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Piezoelectricity in Collagen Films

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Summary. Tissue collagen exhibits several levels of structural organization, and this complicates efforts to determine the origin of its piezoelectricity. We made collagen films—by evaporation and electrodeposition from solution—and examined the relation between collagen's piezoelectricity and its electron microscopic appearance. We found that the electrodeposited films were more organized and exhibited higher piezoelectric coefficients than the evaporated films. Despite this, the evaporated films were piezoelectric, thereby suggesting that the effect originates either at the level of the tropocollagen molecule or, at most, with aggregated structures no larger than 50 Å in diameter.

Key words: Piezoelectricity — Collagen.

Introduction

Tissues containing the structural protein collagen are piezoelectric [1, 2]; but collagen exhibits levels of structural organization from the fiber to the tropocollagen molecule, and little is known concerning the level of origin of its piezoelectricity. Collagen can be reconstituted from solution as a thin film—by evaporation or by electrodeposition—and in this form it exhibits one of its simplest structural patterns. This study was undertaken to determine whether such films are piezoelectric.

Methods

An electrodeposited and evaporated film was made from each of three batches of collagen solution. The origin of the collagen and details of its solubilization have been described previously. Briefly, citrate-buffered monomeric collagen solution was made from calfskin corium and then dialyzed to an ionic strength of 0.04 [3]. The electrodeposited films were made by inserting a carbon cathode and a platinum anode in the solution and applying 10 square wave pulses, each of which was 1000 V and lasted 10 ms; the current was not measured. A film was formed only at the cathode. The evaporated films were made by spreading the solution on an aluminum plate and allowing the liquid phase to evaporate at room temperature and humidity—the process required several days.

The films were vacuum dried at 100°C for 2 h and stored in a desiccator. Piezoelectric measurements were performed as follows [4]. Electrodes were attached to the faces of the film, which was then clamped on one end to an electromagnetic vibrator and on the other to a frame which could be adjusted to give an appropriate tension to the film. A 10-Hz longitudinal vibration was applied, and the resulting electrical polarization and its angular relationship to the applied stress were measured. Rotating the film made it possible to find the direction which yielded the largest value of the piezoelectric coefficient d′, and the loss angle tan δ.

Specimens of each of the films were examined by electron microscopy. For transmission microscopy the films were fixed in glutaraldehyde and osmic acid, embedded, sectioned, and stained with uranyl acetate. In addition, portions of the films were mechanically dispersed in water and deposited directly on grids, without sectioning, and were stained with sodium phosphotungstate (pH 7.4) [5]. For scanning electron microscopy, portions of the films were coated with Pd-Au alloy.

Results

A weak piezoelectric effect was found in each of the films examined (Table 1). The piezoelectric coefficient was always greater, and tan δ was always smaller in the electrodeposited films prepared from the same batch of collagen. Because the piezoelectric coefficient reflects a material's structural organization, these differences led us to examine the electron microscopic appearance of both kinds of films.

By transmission electron microscopy the films showed a clear difference in structure and texture. The electrodeposited films appeared in many areas

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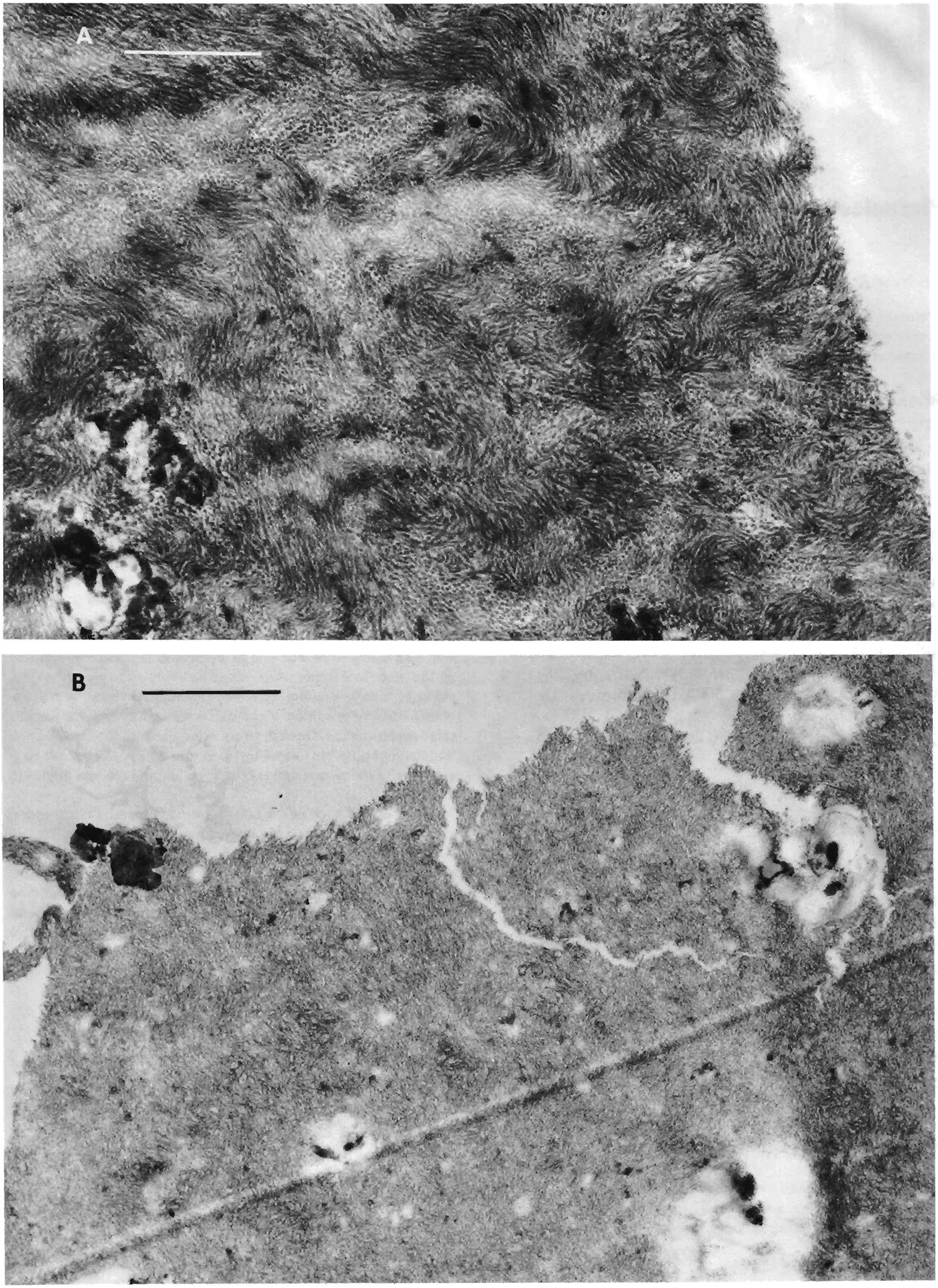

