Review Article

Stretched exponential relaxation of piezovoltages in wet bovine bone

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A B S T R A C T

It is important to determine the amplitude and variation characteristics of piezovoltage in wet bone, which can, in turn, be taken as a basis for studying whether electrical signals induced by external forces can affect the growth of bone cells. This work measured the characteristics of piezoelectric effects under dynamic and static loading. The results show that the variations of piezovoltage in wet bone in both loading and load holding periods follow a stretched exponential relaxation law, and the relaxation time constants of the piezovoltages are much larger than those of dry bone. This finding means that the active time of piezovoltage in wet bone is much longer than that of dry bone. Regardless of the loading and load holding processes, continuously increasing deformation in wet bone caused piezoelectric charges to be continuously induced and increased the dielectric constant of wet bone along with the deformation process. In general, compared with piezovoltage in dry bone, that in wet bone had lower amplitude and could exist for a longer duration. It can be inferred, therefore, that piezoelectricity might create coupling with the streaming potential in bone by changing the thickness of the double electrode layer.

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1. Introduction

Study of bone remodeling based on Wolf’s Law has been extensively reported (Ahn and Grodzinsky, 2009; Christen et al., 2012; Carrey, 2003; Qin and Ye, 2004; Skerry et al., 1988). The core of Wolf’s law is that the stresses in bone can stimulate the cell growth or remodeling of bone (Anderson and Eriksson, 1970; Fukada and Yasuda, 1964; Qu et al., 2006; Steinber et al., 1968; Yokota and Tanaka, 2005). Moreover, piezoelectricity and streaming potentials, also called stress generated potentials (SGPs), have been reported to exist in bone (Ahn and Grodzinsky, 2009; Anderson and Eriksson, 1970; Fukada and Yasuda, 1964). The question has arisen as to whether the electrical potential generated by stresses can stimulate the growth of bone cells instead of the stresses. The aim of this study is to variation characteristics of piezovoltage in wet bone.

During recent decades, reports have appeared in the literature on the effects of SGPs on bone remodeling. Fernandez et al. (2012) presented mathematically the distributions of piezoelectric charges on bone surfaces under different force boundary conditions. Qin et al. (2005) developed a theoretical solution for analyzing the surface remodeling of thermopiezoelectric bones. Minary-Jolandan and Yu (2010) studied the piezoelectric properties of collagen in bone at nanometer scale, and confirmed that the shear deformation of collagens arose from their inverse piezoelectric effect. Hou et al. (2011) measured the piezovoltages of bone samples through a three-point bending experiment and found that the variation of piezovoltage with time followed a stretched exponential relaxation law. Most of these studies were concerned with dry bones. For practical applications, a more meaningful study is needed to investigate the piezoelectric properties of wet bone, which is closer in nature to living bone. As far as we know, most previous studies have concentrated on measuring the piezoelectric coefficients of wet bone (Anderson and Eriksson, 1970; Bur, 1976). However, piezoelectric coefficients may be different at various points in a nonhomogeneous bone, and such differences would complicate measurement. To bypass this problem, this study analyzes the waveform variations of piezoelectric voltage in wet bone by measuring those voltages under static and dynamic loadings. The measured results show that the variation of piezovoltage with time follows the stretched exponential law during both loading and load holding processes. The results indicate that the viscoelasticity of wet bone plays an important role in piezovoltage relaxation. During loading and load holding processes, the deformation of bone occurs continuously due to the viscoelasticity of wet bone, which leads to the variation of piezocharges.

2. Materials and methods

The experimental procedure we employed in this work is similar to that used in Xu et al. (2013). Fig. 1 shows the experimental setup for measuring bone piezovoltage. Thin circular plate samples used were harvested from the mid-diaphysis of dry bovine (age 2–3 years) tibias, with their surfaces parallel to the axis line and facing the radius direction (Fig. 1(a)). These samples were 15 mm in diameter and 1.5 mm in thickness.

In our experiment, the sample was first immersed in phosphate-buffered saline solution (0.145 M, pH 7.3) (Garon et al., 2002; Quenneville et al., 2004) and was fixed in the plexiglass reservoir as a circular plate with a clamped edge. Then, a force was applied at the centroid of the upper sample surface via a plexiglass force bar installed in an Instron E1000 universal test machine. When the bone sample was bent by the force, piezoelectric charges were induced and distributed on both its surfaces. The ions in the buffer solution gave the solution weak conductivity; hence the two platinum/iridium electrodes could sense or test the voltage without touching the bone. Because the head stage has very high input impedance there is almost no electric current passing through the solution into the head stage. That is, the solutions above the upper surface and below the down surface have the same electric potentials as those at the upper and down surfaces respectively, which means that the electrodes can test the surface potentials of the sample without touching the surfaces.

