predict that the recrystallisation mode depends on the calcium level of the solution; a low calcium concentration favours crystallisation on existing damaged crystals whilst higher

concentrations may support nucleation and new crystal formation[54]. Other agents possess similar surface remineralisation efficacy, including fluoride, CPP-ACP, f-TCP and n-HA[53,55–57]. The mineral gain in the surface zone can be measured as the recovery of surface microhardness resulting from the micro-structure reinforcement by the newly formed crystals. A recent in-vitro pH-cycling study by Wang et al. comparing bioglass, CPP-ACP and fluoride on surface remineralisation suggested that treatment with these agents yielded significantly greater surface hardness recovery than in controls, which correlated well with morphological observations by SEM[58]. Caution is required as techniques such as SEM and microhardness are surface characterisations only. The Knoop microhardness indenter, for example, produces indentations with limited penetration to approx. 1.5 µm[59]. Thus the interpretation of these results must be made with care when extrapolating to the clinical situation.

The sub-surface body of the incipient carious lesion accounts for its bulk and mineral infill in this part is crucial to successful long term lesion repair. Some pH-cycling studies showed that by increasing the concentration, fluoride could diffuse through the depth of the lesion and initiate a deeper remineralisation[60]. Fluoride tablets (4350 ppm) exhibited extensive remineralisation from the base of lesions, resulting in significant reduction in lesion depth, when compared to 1450 ppm fluoride. Polarised light microscopy also showed an increased translucent zone after high-dose fluoride application, representing mineral infill in the inner part of the lesion. However, a recent in-situ study suggested the opposite. Microradiographic observations by Amaechi et al. indicated that HA induced a more homogeneous remineralisation throughout the lesion depth than 500 ppm fluoride, where remineralisation occurred mostly in the surface zone[61]. Nevertheless, both studies exhibited clear lamination after treatment, implying that the diffusion pathways in the lesion surface were blocked by the superficial mineral deposition. This is in agreement with previous studies[62].

Similarly, CPP-ACP claims to possess subsurface remineralisation capabilities[63]. Longitudinal microhardness and microradiography suggested that CPP-ACP could favour more subsurface mineral regain than other agents including calcium silicate and fluoride[57,64]. However, it was noted that the

lesion surface remineralised by CPP-ACP exhibited comparably weaker mechanical properties. The conjecture was that newly precipitated mineral by CPP-ACP was less acid-resistant, hence the pathways for ion diffusion remained open. A comparative study, on the other hand, suggested that CPP-ACP and fluoride were not superior to HA in remineralising the subsurface lesion; all mineral gain followed an outside-inwards direction and predominately took place in/on the lesion surface[65]. These contradictory findings may arise from the disparities in study design, including concentration of the agent, study duration, type of study, etc.

CHALLENGES FOR REMINERALISATION

Deep mineral gain within the body of the carious lesion remains the challenge for remineralisation strategies. The porosity of the lesion surface zone is relatively small and can be more easily occluded when topical agents react and readily deposit on the surface, thus hindering ion penetration into the lesion depth. The lesion surface is arrested at the sacrifice of the lesion body in which the caries process and sub-surface damage may still continue. The salivary pellicle, a tenacious semi-permeable organic layer on the enamel surface, retards transportation of ionic matter across the enamel[66]. In-vitro studies have demonstrated that this pellicle could inhibit remineralisation on artificially formed WSLs[67]. It is therefore the delivery of calcium and phosphate ions into the depths of a lesion that controls remineralisation. Figure 2 demonstrates the influence of mineral ions (concentration and exposure time) on the remineralisation of incipient enamel lesions.

Some agents, such as self-assembling peptides and CPP-ACP, claim to possess the potential for subsurface remineralisation. The mechanisms were discussed earlier. Although CPP-ACP has been shown to boost subsurface remineralisation, a recent study found that extended application time with CPP-ACP failed to produce additive remineralisation effect[64]. Pre-conditioning the lesion surface to create better conditions for diffusion may provide an alternative route. Self-assembling peptides require conditioning through use of oral prophylaxis to remove biofilms and existing mineral deposits

which may prevent peptide monomers from penetrating[68]. Meanwhile, acid etching the lesion surface could also be considered to improve diffusion. Similar efforts have also been reported for other agents. For example, subsurface remineralisation using a bioglass slurry was enhanced by surface pre-conditioning with air-abrasion using bioglass particles modified by polyacrylic acids[69]. Mineral depositions were observed at approximately 40 µm depth. Likewise, subsurface remineralisation, to some degree, was enhanced by surface pre-conditioning with chitosan before bioglass application[70]. However, a dense layer consisting of newly deposited minerals was found to cover the lesion surface after the remineralisation regime. However, will this level of subsurface remineralisation be effective long-term? Perhaps these surface pre-conditioning techniques may require continuous / multiple applications by oral healthcare professionals, making home-based treatment difficult. Thus, designing an effective method for mineral ion penetration is paramount for new effective remineralisation strategies.

