Discrimination between Intact and Decayed Pulp Regions in Carious Teeth by ADC Mapping

Ksenija Cankar a, Lidija Nemeth b, Franci Bajd c, Jernej Vidmar a, c, Igor Serša c, d

a Institute of Physiology and b Department of Dental Diseases and Normal Dental Morphology, Medical Faculty, University of Ljubljana, and c Laboratory for Magnetic Resonance Imaging, Jožef Stefan Institute, Ljubljana, Slovenia; d Department of Biomedical Engineering, Kyung Hee University, Seoul, South Korea

The infectious disease of dental caries results in diverse lesions that may affect hard dental tissues and dental pulp. Several histopathological examinations of pulp sections of teeth extracted due to carious pulpitis showed that pulpal reactions may differ significantly. The reactions ranged from minimal inflamations to marked infiltrations of the pulp tissue [Martin, 2003]. Therefore, an assessment of pulp tissue pathosis before any treatment decision is of upmost importance [Zero et al., 2011]. Thus far not many endodontic studies have focused on issues relevant to pulp diseases, their diagnosis, treatment and monitoring. In particular, there still exist many controversies regarding the clinical management of diseases...
associated with the vitality of dental pulp [Bergenholtz and Spangberg, 2004]. However, it is reasonable to assume that the condition of the pulp plays a decisive role in the outcome of conservative pulp-saving treatment [Reeves and Stanley, 1966; Langeland, 1987; Mjör, 2002].

Factors determining the regenerative properties of the pulp, such as remaining dentin thickness and degree of pulp inflammation, are important in the prevention of treatment complications [Murray et al., 2002b]. Present diagnostic modalities, i.e. clinical symptoms and periapical radiographs, enable a relatively inaccurate assessment of the inflammatory status of the pulp related to caries, while probing and bitewing radiographs might be helpful only in estimating the hardness and thickness of dentin over the pulp, respectively. Consequently, the choice between two treatment options, i.e. pulp capping and pulpotomy, is controversial for adult dentition [Cvek, 1978; Lim and Kirk, 1987; Maryniuk and Haywood, 1990; Stanley, 1998; Schuurs et al., 2000; Ward, 2002]. Unfortunately, there are no reliable methods available for a preoperative assessment of inflammation progression [Seltzer et al., 1963]. This is mainly due to a lack of sensitive diagnostic techniques that could help identify the threshold between reversible and irreversible (decayed) inflammatory regions of the pulp [Bergenholtz, 1986].

Several feasibility studies have shown that magnetic resonance imaging (MRI) is suitable for dental applications, enabling with high sensitivity and specificity the assessment of caries lesions and dental pulp. Specifically, caries lesions can be visualized due to the MRI signal rise in the porous impaired dentin [Tymofiyeva et al., 2010]. In dentistry, high-resolution dental MRI has been used to visualize and quantify carious lesions three-dimensionally, including approximal and occult caries lesions, and to measure the minimum distance to the dental pulp [Tymofiyeva et al., 2009]. Advanced MRI techniques, such as SWIFT MRI, have also been used for simultaneous three-dimensional hard and soft tissue imaging of teeth within clinically relevant scanning times [Idiyatullin et al., 2011].

Diffusion-weighted imaging (DWI) with its mapping of the apparent diffusion coefficient (ADC) is an advanced MRI technique highly sensitive to the microscopically incoherent motion of water, which allows the quantification of water ADC in tissue. In addition, ADC mapping is susceptible to several cellular changes and tissue abnormalities [Le Bihan et al., 1986; Beaulieu et al., 1993]. Our previous study showed that ADC maps of carious teeth enabled reliable differentiation between intact and decayed regions of dental pulp [Vidmar et al., 2012].

In the present study, the influence of demineralization in hard dental tissues to adjacent pulps was assessed by using ADC mapping. ADC maps were compared in relation to standard International Caries Detection and Assessment System (ICDAS) scores in order to evaluate the potential of ADC mapping for quantifying the inflammatory status of the pulp and the changes in distribution of the ADC values along the dental pulp in response to caries progression.