The voltage signals were sent to a bioamplifier (BMA-931, CWE Inc., USA) via an ultra-high-input impedance (over 10$^{12}$ Ω) head stage (HS) (Super Z, CWE Inc., USA). The amplified voltage signals were in turn recorded by a computer connected to the bioamplifier.

It should be pointed out that the piezovoltage in wet bone is very weak and easily affected by the ambient electromagnetic field. To avoid this effect, the apparatus used was placed in a double-layer shield box. The inner shield layer was connected to the common ends of all the devices and the outer layer was connected to the earth (with zero voltage).

3. Results

The results shown in Figs. 2–5 were obtained from experiment as follows. A total of six samples were used to ensure reliable and repeatable results. Testing was performed after the samples had been immersed in the solution for over 12 h. A trapezoidal loading profile was used in which the maximum load was 18 N and the initial load was 0 (Fig. 2(a)). The duration time, $t_0$, for the linear increase of the load from its initial state to the maximum (or vice versa), was 0.25 s, 0.5 s, 1 s and 3 s respectively. The loading profile obviously involved both dynamic and static processes. The loading and unloading processes were dynamic, with the load increased or decreased uniformly, whereas load holding was a static process.

Fig. 2(b)–(e) shows some typical plots for the variation of piezovoltage with time for sample No. 1. Each plot corresponds to a loading profile with a given loading duration time $t_0$ and has negative and positive pulses corresponding to the loading and unloading processes, respectively.

The peak point of these plots always occurred during loading changes. It can be seen that the piezovoltage started to decay (or decrease) towards zero once the loading or unloading process came to an end. For the loading durations $t_0=0.25$ s, 0.5 s, 1 s and 3 s, the peak piezovoltage values of sample 1 are shown in Table 1 as $-4.36$, $-3.96$, $-3.16$, and $-1.9$ mV, respectively. These results mean that piezovoltage value
increased along with an increase in the loading duration. The remaining 5 samples are also found from Table 1 to have similar peak values of piezovoltage.

The pattern of the piezovoltage plot at different loading durations is that the pulses represent the dynamic processes and relaxation occurs during load holding.

Table 1 lists the peak piezovoltages of the six samples corresponding to 4 loading and unloading durations. Compared with the corresponding value for $t_0 = 0.25$ s, the average piezovoltage for $t_0 = 0.5$ s is 80%; for $t_0 = 1$ s is 57%; and for $t_0 = 3$ s is 30% of that for $t_0 = 0.25$ s.

4. Discussion and exponential law of piezovoltage

As in the procedure used for dry bone (Hou et al., 2011), in the loading process with wet bone, the piezovoltage decay or relaxation here also followed a stretched exponential law as

$$V(t) = KF(t) \exp(-t/\tau)^{\beta_1}$$

$$F(t) = F_0 \exp(-t/\tau_d)$$

where $t$ is the time, $\beta_1$ ($0 < \beta_1 < 1$) is the stretching exponent, $\tau_d$ is a time constant for the loading and unloading processes, $K$ is a proportional coefficient denoting the proportional relationship between the load and piezovoltage, and $F(t)$ is the load profile function with $F_0$ being the maximum load and $t_0$ the loading or unloading time. The piezovoltage follows a typical exponential law in load holding:

$$V(t) = V_0 \exp(-t/\tau)$$

$$V_0 = KF_0 \exp(-t_0/\tau_d)$$

where $V_0$ is the piezovoltage value at the end of loading and $\tau$ is the relaxation time constant in the load holding process.

As explained in Hou et al. (2011), Fu et al. (2012), the physical meaning of Eqs. (1) and (2) is that the piezoelectricity of bone arises from shear deformation between the collagen fibrils in bone. That is, when two adjacent fibrils slip mutually, a piezo-charge appears. Because collagen fibrils distribute randomly, the shear stresses in these fibrils may also be different, such that different piezovoltages within an area and to the material may have different time constants.

That is the reason why the piezovoltage decay follows the stretched exponential law. On the other hand, in the load holding process, deformation of dry bone stops and there is no slippage between collagen fibrils, in which case the decay pattern follows a typical exponential law. That is, as long as bone deforms continuously, piezocharges will be induced and the decay process in piezovoltage follows a stretched exponential law. Here we employed the same method as that in Hou et al. (2011) to analyze the measured data of wet bone. We found that the results were to some extent different from those for dry bone. Figs. 3 and 4 are the fitted results of sample No. 1 for loading and unloading processes respectively. The black curves are the measured piezovoltage and the red curves are the fitted ones.

