Transport of water in human dentine

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10.1. SUMMARY

Sooner or later, nearly all teeth fall victim to dental caries. During dental caries parts of the inorganic and organic fraction of the enamel and dentine are transported out of the tooth and thus research on transport in teeth is fundamental in understanding the caries process.

The global goal of this thesis is to obtain information about the mechanisms involved in the transport of compounds through human dentine. As the water phase in dentine is an evident route for transport we decided to make it the subject of our investigation. The specific aims of this study are:

1) To obtain knowledge about the mechanisms involved in the transport of water in human dentine.
2) To determine the water distribution in dentine.
3) To determine the degree to which the water is bound at the different locations.
4) To study the influence of dentine structure on the transport of water.

The literature on transport phenomena in dentine is surveyed in chapter 2. Three aspects seem to determine the transport: 1) The structure and composition of the dentine; 2) The preparation of the dentine surface; 3) The forces that cause the transport.

Chapter 3 describes the water uptake during rehydration by freeze-dried human dentine sections, that were cut out of the crowns of third molars, perpendicularly to the dentinal tubules. On the basis of a comparison of three theoretical models with experiments it is concluded that the water uptake can be described by a combination of the capillary suction of water into the dentinal tubules and the diffusion of water into the mineralized matrix in the direction parallel to the tubules. Using this model the diffusion coefficient of water in intertubular dentine is calculated as $(1.74 \pm 0.42) \cdot 10^{-10}$ m^2/s. These calculations also show that $(75.2 \pm 1.5)\%$ of the dentine water is in the tubules and thus $(24.8 \pm 1.5)\%$ is in the mineralized matrix.

The water uptake of dentine sections during rehydration at four temperatures, 10°C, 25°C, 40°C, and 70°C is investigated in chapter 4. Using the theoretical uptake model from chapter 3 the diffusion coefficient of water in intertubular dentine is obtained as a function of temperature. With the Arrhenius relation the activation energy E_a for the diffusion of water in intertubular dentine is calculated, $E_a = (29.5 \pm 2.2)$ kJ/mole, which is of the order of the strength of a hydrogen bond. It is concluded that transport of water in the intertubular dentine is most likely a hopping of water molecules along the surfaces of the collagen and/or mineral, each jump involving the breaking of one hydrogen bond.

In order to study the influence of dentine structure on the transport of water, this structure is varied by fixing the dentine in glutardialdehyde (GDA) at pH 7. Firstly, the effect of the fixing on dentine stucture is studied (chapter 5). Microhardness indentation lengths are measured on glutardialdehyde (GDA) fixed and unfixed

human dentine. GDA-fixed dentine is found to be softer than unfixed dentine. The indentation length increases with approximately 10% after fixation in a buffered 5% GDA solution with pH 7. Using this effect the penetration of GDA in dentine is investigated by measuring the variation of indentation length in the direction of the penetrating GDA as a function of fixation time. Describing the penetration as diffusion in a plane sheet a diffusion coefficient of $5.4 \cdot 10^{-13}$ m^2/s is calculated. It is speculated that transport of GDA into dentine is probably by diffusion in the direction of the tubules, in combination with a reaction of GDA with the collagen. Dimensional changes of dentine due to fixation with GDA are also measured. After fixation, dentine expands in both the direction parallel (approximately 0.4%) and perpendicular (approximately 2%) to the tubules. It is concluded that the decrease in dentine hardness after fixation is most likely due to the expansion of the dentine.

In chapter 6 the influence of GDA-fixation on the diffusion of water into dentine is investigated. Using the transport model of chapter 3, the diffusion coefficient of water into GDA-fixed intertubular dentine is calculated as $5.20 \cdot 10^{-10}$ m^2/s. This value is three times larger than the diffusion coefficient in unfixed dentine and indicates that fixation makes the dentine structure more permeable.