**Materials and Methods**

**Human Teeth**

In the study, 38 extracted human teeth (26 molars and 12 premolars) were MRI scanned, the reason for extraction being orthodontic or surgical. The teeth included in the study were obtained from volunteers aged between 20 and 40 years. There was no statistically significant difference in age among groups of teeth with ICDAS scores of 0, 3 and 6. Before extraction all caries lesions were clinically examined using the visual-tactile method and recorded as active following the scores and criteria used by Ekstrand et al. [1998]. The teeth were immersed in a physiological solution (pH ≈7.4) immediately after extraction and subsequently stored in a neutral buffered 10% formalin solution (Sigma-Aldrich, Germany) at 8°C in order to avoid autolysis in the pulp [Vidmar et al., 2012]. In order to prevent dental pulp desiccation during long scanning time as well as to visualize the tooth surface, the teeth were coated with the two-component silicone dental impression material Exaflex® (GC, Japan) prior to MR acquisition. The firm and waterproof coating remained hardened from immediately after the administration of the catalyst to the base and had a low MRI signal. Subsequently, the teeth were inserted into Teflon tubes of 12 mm diameter and 25 mm height and were then placed centrally into a 15 mm radiofrequency probe (fig. 1a).

**Examination of Teeth and ICDAS Severity Scores**

Dental examinations with the detection of caries lesions were interpreted by two independent clinical examiners using the ICDAS severity scores as described [Jablonski-Momeni et al., 2008]. Both examiners had extensive clinical experience in restorative dentistry and did not have access to any other information, such as patient identity or clinical history; any histopathological evaluations of the post extraction teeth were unavailable. The teeth were assessed using conventional probing, radiography or fiberoptic transillumination and were classified according to the seven-grade ICDAS severity scale: score 0 = sound dental surface; score 1 = first visual change in enamel; score 2 = distinct visual changes in enamel; score 3 = localized enamel breakdown; score 4 = underlying discolored dentin with or without localized enamel breakdown; score 5 = distinct cavity with visible dentin; score 6 = extensive distinct cavity with visible dentin. Only teeth without caries (ICDAS score 0) and teeth with occlusal caries of two different ICDAS scores (3 and 6) were included in the study. The selection of ICDAS scores (0, 3 and 6) was based on the results of our previous study, according to which the greatest differences in ADC distributions of dental pulps were expected for these scores.
**MRI Procedures**

The teeth were scanned by a 2.35 T MR scanner consisting of a horizontal bore superconducting magnet (Oxford Instruments, Oxfordshire, UK) equipped with accessories for MR microscopy (Bruker, Ettlingen, Germany) with maximum imaging gradients of 300 mT/m and a Tecmag spectrometer (Houston, Tex., USA). The DWI was based on the pulsed-field gradient spin-echo technique [Stejskal and Tanner, 1965] with two 11 ms gradient pulses positioned symmetrically with respect to the refocusing radiofrequency pulse (δ = 11 ms, Δ = 18 ms) and with the following parameters: TE/TR = 34/1,300 ms, imaging matrix 256 × 128 × 8, field of view 30 mm × 15 mm × 10 mm. Corresponding in-plane resolution was equal to 120 μm per voxel, while the slice thickness was 1.9 mm. DWI was performed with four different diffusion gradient amplitudes that corresponded to the diffusion weighting coefficients (b-factors) of 0 s/mm², 132 s/mm², 317 s/mm² and 635 s/mm²; the total scan time of the DWI method was 90 min. The corresponding ADC map was calculated from the acquired diffusion-weighted images using the MRI Analysis Calculator plugin (Karl Schmidt, Harvard University, USA) of the ImageJ (National Institutes of Health, USA) image processing software.
Image Analysis

The teeth were analyzed by ADC mapping to obtain ADC distributions along dental pulps, i.e. from occlusal to apical plane. The distributions were obtained from ADC maps by calculating an average ADC value of all pixels having equal radial distance from the occlusal side for sound teeth or from the deepest pit of the caries lesion for teeth with caries. In order to compare ADC profiles of different teeth (with different pulp lengths) the profiles were considered as functions of relative pulp lengths x, i.e. distances from the occlusal side of the pulp normalized to the pulp length (fig. 1b). Therefore, values $x_{\text{decayed}}$ and $x_{\text{intact}}$ correspond to the relative distances from the occlusal side of the pulp to the end of the decayed region and to the end of the transition region, respectively. The analysis was performed by the corresponding ImageJ macro program.