The fitting results show that the variation of piezovoltage of wet bone still follows the stretched exponential law, and it also follows the same relaxation law (1), rather than the typical exponential law (2) in the load holding process found with dry bone. In this case, $V(t)$ in Eq. (2) is changed as

$$V(t) = V_0 \exp(-t/\tau)^{\beta_2}$$

where $\beta_2$ is the second stretching exponent for the load holding process.

Taking sample No. 4 as an example, Fig. 5 illustrates the measured and fitted piezovoltages in the loading and load

![Fig. 1 - Schematic diagram of measurement setup.](image-url)
holding processes. These results show the same trends as those in Fig. 3.

Table 2 lists the fitted characteristic parameters of all six samples. For comparison, the characteristic parameters of dry bone reported in Hou et al. (2011) are also listed.

It can be seen from Table 2 that the stretching exponents $\beta_1$ of wet bone for the loading process are greater than those of dry bone ($p<0.05$), and are closer to 1. The results indicate that the time constants of piezovoltage decay of the wet collagen fibrils in different directions tend to be the same. This may be due to the ability of the water molecules in wet bone to reduce the frictional resistance between fibrils, which is equivalent to reducing the effect of shear stresses. Although the shapes and deformation modes of the samples used are different from those in Hou et al. (2011), their stress levels are not significantly different. The shear stress in dry bone at the measured location was about 1.1 MPa, inducing 3.5–4 mV of piezovoltage, and the average shear stress (the maximum stress surrounding the load point) in wet bone is 2.39 MPa, inducing 6 mV of piezovoltage. This result indicates that the average voltage produced by unit stress is similar.

It can be seen from Table 2 that a significant difference exists in the stretching exponent $\beta_2$ between dry bone and wet bone ($p<0.05$). $\beta_2$ for dry bone is equal to 1 (Hou et al., 2011) and that for wet bone is averaged at 0.727. The $\beta_2<1$ means that the piezovoltage decay in wet bone also has a stretched exponential behavior in the load holding process, which implies that piezo-charges are being continuously induced due to the changes of stresses. It is most likely that the viscoelasticity of wet bone (Johnson et al., 1980; Lakes et al., 1977; Singh and Saha, 1984) cause deformations to arise during load holding, and the charges are induced continuously.

During the loading process, the maximum of the time constant $\tau_d$ is 2464 ms (sample 1) and the minimum is 755 ms (sample 4), which is about two orders of magnitude greater than the average maxima of dry bone ($p<0.05$). Similarly, the time constant $\tau_c$ is also greater than that of dry bone in the load holding process ($p<0.05$), although the difference of $\tau_c$ between dry bone and wet bone is not as significant as that of $\tau_d$. The average $\tau_c$ for wet bone is 475 ms compared to the average for dry bone of 88.5 ms, which does not exceed 0.5 order in magnitude.

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**Fig. 2** – Loading and piezovoltage waveform for sample No. 1. (a) Loading waveform, (b) piezovoltage waveform for $t_0=0.25$ s, (c) piezovoltage waveform for $t_0=0.5$ s, (d) piezovoltage waveform for $t_0=1$ s, (e) piezovoltage waveform for $t_0=3$ s.
To understand the effect of stretching exponents, a special fitting curve with blue color is plotted in Fig. 3(a), which fits the load holding process. The curve has the same fitting parameters as the practical fitting curve (Red curve) except the stretching exponents $\beta_2$. The practical $\beta_2$ is 0.71, while the special fitting curve takes the $\beta_2$ equal to 1, which returns to a typical exponential fitting. Fig. 3(a) shows that the special curve does not coincide well with the measured curve (black color) as the stretching exponents fitting. This is the substantial action of the stretching exponents.

It is noted that a sample with positive and negative polarization charges on both its surfaces and its bulk can be equivalent.
to that of a parallel plate capacitor (Johnson et al., 1980; Singh and Saha, 1984), the capacitance of which is proportional to the dielectric constant of bone. Since the dielectric constant of wet bone is greater than that of dry bone (Lakes et al., 1977; Marzec and Warchol, 2005; Saha et al., 1984), the corresponding $\tau_d$ and $\tau_c$ of wet bone increase naturally with respect to those of dry bone. Their physical meaning is that unlike charges on the two surfaces of a sample discharge through the sample bulk slowly. Although the ions in the buffer solution decrease the resistance of the bulk of bone, causing the charges to discharge quickly or the piezovoltage to decay quickly, the tendency of capacitance increase does not change. The issue to which attention should be paid is why $\tau_d$ and $\tau_c$ in wet bone increase with different amplitudes in relation to those in dry bone.

