How is residual stress/strain detected in bone tissue?

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Abstract
 Recent experimental approaches were reviewed to the residual stress/strain in bone tissue using X-ray diffraction (XRD) techniques. After a brief introduction of the experimental methods and obtained results, we discussed the generation mechanisms and biomechanical implications of the residual stress/strain in bone tissue. Strain gauge approaches provided the existence of residual stresses in the bone at the whole bone level. XRD approaches have also provided the existence of residual stresses at the tissue and mineral phase levels. The residual strains at the mineral phase related to the degree of orientation of the HAp crystals. The distributions of residual stress were obtained around the surface and along the radial depth of the diaphysis of quadrupedal extremities. The correlation between the residual stress and the osteon structures was indicated and the difference of residual stress with growth was revealed. It would appear that the residual stress state might be generated by the indeterminate structure in the hierarchical structures of the bone tissue relating to bone adaptation with the bone formation and reconstruction process. A long-term study is needed to better understand the generation and biomechanical implications of residual stress in bone tissue throughout the hierarchical structure during maturation and aging.

Key words : Biomechanics, Bone, Residual stress, Hierarchical structure, X-ray diffraction, Hydroxyapatite crystal

1. Introduction

Bone tissue is subject to in vivo stresses that are static stresses due to the body weight and cyclic, dynamic stresses due to the daily movement, and is optimized for the mechanical environments with reconstructing its structure, generally called as “functional adaptation of bone”. Stress measurements of bone are essential for evaluating the risk of bone fracture, the cure of bone diseases (e.g., osteoporosis), bone turnover, and the bone adaptation. Few studies have reported the existence of residual stresses in bone tissue. The residual stress is defined as the stress that remains in the tissue without any external forces. The stress will be one of the important factors to understand the bone strength and bone adaptation. It is well known that living tissue, as blood vessels, is subject to residual stresses (Fung, 1990). In blood vessel wall, compressive residual stress exists in the inner wall and tensile stress exists in the outer wall along the circumferential direction, suggesting that the residual stresses decrease high in vivo stress applied to the inner wall due to the blood pressure. It appears that the residual stress in living tissue plays an important role in the mechanical strength. However, the residual stresses in the bone tissue have not been fully investigated and elucidated.

Researches attempted to measure the strain of bone tissue by invasive procedures using strain gauges glued to the bone surface (Al Nazer et al., 2012). For instance, large strain was obtained in vivo as \(9.096 \times 10^{-6}\) on the human medial tibia during basketball rebounding (Milgrom et al., 2000), also large strain rate was measured in vivo as \(-85,500 \times 10^{-6}/s\) at the human distal radius during forward fall from standing, landing on extended hands (Földhazy et al., 2005). Tanaka and Adachi (1994) measured the changes in the strain gauge values attached to the surface of rabbit tibia in situ by cutting the fibula, suggesting the existence of residual stresses in the tibia-fibula indeterminate structure. Adachi et al. (1998) used strain gauges bonded onto the cortical surface of bovine coccygeal vertebrae aligned in the
cephalocaudal and circumferential directions and reported the changes in strains induced by removal of the end-plate and the cancellous bone. The results suggested the existence of compressive residual stress in the cortical bone and tensile residual stress in the cancellous bone in both the cephalocaudal and the circumferential direction. However, strain gauge approaches have some limitations; it requires specimen destruction processes (i.e., cutting and boring) and provides residual stresses in indeterminate structure at whole bone level.

As shown in Fig. 1, bone has a hierarchical structure, spanning from the macrostructure at several millimeters or whole bone level, the microstructure at several hundred micrometers level, including osteons and Haversian canals in the cortical bone, to the nanostructure at hydroxyapatite (HAp) crystals and collagen fibrils level. It is well known that bone is usually replaced by new tissue (Currey, 2002; Fung, 1990). Since the new tissue develops under in vivo loadings as the non-deformed state, an indeterminate structure may be generated by the difference of the deformation between the old and new phases. The mechanical properties (e.g. elastic modulus) are also different in these phases (Gibson et al., 2006; Rho et al., 1999). Because of such non-uniform structures, residual stress/strain will remain in the bone tissue even without external forces being applied. Therefore, to fully understand the residual stresses in the bone tissue, it is important to investigate the residual stress/strain related to the indeterminate structure in the hierarchical structures.

