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Assessment of structural changes of human teeth by low-field nuclear magnetic resonance (NMR)

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Abstract

A technique of low-field pulsed proton nuclear magnetic resonance (NMR) spin relaxation is described for assessment of age-related structural changes (dentin and pulp) of human teeth *in vitro*. The technique involves spin–spin relaxation measurement and inversion spin–spin spectral analysis methods. The spin–spin relaxation decay curve is converted into a T_2 distribution spectrum by a sum of single exponential decays. The NMR spectra from the extracted dentin-portion-only and dental pulp-cells-only were compared with the whole extracted teeth spectra, for the dentin and pulp peak assignments. While dentin and pulp are highly significant parameters in determining tooth quality, variations in these parameters with age can be used as an effective tool for estimating tooth quality. Here we propose an NMR calibration method—the ratio of the amount of dentin to the amount of pulp obtained from NMR T_2 distribution spectra can be used for measuring the age-related structural changes in teeth while eliminating any variations in size of teeth. Eight teeth (third molars) extracted from humans, aged among 17–67 years old, were tested in this study. It is found that the intensity ratio of dentin to pulp sensitively changes from 0.48 to 3.2 approaching a linear growth with age. This indicates that age-related structural changes in human teeth can be detected using the low-field NMR technique.

Keywords

NMR; spin–spin relaxation; NMR system; tooth; dentin; pulp

1. Introduction

The tooth is an amazing sensory organ. The outside of the tooth, the enamel, is the hardest tissue in the human body. The layer of the tooth surrounded by the enamel is called dentin. The tooth pulp lies in the center of the tooth. Enamel and dentinal structures are keys to understand the relationship between mechanical loading and the creation of cervical lesions. Dentin structure more closely resembles bone than it does enamel, and is slightly more calcified than bone (75% versus 65%). Dentin is made up of hydroxyapatite crystals (70%), organic matter (20%) and water (10%) [1]. Dentin, however, is ‘a stronger substance in tension’ than enamel [2]. When the tensile stress of enamel is exceeded, fracturing of enamel rods can occur and create an environment for the potential formation of wedge-shaped lesions. The dental

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pulp is soft connective tissues containing dental pulp cells, nerves, arteries, veins and lymph vessels. It is located at the core of the tooth. Decrease in dental pulp cell density can reduce pulp repair activity after restorative treatments.

Currently, the number of older patients requiring restorative dental treatment increases due to improvement in oral health. Several investigations have reported age-related changes in the pulp–dentin complex [3–9]. Murray *et al* [10] described changes in pulp cell density and dentinal thickness with aging from extracted human teeth using histomorphometrical measurements. Unfortunately, the major disadvantage of histomorphometry is that it is essentially a surface measurement. The only way to obtain three-dimensional data is by serial sectioning, which is destructive, time consuming and prevents further direct studies on the dynamics of the process. Although x-ray and micro-CT are routinely used to image and analyze tooth quality [11], they have not been successfully applied to an assessment of dental pulp changes. On the other hand, the non-destructive and non-invasive NMR technique can be used to directly detect the changes of dentin and pulp simultaneously, and provide the bulk of tooth quality information.

Magnetic resonance imaging (MRI) techniques have been widely applied in biomedical research, but are generally limited to the study of soft tissue or of gross skeletal structures of the bone even using high-resolution MRI images [31,32], although signal intensity changes of age-related pulp cavity in teeth have been studied by MRI analysis [12]. However, no current MRI technology has successfully obtained images of human teeth dentin due to the resolution limitation.

Recently, nuclear magnetic resonance (NMR) proton spin–spin (T_2) or spin–lattice (T_1) relaxation time measurements and analytical processing techniques have been used to determine microstructural characteristics of various types of fluid-filled porous materials [13–17]. This method has been used to quantify the porosity, pore size distribution and permeability in oil reservoirs where the pores in the rock structure range in size from sub-micron to sub-millimeter. More recently this technique has been developed and applied to determine the porosity and pore size distributions in bone by Ni *et al* [18–20]. Since teeth are comprised of fluid-filled porous materials, we applied this rapid, non-destructive and non-invasive technique to detect and quantify age-related tooth structural changes particularly for both dentin and pulp based on broadband pulsed NMR.