![Diagram](image)

**Fig. 5** – Fitting functions for different $t_0$ for sample No. 4. (a) Measured voltage and fitted curve at $t_0=0.25$ s and $F=18$ N, (b) measured voltage and fitted curve at $t_0=0.5$ s and $F=18$ N, (c) measured voltage and fitted curve at $t_0=1$ s and $F=18$ N, (d) measured voltage and fitted curve at $t_0=3$ s and $F=18$ N.

**Table 1** – Peak values of piezovoltage of six samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.25 s</th>
<th></th>
<th>0.5 s</th>
<th></th>
<th>1 s</th>
<th></th>
<th>3 s</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>-4.36</td>
<td>4.3</td>
<td></td>
<td>-4.09</td>
<td>3.97</td>
<td></td>
<td>-5.96</td>
<td>5.72</td>
</tr>
<tr>
<td>Unload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-3.16</td>
<td>2.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-3.96</td>
<td>3.63</td>
<td></td>
<td>-2.81</td>
<td>2.90</td>
<td></td>
<td>-4.85</td>
<td>4.72</td>
</tr>
<tr>
<td></td>
<td>-3.36</td>
<td>3.54</td>
<td></td>
<td>-5.96</td>
<td>5.72</td>
<td></td>
<td>-4.39</td>
<td>4.31</td>
</tr>
<tr>
<td></td>
<td>-3.16</td>
<td>2.88</td>
<td></td>
<td>-3.66</td>
<td>3.54</td>
<td></td>
<td>-2.84</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>-4.08</td>
<td>3.91</td>
<td></td>
<td>-1.70</td>
<td>1.88</td>
<td></td>
<td>-1.93</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>-4.10</td>
<td>3.94</td>
<td></td>
<td>-1.90</td>
<td>1.71</td>
<td></td>
<td>-1.51</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>-6.14</td>
<td>5.91</td>
<td></td>
<td>-1.99</td>
<td>1.89</td>
<td></td>
<td>-1.72</td>
<td>1.74</td>
</tr>
</tbody>
</table>

**Table 2** – Fitting parameters for dry bone and wet bone.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fitting parameters for dry bone (Hou et al., 2011)</th>
<th>Fitting parameters for wet bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_1$</td>
<td>$K$</td>
</tr>
<tr>
<td>1</td>
<td>0.299</td>
<td>-0.272</td>
</tr>
<tr>
<td>2</td>
<td>0.302</td>
<td>-0.295</td>
</tr>
<tr>
<td>3</td>
<td>0.305</td>
<td>-0.212</td>
</tr>
<tr>
<td>4</td>
<td>0.276</td>
<td>-0.190</td>
</tr>
<tr>
<td>5</td>
<td>0.286</td>
<td>-0.214</td>
</tr>
<tr>
<td>6</td>
<td>0.291</td>
<td>-0.213</td>
</tr>
<tr>
<td>Mean</td>
<td>0.291</td>
<td>-0.233</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.038</td>
</tr>
</tbody>
</table>


It is noted from the report in Hou et al. (2011) that the dielectric constants of dry bone might increase during its shear deformation and do not change when the shear deformation stops. From the above results, we can discuss the reason for wet bone. The elastic modulus of wet bone decreases in relation to dry bone (Rho and Pharr, 1999) and the wet bone has significant viscoelasticity (Abdel-Wahab et al., 2011; Fan and Rho, 2003). Its creep behavior increases with its moisture (Screen, 2008). The viscoelastic creep behavior of bone during mechanical loading is determined by considering both water and collagen (Yamashita et al., 2001). The water molecules play an important role for the viscoelasticity of bone. When a stress is applied to collagen fibrils, they may straighten or shorten, and slide with respect to one another. This process makes the water molecules surrounding the fibrils move in the microspaces around the fibrils. It is known as ‘rearrangement’ in the report (Shen et al., 2011) and the related viscoelastic behaviors were affected by the rearrangement influences in some extent. Based on the above description, we can conclude that the shear deformation of wet bone is greater than that of dry bone under the same shear stress.