Fig. 1. Transmission electron micrographs of electrodeposited (**A**) and evaporated (**B**) collagen films after fixation, embedding, and ultramicrotomy. Bars indicate 1 μm. The 1000–10,000 Å groups of parallel fibrillar elements were seen only in the electrodeposited films

Table 1. Piezoelectric coefficient and loss tangent of collagen films

Batch	Sample	d′ (10^{-10} cgsesu)	tan δ
1	Evaporated	6.7	0.090
	Electrodeposited	23	0.043
2	Evaporated	3	1.16
	Electrodeposited	13	0.22
3	Evaporated	6.3	1.22
	Electrodeposited	9.5	0.063

Each pair of films was prepared from a different batch of collagen solution. Measurements made at 25°C

to be composed of parallel filaments about 300 Å in diameter, organized in loosely associated groups 1000 to 10,000 Å in extent (Fig. 1A). Typically, the groups were randomly organized with respect to one another, but occasionally suggested crude lamellae. The evaporated films, on the other hand, appeared amorphous at moderate magnification and showed minute filaments at higher magnification (Fig. 1B). The largest such filaments were about 50 Å in diameter and were almost completely disorganized. Thus the electrodeposited films showed a fair degree of supermolecular organization in contrast to the evaporated films which appeared to be composed mostly of disorganized units of molecular dimensions.

The mechanically dispersed evaporated films were very soluble and yielded structureless coating when dried on the grids. The dispersed electrodeposited films were mostly filamentous and contained a few collagen fibrils of 500–2000 Å in diameter; band periodicities of 630–680 Å which exhibited the fine structure of normal collagen were present. Unlike the dense structures found in tissue, these fibrils were loosely aggregated; but even so, their presence in the electrodeposited films indicated a higher level of organization in the electrodeposited as compared to the evaporated films. By scanning electron microscopy all films generally lacked organization (up to 5000×). Occasionally the surface of the electrodeposited films showed a rippling suggestive of an underlying fibrous structure, but the evaporated films were always devoid of texture.

The electrodeposited films were formed using arbitrary electrical conditions. There are many other voltage wave forms which can precipitate collagen [6, 7]; the structure and properties of these precipitates and their relation to the forming voltages and currents are unknown and require further study.

In summary, the piezoelectric effect has been found in electrodeposited and evaporated collagen films. The magnitude of the effect was small in both kinds of films, but was consistently greater in the electrodeposited films. Various electron microscopic studies confirmed the existence of a greater degree of organization in the electrodeposited films, but the existence of piezoelectricity in evaporated films—which are essentially structureless down to 50 Å—suggests that at least part of the piezoelectric behavior in collagen arises form units far down on the structural hierarchy, perhaps the tropocollagen molecule itself.

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References

1. Fukada, E., Yasuda, I.: On the piezoelectric effect of bone, J. Physiol. Soc. Jpn. **12**:1158–1162, 1957
2. Marino, A.A., Soderholm, S.C., Becker, R.O.: Origin of the piezoelectric effect in bone, Calcif. Tissue Res. **8**:177–180, 1971
3. Kahn, L.D., Witnauer, L.P.: Solubilization of calfskin collagen in citrate buffer with the use of automated equipment, J. Appl. Polymer Sci. **13**:141–148, 1969
4. Fukada, E., Date, M., Hara, K.: Temperature dispersion of complex piezoelectric modulus of wood, J. Appl. Physiol. Jpn. **8**:151–158, 1969
5. Spadaro, J.A., Becker, R.O.: Effects of thermal denautration on metal binding and ultrastructure in collagen fibrils, Biochem. Biophys. Acta **263**:585–592, 1972
6. Rorer, F.P., Carroll, R.J., Kahn, L.D.: Electron microscopy of collagen film surfaces, J. Am. Leather Chem. Soc. **68**:107–111, 1973
7. Marino, A.A., Becker, R.O.: The effect of electric current on rat-tail tendon collagen in solution, Calcif. Tissue Res. **4**:330–338, 1970

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