The speed of ion release is another challenge especially for toothpastes and mouthwash carriers. Toothpastes cannot release active ingredients until dispensed and having interacted with water or saliva. Other factors such as toothbrush head geometry and filament size, saliva quality and secretion rate are further complexities to consider[71]. Mouthrinse has better ion-release potential. However, retention of such mineralising agents remains an issue. The active phase of these agents is transient after brushing or rinsing, because of the diluting effect caused by abrasive challenges by tongue, cheek movement, and/or pH change[72,73].

THE FUTURE OF BIOMIMETIC REMINERALISATION

In recent years, attention has been drawn to the biomineralisation strategy for carious lesion repair, which aims to incorporate enamel proteins such as amelogenin to induce remineralisation. Tooth enamel is the hardest tissue in the human body and this unique characteristic is reportedly due to its delicate 7-level hierarchical structure[74]. Current commercialised remineralisation agents can achieve

mineral gain in the lesion, but to the best of the authors' knowledge seldom replicate the hierarchical structure of enamel during remineralisation. Amelogenins constitute 90% of matrix proteins involved in the process of enamel growth and the self-assembly ability is thought to be pivotal in forming the complicated hierarchical structure of enamel[75]. Molecular mechanisms include selective binding to specific crystal facets to regulate orientated crystal growth and reaction with ACP to form intermediate pre-nucleation clusters that transform into organised crystals in subsequent stages[75]. Remineralisation efficacy of amelogenin and its derivatives have been investigated. A hydrogel composed of chitosan-amelogenin rP172 was found to biomimetically form mineral depositions with enamel-like crystals on both the surface and the subsurface of acid-etched and artificial carious lesions after pH-cycling for seven days[76,77]. Leucine-rich amelogenin peptide, an amelogenin derivative, has also been found to reduce lesion depth and facilitate biomimetic reconstruction of enamel[78]. Meanwhile, there are certain concerns regarding amelogenin strategy, including the extraction and storage of amelogenins and duration required for the treatment[11]. However, clinical evidence is sparse, hence requiring more research to prove clinical efficacy.

CONCLUSIONS

Remineralisation of early carious enamel lesions has seen promising progress in recent decades. Fluoride, calcium phosphate and biomimetic approaches and their combinations could successfully repair the surface and, to some degree, the subsurface zones of such incipient carious lesions. The lack of sufficient remineralisation in the lesion body remains the greatest challenge for all commercialised mineralising agents. New biomineralisation strategies employing amelogenin and its derivatives may provide an alternative route which has the potential to restore the hierarchical structure of tooth enamel. Extensive clinical investigations are demanded to prove the clinical benefits for these remineralisation agents.

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Table 1 Summary of non-fluoride based and biomimetic remineralisation technologies for enamel caries management (at the time of publication)

Technology	Remineralising mechanism	Advantages	Commercial products (company name)
Calcium-phosphate systems			
CPP-ACP	CPP amino acid sequences stabilise ACP to prevent crystal growth and provide mineral ions for later remineralisation	CPP-ACP can penetrate into subsurface and remineralise deep lesion	Tooth Mousse™/ MI Paste™ crème/ Recaldent™ (GC Corp.)
Bioglass (NovaMin)	Bioglass particles release Ca^{2+} and PO_4^{3-} as well as increase the pH via surface ion exchange	Fast release of ions and increase of pH can induce rapid deposition on lesion surface	Sensodyne™ Repair/ Oravive™ (GlaxoSmithKlne)
ACP (Enamelon)	Unstable ACP releases bioavailable mineral ions before transformation to stable crystal phases	Bioavailable Ca^{2+} and PO_4^{3-} could transiently favour subsurface remineralisation	Enamelon™ (Premier Dental)
n-HA	n-HA particles bind to enamel defects, attracting Ca^{2+} and PO_4^{3-} to grow or acting as a reservoir	It has excellent biocompatibility and bioactivity due to similar morphology, structure and crystallinity to enamel crystals	Remin Pro™ (VOCO) Biorepair™ (Coswell)
f-TCP	f-TCP prevents premature reaction between Ca^{2+} and fluoride, providing Ca^{2+} for remineralisation	f-TCP can act as a targeted low-dose delivery system for remineralisation	Clinpro™ Toothpaste (3M)
Biomimetic systems			
Self-assembling peptides	SAPs act as an analogue to enamel matrix proteins to guide oriented apatite growth	SAP can diffuse into lesion body and attract ions for subsurface remineralisation	Curodont™ Repair (Credentis)
Amelogenin	Amelogenin proteins selectively bind to crystal facets to regulate oriented growth and react with ACP to form clusters that can later transform to HA	True biomineralisation with hierarchical structure is possible	Not available