In this study, we have applied the NMR relaxation technique to assess age-related tooth quality *in vitro* by quantifying changes in dentin and pulp simultaneously. A preliminary study on age-related teeth structural changes using NMR was presented at 2005 Summary Bioengineering Conference [33]. The major hypothesis in this paper was whether noninvasive NMR relaxation time measurements could be used to characterize age-related changes in dentin and pulp, and to predict tooth quality. Specifically, we tested whether age-related tooth changes result in an alteration of the NMR spin–spin (T_2) relaxation time signal due to the structural changes in the tooth matrix. This signal can be further processed to produce a T_2 relaxation distribution spectrum related to dentin and pulp as well as their interfaces, and their derived parameters can be used as descriptors of age-related tooth changes. In this study, the proton liquid-like NMR spin–spin (T_2) relaxation decay signal was obtained from the Carr–Purcell–Meiboom–Gill (CPMG) NMR spin echo train method [21,22], then the relaxation decay signal was converted to T_2 relaxation distribution spectra describing the size domain of dentin and pulp. Therefore, we can calibrate the intensities in NMR inversion T_2 relaxation distribution spectra corresponding to the amount of dentin and pulp related to the age.

While dentin and pulp are highly significant parameters in determining tooth quality, variations in these parameters with age provide a highly accurate method of estimating age and have been

studied [5–7]. The pulp–dental complex is capable of responding to varieties of stimuli over time. These stimuli can be physiological, related to normal stresses, and also pathological, a result of cavities, tooth surface loss, or restorative treatment [23]. Although it is generally accepted that changes occur in the functioning of teeth from the time of eruption to old age, there are conflicting views on the details of these changes [24]. One of the reasons for this conflict is that interpretations of findings have been based on a study of tooth sections, without systematic consideration of age or the type of tooth from which the sections were made [25]. In addition, tooth size varies from person to person based on parameters such as height and weight. A nondestructive, potentially noninvasive assessment of these measures of tooth quality may lead to accurate predictions of age-related health.

Here, we propose an NMR calibration method ‘NMR standard estimation’—the ratio of the amount of dentin to the amount of pulp obtained from NMR T_2 distribution spectra that can be used to measure the age-related structural changes in teeth. We are cognizant of the biological and physiological variability manifest in teeth size variations, but feel that this kind of NMR standard estimation—the ratio of the amount of dentin to the amount of pulp from the NMR T_2 inversion spectrum—can be used to determine age-related structural changes in teeth and eliminate any variations in size of teeth.

2. Materials and methods

2.1. Sample population and preparation

All experimental procedures involving the use of human tissues were reviewed and approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio (UTHSCSA), TX USA (IRB Protocol # HSC2007666N). Compared to the first and secondary molars, the third (wisdom) molar is often used for studies because oral biological functions are generally not affected by extraction of the third molar [34,35]. Eight third molars clinically extracted with the ages from 17 to 67 years old were collected from UTHSCSA and used for this study. The extracted third molars were kept in a phosphate buffered saline (PBS) solution.

2.2. NMR measurement

A 0.540 MHz broadband NMR system (designed and fabricated by Southwest Research Institute) was set up at a proton frequency of 27 Hz for this study. A 0.5 inch diameter RF coil was used to contain the tooth samples in the experiment. ^1H spin–spin (T_2) relaxation profiles were obtained by using the NMR–CPMG spin echo method using a $6.5\ \mu\text{s}$ 90° pulse, a 500 μs delay between 90° and 180° pulses and 10 s for the cycle delay time. For each T_2 profile, 1000 echoes were acquired in one scan and 64 scans were used. Thus, one scan had repeated 1000 echoes within a time of about 1 ms. This, plus the sequence repetition time of 10 s, made the total time for one scan about 11 to 12 s [18,20].

2.3. Preparation of pulp cells and dentin portion for NMR measurements

Dental pulp cells were extracted from the teeth of different ages with signed informed consent as previously outlined [26]. Minced fragments of soft tissues from the pulp chambers were digested with $3\ \text{mg}\ \text{ml}^{-1}$ of collagenase type I and $4\ \text{mg}\ \text{ml}^{-1}$ of neutral protease (Worthington Biochemical Corporation, Lakewood, NJ) for 1 h at 37°C . The digested cells were filtered with $70\ \mu\text{m}$ nylon of cell strain (BD Falcon, Bedford, MD) and grown in $35\ \text{cm}^2$ tissue culture dishes containing Dulbecco's modified Eagle's medium (DMEM; GIBCO) with 10% fetal bovine serum and 100 U/penicillin/streptomycin. The next day, cell numbers were counted using a Coulter counter by direct cell counting (Beckman Coulter, Inc., Fullerton, CA). For measurement of cell volumes, the cell populations were treated with 0.1% trypsin, harvested from the wells and spun down. The supernatant was removed carefully and cell pellets obtained.