The shrinkage of dentine and decrease in dentine mass after drying is measured in chapter 7. Three drying procedures are used viz., freeze-drying and drying under a flow of nitrogen gas at 60°C and 100°C. To determine if any irreversible changes are induced by drying, the dentine is rehydrated and during rehydration the swelling of dentine dimensions is measured and after the rehydration is complete, the dentine mass is weighed. Dentine mass is found to decrease with 10.2% after freeze-drying, with 9.0% after drying at 60°C and with 10.5% after drying at 100°C. After all three drying methods, dentine shrinkage is roughly equal. Linear dimensional changes are 1.7-2.0% in the plane perpendicular to the tubules and 1.4—1.7% in the direction of the tubules. In comparing the rate of swelling during rehydration with the water uptake model of chapter 3 it is concluded that linear changes in the direction of the tubules are probably due to shrinkage/swelling of the lamina limitans (a membrane lining the inner wall of the tubules) while changes in the perpendicular direction probably are due to shrinkage/swelling of the collagen fibers in the mineralized matrix. During rehydration, freeze-dried dentine and dentine dried at 60°C regain their original wet dimensions and mass, dentine dried at 100°C shows some permanent shrinkage and mass loss, probably due to some irreversible changes in the dentine matrix.

Chapter 8 presents a theoretical model that describes the dependence of the hydraulic conductance of dentine disks on the decrease in dentine tubule radius and tubule density in the pulp-enamel direction. A parameter that describes the decrease in tubule radius is calculated by fitting the model to experimental results from the literature.

In the general discussion (chapter 9) an attempt is made to relate these *in vitro* experiments to dentine caries. A model in which peritubular dentine dissolves in the water phase in the dentinal tubules and the intertubular dentine dissolves by transport of mineral ions in the direction parallel to the tubules is proposed.

10.2. SAMENVATTING (SUMMARY IN DUTCH)

Vroeg of laat vallen bijna alle natuurlijke gebitselementen ten prooi aan de ziekte tandcariës. Gedeelten van de anorganische en organische component van het glazuur en dentine verdwijnen tijdens het cariësproces uit de tand en ten gevolg hiervan ontstaat een lesie (gaatje). Transportprocessen zullen een belangrijke rol vervullen in deze lesievorming, waaruit volgt dat onderzoek aan transport in tanden fundamenteel is voor begripsvorming omtrent het cariësproces.

In dit proefschrift wordt gepoogd kennis te verwerven over de factoren die het transport in mensdentine bepalen. Omdat het water in dentine een voor de hand liggende weg is waarlangs transport kan plaatsvinden, is besloten deze waterfase te onderzoeken. De specifieke doelen van dit onderzoek zijn:

1) Inzicht te verschaffen omtrent de mechanismen van water transport in mensdentine.
2) Het bepalen van de locaties in dentine waar het water zich bevindt.
3) Het bepalen van de mate van gebondenheid van het water op deze locaties.
4) De invloed te onderzoeken van de dentine structuur op het transport van water.

In hoofdstuk 2 wordt een overzicht gegeven van de literatuur betreffende transport in dentine. Drie aspecten lijken belangrijk: 1) De structuur en samenstelling van het dentine; 2) De manier waarop het dentine oppervlak waardoor het transport plaatsvindt is geprepareerd; 3) De oorzaken van het transport.

In hoofdstuk 3 wordt de water opname gewogen van gevriesdroogde dentineplakjes gedurende rehydratie als functie van de rehydratietijd. Deze plakjes zijn gezaagd uit derde molaren, in een vlak loodrecht op de dentinekanaaltjes. Er worden drie theoretische transportmodellen ontwikkeld om de wateropname te beschrijven. Aan de hand van de vergelijking van deze modellen met de experimenten wordt geconcludeerd dat de wateropname beschreven kan worden door een combinatie van twee processen. Het eerste proces is de wateropname ten gevolge van capillaire opzuiging in de dentinekanaaltjes, het tweede proces is de diffusie van water in de gemineralizeerde matrix, in de richting van de dentinekanaaltjes. Met dit model is de diffusiecoëfficiënt van water in intertubulair dentine berekend, $(1.74 \pm 0.42)\ .\ 10^{-10}\ m^2/s$. Uit dit model volgt tevens dat $(75.2 \pm 1.5)\%$ van het dentinewater in de kanaaltjes zit en dus $(24.8 \pm 1.\%)\%$ zich in de gemineralizeerde matrix bevindt.