Tooth Histology

Extracted teeth (intact and severely decayed) were fixed in 4% neutral buffered formalin for 24 h and in 70% alcohol for 12 h. Decalcification took place in 10% HNO$_3$ for 10 h at room temperature followed by decalcification in 10% ethylenediaminetetraacetic acid for 60 days at 37°C. After embedding in paraffin and orienting the tissue in the sagittal plane of the long axis, serial 5-μm-thin sections were cut parasagitally and stained with hematoxylin and eosin. The dental tissues of the entire tooth were observed with a light microscope (Reichert, Austria) at 100× magnification. The inflammatory reaction was assessed according to the extent of the inflammatory infiltrate [Six et al., 2000].

Model of Dental Pulp Response to Caries

A phenomenological mathematical model for dental pulp response to caries is proposed (fig. 1b). The model assumes that a dental pulp response to caries progression is a three-modal process. The modes are the decayed pulp region, the transition region between the intact and decayed pulp and the intact dental pulp. All three modes were modeled by a linear regression

$$ADC(x) = \begin{cases} ADC_{\text{intact}} - ADC_{\text{decayed}}, & x < x_{\text{decayed}} \\ ADC_{\text{decayed}} + ADC_{\text{intact}} - ADC_{\text{intact}}(x_{\text{intact}} - x_{\text{decayed}}), & x_{\text{intact}} \leq x < x_{\text{intact}}(x_{\text{intact}}), \\ ADC_{\text{intact}}, & x \geq x_{\text{intact}} \end{cases}$$

Here the parameters $ADC_{\text{decayed}}$ and $ADC_{\text{intact}}$ correspond to the ADC values of decayed and intact dental pulp regions, respectively, while $x_{\text{decayed}}$ and $x_{\text{intact}}$ are normalized radial distances of the end of decayed and the beginning of intact dental pulp regions measured from the deepest pit of the caries lesion, respectively (fig. 1b). In teeth without caries only $ADC_{\text{intact}}$ was measured as an average ADC value along the pulp. The model analysis given by equation 1 was performed for each tooth included in the study, yielding a set of $ADC_{\text{decayed}}$ and $ADC_{\text{intact}}$ values of ICDAS scores 0, 3 and 6 that was then used for statistical analysis. Previously obtained experimental ADC distributions along dental pulps of teeth with the three different ICDAS scores 0, 3 and 6 were analyzed for the best fit with the proposed three-modal model using the Origin software package (Origin Lab Corporation, Northampton, Mass., USA).

Statistical Analysis

Differences among ADC values of intact and decayed pulp regions of different ICDAS scores (0, 3 and 6) were analyzed for statistical significance using two-tailed, homoscedastic Student’s t test.

Results

Figure 2 shows a comparison between ADC maps and histological sections of the same slice across two representative teeth, one intact and another severely decayed. ADC maps have good contrast between intact and decayed pulp regions. Assessment of pulp decay based on ADC mapping (fig. 2a, e) is also in a good agreement with histological results (fig. 2b–d, f–h). Specifically, ADC maps, which are sensitive to water mobility, clearly depict high cell compaction at the odontoblastic layer as an interface region with reduced ADC values. In the rest of the pulp, the cell arrangement is less compact and water more mobile so that ADC values are higher. Severely decayed teeth have an affected odontoblastic layer, significant progression of pulp inflammation and demineralization of dentin (fig. 2f–h). All these can be efficiently detected by ADC maps that have reduced ADC values in inflamed pulp regions and increased ADC values in the demineralized hard dental tissues (fig. 2e, arrow). No layer with reduced ADC values can be seen at the interface between the pulp and dentin due to impairment of the odontoblastic layer.

Figure 3 depicts ADC maps in color-coded images of three representative teeth with different ICDAS scores ranging from ICDAS 0 (intact tooth) over ICDAS 3 (localized enamel breakdown) to ICDAS 6 (extensive cavity with visible dentin). The teeth orientation is buccal – palatal for upper teeth and buccal – lingual for lower teeth. The outline of the teeth, shown as a blue contour, was determined from the interface between the hard dental tissues with no MR signal and the impression material yielding a weak MR signal. In the ADC maps, regions of slow water diffusion (blue, $ADC \approx 0.5 \times 10^{-9}$ m$^2$/s) in decayed soft dental tissue, regions of intermediate water diffusion (red, $ADC \approx 1.3 \times 10^{-9}$ m$^2$/s) in intact soft tissue as well as regions of fast water diffusion (yellow, $ADC \approx 2 \times 10^{-9}$ m$^2$/s) in demineralized hard dental tissue (regions encircled in red) can clearly be seen. The average ADC values of decayed and intact pulp regions with ICDAS scores 0, 3 and 6 are presented in table 1. Differences between intact and decayed pulp regions were found to be statistically significant ($p < 0.05$) for all cases. Differences between decayed pulp regions of ICDAS scores 3 and 6 were also found to be significant ($p = 0.034$). No statistically significant differences among different intact pulp regions of ICDAS scores 0, 3 and 6 were found ($p > 0.05$).