Then, 100 μl of PBS was added to the cell pellets to re-suspend the cells. The whole volume (cells plus 100 μl of PBS) was measured and cell volume was obtained by subtracting 100 μl of PBS from the whole volume. In addition, we used a diamond saw to cut the teeth and extract the dentin up portion (above the pulp chamber) from the third molar tooth for the dentin-only NMR measurement.

2.4. SEM measurements

The SEM analysis for dentin portion was performed after the NMR measurements. Each specimen was mounted in plastic and transverse sections were cut using a diamond saw and then ground and polished to approximately 80 μm thick. The sections were then imaged using an optical microscope at magnifications of 300 $\times$. Standard stereological techniques were used to characterize specimen pore size from the two-dimensional sections. Such measurements were repeated at four random locations of the cross-section.

2.5. Tooth mechanical cyclic loading test

In the tooth cyclic loading test, the sample was subjected to lateral cyclic loading at an angle of about 45°, at a force of 20 N (about 2 kg), with a frequency of 2 Hz and a total of 40 000 cycles. The diagram of the test machine is shown in figure 5.

2.6. Relationship between NMR data and pore size

The essential feature for an NMR experiment is that the transient NMR signal from solids decays very fast (decay time constant T_2 is normally less than 100 μs), while it takes much longer to decay in liquids. In porous media, the total amplitude of the transient hydrogen NMR signal is representative of the liquid phase inside the pores [27].

In low-field NMR, at the fast diffusion limit (diffusion effect is negligible), the relaxation rate $1/T_2$ is proportional to the surface-to-volume (S/V) ratio of the pore [28]:

$$1/T_2 = \rho(S/V)_{\text{pore}} \quad (1)$$

where ρ is the surface relaxivity—a measure of the pore surface's ability to enhance the relaxation rate—which falls within a reasonably narrow band. For compact bone material, it ranges roughly from a micron to tens of microns per second [18,29].

In the CPMG [21,22] NMR sequence (90°– t –180°–echo–delay) for spin–spin relaxation measurement, for a fluid contained in a single pore size, the echo following the 180° rotation of the magnetization vector is given by

$$M(t) = M_0 \exp(-t/T_2) \quad (2)$$

where M_0 is the magnetization of the nuclei at equilibrium and $M(t)$ is the observed magnetization at a variable delay time t , between the 90° and 180° measurement pulses. For porous teeth, the observed nuclear magnetization (NMR signal) depends on T_2 (i.e. pore size) of all pores. As shown in equation (1), the NMR relaxation time is proportional to pore size, and it is known that teeth and bones have distributions of pore sizes. This implies that NMR transverse relaxation (T_2) data can be expressed as a sum of exponential functions:

$$M(t) = \sum_i M_i \exp(-t/T_{2i}) \quad (3)$$

where M_i is proportional to the number of spins that relax with a time constant T_{2i} . $M(t)$ is the NMR magnetization decay from fluid saturated teeth and compact bone. Equation (3) can be inverted into a T_2 relaxation time distribution. Thus, instead of estimating a single relaxation time from a magnetization decay, it is necessary to estimate a spectrum or distribution of relaxation times $M(T_{2i})$. Since T_2 depends linearly on pore size, the T_2 distribution corresponds to pore-size distribution, with the longer relaxation times being from larger pores [18, 30].

3. Results and discussions

Figure 1 shows an example of NMR–CPMG relaxation decay data for extracted human tooth samples (third molars) from persons aged 43 and 17 years, respectively. Using the T_2 relaxation data as shown in figure 1, the obtained inversion T_2 relaxation distribution patterns for the 17, 25, 37, 39, 43, 45, 52 and 67 year old teeth are shown in figure 2(A–H). The T_2 relaxation times are distributed in a wide range, from sub-millisecond to second, thus indicating a wide range of pore sizes with the longer T_2 relaxation time corresponding to larger pore sizes. Comparing the old tooth to young tooth, the volume fraction of larger pore size (pulp) is significantly reduced for the older tooth, since the volume fraction of the median and small-diameter pores is increased due to age-related changes. These data demonstrate that age-related structural changes in teeth can be detected by broadband pulsed NMR.

