Mechanical characterization of human dentin

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Dentin is a complex hydrated biological composite consisting of:
- about 50 vol% mineral in the form of apatite,
- 30 vol% organic matter (largely type I collagen),
- about 20 vol% fluid.

Other non-collagenous proteins and other organic components are also present in small amounts.
Outlook

• Critical review of literature data

• Experimental tests:
  1- local mapping of mechanical properties
  2- anelastic behaviour
Critical review of literature data

Several experimental techniques have been used to measure the mechanical properties of human dentin that exhibits a behaviour like a functionally graded material depending on tubule density and orientation.

The work described and critically analyzed the characteristics of dentin on the basis of scientific literature:

- Young’s modulus
- Shear modulus
- Poisson’s ratio
- Yield and ultimate stress
- Hardness
- Fatigue
- Fracture toughness
- Creep
- Stress relaxation
- Damping
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Sample preparation

Human molars were extracted from individuals (males 55-70 years old) as part of their dental treatment.

After disinfection by immersion in a solution of sodium hypochlorite in water for about 12 hours, they were longitudinally sectioned in order to obtain 0.8 mm-thick slices. From these sections bar-shaped samples (length $L = 13\div 16$ mm) have been cut for MS measurements.

Dentin density is different from point to point decreasing from the outer part to the inner one thus mechanical properties are not homogeneous.

Therefore, elastic modulus $E$ and $Q^{-1}$ obtained in present experiments represent average values.
Micro-hardness

Instrumented Indentation
Mechanical Spectroscopy

The samples, mounted in free-clamped mode have been tested using the method of frequency modulation.
Damping factor $Q^{-1}$

\[ d = \frac{1}{k} \ln \frac{A_n}{A_{n+k}} \]

\[ Q^{-1} = \frac{d}{\pi} \]

Dynamic modulus $E$

\[ E = \frac{48\pi^2PL^4}{m^4h^2f^2} \]

- $f$: resonance frequency
- $m$: constant ($m = 1.875$)
- $P$: material density
- $L, h$: length and thickness of the sample
Two sets of samples.

1- Heating-cooling cycles from 300 to 673 K with constant heating rate of $3.33 \times 10^{-2} \text{ K s}^{-1}$. Thermo-gravimetric analysis (TGA) were performed up to 693 K on samples submitted to the same heating used in MS to assess possible weight loss.

2- Isothermal experiments at 300 K with increasing strain amplitudes. The samples, previously kept for 36 hours in the spectrometer chamber under a vacuum of $10^{-7} \text{ bar}$, were submitted to a strain ramp from $7 \times 10^{-6}$ to $6 \times 10^{-4}$, kept at the maximum strain for 20 minutes and finally brought to the initial condition. Successive measurement runs were made on the same samples.
To assess possible structural changes occurring after heating-cooling cycles, X-ray diffraction (XRD) experiments were performed by using the Co K-α radiation ($\lambda = 1.78897 \text{ Å}$).
Experiments below room temperature

Repeated cooling-heating cycles on the same samples, from 300 K to 100 K and vice versa. For comparison some dehydrated samples were tested too.

DSC investigations were made on a Neztsch Phox 200 PC instrument with heating rate of 6 K/minute.
Experiments at high temperature

From 300 to 373 K modulus exhibits an increasing trend while $Q^{-1}$ behaves in the opposite way. Heating up to 373 K affects only residual water present in the pores. If the samples are cooled down from 373 K and rewetted, original characteristics are restored thus the process is reversible.

Above 373 K a broad and asymmetric $Q^{-1}$ maximum at 523 K is observed. Modulus decreases and exhibits two slope changes around 473 and 573 K. The asymmetric shape of $Q^{-1}$ maximum is due to the overlapping of two different maxima.

These phenomena are not present during cooling to room temperature and successive cycles.
TGA: weight loss with two stages corresponding to the ascending (1) and descending (2) parts of the $Q^{-1}$ maximum.
Peaks of the mineral phases: hydroxylapatite $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, fluorapatite $\text{Ca}_5(\text{PO}_4)_3\text{F}$, chlorapatite $\text{Ca}_5(\text{PO}_4)_3\text{Cl}$ and carbonate-apatite $\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3\text{F}$.

XRD spectrum does not substantially change after the heating-cooling cycles.
Start of collagen denaturation

Significant collagen denaturation

Breaking of proteins

Degradation and combustion of collagen

\[ Q^{-1} \times 10^2 \]

\[ E \text{ (GPa)} \]

Temperature (K)
Granato-Lücke string model

The model describes the changes of dynamic modulus and $Q^{-1}$ of metals in terms of string density and mean distance between pinning points.

$$Q^{-1} \propto \rho l^4 \omega$$

$$\frac{\Delta G}{G} \approx -\beta \rho l^2$$

$\beta$ constant,
$\rho$ density of vibrating strings,
$l$ average string length,
$\omega/2\pi$ vibration frequency
The concept that energy loss and modulus depend on the number of oscillating strings and on their mean length is of general validity and can be used to explain the anelastic phenomena of dentin.
Damping behaviour can be explained by considering the specific structure of dentin.