Average ADC distributions along the dental pulps of the three representative ICDAS groups (score 0, 3 and 6) are shown in figure 4 by experimental data points (squares)
and the model function (three segment lines) for parameters in table 1. The parameters were obtained as an average of best fit parameters to individual ADC distributions along the pulp. From table 1 it can be seen that ADC values of intact pulp regions are all approximately identical to $1.0 \times 10^{-9}$ m$^2$/s with all ICDAS scores (0, 3 and 6) while ADC values of decayed pulp regions are much lower and decrease with increasing ICDAS score ($0.9 \times 10^{-9}$ m$^2$/s with ICDAS 3 vs. $0.74 \times 10^{-9}$ m$^2$/s with ICDAS 6). The transition region width ($x_{\text{intact}} - x_{\text{decayed}}$) is practically identical with both tested carious ICDAS scores (19% with ICDAS 3 vs. 16% with ICDAS 6). However, the relative decayed tissue depth ($x_{\text{decayed}}$, fig. 4b, c) significantly increases with caries progression (3 with ICDAS 3 vs. 46% with ICDAS 6).
In the present study ADC mapping was employed for the functional assessment of hard and soft dental tissues in carious teeth. Specifically, it was employed for identifying intact and decayed dental pulp regions as well as for the changes in ADC distributions along the dental pulp in response to caries progression. The reduction of ADC values in the decayed dental pulp provides only indirect experimental evidence for a possible ongoing tissue response. Therefore, complementary histological analysis of intact and severely decayed teeth was performed in order to support ADC mapping results. ADC mapping was found to be highly sensitive to several changes in dental tissues and enabled a semiquantitative assessment of affected hard and soft dental tissues. ADC maps of teeth with ICDAS score 0 were mainly uni-

### Table 1: Best fit parameters of the model in equation 1 to experimental ADC distributions along the dental pulp

<table>
<thead>
<tr>
<th>ICDAS score</th>
<th>$x_{\text{decayed}}$</th>
<th>$x_{\text{intact}}$</th>
<th>$ADC_{\text{decayed}}$</th>
<th>$ADC_{\text{intact}}$</th>
</tr>
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<tbody>
<tr>
<td>0 (n = 14)</td>
<td>1.05±0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (n = 11)</td>
<td>3±6</td>
<td>22±13</td>
<td>0.89±0.15</td>
<td>1.01±0.08</td>
</tr>
<tr>
<td>6 (n = 13)</td>
<td>46±21</td>
<td>62±20</td>
<td>0.74±0.18</td>
<td>1.02±0.16</td>
</tr>
</tbody>
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$x_{\text{decayed}}$ = Distance from the occlusal side of the pulp to the end of the decayed region normalized to the pulp length. $x_{\text{intact}}$ = Distance from the occlusal side of the pulp to the end of the transition region normalized to the pulp length. $ADC_{\text{decayed}}$ = Average ADC value in the decayed pulp region. $ADC_{\text{intact}}$ = Average ADC value in the intact pulp region.

**Discussion**

In the present study ADC mapping was employed for the functional assessment of hard and soft dental tissues in carious teeth. Specifically, it was employed for identifying intact and decayed dental pulp regions as well as for the changes in ADC distributions along the dental pulp in response to caries progression. The reduction of ADC values in the decayed dental pulp provides only indirect experimental evidence for a possible ongoing tissue response. Therefore, complementary histological analysis of intact and severely decayed teeth was performed in order to support ADC mapping results. ADC mapping was found to be highly sensitive to several changes in dental tissues and enabled a semiquantitative assessment of affected hard and soft dental tissues. ADC maps of teeth with ICDAS score 0 were mainly uni-
form with ADC values well above the $1.0 \times 10^{-9} \text{ m}^2/\text{s}$. This result is expectable as all teeth exhibited sound surfaces without any evidence of caries lesions [Jablonski-Momeni et al., 2012]. ADC values in hard dental tissues increased with the progression of caries. This can be explained by the increased porosity that accompanies enamel breakdown and the disruption of dentin organization of impaired hard dental tissues.

