QUASI-STATIC CHARGE INTERACTIONS IN BONE

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Summary

Bones respond to mechanical forces by modifying their architecture. Mechanical forces also result in at least two electromechanical signals in bone — streaming potentials and piezoelectric polarization. Observations of bone's parallel architectural and electrical responses to forces have led to suggestions that one or both of the signals mediate the osteogenic response by directly triggering bone-cell activity. This study was undertaken to determine whether piezoelectric polarization could alter bone-cell physiological function.

Piezoelectric and non-piezoelectric forms of the polymer polyvinylidene fluoride were implanted in rats, and the effect on periosteum and bone was studied histologically. More bone formation and periosteal reaction occurred in association with the piezoelectric form of polyvinylidene fluoride. The effects were statistically significant at 1–6 and 1–2 weeks postoperatively for bone and periosteum, respectively. Nether mechanical nor chemical factors could account for the results, which therefore must have been due to the quasi-static piezoelectric polarization (about 90 pC/cm^2). The osteoprogenitor cell but not the mesenchymal cell (its less differentiated precursor) was capable of responding to the polarization.

Introduction

The mechanical forces normally applied to living bones affect both their architecture and composition [1] and also result in the production of electrical signals. Streaming potentials arise from kinetic modification of the electrical potential at the slip plane [2], and piezoelectric polarization occurs as a consequence of rotation of peptide-bond dipoles [3]. Streaming potentials are the physical basis of the electrical signals observed in bone, tendon, and cartilage subjected to mechanical forces under physiological conditions [4]. The piezoelectric signal is not usually measured under such conditions because the induced polarization is rapidly neutralized by ions in the bulk fluid. Observations of bone's parallel architectural and electrical responses to applied forces

have led to suggestions that one or both of the electromechanical signals mediate osteogenesis. In the case of piezoelectricity, the growth could occur in response to the surface polarization itself, or to the subsequent neutralization-ion kinetics [5] which occur probably on a microsecond timescale.

Several attempts have been made to provide affirmative evidence of the biological activity of piezoelectrically induced polarization. In one approach, piezoelectric materials including lead molybdate, quartz, barium titanate, tourmaline, and cholesterol were introduced as finely divided dust into the peritoneal cavity and lungs of rats and rabbits [6], and marked fibrotic reactions were observed. Non-piezoelectric control dusts including ground glass and silicon carbide produced significantly less fibrosis. The authors concluded that the difference in the biological response was due to piezoelectricity, but since the two groups of substances differed chemically (and perhaps physically in uncontrolled physical properties such as average particle size), factors other than induced surface charge may have been responsible for the observed difference.

Inoue et al. [7] sutured a film of the piezoelectric material poly-γ-methyl-L-glutamate (PMG) to the quadriceps tendon and biceps femoris in rats. Motion of the joints was expected to result in generation of a piezoelectric polarization, and femoral osteogenesis was observed. As a control, PMG was implanted in the contralateral limb but left unsutured, and no bone growth was observed. Although the foreign-body reaction to the implant was probably bilaterally identical (because the same material was implanted on each side), the model did not control for mechanical effects. The observed osteogenesis may have been due to mechanical stimulation of the periosteal surface of the bone, irre-spective of the polarization.

Recently, the polymer polyvinylidene fluoride became available in two chemically identical forms, only one of which is strongly piezoelectric [8]. This study was undertaken to determine whether a piezoelectrically induced surface charge in polyvinylidene fluoride could alter bone-cell function.

Methods

Polyvinylidene fluoride is a fluorocarbon polymer that can be made piezoelectrically active by mechanical extension and electrical poling of the melt-solidified form [8]. Poled polyvinylidene fluoride (PVDF) exhibits the largest piezoelectric coefficients of all the known piezoelectric polymers [8]. Pertinent physical characteristics of the PVDF employed in this study (Solvay & Cie, Brussels) are listed in Table 1. As a control, we used polyvinylidene fluoride (Solvay & Cie., Brussels) that had been subjected to mechanical extension but not poled (PVDFc). Both PVDFc and PVDF were in the beta crystal form, and differed in that only the latter exhibited a strong preferential dipolar alignment. PVDFc was weakly piezoelectric. Its piezoelectric coefficients were not