Fibres, fibrils and molecular chains forming the helix structure are like strings able to oscillate under an external applied stress.

The strings are subjected to a complex system of constraints, i.e. bonds fiber-fiber, fibril-fibril and helix structure, which are progressively modified as temperature increases with a consequent effect on the anelastic properties of the organic phase.
• The ascending part of maximum can be ascribed to the loss of water located in the gaps between fibres and between fibrils. Despiralization of helix structure of collagen molecule occurs so molecules become free to oscillate and to contribute to the damping. The increased number of oscillating strings leads to $Q^{-1}$ increase and $E$ decrease.

• When temperature further increases (above 523 K) the water loss regards that inside the fibrils, i.e. water that guarantees the continuity of the peptide chain, so fibrils degrade and the chain is broken in fragments. Damping decreases owing to the shorter length of the vibrating strings.

At 573 K combustion occurs further contributing to decreasing the damping and producing the second slope change of modulus.
Experiments at increasing strain

In each strain ramp $Q^{-1}$ progressively increases while modulus decreases. The effects are not permanent because original values are recovered if strain is decreased to the initial value.

The polypeptide chains forming the triple-helix are taken together by weak H-bonds.

The anelastic behaviour is attributed to the progressive rupture of H-bonds as strain increases with consequent increase of the mean length $l$ of vibrating strings (segments of polypeptide chains).

As strain amplitude comes back to the initial value, H-bonds reform so anelastic properties are restored.
H-bonds between the polypeptide chains of triple helix
Experiments at low temperature

In the 1st cycle (A) a broad $Q^{-1}$ maximum centred at $\sim 210$ K is observed. The maximum is still present in the 2nd (B) and 3rd (C) cycles but its intensity is lower.

As temperature decreases modulus increases with a variation of about 20% at 100 K.
Phase diagram of water
Cubic Ice

Cubic ice crystals

Diagram of cubic ice structure and crystals.
Structure consisting of alternating sheets of hexagonal ice and cubic ice

Amorphous forms of ice

- At the lowest temperatures examined here ice can assume also an amorphous structure. Two forms of glassy water have been extensively investigate: low-density amorphous (LDA) ice and high-density amorphous (HAD) ice.

- More recently, another variety, very-high-density amorphous (VHDA) ice, has been also discovered as a distinct form of glassy water.
a) 
Vapor → LDA

Liquid

~55 kbar, 77K → ~5 kbar, < 130 K → ~11 Kbar, 77K

LDA

HDA

1 bar, ~120K → Isochoric, 140K

Ice Ih

1 bar, 127K → 1 bar, 127K

b) 

LDA

136K → VVL water

150K → Ice Ic
In heating stage of all the cycles two maxima, M1 at 155 K and M2 at 178 K, are observed. The intensity slightly decreases in the cycles following the first one.

Above 200 K, $Q^{-1}$ progressively increases and damping values are lower in the cycles after the first one.

Modulus increases with temperature and exhibits slope changes in correspondence of M1 and M2.

The samples previously heated at 673 K behave quite differently from the other ones: damping and modulus variations are very low and there are no $Q^{-1}$ maxima.
Differential Scanning Calorimetry (DSC)
M1 and M2 maxima were not found in samples previously heated to 673 K (dehydrated) thus they seem connected to the presence of water.

Since they appear at the same temperatures in successive thermal cycles their origin is attributed to the phase transformations occurring to ice by heating.

The M1 temperature exactly corresponds to that of the transformation of LDA ice into ice $I_C$ at about 150 K. This correspondence supports the hypothesis that a small part of water present in dentin could solidify directly to LDA ice during cooling.

LDA ice transforms to cubic ice during heating and is then responsible for M1.

The maximum M2 at 178 K is due to the transformation of the metastable ice $I_C$ to ice $I_h$. 
Conclusions

• **Above room temperature.** As temperature increases a broad and asymmetric $Q^{-1}$ maximum at 523 K has been observed; it is no more present during the successive cooling to room temperature testifying the occurrence of an irreversible transformation. Two stages have been identified, which basically correspond to the ascending (stage 1) and descending (stage 2) parts of the maximum. The results have been related to loss of fluids and degradation of collagen.

• A set of MS tests have been carried out vs. strain amplitude at room temperature. In each strain ramp $Q^{-1}$ progressively increases while modulus decreases. Recovery of original anelastic properties occurs when strain decreases to the initial value. The phenomenon has been ascribed to the breaking of weak H-bonds between polypeptide chains forming the triple-helix in collagen with consequent increase of the mean length of vibrating chain segments.

• **Below room temperature**. In the cooling stage of the first cycle $Q^{-1}$ exhibits a very broad maximum due to a series of phase transformations involving water present in the pores, in the interstices between fibres, between fibrils and inside collagen triple helix. The formation of ice Ih produces permanent damages to the dentin structure (rupture of fibres and fibrils) leading to a decrease of maximum intensity in the following cycles.

• In the heating stage of all the cycles two maxima, M1 and M2, have been observed around 155 K and 178 K, respectively related to the transformation of low-density amorphous (LDA) ice into cubic ice $I_C$ and to that of ice $I_C$ into ice Ih.
Papers