A decrease in tissue ADC values at the caries-pulp interface could be attributed to restricted water mobility due to increased tissue compaction associated with cellular and subcellular barriers. This is most likely to occur as a consequence of an affected odontoblastic layer, calcitroaumatic response and accumulation of exudate cells [Brannstrom and Lind, 1965]. Therefore, regions of low ADC values may indirectly indicate decayed regions of the dental pulp. In the study, ADC values at the caries-pulp interface consistently decreased with increased ICDAS scores (3 and 6). This may be explained not only by the extent of the caries progression (minor in ICDAS score 3 as compared to ICDAS score 6), but also by more successful regeneration and reparation processes in hard and soft dental tissues, which slow the progression of caries [Nemeth et al., 2006]. In addition, with high ICDAS scores, a proliferation of chronic inflammatory tissue in response to caries could also result in increased extracellular and intracellular tortuosity [Ekstrand et al., 1995; Kitasako et al., 2002; Murray et al., 2002a]. The ICDAS score 3 and 6 criteria do not preclude pulp involvement. However, according to our measurements, ADC mapping is highly sensitive to differences in water mobility and can detect caries-induced changes in pulps of carious teeth. As can be seen from table 1, ADC value decrease in the decayed pulp progresses with ICDAS scores. The changes are also consistent with findings shown in histological sections of representative intact and severely decayed carious teeth (fig. 2). More elaborate preclinical MRI studies of carious teeth including teeth of all ICDAS scores would enable more precise determination of an ADC cut-off value for irreversible pulpitis. Thus it would be possible to predict the chance of pulp inflammation recovering versus progression to pulp necrosis.

To avoid problems with variable teeth sizes, ADC profiles were compared in the relative scale, i.e. in distances from the occlusal side of the pulp (side of caries origin) that were normalized to the total pulp length. The approach is also supported with ICDAS score criteria, in which the score is proportional to the relative (not absolute) volume of the decayed tissue with respect to the tooth volume. Statistical analysis of best fit model parameters confirms good agreement between the experimental ADC spatial profiles and the model given in equation 1. The results of the best fit model function in figure 4b and c and in table 1 indicate that the transition region width $(x_{\text{intact}} - x_{\text{decayed}})$ is approximately constant with ICDAS score, however, the relative depth of the decayed region $(x_{\text{decayed}})$ progresses with ICDAS score. This is consistent with the assumption that the permeability of dentin seems to be critical in determining the quantity of incoming antigens and subsequently the magnitude of the reactions operational in the underlying pulp [Jontell et al., 1998].

The age of the subjects donating the teeth and activity of caries lesions may influence pulp reaction to caries [Kim in Trowbridge, 1994; Mjör, 2002]. The findings regarding influence of age on decrease of immunological function of the dentin-pulp complex are controversial [Izumi et al., 2002; Murray et al., 2002c]. For these reasons, the conclusions of the present study are limited to young subjects and active carries lesions.

Only two extreme cases of teeth, one intact and one severely decayed, were analyzed histologically. This was mainly because histological analysis of teeth is demanding and time-consuming as it involves prolonged decalcification of hard dental tissues, tissue sectioning (possible tissue loss) and staining [Jablonski-Momeni et al., 2008].

While lower resolution multi-slice ADC mapping was less convenient for the localization and assessment of demineralized hard dental tissues, it was more sensitive for the discrimination of different soft tissue responses to caries over all three ICDAS scores (0, 3 and 6). Its use produced a nearly perfect linear relation to ICDAS scores, i.e. the higher the average ADC value, the lower the ICDAS score [Vidmar et al., 2012]. It must also be noted that the time involved for ADC mapping – equal to one and a half hours in our study – is still too long for in vivo use. The experiment time can be significantly shortened by using fewer slices, less different b-values of DWI and by using another (faster) imaging modality for DWI instead of the spin-echo method.

In conclusion, ADC mapping enables functional assessment of dental tissue response to caries as it provides water mobility information of the soft dental tissue response to caries. MRI results were in a good agreement with findings in histological sections of identical teeth. The study confirmed the relevance of ADC mapping for the discrimination and localization of the intact and decayed regions in dental pulps of carious teeth. However, prospective MRI studies in vivo are needed to demonstrate the feasibility of ADC mapping in the assessment and monitoring of pulp vitality before and after dental treatment or after tooth injury.
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Disclosure Statement

The authors state that they have no conflict of interest.

References


