Biomedical engineering

Effect of various storage methods on the dielectric properties of compact bone

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Abstract—*In the study the effects of various storage environments on the dielectric properties of bone were evaluated. Fresh cortical bone specimens from canine femora and tibiae were prepared and divided into three groups, with one group maintained at room temperature (24°C), a second group stored in a refrigerator at 3°C, and a third group stored in a freezer at −10° to −20°C. In each group, both the resistance and the capacitance decreased with time, the percentage change being largest for the samples stored in the freezer. This suggests that storage of bone specimens in a refrigerator or freezer with repeated thawing at room temperature does affect the dielectric properties of bone, the effect being dependent on the method of storage.*

Keywords—*Bone, Bone impedance, Capacitance, Dielectric properties, Electrical properties, Resistance, Storage medium*

Med. & Biol. Eng. & Comput., 1988, **26**, 199–202

1 Introduction

ALTHOUGH ORTHOPAEDIC surgeons increasingly use electrical stimulation to treat nonunions and congenital pseudoarthrosis, the mechanism of action of bioelectricity is still unknown. Several authors have suggested that electromechanical behaviour of bone provides the transduction mechanisms for stress-induced remodelling of bone tissue (BASSETT, 1971; SINGH and SAHA, 1984; GRODZINSKY, 1983). For a better understanding of the role of electrical stimulation in bone remodelling and for an analysis of the distribution of direct or induced current in bone, we need accurate data on the dielectric properties of bone. Although some investigators have measured electrical properties *in vivo*, such measurement creates uncertainties regarding the current paths between a pair of electrodes placed in such a material and the nature of the tissue/electrode interface (SAHA *et al.*, 1981). Therefore *in vitro* measurement techniques on standardised bone specimens have mostly been used to characterise the dielectric properties of bone (SINGH and SAHA, 1984).

With *in vitro* measurement methods, it is important to know how various factors and parameters affect the measured value. Previously, REDDY and SAHA (1984) have shown that the dielectric properties of bone are anisotropic in nature and frequency-dependent. SAHA *et al.* (1984) have shown that the electrical properties of bone are dependent on the moisture content, temperature, pH, time of exposure to the air and measurement procedures. Other authors (CHAKKALAKAL and JOHNSON, 1981; KOSTERICH *et al.*, 1984; SINGH and SAHA, 1984) have shown that the electrical properties of bone are dependent on the conductivity of the immersion fluid or preserving solution, and/or the principles and techniques of measurement. However, the effect of the environment in which the bone sample is stored has not been properly investigated.

Studies on the effects of storage environment on the mechanical properties of bone have shown that alcohol or formalin produce a slight effect, whereas freezing produces none (MCELHANEY *et al.*, 1964; SEDLIN, 1965; SEDLIN and HIRSCH, 1966). However, no such information exists on the effect of storage techniques on the electrical properties of bone.

Because refrigeration or freezing is the usual storage method in studies on the dielectric and piezoelectrical properties of bone, it is important that the effect of this storage technique on the dielectric properties of bone be understood. The objective of this study was to evaluate and determine whether or not refrigeration or freezing changes the dielectric properties of bone and to compare these results with other types of storage environments. Three storage environments were evaluated: storage at room temperature, in a refrigerator, and in a freezer.

2 Methods and procedures

Canine femorae and tibiae were used in the study. The bones were removed soon after the sacrifice of the animal and wrapped in towels soaked in lactated Ringer's solution to prevent them from drying. Specimens two to three centimetres long were then machined from the mid-diaphysis of each bone (Fig. 1). Each specimen was then further machined in the axial direction to produce two to four matched specimens from each bone (Fig. 1). Additional grinding and polishing were performed as needed to provide appropriate surfaces on all faces of the specimens. In a preliminary study, the specimens had been left intact after the cylindrical sample had been cut in order to reduce the machining time; however, it was found that proper preparation, cleaning of debris and measurements were facilitated by additional machining of the specimens. This

This paper was presented in part at the Fourth Southern Biomedical Engineering Conference at Jackson, Mississippi, USA, 11th–12th October 1985.