X-ray diffraction (XRD) is an alternative promising tool to obtain measurements of the stress/strain in bone tissue, because X-rays have nondestructive and noninvasive properties (Tadano and Giri, 2011). It is possible to obtain the distribution of the stress/strain state in the tissue because X-rays have can pass through the tissue and can be collimated locally. Some studies reported the deformation of hydroxyapatite (HAp) crystals in bone tissue under external loads using X-ray diffraction techniques (e.g., Borsato and Sasaki, 1997; Almer and Stock, 2005, 2007; Fujisaki et al., 2006; Gupta et al., 2006; Fujisaki and Tadano, 2007; Tadano et al., 2008; Akhtar et al., 2008a, 2008b, 2011; Giri et al., 2012; Singhal et al., 2012; Yamada et al., 2013b, 2014b). It has been shown that the distance between the lattice planes of the HAp crystals changes proportionally to the deformation of the bone tissue (Fujisaki and Tadano, 2007). The HAp crystal strain can be calculated by the deformation of the interplanar spacing compared with a reference state (Fujisaki et al., 2006; Tadano et al., 2008). Based on this, the residual stress/strain in the bone tissue is measured by XRD.

In this article, we review recent experimental approaches to reveal the residual stress/strain in bone tissue by the methods based on XRD. After a brief introduction of the experimental methods and obtained results, we discuss the generation mechanisms and biomechanical implications of the residual stress/strain in bone tissue.

2. X-ray diffraction for bone tissue

Bragg’s law, the fundamental equation for X-ray diffraction, is expressed as in Eq. (1)

\[ 2d \sin \theta = n \lambda \]  

(1)

Fig. 1 Five levels of hierarchical structure in cortical bone; (I) Macrostructure level (10 mm to several cm), or whole bone level, consisting of cortical and trabecular bone types. (II) Mesostructure level (0.5–10 mm), or cortical bone level. (III) Microstructure level (10–500 µm), single osteon and interstitial lamella level in cortical bone. (IV) Sub-microstructural level, single lamella level (1–10 µm). (V) Nanostructure level (<1 µm), multiphase nanocomposite consisting of an organic phase, an inorganic phase and water. Reprinted from Tadano and Giri (2011), with permission from TAYLOR & FRANCIS INFORMA UK LTD.
where $\theta$ and $d$ are the Bragg angle and the interplanar spacing at a specific lattice plane ($hkl$) of HAp crystals in bone tissue, respectively.

The HAp crystal strain $\varepsilon^H$ is defined as in Eq. (2) using the changes in the interplanar spacing of the crystals, where the subindex 0 indicates the values at the non-strained state.

$$\varepsilon^H = \frac{d - d_0}{d_0}$$

(2)

3. Residual stress/strain of mineral phase in bone tissue

Some researches attempted to obtain the stress/strain state of the mineral phase in bone by using XRD (Almer and Stock, 2005, 2007; Giri et al., 2008; Stock et al., 2011; Hoo et al., 2011).

Almer and Stock (2005, 2007) detected the stress state of mineral phase inside a beagle fibula and a canine fibula under in situ compressive loading by XRD with high-energy synchrotron X-rays (80.7 keV). The deviatoric stress of mineral phase $\sigma^H_x$ of the bone was calculated from deviatoric strains of HAp crystals ($\varepsilon^H_x$ and $\varepsilon^H_y$) and the Kröner–Eshelby model as in Eq. (3)

$$\sigma^H_x = \frac{1}{S_x / 2} \left[ \varepsilon^H_x - \frac{S_1}{S_2 / 2 - 3S_1} (\varepsilon^H_x + 2\varepsilon^H_y) \right]$$

(3)

where $x$ axis was the bone axis and the loading direction in the experiments. The $y$ axis was the circumferential direction. $S_1$ and $S_2/2$ were X-ray elastic compliances for a specific lattice plane of the HAp crystals in the model. Additionally, $\varepsilon^H_y$ and $\varepsilon^H_z$ were assumed to be equal here.