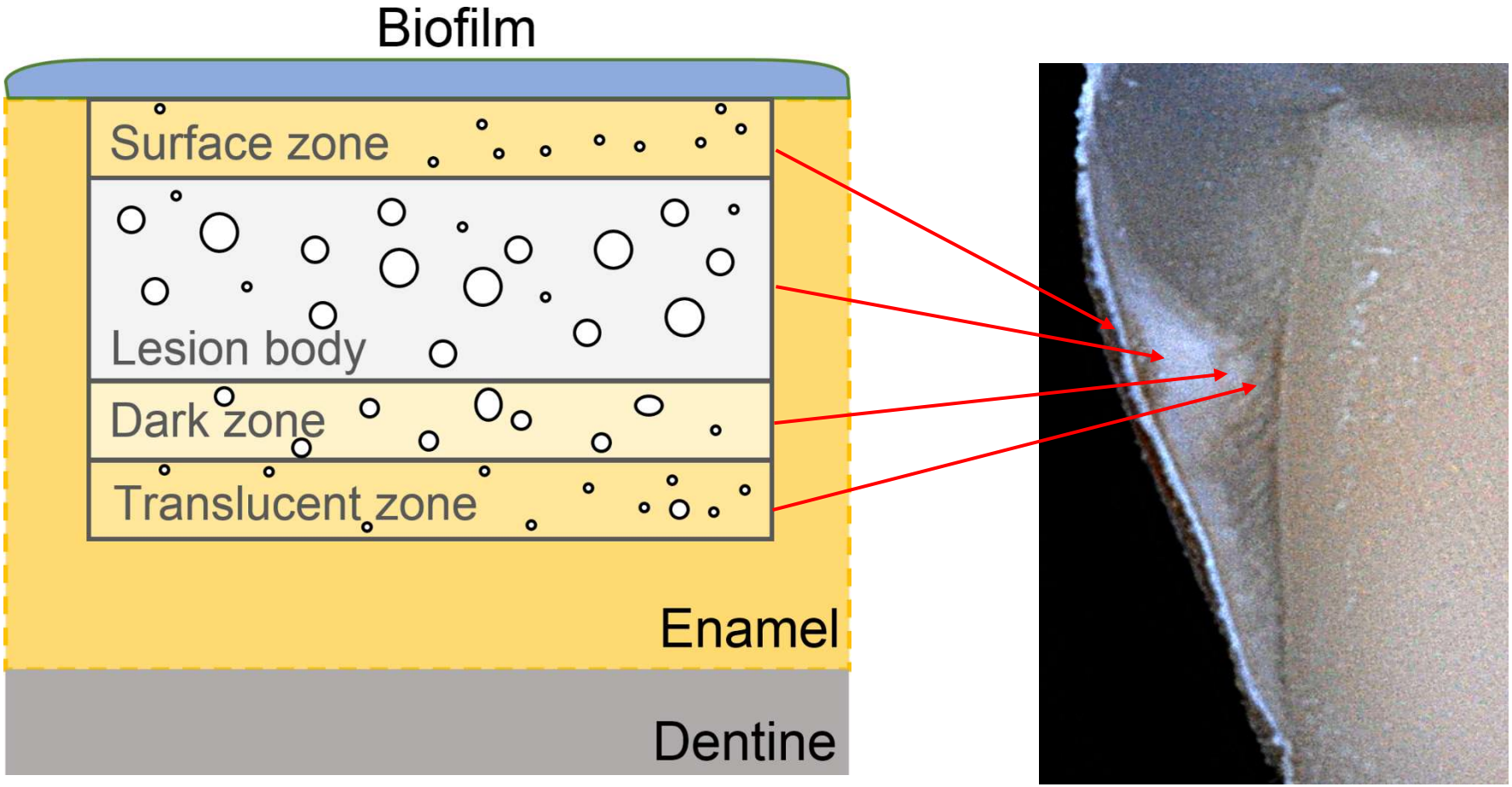

Figure 1 Basic histology (left) and clinical example (right, courtesy of L. Mackenzie) of an early enamel carious lesion. The enamel surface is covered by a layer of dental plaque biofilm, under which lies the surface zone with relatively small porosities which account for 1-2% vol. In contrast, the lesion body is more extensively demineralised with larger porosities (25-50%). Succeeding is the dark zone (porosity 5-10%) which shows a positive birefringence. Translucent zone has a similar porosity to the surface zone (1-2%) but the pore size is relatively larger which allows small molecules such as 2-chloronaphthalene or quinoline to penetrate[52].