First received 19th March and in final form 15th September 1987

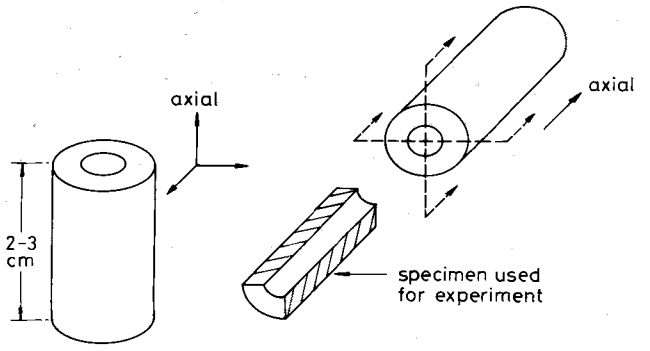

Fig. 1 *Machining scheme for preparing the bone specimens*

also provided, with less time and effort, a larger sample for the statistical analysis.

During the entire machining process, the bones were kept moist. After machining, a total of 11 specimens were individually placed in containers with lactated Ringer's solution (pH 6·5) and a bacteriostatic agent. After the specimens were prepared, the resistance and capacitance were measured using an LCR meter (HP model 4262A) as described before (SAHA *et al.*, 1984) (Fig. 2). All measurements were made at 1 kHz. The initial measurement was made approximately $2\frac{1}{2}$ hours after the sacrifice of the animal; these resistance and capacitance values were used to normalise the electrical properties measured subsequently. Measurements were then repeated several times throughout the day. At the end of the first day, the samples were divided into three groups. The first group was maintained at room temperature (24°C); the second group was stored in a refrigerator at 3°C; and the third group was stored in a freezer at $-10°$ to -20°C. The next day the bone samples from the second and third groups were removed from their storage environments and allowed to thaw and equilibrate to room temperature. Then the resistance and capacitance of all specimens were measured repeatedly through the course of the day. The procedure was repeated for up to four days, with the times at which the bone specimens were removed from their environment and returned being the same on all four days.

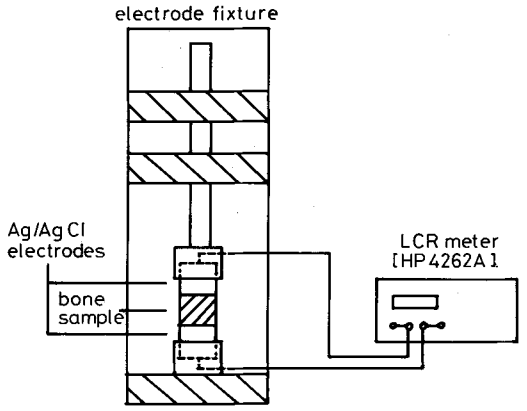

Fig. 2 *Experimental setup for measuring the dielectric properties of cortical bone specimens*

Initially the room temperature, refrigerator and freezer groups were composed of four, three and four machined compact bone specimens, respectively, all samples being from the same dog. Repeated readings at different time intervals up to 5 days were taken on these 11 bone samples. Most of our results and analysis are based on the readings on these bone samples. However, to verify our results, four additional bone samples from a second dog were tested similarly, measuring only the resistance values. These four specimens were maintained at room temperature only throughout the 100 h observation period. Table 1 shows the total number of bone samples tested in each group and the exact times of measurements throughout the five day observation period.

The electrical properties were measured using chlorided silver metal electrodes in the setup shown in Fig. 2. Surface moisture was removed from the bone prior to measurement, and a layer of conductive gel (Aquasonic 100, Parker Lab.) was applied to the bone surfaces and to the electrodes. All measurements were made in the axial direction only. Because of the effect of exposure time (SAHA *et al.*, 1984), the amount of time between the removal of the sample from the solution and the measurement was kept constant for each measurement.