In the experiments, the diffracted X-rays transmitted through the bones were detected, and the stress/strain in the mineral phase was the weighted average of the X-ray pathway from the irradiated surface to the opposite surface of the diaphysis. As a result, internal stress of the mineral phase was proportionally increase with applied tissue stress, as shown in Fig. 2 (Almer and Stock, 2005). Under no external load, compressive residual stresses were observed in the mineral phase inside the bone specimen along the bone axis on the order from -60 to -200 MPa. The amount of residual stresses in the mineral phase was different between (222) planes and (002)/(004) planes. Furthermore, it was also noted that the amount of residual stress in the mineral phase decreased with immersion time in saline solution (Almer and Stock, 2007).

Giri et al. (2008) noted the site-specific residual strain around the small hole, called foramen, in bovine metacarpals and femurs detected by XRD (Fig. 3). The diffracted X-rays penetrated the sliced specimens and then were detected by an imaging plate (IP), which is a two-dimensional X-ray detector. The traveling direction of the diffracted X-rays...
depends on the direction and the displacement of the lattice planes in the HAp crystals. The measurements assumed the plane stress state and Poisson’s ratio as 0.29. Residual strains were found to exist around the foramen in the specimens and trends of residual strains were mostly consistent with the degree of orientation of the HAp crystals, which may reinforce the tissue around the hole (Giri et al., 2007). It may be able to explain the response behavior of bone to the mechanical loading history near the foramen.

4. \textit{Sin}^2\psi \textbf{method for residual stress in the surface region of the bone tissue}

Todoh et al. (2000) reported to detect the residual stresses in bone tissue at the tissue level by calculating the HAp crystal strain measured with XRD. The surface region (up to 200 µm depth) of the cortical bone specimens from bovine femurs was examined. Tadano and Okoshi (2006) also investigated the residual stresses in rabbit tibiofibula. In these studies, the HAp crystal strain was calculated from the displacement of the lattice plane with reference to the lattice plane in bone powder as non-strained specimens. However, the lattice planes of the bone powder were much influenced by the particle size (Tadano and Okoshi, 2006). The non-strained state of the bone tissue was not easy to decide.

Fig. 3 (a) A foramen specimen in a bovine femur. (b) XRD setup. (c) Degree of HAp crystal orientation for the bone axis \(<\cos^2\beta>\) around the foramen. (d) Residual strain of the (211) planes in the HAp crystals along the bone axis (\(×10^{-6}\)). Reprinted from Giri et al. (2008), with permission from Elsevier.

Fig. 4 Interplanar spacing \(d\) of HAp crystals at the bone surface in cortical bone tissue under (a) non-strained and (b) tensile loading states. The variation in \(d\) with orientation \(\psi\) normal to the lattice planes of the HAp crystals is shown in polar coordinates, and the lengths and directions of the vectors in the diagrams show the interplanar spacing and plane-normal direction, respectively. In the non-strained state the interplanar spacing is \(d_0\). Under tensile loading horizontal to the surface of the specimen, the lattice plane deforms and the interplanar spacing of the lattice planes oriented in the loading direction is the largest and that oriented normal to the surface the smallest (\(d_1 < d_2 < d_3\)). Reprinted from Yamada et al. (2011a), with permission from Elsevier.
An XRD technique based on the \( \sin^2 \psi \) method solves the problems on the non-strained state. The angle of inclination \( \psi \) is defined as the angle between the normal direction of the specimen surface and the diffracted lattice plane. As suggested in Fig. 4a, in the non-strained state the interplanar spacing is \( d_0 \). Under tensile loading horizontal to the surface of the specimen, the interplanar spacing in lattice planes under tensile stress in the \( \psi = 90^\circ \) direction is larger than that in the \( \psi = 0^\circ \) direction (Fig. 4b). The relation between \( d \) and \( \psi \) is affected by the intensity of the stress. Based on this, residual stress is measured from the variation of the interplanar spacing of HAp crystals without a comparison against non-strained samples or information on the interplanar spacing in the stress direction (Yamada and Tadano, 2010).