TABLE 1

Properties of polyvinylidene fluoride

	PVDF	PVDFc
Chemical formula	$(CH_2\text{-}CF_2)n$	$(CH_2\text{-}CF_2)n$
Thickness (μm)	25	25
Piezoelectric coefficient (pC/N)		
d_{31}	9	0
d_{32}	9	0
d_{33}	17	0
Volume resistivity (Q m)	5×10^{14}	5×10^{14}
Relative dielectric constant	11	11
Ultimate tensile strength (MPa)	190	190

Data supplied by Solvay & Cie., Brussels, Belgium.

directly measured, but from voltage measurements on films under standardized conditions we estimated them to have been 1–2 orders of magnitude smaller than those of PVDF.

Our hypothesis was that piezoelectrically induced surface charge on PVDF could alter the physiological status of osteoprogenitor cells — the immediate precursors of osteo-blasts (bone-building cells) located in the periosteal sheath that covers bones. To test this hypothesis, it was necessary to control for the chemical composition of the implant, the amount of mechanical stimulation delivered to the bone under study, the possible effect of a leachable substance contained only in the PVDF (since it was subjected to the further processing step of poling), and the particular cell type suspected of being sensitive to the piezoelectric polarization. Incorporation of these controls required the use of three distinct animal models. In each, the implant procedure was performed bilaterally— PVDF on one side, and PVDFc on the contralateral side. In addition, since it was not convenient to measure the actual stress applied to the implanted material after its attachment to the musculoskeletal system (or the resulting strain), we used both methods of attachment previously reported to successfully load the implant [7].

Female Sprague-Dawley rats, 8-10 weeks of age were used in all studies. In Group A, an incision was made over the anterior tibia and about 1 cm of the attachments of the tibialis anterior and tibialis posticus were incised. The muscles were retracted to expose the tibia and the interosseous membrane, which was incised. Polyvinylidene fluoride film, 4 mm wide, was wrapped around the bone (with an overlap of approximately 1/4 turn), and secured in place using 5-0 Mersilene suture wrapped outside the film and knotted (Fig. 1). The film was positioned on the bone using a leader of 4-0 Mersilene suture knotted at one end of the film. The portion of the implant containing the knot was cut and discarded prior to closure of the skin. This procedure ensured that the
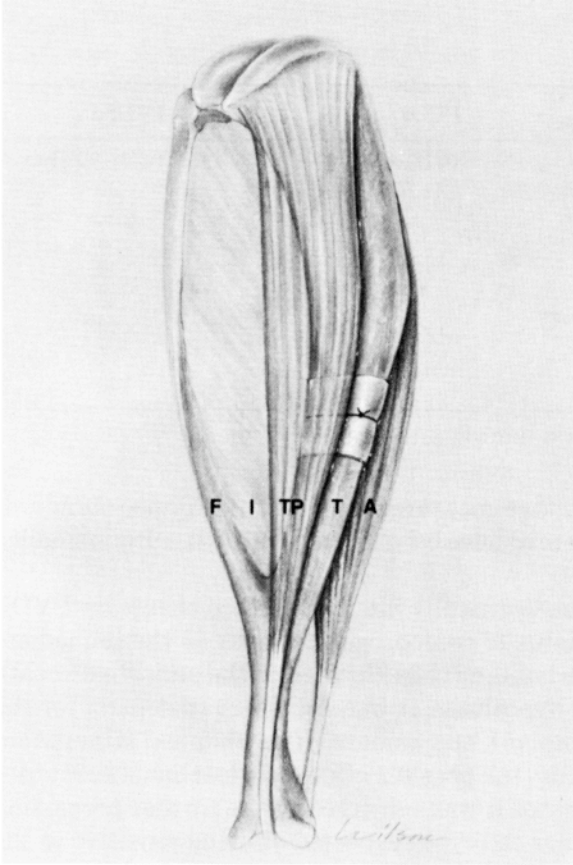
Fig. 1. Polyvinylidene fluoride film implanted in Group A rats. F, fibula; I, interosseus membrane; TP, tibialis posticus; T, tibia; A, tibialis anterior.

surface of the implanted film was not marred by contact with surgical instruments. The skin was sutured with 6-0 coated Vicryl.