3 Results

Fig. 3 shows the normalised resistance against time for the three groups of bone specimens maintained in three storage environments for each of the five days. The values

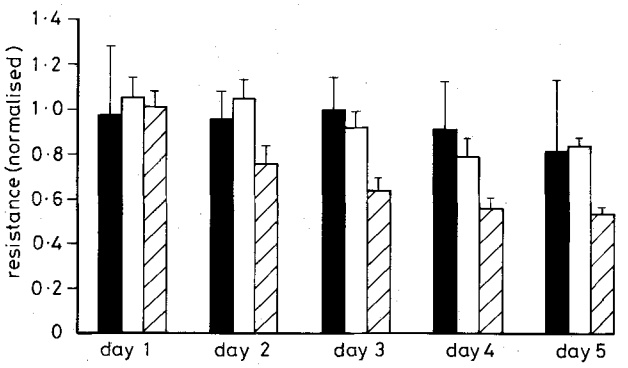

Fig. 3 *Normalised resistance (mean ± 1 SD) of the bone specimens stored in different environments as a function of storage time*
■ *room temperature (n = 4)*
□ *refrigerator (n = 3)*
▨ *freezer (n = 4)*

for each day were calculated as the mean for the hourly readings for that day. For day 1, no significant difference in the specific resistance was found among the groups ($p > 0{\cdot}05$). For days 2–4 there was also no statistically significant difference between the room temperature and refrigerator groups, but there was a significant difference between the freezer and the room temperature or refrigerator groups ($p < 0{\cdot}01$). There was also a significant difference between the freezer and the room or refrigerator samples for day 5 ($p < 0{\cdot}05$). The resistance for the group stored at room temperature showed no significant decrease ($p > 0{\cdot}01$) until day 5, yet this group had a larger variance than the other groups. The group stored in the refrigerator showed a significant decrease ($p < 0{\cdot}05$) only for days 3 and 4. The third group, that was stored in the freezer, showed a significant decrease ($p < 0{\cdot}01$) for each day except for day 5. The resistance of one sample at room temperature began to increase at day 5, whereas that of the other specimens continued to decrease, this being the reason for the large standard deviation noted. The reason for this increase is still unknown.

From Fig. 3, it appears that the change in resistivity was minimum for the specimens stored at room temperature; thus this may be the preferable mode of storage. To obtain increased confidence in the measured data on the resistance of the room temperature group, four additional compact bone specimens from another canine femur were tested as described before. The change in normalised resistance for all eight bone specimens Table 1 as a function

Table 1 Number of specimens tested at different time intervals

Time of Measurement		Number of specimens in each group		
Days	Hours	Room	Refrigerator	Freezer
1	2·33	4	3	4
	2·58	4*		
	3·67	4	3	4
	6·33	4	3	4
	7·33	4*		
2	23·67	4*		
	25·67	4*		
	27·33	4	3	4
	28·33	4	3	4
	28·67	4*		
	29·33	4	3	4
	30·33	4	3	4
	30·67	4*		
3	49·00	4*		
	49·33	4	3	4
	51·00	4*		
	52·33	4	3	4
	53·33	4	3	4
	54·33	4	3	4
	55·67	4*		
4	72·50	4*		
	73·00	4	3	4
	75·00	4, 4*	3	4
	77·00	4, 4*	3	4
	79·00	4, 4*	3	4
5	96·00	4*		
	99·00	4	3	4
	99·50	4	3	4
	100·00	4*		
Total observations		128	51	68