As shown in figure 2, there are about three peaks displayed in the relaxation distribution spectra, and we need to decide how to assign the peaks. It is better to separate the enamel, dentin and pulp components. Thus, we can measure the separated dentin and pulp individually by NMR, and then compare the obtained dentin- or pulp-only relaxation distribution spectra with the whole-tooth NMR relaxation distribution spectra. With this method, the peaks in relaxation distribution spectra from the whole tooth can be assigned. Figure 3 shows a 37 year old sample (third molar) of an NMR T_2 relaxation spectrum from the extracted dentin and an NMR relaxation spectrum from the whole tooth before extracting the dentin. This provides evidence that the first peak (from left to right) is the dentin peak in the relaxation distribution spectrum.

Meanwhile, pulp cells were extracted from the same tooth. Figure 4 displays the inversion relaxation distribution data for only the extracted pulp tissue from this tooth sample. This spectrum proves that the third peak (from left to right) in the whole tooth spectrum is the pulp portion. Here we assume that the middle peak of the spectrum is mainly contributed from the interface between the dentin and pulp. Figure 5 shows the diagram of the mechanical test machine, and figure 6 shows the tooth T_2 distribution spectra comparison before and after the mechanical cyclic loading test. Before and after loading, spectral differences could be detected which is assumed due to the interface damage formed in the regions between dentin and pulp. The increased peak intensity (second peak from left-to-right or middle peak in the whole tooth) may be caused by the fluids inside the damaged interface. The third peak (pulp) is not included in figure 6. More detailed information in this region depends on a further study, and is not discussed in this paper. Meanwhile, figure 7 shows the SEM pictures for the two different age dentin-only samples. According to the measurements both of the average pore diameters are in the ranges of 1–2 μm which are consistent with the literature [36]. However, there is no clear evidence to distinguish the changes from the different ages.

Following are the deconvolution results from the spectra of A–H in figure 2. The estimated ratios of intensity of the dentin peak to the pulp peak from 17, 25, 37, 39, 43, 45, 52 and 67

year old teeth are 0.48, 0.71, 0.94, 1.1, 2.2, 2.4, 2.8 and 3.2, respectively. The correlation between the ratios with age is shown in figure 8. It is found that the intensity ratio of dentin to pulp sensitively changes approaching a linear correlation with age ($y = a \cdot x + b$, where $a = 0.0626$, $b = -0.8183$, with $r^2 = 0.86$). Therefore, the NMR standard estimation of the ratio of the amount of dentin to the amount of pulp obtained from NMR inversion T_2 distribution spectra can be used as a good measure to determine age-related structural changes in teeth.

4. Conclusions

A 'low-field' methodology has been described for estimating age-related microstructural changes in human teeth. This study has demonstrated that the nondestructive and noninvasive 'low-field' methodology can be used to detect age-related dentin and pulp changes rapidly and simultaneously. In this study, the proposed 'NMR standard estimation' method—the ratio of the amount of dentin to the amount of pulp from T_2 inversion spectrum—is a very sensitive and effective technique to measure age-related microstructural changes in teeth. In addition, the advantage of this technique over existing methods is that dentin and pulp information is based on volume (or total amount) measurement, not a few two-dimensional slices from a specific section of the sample. Currently, there are no nondestructive methods that can determine dentin and pulp simultaneously. Furthermore, full development of this technology with an *in vivo* study is needed.

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Figure 1. NMR-CPMG spin relaxation decay signals from 17 year old and 43 year old extracted human teeth.

Figure 2.

Inversion T_2 relaxation time spectra from the extracted human teeth: (A) 17 year old, (B) 25 year old, (C) 37 year old, (D) 39 year old, (E) 43 year old, (F) 45 year old, (G) 52 year old, (H) 67 year old.

Figure 3.
Top: NMR inversion T_2 spectrum from the extracted dentin of the tooth. Bottom: NMR inversion T_2 spectrum from the whole tooth.

Figure 4.
NMR inversion T_2 spectrum obtained from the *extracted tooth pulp tissue*.

Figure 5.
Teeth cyclic loading test apparatus.

Figure 6. The first and second peaks (left to right) in inversion T_2 relaxation spectra obtained from the whole tooth: (A) after cyclic lateral loading test; (B) before test.

Figure 7.
SEM photographs from two different age dentins.

Figure 8.
The correlations between the intensity ratios of dentin peak to pulp peak among 17–67 year old teeth.