In group B rats, a longer incision was made along the anterior aspect of the tibia, and the incision was pulled laterally to expose the posterior border of the tibialis anterior. The muscle fascia was cut, and the tibialis anterior was loosened by blunt dissection down to the tibia. About 1 cm of the tibial attachment was cut, and incisions were made through the interosseous membrane and between the tibia and the tibialis posticus as described above. A 4-mm strip of polyvinylidene fluoride (3 cm long) was passed through the incision made in the fascia behind the tibialis anterior, and pulled through the incision between the tibialis anterior and the tibia. The film was looped around the anterior tibia, passed through the interosseous membrane, and sutured at the ends to

the tibialis anterior and the extensor digitorum longus using 5-0 Mersilene (Fig. 2). The skin was then sutured using 6-0 coated Vicryl.

In group C rats, a 1-cm incision was made through the skin and muscle over the lateral femur, and polyvinylidene fluoride film (0.3 × 0.7 mm) was placed on the exposed periosteum (but not fixed to the bone). The muscle and skin were then closed with 6-0 coated Vicryl. This model permitted evaluation of the possibility that osteoprogenitor cells might respond to a leachable substance in PVDF, PVDFc, or both. In the same animals, polyvinylidene fluoride was implanted in a surgically created pouch in the spinodeltoideus muscle. This model permitted evaluation of the possibility that mesenchymal cells — a less differentiated form of the osteoprogenitor cells, found in many tissues including bone — were capable of responding to quasi-static surface polarization. In Groups A, B and C, flexion and extension of the joint distal to the
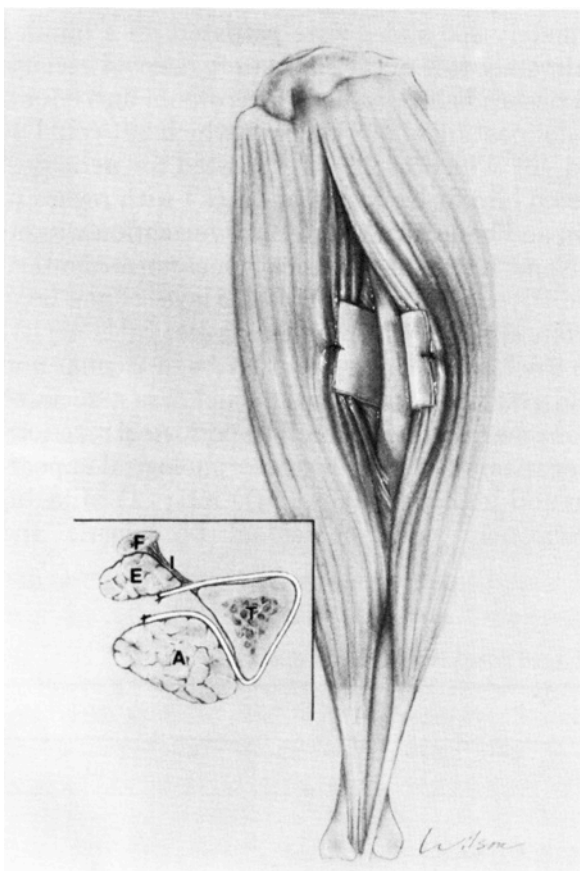

Fig. 2. Polyvinylidene fluoride film implanted in Group B rats. F, fibula; E, extensor digitorum longus; I, interosseus membrane; A, tibialis anterior; T, tibia.

implant was assumed to create a piezoelectric polarization on the surface of the PVDF. The relationship between the polarity of the polarization and the local tissue was not controlled in any Group. Group D consisted of 5 non-implanted control rats.