A coordinate system is fixed at the bone surface, and the \( x, y, \) and \( z \) axes correspond to the bone axis, circumferential, and radial directions, respectively. The \( \psi \) is defined as the angle in the \( x-z \) plane. Cortical bone in the diaphysis of the extremities is considered as an orthotropic compound. It is assumed that the residual stress state in the measurement region is a plane stress, because the X-rays generated by X-ray tubes only penetrate up to 100 \( \mu \)m into the specimens and only the outermost region is measured. The relationship between bone tissue stress \( \sigma \) and HAp crystal strain \( \varepsilon^H \) in the bone is assumed to be described by Eq. (4)

\[
\begin{pmatrix}
\varepsilon_x^H \\
\varepsilon_y^H \\
\varepsilon_z^H
\end{pmatrix} =
\begin{pmatrix}
\frac{1}{E^*} & -\frac{v^*}{E^*} & -\frac{v^*}{E^*} \\
-\frac{v^*}{E^*} & \frac{1}{E^*} & -\frac{v^*}{E^*} \\
-\frac{v^*}{E^*} & -\frac{v^*}{E^*} & \frac{1}{E^*}
\end{pmatrix}
\begin{pmatrix}
\sigma_x^H \\
\sigma_y^H \\
\sigma_z^H
\end{pmatrix}
\tag{4}
\]

where \( E^* \) and \( v^* \) are X-ray elastic constant and X-ray Poisson's ratio respectively, and these are the elastic properties between the bone tissue stress and the HAp crystal strain. The bone tissue stress \( \sigma^H \) in the bone axis is calculated with the relationship between \( \varepsilon^H \) in the \( \psi \) direction and \( \sin^2 \psi \) as in Eq. (5).

\[
\sigma_x^H = \frac{E^*}{1 + v^*} \frac{\partial \varepsilon_y^H}{\partial (\sin^2 \psi)}
\tag{5}
\]

The bone tissue stress \( \sigma \) in the bone axis is described with \( \theta \) and \( \psi \) using Eqs. (1) and (2) as in Eq. (6)

\[
\sigma_x = -\frac{E^*}{2(1 + v^*)} \frac{\pi}{180} \cot \theta_0 \frac{\partial (2\theta)}{\partial (\sin^2 \psi)} = K \frac{\partial (2\theta)}{\partial (\sin^2 \psi)}
\tag{6}
\]

where \( K \) is the stress constant.

Therefore, the residual stress \( \sigma_x^H \) in the bone axis is calculated from the variation of the interplanar spacing of HAp crystals in the \( x-z \) plane by using X-ray diffraction. The calculated stress is the deviatoric stress because the hydrostatic deformation resulting from hydrostatic stress is not detected. The residual stress \( \sigma_y^H \), in the circumferential direction is also calculated from the variation of the interplanar spacing of HAp crystals in the \( y-z \) plane. To measure \( \partial (2\theta)/\partial (\sin^2 \psi) \), rotating and tilting the sample is required with a conventional X-ray diffraction instrument and a scintillation counter. To determine \( K \), a four-point bending test of a thin bone specimen with X-ray irradiation was conducted and the relationship between applied bone tissue stress \( \sigma^H \) and \( \partial (2\theta)/\partial (\sin^2 \psi) \) was measured as a calibration (Yamada and Tadano, 2010; Yamada et al., 2011a; Yamada and Tadano, 2013a).

Using this method, adult bovine femurs were examined (Fig. 5a, Yamada and Tadano, 2010). The diaphyseal specimens were 50 mm long in the bone axis. The bone marrow and the soft tissue around the surfaces were removed, and the specimens were air-dried. The X-ray diffraction profile from the (211) planes of HAp crystals, which included (112) and (300) planes, was detected by using an X-ray diffractometer with characteristic Mo-Ka X-rays (\( \lambda = 0.071 \) nm). The residual stresses were measured along the bone axial and circumferential directions around the specimens.