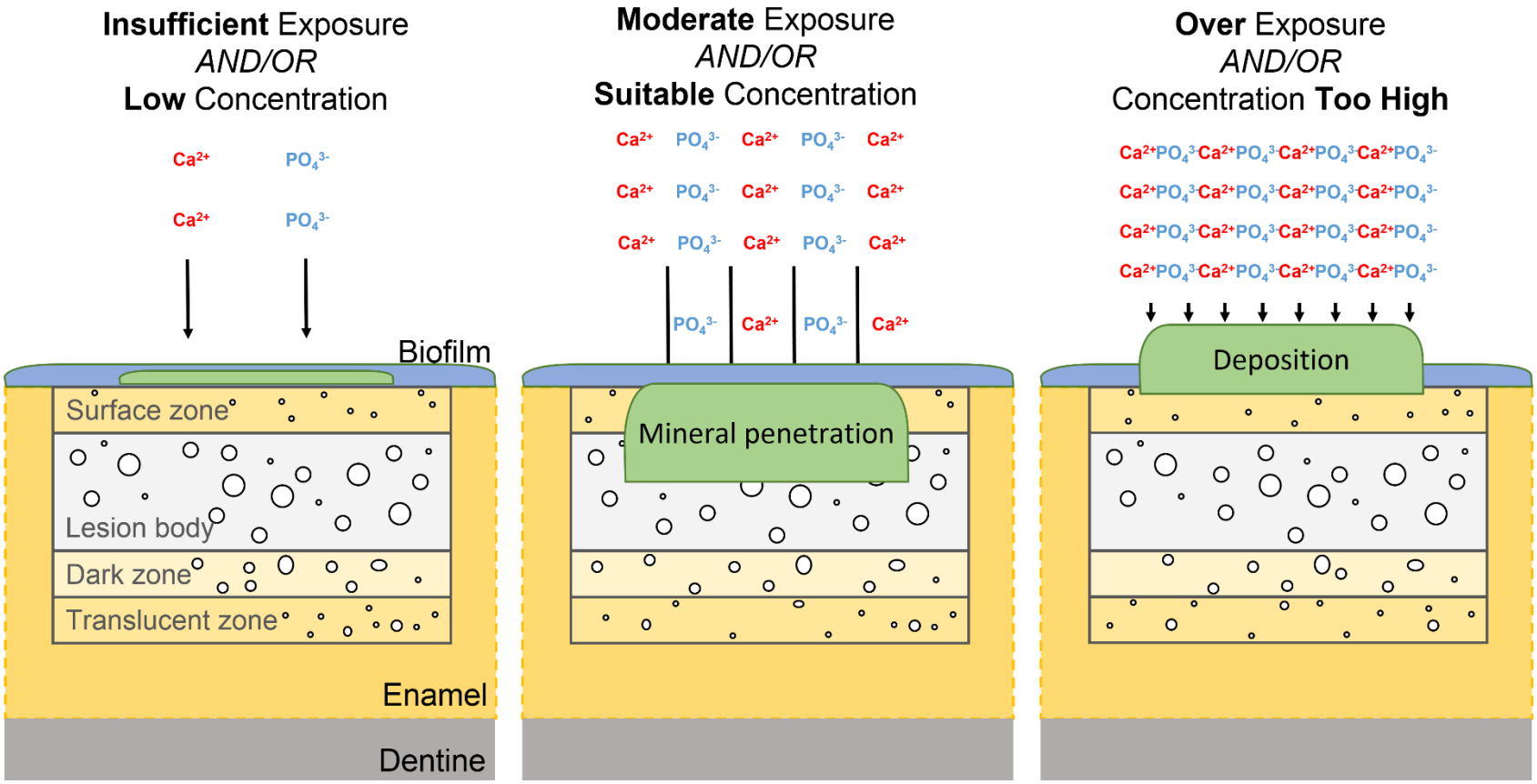

Figure 2 Illustration of basic mechanisms of remineralisation of the early carious lesion depending on calcium and phosphate exposure time as well as concentration in the biofilm adjacent to the tooth surface. The biofilm can reserve a portion of mineral ions, therefore providing buffering capacity and allow for an extended remineralisation. Excessive exposure time and/or concentration of mineral ions (left) would result in mineral deposition primarily on

the surface of the lesion whilst the subsurface remains untreated and the penetration of ions is hindered by the deposition. In comparison, insufficient exposure time and/or concentration (right) would result in small amount of mineral deposition on the surface. If the exposure time and/or concentration are controlled to be moderate, the subsurface of the lesion would be remineralised along with the surface, and the penetration of calcium phosphate ions would be allowed into depth.