The rats were sacrificed (T–61 euthanasia solution) at predetermined times following surgery (Table 2), and the implant and adjacent tissue was removed and fixed in buffered formalin. The specimens containing bone were decalcified (Cal-Ex), dehydrated in a graded series of alcohols, embedded in wax, sectioned at 10 micrometers, and stained with hematoxylin and eosin. The muscle specimens from the Group C rats were prepared similarly except for the omission of the decalcification step.

In Groups A and B, 8–12 slides were made from representative regions of the specimen, with each slide containing 2–3 sections. Approximately 10% of the specimens were lost, either because of failure to recover the actual implant site, or because they were destroyed during decalcification or sectioning. From the remaining specimens, approximately 900 slides were prepared. In a blinded preliminary evaluation, approximately 45% of the slides were rejected because they exhibited too little of the tissues being studied (periosteum and bone). This procedure resulted in the delineation of 508 slides of which 501 could be evaluated for bone growth and 467 which could be evaluated for periosteal reaction. Each slide was evaluated blindly by one of us (E.G.) with regard to the extent of periosteal reaction and bone formation. Bone formation was divided into four categories: (1) None, histomorphological appearance identical with that seen in non-implanted (Group D) rats; (2) Mild, a layer of new bone less than 40 yin thick in immediate apposition to preexisting cortical bone; (3) Moderate, new bone 40–80 µm thick exhibiting several layers of lacunae and osteocytes; (4) Marked, osteoid greater than 80 µm in thickness associated with capillaries, lacunae, and bone marrow formation. The periosteal reactions were also divided into four categories: (1) None, histomorphological appearance identical with that seen in non-implanted (Group D) rats; (2) Mild, increased thickness of the periosteum with occasional fibroblastic and

TABLE 2

Number of rats sacrificed in each implanted group at various postoperative times

Group	Postoperative time (weeks)					
	1	2	3	4	6	10
A	4	5	4	6	5	—
B	6	5	5	5	5	—
C	—	—	5	—	—	15

Group D consisted of 5 non-implanted control rats.

lymphocytic reaction; (3) Moderate, a 50-100% increase in periosteal thickness and a greater presence of lymphocytes; (4) Marked, periosteal thickness greater than 100% of the periosteal thickness in the control (Group D) rats accompanied by dense lymphocytic infiltrates.

Since 10% of the specimens were lost during processing, paired analysis of the data from Groups A and B was not possible in 20% of the animals in both groups. Additionally, the number of slides accepted for detailed evaluation (6.0 ± 2.3) varied with the specimen. These factors necessitated an unpaired analysis of the histological evaluations. The data were evaluated using the chi-square test with a 4 X 2 contingency table. To satisfy the requirements of the test regarding numbers of observations in each category, it was necessary to combine the data from Groups A and B. A 3 X 2 or 2 X 2 contingency table was formed by combining categories if the expected frequency in one of the cells was less than 5. The Yates continuity correction was applied in the case of the 2 X 2 table. The level of significance chosen was $P < 0.05$. The null hypothesis was that the effects of PVDF and PVDFc on periosteal proliferation and bone growth (occurring by virtue of their ability to elicit a foreign-body response, supply mechanical stimulation to the periosteum, and secrete leachable agents into the adjacent tissue) were equal to one another.

In Group C the polyvinylidene fluoride was lost in processing in 18 of 40 muscle specimens, and 9 of 40 femur specimens; 6-7 representative slides (2–3 sections per slide) were prepared from each of the remaining specimens as described above.
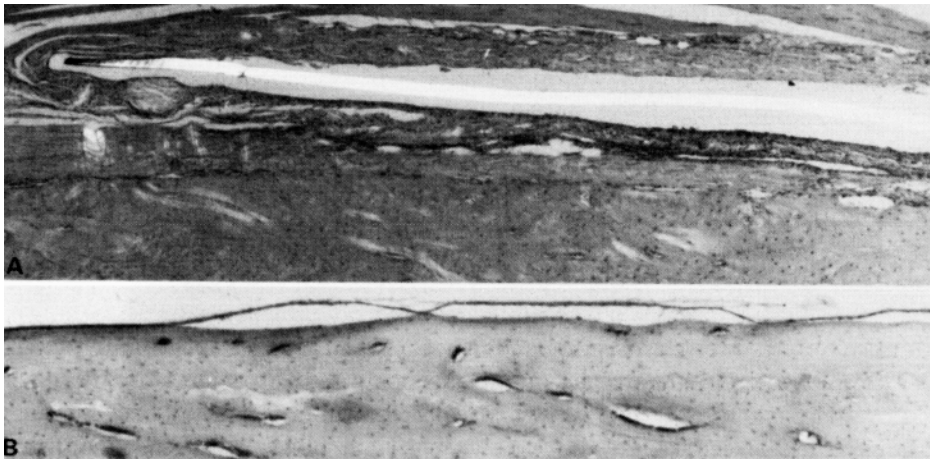