Figure 5b shows an example of residual stress distributions in the bone axial and the circumferential directions in
the middle section of the femoral diaphysis. The residual stresses in the bone axial direction were everywhere tensile. The stresses in the circumferential direction were smaller than in the bone axial direction and some positions were close to zero. After the measurements, a diaphysis specimen was cut 2 mm above, in the upward direction from, where the stress measurements were made. Here the residual stress after the cutting was lower than before the cutting. It suggests that residual stress was released and that a new equilibrium was established in the bone, and that there is residual stress in the bone axial direction at the surface of the bovine femoral diaphysis.

Furthermore, diaphyseal surface of the extremities from adult rabbits were examined (Fig. 6a, Yamada et al., 2011a). Three femur, three tibia/fibula, three humerus, and three radius/ulna specimens were used in the experiment. The specimens were 60 mm long in the bone axial direction. The bone marrow and the soft tissue around the surfaces were removed and the specimens were kept in saline until just before the X-ray measurements. The residual stresses along the bone axis were measured at the bone surface of the anterior and posterior positions in each extremity using an X-ray diffractometer with characteristic Mo-Kα X-rays. The specimens were air dried after the X-ray

![Fig. 5](image-url) Fig. 5 (a) Diaphyseal specimens of bovine femurs. (b) Residual stresses in the middle section of a diaphysis of the bone axial and the circumferential directions. After the same specimen was cut 2 mm above, in the upward direction from, where the stress measurements were made. A=anterior, AL=lateral anterior, L=lateral, LP= lateral posterior, P=posterior, PM=medial posterior, M=medial, and MA=medial anterior. Reprinted from Yamada and Tadano (2010), with permission from ASME.

![Fig. 6](image-url) Fig. 6 (a) Diaphyseal specimens of rabbit extremities. Measurement positions were anterior and posterior at the center of the diaphyses. (b) Typical microscopic images at the X-ray measurement positions in the hindlimb bones of the same limb. The dashed lines show a depth from the surface of about 100 µm, and the black arrows indicate osteons included wholly or partly in the region. Reprinted from Yamada et al. (2011a), with permission from Elsevier.
measurements and then they were cut out, to observe the microstructures at the X-ray measurement position in the transverse cross-section of the specimens (Fig. 6b). The number of osteons in the region was counted and the osteon population density (OPD) was calculated. The osteons were quantified to secondary osteons (Haversian systems) and primary osteons.

Figure 7a shows the distribution of residual stress in the rabbit limb bones. Tensile residual stresses were also observed at the bone surface. The hindlimb bones were subject to tensile residual stress 1.4 times higher than that in the forelimb bones. In the femur and humerus, the OPD in the anterior positions were larger than that in the posterior positions. In the tibia, the OPD in the posterior position was larger than that in the anterior position. Overall, more osteon structures were observed in the positions subjected to higher residual stresses. As shown in Fig. 7b, there was a statistically positive correlation between the residual stress and the osteon population density (OPD) \( r = 0.55, p < 0.01 \).

5. XRD-IP system based on the \( \sin^2 \psi \) method

The \( \sin^2 \psi \) method required a complicated experimental setup, long irradiation time, and limitations of the sample size in the longitudinal direction because rotating and tilting the sample is required to obtain the distribution of the HAp crystal deformation. The method could not be directly applied to the measurements of residual stress distribution \textit{in situ} and \textit{in vivo}. To profoundly enhance the investigating of residual stress distribution in bone tissue, it is necessary to develop an improved method that features a simple setup without limitations on the sample size and shape.

Imaging plate (IP) can obtain the distribution of the diffracted X-rays from the HAp crystals with only one irradiation. The distribution of the HAp crystal deformation is then calculated from the XRD pattern detected via the IP. A measurement system using an XRD technique with the IP set in the reflection side (XRD-IP system) has been proposed for obtaining the surface distribution of residual stress in the diaphysis of extremities without limitations of the sample size and long irradiation time (Yamada et al., 2014a).

