

Piezoelectricity and Autoinduction

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We have previously reported the existence of piezoelectricity in bone and bone matrix,³ and have suggested its importance in the processes of modeling and remodeling.² A third type of bone growth called autoinduction has been extensively studied over the past decade by the Urist group.⁴⁻⁶ Autoinduced bone growth is the *in vivo* production of bone and bone marrow, following the implantation of some tissue able to elicit the response. Many kinds of tissues have been successfully employed, and the most thoroughly studied is demineralized bone matrix. It occurred to us that the piezoelectric nature of demineralized bone might be related to autoinduction. One possibility is that the implant, which is necessarily subject to quasi-periodic mechanical deformation, produces time varying, fixed surface charge distribution which can be "read" by the mesenchymal cells of the host. Another more subtle possibility is that the structural organization of bone matrix which accounts

for its piezoelectricity also accounts for autoinduction, independent of any applied mechanical strain. Considering that all of the otherwise diverse tissues that elicit autoinduction contain the protein collagen which is piezoelectric, the existence of a link between the 2 phenomena seemed to warrant study.

The inductive activity of the implant is quantified by measuring the volume of new bone per unit volume of implanted matrix.⁶ One variable in this system is the number of days the matrix is exposed to the demineralizing acid solution before implantation. When the acid employed is 0.6 HCl the changes in inductive activity have been reported⁶ and are reproduced in Table 1. It can be seen that continued exposure of the bone matrix destroys its inductive property. A measurement of the piezoelectric constant of bone matrix exposed to HCl solution for varying lengths of time would serve to determine whether piezoelectricity is related to autoinduction. If the piezoelectric constant were independent of the time of acid treatment, the 2 phenomena would be effectively disassociated. Experimental results described below show that in fact this is the case.

An independent but somewhat related line of inquiry concerns the effect of demineralization employing HNO₃. Bone matrix prepared using HNO₃ uniformly fails to act as an inductive substitute.^{4, 5} Thus piezoelectric measurements following treatment with HNO₃ should provide complementary data to that discussed above.

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TABLE 1. Changes in Inductive Activity of Bone Matrix Substrates as a Function of Demineralization Time at 25 C⁵

<i>Solution</i>	<i>Matrix Exposed in Solution, Days</i>	<i>Volume of New Bone in mm³/6.0 mm² Matrix</i>
0.6 M HCl	1-3	5
0.6 M HCl	4-9	4.5-4.0
0.6 M HCl	10-14	3.5-3.0
0.6 M HCl	15-30	0.5-0.0

METHODS

The piezoelectric measurements described here were made with samples cut from a single male bovine femur. The animal was between 3 and 4 years old when slaughtered and the bone was obtained shortly after death. Measurements were made using a slightly modified version of Fukada's method,¹ employing the converse effect. The diaphysis was cleaned of all adhering soft tissue and degreased in acetone for 24 hours. Individual samples having nominal dimensions of 10 × 5 × 2 mm were then cut in a manner to permit measurement of the piezoelectric coefficient d_{14} . The size of the samples required precluded the use of rabbit or rat bone as was employed in the autoinduction experiments.^{4, 6} We assume that bovine bone is capable of autoinduction.

The samples were demineralized in either 0.6 M HCl or 0.6 M HNO₃ at room temperature for varying times. Bone-weight to acid-volume ratios were as given by Urist.⁵ The samples were suspended in the acid in such a manner as to permit maximum exposure to the acid. The solutions were stirred gently and continuously. Following demineralization the samples were rinsed in distilled water and air dried under slight pressure. The pressure was applied by a specially constructed screw-type clamping apparatus, and was necessary in order to prevent the dried sample from warping or distorting. Accompanying demineralization were small changes in all linear dimensions of the samples and for convenience all calculations of d_{14} were made employing the linear measurements of the original bone sample. Electrodes were attached to the 2 faces of the air-dried

sample with silver paint. Initial observation of the piezoelectric constant of bone matrix revealed that it decreased continuously with increasing water content. To provide a uniform baseline water content for all samples, they were dried at 100 C for 24 hours. All measurements were made at room temperature in a time sufficiently short so as to precede reabsorption of atmospheric water vapor.

RESULTS AND DISCUSSION

The value of d_{14} of bovine matrix as a function of the time of HCl and HNO₃ demineralized is given in Table 2. The first entry in Table 2 is d_{14} of whole bone, before any acid treatment. The large increase on day 1 is entirely expected and results from removal of the mineral, which is non-piezoelectric but has a high modulus of elasticity.³ Subsequently, within experimental error, d_{14} is independent of the time of acid treatment. At day 15 the HCl samples were completely gelatinized and no measurements could be made. The relatively large standard deviations of the demineral-

TABLE 2. The Average Piezoelectric Constant, d_{14} , and Standard Deviation of Bone Matrix as a Function of Demineralization Time

<i>Days</i>	<i>d₁₄*</i>	
	<i>HCl</i>	<i>HNO₃</i>
0	0.53 ± 0.072	
1	8.6 ± 1.2	7.7 ± 1.8
2	7.0 ± 1.6	6.6 ± 0.9
3	6.8 ± 1.4	6.8 ± 1.3
5	6.7 ± 2.3	6.0 ± 0.6
7	7.0 ± 2.0	6.5 ± 1.0
12	6.7 ± 1.7	7.4 ± 1.4
15		6.7 ± 1.1

* (× 10⁻⁸ cgs esu).

Note: All values are averages of 5 samples except for day 0 (undemineralized bone) which is an average of 14 samples.

ized sample as compared to the whole bone samples is a reflection of the sample distortion that unavoidably occurs following acid treatment.

Prolonged treatment with HCl destroys the autoinduction property of bone matrix, however such treatment does not affect its piezoelectric constant d_{14} and therefore the 2 phenomena are unrelated. In addition, treatment with HNO_3 yields bone matrix which is not capable of inducing bone formation and yet its d_{14} is identical with the value for HCl demineralized bone matrix. This reinforces the conclusion reached above.

Beyond the proposition that autoinduction is unrelated to piezoelectricity, these measurements suggest that it is not directly associated with the collagen. The ultimate source of the piezoelectric effect in collagen is unknown, although presumably it results from some periodic bonding property associated with its structure. Prolonged HCl treatment ultimately produces a gelatin, and yet no changes are detected in d_{14} up to the point of complete protein breakdown as occurred at day 15. Since collagen matrix structure as determined by the d_{14} measurement is intact through at least day 12 of HCl demineralization, a time at which there is decreased inductive capacity, it is reasonable to conclude that inductive capacity is associated with some substance other than collagen. The same comment is even more applicable to HNO_3 demineralized bone. It is interesting to note that Urist has previously arrived at the same conclusion via a completely different path.⁷

SUMMARY

A relationship between 2 phenomena exhibited by bone matrix, piezoelectricity and autoinduction, is unlikely. Autoinduction, demonstrated by others to be diminished following prolonged treatment of the bone matrix with HCl, and altogether absent with HNO_3 demineralized bone matrix, has a relatively constant piezoelectric coefficient, d_{14} , and does not depend on either the acid used or the time of exposure. Since prolonged acid treatment destroys the autoinduction property but does not materially affect the bone collagen matrix, it is reasonable to assume that the bone matrix inductive capacity may be associated with some constituent other than collagen alone.

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