The larger deformation must imply that either the dielectric constant or the time constant increases. During the loading process, elastic deformation and viscoelastic deformation (creep deformation) occur simultaneously. During load holding, elastic deformation remains constant, and only creep deformation increases. The increase in deformation in the loading process is stronger than that in load holding. This explanation is consistent with the finding that \( \tau_d \) increases more significantly than \( \tau_c \).

To determine the viscoelastic property of the wet bone samples used, we conducted an experiment to compare viscoelasticity in wet and dry states. We used the same experimental setup as shown in Fig. 2 to measure the relations between the load (maximum force 18 N) and the bending deflection at the upper surface centroid of the sample. The crosshead displacements were recorded as the deflections of samples and the plexiglass force bar was replaced by a steel bar, in order to eliminate errors caused by the shortening of the plexiglass bar. In our experiment, five samples were employed. We first measured the dry bone samples, then immersed them in buffer solution for over 12 h and subsequently tested them. All five samples were found to have a similar diagram of deflection versus time. Fig. 6 shows typical diagrams of sample 3, in which Fig. 6(a) is for dry bone and Fig. 6(b) wet bone.

To further identify the creep behaviors of dry bone and wet bone, the results of the creep portions were fitted into a curve using the standard viscoelastic model (Shepherd et al., 2011; Wu et al., 2012) and method described in the literature (DeHoff, Anusavice 2004). The fitting function form is

\[
\omega(t) = A + Be^{-t/\tau_c}
\]

where \( \omega \) is the displacement measured at the centroid of the up surface of a sample, \( A, B \) are constants representing the dimensions, elasticities and viscoelasticity of a sample. The creep time constant \( \tau_c \) denotes the ratio of viscosity and elastic modulus of the parallel connected dashpot and spring in the standard model. The \( A, B \) and \( \tau_c \) can be obtained by an exponential fitting through regression analysis.

In Fig. 6, the black curves are the measured displacements. The initial portion of the diagrams corresponds to the loading process, during which the deformation of wet bone is observed to be much greater than that of dry bone, which implies that the elastic modulus of wet bone is lower than that of dry bone. The subsequent portion of the diagrams represents the load holding process. The red curves represent fitting plots. The fitting creep time constant of the dry bone is 6.63 s (\( R^2=0.969 \)) and the wet bone is 11.14 s (\( R^2=0.998 \)), which means that the viscoelastic deformation or creep of wet bone is much larger than that of dry bone.

5. Conclusion

The results we present support the following conclusions. In both the loading and load holding processes, wet bone deformation increases continuously due its viscoelasticity. Piezoelectric charges are in turn induced successively and the relaxation of piezovoltages in the two processes follows the stretched exponential law. A further consequence is that the dielectric constant or the equivalent capacitance of wet bone obviously increases during the two processes. Therefore, the value of the time constants \( \tau_d \) and \( \tau_c \) increases relative to dry bone. Since the deformation rate in the loading process is much greater than that in load holding, the increase in the amplitude of \( \tau_d \) greatly exceeds that of \( \tau_c \). An interesting comparison is that the creep of wet bone increased about 5.2 times relative to dry bone, whereas the time constant \( \tau_c \) increased 4-fold. Although we cannot confirm absolutely
the one-to-one dependence between creep deformation and $\tau_c$, they have a corresponding relationship to some extent.

Intuitively, the ions in the buffer solution can cause unlike piezocharges in bone to be neutralized quickly; the piezoelectric property then becomes weak and even disappears. Actually, the piezoelectric property of wet bone may not be weaker than that of dry bone and its relaxation time may be even longer. This factor suggests an association with streaming potentials in bone. In fact, the streaming potentials exist in the microchannels of bone (Anderson and Eriksson, 1968; Xu et al., 2011). At the interface of solid bone and fluid in microchannels, electric charges on the bone surface attract counterions in the liquid that accumulate near the interface, forming a so-called electrical double layer (Anderson and Eriksson, 1968). When external loads are applied to the bone, it deforms and causes fluid pressure to build up in microchannels such as the Haversian, Volkmann canals, canaliculi and lacunae. The pressure gradient in different portions of the microchannels drives fluid flow to flow through them. Thus, when the bone fluid under pressure flows tangentially along the interface, a streaming potential gradient appears along the fluid flow direction (Anderson and Eriksson, 1968; Xu et al., 2011). If electric potential has an influence on the growth of bone cells, the positive explanation is that piezoelectric charges might change the thickness of the electric double layer and then affect the distribution of streaming potentials in the microchannels of bone. That is, there is coupling effect between piezovoltages and streaming potentials. Of course, piezovoltages might only influence bone cells. That is one of the highlights of this study.

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