Figure 8 shows the measurement setup of the XRD-IP system. The diaphysis specimen is irradiated with characteristic X-rays and the resulting diffracted X-rays are detected by the IP in the reflection side. The \( x' \)-\( y' \)-\( z' \) coordinate system is defined as the X-ray coordinate system and the incident X-rays enter the specimen along the \( y' \)-axis. The \( x' \)-axis corresponds to the \( x \)-axis and the angle between the \( y' \)- and \( y \)-axes is set to 7.25°, which is the Bragg angle \( \theta_b \) of the (211) lattice planes in the HAp crystals in a non-strained state. The \( X' \)-\( Y' \)-\( Z' \) coordinate system is fixed at the IP surface and the \( Y' \)-axis corresponds to the \( y' \)-axis. The \( 1 \)-, \( 2 \)-, and \( 3 \)-axes are the principal axes and the \( 3 \)-axis corresponds to the \( z \)-axis. The residual stress state in the measurement region is assumed as a plane stress. The angle \( \psi \) is defined as the angle between the \( 1 \)- and \( x \)-axes.

The XRD pattern, which was a portion of the Debye ring of (211) lattice planes in HAp crystals, was detected by the IP. The direction of \( \epsilon_{y'} \) was slightly inclined at an angle of \( \Delta \phi \) from the \( x \)-\( z \) plane toward the \( y \)-axis with increasing \( \beta \) (Fig. 8b). Because \( \Delta \phi \) was presumed as negligibly small, \( \cos(\psi + \Delta \phi) \) and \( \sin(\psi + \Delta \phi) \) were approximated as \( \cos^2 \psi \) and \( \sin^2 \psi \), respectively. Then, the bone tissue stress \( \sigma_i \) in the bone axis is calculated based on Eq. (6). Therefore, the residual stress is calculated via the portion of the Debye ring of (211) lattice plane in HAp crystals detected by the IP in the reflection side.
6. Residual stress distribution along the radial depth of cortical cylinders by synchrotron

The residual stress distribution in the deeper region of the diaphysis of extremities is not measured using the previous methods based on the $\sin^2\psi$ method. An alternative method for measurement of residual stresses in deeper regions of the diaphysis has been proposed using synchrotron white X-rays (Yamada et al., 2011b).

A coordinate system is fixed at each measurement location inside the bone and the $x$, $y$, and $z$ axes are defined as the principal axes. It is assumed that the diaphysis is not subject to residual stress in the radial direction ($\sigma^B_z = 0$). Under the assumptions, Eq. (6) is satisfied.

The energy-dispersive X-ray diffraction with synchrotron white X-rays is used to measure the $d$–$\psi$ relationship. The relationship between the wavelength $\lambda$ and energy $W$ of X-rays is expressed as in Eq. (7), where $h$ is Planck’s constant and $c$ is the speed of light, and the relationship between $d$ and $W$ is expressed as in Eq. (8).

$$\lambda = \frac{hc}{W} \quad (7)$$

$$d = \frac{hc}{2W \sin \theta} \quad (8)$$

The $d$ is measured from the energy $W$ of the diffracted X-rays when $\theta$ is fixed. Therefore, the $\sigma_x$ at each measurement locations inside the cortical bone is calculated form Eq. (9).

$$\sigma^B_x = \frac{E^*}{d_0(1 + \nu^*)} \frac{hc}{2 \sin \theta} \partial(1/W^2) \partial(\sin^2 \psi) \quad (9)$$

To understand the force equilibrium of residual stresses inside the bone, the residual stress distribution along the radial depth of bovine femurs was investigated using the method (Yamada et al., 2011b). Furthermore, to investigate the effects of growth on the distribution, different animal ages were examined (Yamada and Tadano, 2013a). Diaphyseal specimens were obtained from less-than-one-month-old (Group Y) and two-year-old (Group M) bovine femurs. The bone marrow and the soft tissue around the surfaces were removed and the specimens were air-dried. The synchrotron white X-ray diffraction was performed at the BL28B2 beamline of SPring-8, a large synchrotron radiation facility managed by RIKEN and JASRI. The diaphysis specimens from Group Y were measured at 0.5-mm intervals from the outer surface to the deeper region of the specimens at four positions: anterior, posterior, lateral, and medial. The Group M samples were measured at 1-mm intervals.