Fig. 3. (A) Photomicrograph taken with polarized light showing the position of PVDFc in relation to the adjacent tissue after six weeks. The PVDFc (bright white line) is covered by a fibrous capsule which separates it from muscle and bone. Both bone and periosteum exhibited a "Mild" reaction. (B) Bone from an unimplanted rat. The periosteum became slightly elevated in some areas during processing of the specimen. (A) and (B), hematoxylin and eosin, X 40.
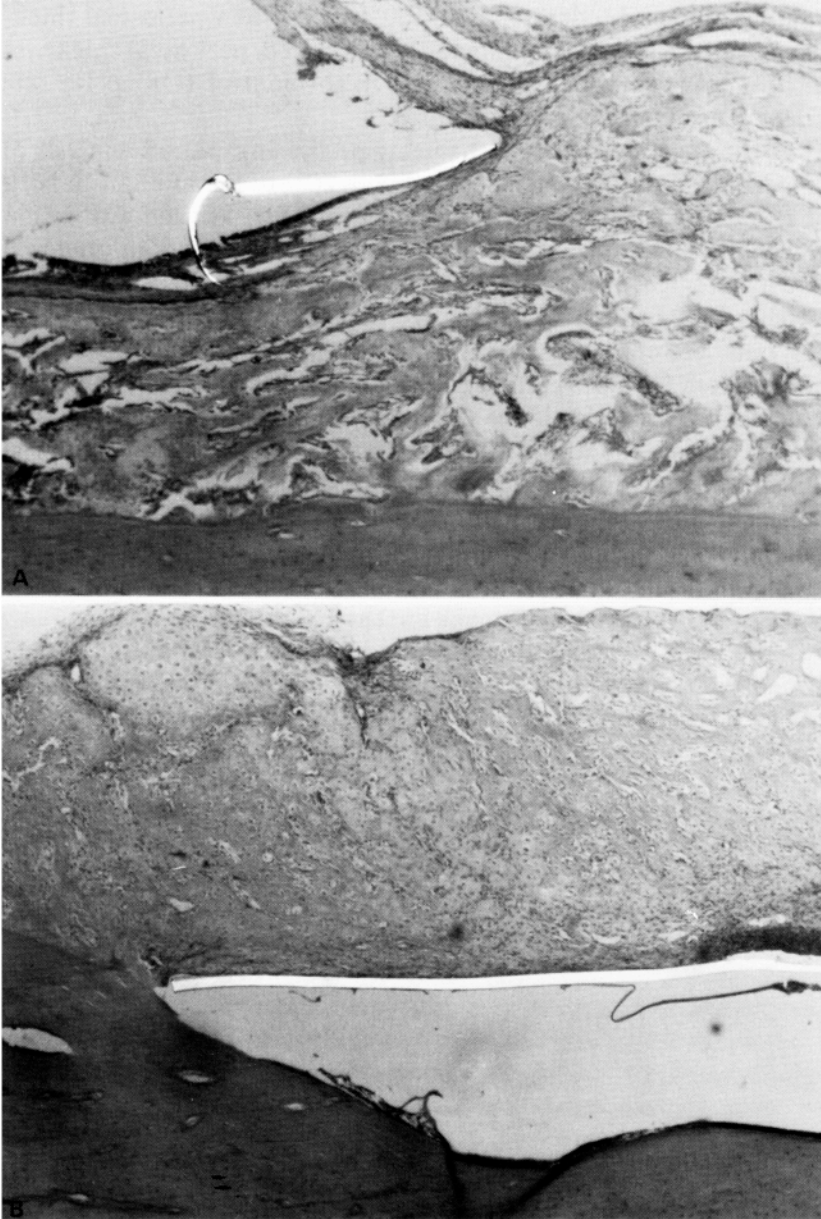
Fig. 4. Photomicrographs of PVDF-implanted rats taken with polarized light. (A) A "Marked" osteogenic response occurred below the implanted film after 14 days. (B) A "Marked" osteogenic response occurred above the implanted film after 7 days. (A) and (B), hematoxylin and eosin, X 40.
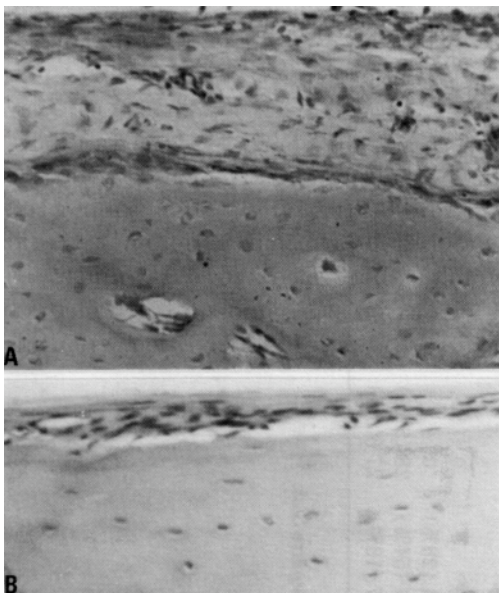
Fig. 5. (A) Marked periosteal reaction in a PVDF-implanted rat after 14 days. (B) Unimplanted control rat. (A) and (B), hematoxylin and eosin, X 125.