* denotes additional samples from a second dog to verify earlier results

of time, is plotted in Fig. 4. The equation of the regression line as shown in Fig. 4 is

$$R_N = 1{\cdot}04 - 0{\cdot}00156t \quad (1)$$

where

R_N = normalised resistance

t = time in hours

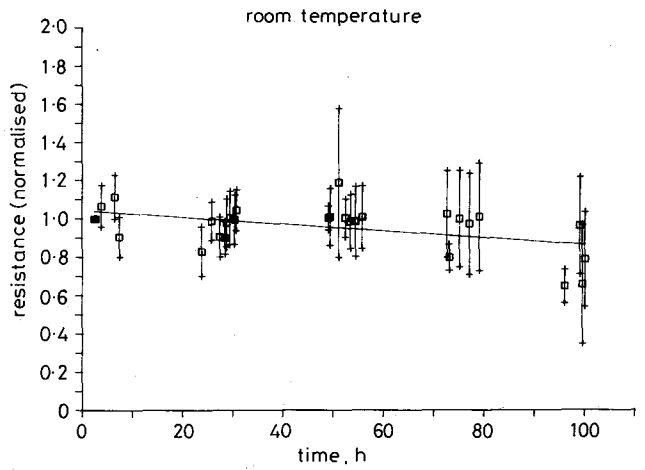

Fig. 4 *Normalised resistance (mean ± 1 SD) of compact bone specimens maintained at room temperature, as a function of the time of measurement (r = 0·2213 and n = 128)*

Fig. 4 shows that there was a small linear decrease with time in the resistance of the bone samples maintained at room temperature. This decrease was 3·7 per cent per day.

Fig. 5 shows the normalised capacitance against time for the three groups of specimens for each of the five days. The values for each day were calculated by the same method as that used for calculating the resistance. Again, no statistical difference in the specific capacitance values was found ($p > 0{\cdot}05$) between the three groups for day 1. For days 2–5, the group at room temperature, the group stored in the refrigerator, and the group stored in the freezer were all significantly different from each other ($p < 0{\cdot}05$), except for day 4 when the room temperature group and the group

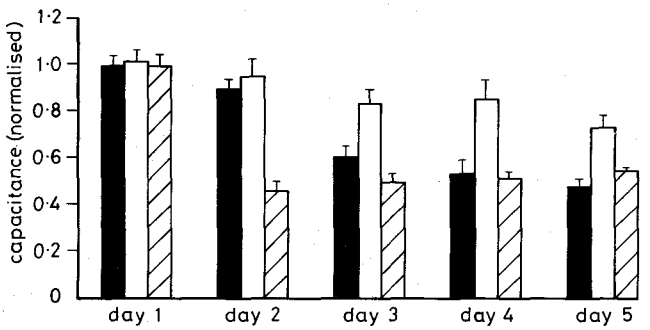

Fig. 5 *Normalised capacitance (mean ± 1 SD) of the bone specimens stored in different environments as a function of storage time*
■ *room temperature (n = 4)*
□ *refrigerator (n = 3)*
▨ *freezer (n = 4)*

stored in the freezer were not significantly different. The group stored at room temperature showed a significant decrease at $p < 0{\cdot}05$ for each day, 1–5. The group stored in the refrigerator showed a significant decrease ($p < 0{\cdot}05$) for each day, except for days 3 and 4, which were not significantly different ($p > 0{\cdot}05$). The group stored in the freezer showed a significant decrease ($p < 0{\cdot}05$) between day 1 and day 2 and then showed statistically significant increases ($p < 0{\cdot}05$) for days 3 and 5, with day 4 not significantly different from either.

Fig. 6 shows the variation in the capacitance values among several readings taken each day at interval of one hour or more. These variations are relatively small and, as in the case of resistance, they may be caused by increased drying effect or changed room temperature during the day.