Figure 9 shows typical distributions of interplanar spacing of the HAp crystals and residual stress along the bone axis from the outer surface region to the deeper region in a diaphysis specimen from Group M.
stresses inside the diaphysis. In the measurements, it was assumed that the diaphysis specimen in the radial direction was not subject to residual stresses. The distribution of interplanar spacings closely corresponded to the distribution of residual stresses. It suggests that even when the results included the radial component, this component may have little effect on the measured values. Although the amount of surface residual stresses were smaller than the results from the measurements with characteristic X-rays generated by X-ray tubes, it may be affected by the difference of the measured volume at the surface region.

Figure 10a shows typical distributions of residual stress at the anterior part in the diaphysis specimens from Group Y and Group M. In Group Y, residual stresses did not vary with the depth from the surface. There was no significant difference between the surface and deeper regions in Group Y. On the other hand, in the Group M, there was a strong significant difference between the residual stresses measured at the surface region and in the deeper region (Fig. 10b). The value of residual stresses had a positive statistical correlation with the cortical thickness.

7. Limitations of the experiments

Some studies used diaphyseal specimens taken from whole bones. To evaluate the effects of the cutting on the measured stress state, strain gauges glued on the surface of the mid-diaphysis in a thawed whole femur of a mature bovine and the diaphysis specimen with 60 mm long was taken from it. The strain changing in the bone axis were smaller than 50µ. It suggests that the cutting process may have less impact on the residual stress in the measurements.

![Fig. 9](image) (a) Radial distribution of the interplanar spacing $d_{(002)}$ of the (002) lattice plane in the bone axial direction from the outer surface to the inside of the diaphysis specimen at the four parts: anterior (A), posterior (P), lateral (L), and medial (M). (b) Typical distribution of the residual stress in the bone axis from the outer surface to the inside of the diaphysis specimen at the four parts. Reprinted from Yamada et al. (2011b).

![Fig. 10](image) (a) Residual stresses between the two groups at the anterior position. Each value indicates the average of five specimens, and the errors corresponding to their standard deviations. (b) Comparisons of residual stresses between the surface and deeper regions of the diaphysis specimens and the averages of cross-sectional area. The inner region in Group M is located at the 3-mm depth from the surface, and that in Group Y is located at the 1.5-mm depth. Reprinted from Yamada and Tadano (2013a), with permission from Elsevier.
(Yamada and Tadano, 2013a). Although the preparation may release some amount of residual stress from in vivo whole bone, the residual stresses that occurred in these specimens were detected at the tissue level.

In some studies, air-dried specimens were used. The effects of air-drying process might have less impact on the measured deviatoric stresses, although there may be hydrostatic compressive deformation related with the sample volume changes due to the air-drying. In deed, the trend that there were tensile residual stresses around the surface region of the mature bovine femurs (Yamada and Tadano, 2010, 2013a) corresponded to the results of the study with the limb bones of adult rabbits that kept saline solution just before the measurements (Yamada et al., 2011a). However, Almer and Stock (2007) and Tung et al. (2013) noted the effects of hydration/dehydration on the residual stress in the mineral phase. A further study of these effects should be conducted.

Synchrotron X-rays are able to pass through the thick bone specimen like diaphyses and are useful to detect the HAp crystal deformation state inside the bone. Further, the method is also applied to the collagen phase (Almer and Stock, 2005, 2007; Gupta et al., 2006; Dong et al., 2011). However, Singhal et al. (2011) and Tung et al. (2013) pointed out that the residual stress/strain in the mineral phase in bone tissue markedly changed with increasing high-energy X-ray doses, because of the damage at the HAp-collagen interface in the tissue. To study the bone residual stresses, it is important to minimize the X-ray energy and irradiation time.