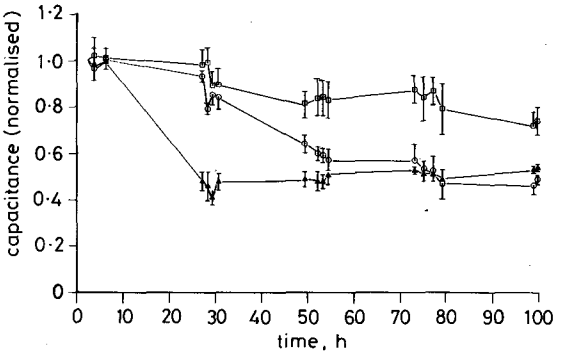

Fig. 6 *Normalised capacitance (mean ± 1 SD) of the bone specimens as a function of the time of measurement*
⊙ *room temperature (n = 4)*
⊡ *refrigerator (n = 3)*
△ *freezer (n = 4)*

4 Discussion

Previously, other authors have reported changes in other physical properties of bone over time when preserved in various ways. STEINBERG *et al.* (1976) found decreases in strain-related potentials in rat femora for 4–7 days after the bone had been excised. ELWOOD and SMITH (1984) have reported decreases in the zeta potentials of bone during storage and found that storage methods using

different fluids could increase or decrease the measured zeta potentials.

KOSTERICH *et al.* (1983) found that the low-frequency conductivity of freshly excised compact bone from rat femora increased by 5–15 per cent over a 50 h period. They explain the change as possibly caused by changes in ionic content or the washout of cellular components from the tissue. They also report that freshly excised bone samples maintained in Hank's Balanced Salt Solution showed only minor changes in permittivity during a period of 50 h. In the present study, in specimens stored in the refrigerator, the resistance was found to decrease by approximately 0·4–16·8 per cent at about 52 h after sacrifice of the animal. However, unlike the findings of KOSTERICH *et al.* (1983), the capacitance was found to significantly change within 48 h after the sacrifice of the animal. It should be pointed out that the fluids used to store bone samples differed between the present study and those by KOSTERICH *et al.* (1983) and ELWOOD and SMITH (1984); the different results obtained in the three studies suggest that tissue fluid may play an important role in the measurement of the electrical properties of bone. Also, it is possible that the parameters of pH and temperature can have an influence on the effect of storage. FUKADA and UEDA (1979) reported that the piezoelectric constants for bone and collagen were temperature dependent.

Although we have reported our results at one frequency (1 kHz), it is possible that the nature of change in resistance and capacitance at other frequencies may be somewhat different, as suggested by the work of KOSTERICH *et al.* (1983; 1984). Previously, we have characterised the electrical properties of compact and cancellous bone as a function of frequency (SAHA *et al.*, 1984; REDDY and SAHA, 1984; SAHA and WILLIAMS, 1986). However, in the present study, our main goal was to determine if different storage methods affect the electrical behaviour of bone and bone tissue. Thus the relationship between the effect of storage method on the electrical properties of bone and the frequency was not investigated.

We have shown that the resistance and capacitance of bone and the rate of change in these parameters are affected by the method in which it is stored. It is possible that most of the effects noticed are caused by the washout of cellular components and changes in ionic content, as suggested by KOSTERICH *et al.* (1983). However, the increase in capacitance noticed for the group stored in the freezer after day 2 cannot be explained easily by these factors. Recent work done in our laboratory with demineralisation of bovine cortical bone suggests a cause; it was found that resistance decreased and capacitance increased with decreasing mineral content. Further studies are in progress to evaluate whether or not change in electrical properties can be minimised by storage methods other than those reported here. We also plan to study the effect of storage methods on frequency dependence of the electrical properties of bone.

Acknowledgment—This research was partially supported by US National Science Foundation Grant ECS-8312680.

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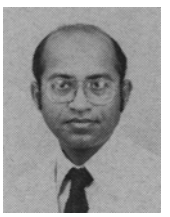
Authors' biographies



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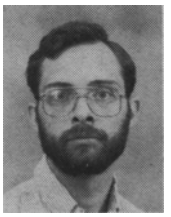

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