8. Generation mechanisms and biomechanical implications of bone residual stress

Strain gauge approaches provided the existence of residual stresses in the bone at the whole bone level. XRD approaches have also provided the existence of residual stresses at the tissue and mineral phase levels. It was investigated that the residual strains at the mineral phase related to the degree of orientation of the HAp crystals (Giri et al., 2008). It also has been attempted to indicate the distributions of residual stresses around the surface and along the radial depth of diaphysis of extremities. The positive correlation between the residual stress and the osteon structures was indicated (Yamada et al., 2011a). Furthermore, the difference of residual stress with growth was indicated (Yamada and Tadano, 2013a). It would appear that the residual stress state might be generated by the indeterminate structure in the hierarchical structures of the bone tissue relating to bone adaptation with the bone formation and reconstruction process.

The young bones were not subjected to residual stresses, whereas larger residual stresses were observed in the mature bones (Yamada and Tadano, 2013a). In addition, the mature bones showed a trend toward tensile residual stresses at the surface region and compressive residual stresses in the deeper region. The results suggest that residual stress is generated during growth, satisfying the equilibrium of forces between the surface and the deeper regions of the diaphysis. The differences in residual stress may be related to mechanical loads applied to bone tissue in vivo during bone formation or reconstruction. In vivo mechanical loading from body weight increases with growth. Because new tissue develops in a nondeformed state under in vivo loading, tensile stresses may occur in the new surface growth and compressive stresses may remain in the older, deeper bone tissue even after any external loadings have been removed. Based on this hypothesis, although the young bones develop at a rapid pace, the large residual stresses may not be generated in the young bones. In deed, bone residual stress/strain at the whole bone level detected in the strain gauge measurements provides the existence of the indeterminate structure at tibia-fibula and cortical-cancellous bone level.

However, because bone tissue has a hierarchical structure, the hypothesis may be quiet simplified. Residual stress showed the positive correlation with osteon structures (Yamada et al., 2011a). The population density and the geometry of osteon structures were correlated with the compression/tension mechanical environments in vivo (e.g., Skedros et al., 2009). The collagen-lamellar organization and mineral crystallite orientation also differ in these regions. In this perspective, these nonuniform structures of the tissue derived from the osteon formation and the internal organization of these entities down to the nanostructural level may explain the spatial differences in residual stress. Furthermore, it is well known that the mechanical and physicochemical properties of bone tissue depend substantially on age (e.g., Raghavan et al., 2012). Such heterogeneity among these properties may produce a locally indeterminate structure in the tissue.

The residual stress being nonuniform may be related to nonuniformities in the mechanical environments in vivo and the resulting functional adaptation of the bone tissue. The residual stress in the bone may be regarded as an epiphenomenon and may be a circumstantial finding of the adapted state. Giri et al. (2008) investigated that the trends of residual strain in mineral phases around the foramen were mostly consistent with the degree of orientation of the HAp crystals that reinforced the tissue. Adachi et al. (1998) discussed that bone residual stresses may work to allow a
stress state to become more uniform. The existence of residual stresses might have the potential to characterize the equilibrium state of mechanical bone adaptation by remodeling. The high tensile residual stresses measured at the bone surface of extremities (e.g., Yamada and Tadano, 2010) might work to reinforce the bone tissue in compressive and bending loadings in vivo. In deed, the surface region of the extremities is under severe mechanical environments. However, the limb bones used in the studies are subjected to too complex loadings (e.g. compression, bending, and torsion) (e.g., Skedros et al., 2009). Gautier et al. (2000) measured the maximum strain in the bone axial direction in sheep tibia during locomotion using strain gauges; and here the anterior part was subject to tensile and the posterior part to compressive stresses, and further, there were also the effects of the torsion in the strains. The measured residual stresses may not be directly related to the tension/compression strain in vivo. To understand the biomechanical implications of bone residual stress, in vivo stress measurements are quite important. The investigations of the residual stresses in the bones that are subject to simple in vivo loadings might be helpful. Furthermore, comparing the residual stresses of intact and adapted bones would provide more direct data for an elucidation of the biomechanical aspects of the bone residual stress.

A long-term study is needed to better understand the generation and biomechanical implications of residual stress in bone tissue throughout the hierarchical structure during maturation and aging.

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