Results

The presence of polyvinylidene fluoride elicited a bone reaction that ranged from a walling-off response (Fig. 3), to massive osteoid formation either below or above the implanted film (Fig. 4). The periosteal reaction similarly exhibited a wide range of histomorphological appearances (Fig. 5).

The numbers of slides in Groups A and B blindly assigned to each growth category are listed in Table 3. The bilateral frequency distributions of degree of bone growth were significantly different at each postoperative interval for which the comparison was made (Table 3). The distributions for periosteal reaction were significantly different only at 1 and 2 weeks postoperatively. The trend of the data is shown in Fig. 6 where the frequencies, converted to percentages, are displayed as bilateral differences. For bone, a consistent pattern of increased bone growth (positive values in the Moderate and Marked growth categories) was observed at each time interval studied. In contrast, the periosteal distribution was skewed in the direction of increased reaction in the Moderate and Marked categories only at the first and second postoperative week.

After 3 weeks, both films implanted in muscle (Group C) were encapsulated by connective tissue 30–40 μm thick. After 10 weeks the capsule thickness was about 60 μm, but again there was no bilateral difference in either the amount or organization of the induced tissue. Occasional foreign-body giant cells were

TABLE 3

Classification of slides (groups A and B combined) by extent of periosteal reaction and bone formation as a function of implant time and presence of electrical poling (our surrogate for piezoelectric polarization). The percentage of slides in each cell is also listed

Growth	Number of slides									
	1 Week		2 Weeks		3 Weeks		4 Weeks		6 Weeks	
Periosteum	PVDFc	PVDF	PVDFc	PVDF	PVDFc	PVDF	PVDFc	PVDF	PVDFc	PVDF
Marked	0	2 (4%)[a]	0	7 (13%)[a]	0	0[a]	0	0	0	0
Moderate	23 (41%)	36 (67%)	16 (38%)	37 (71%)	14 (35%)	14 (36%)	11 (24%)	25 (41%)	13 (23%)	7 (24%)
Mild	18 (32%)	8 (17%)	26 (62%)	6 (12%)	24 (60%)	20 (51%)	23 (51%)	27 (44%)	31 (54%)	20 (69%)
None	15 (27%)	0	0	2 (4%)	2 (5%)	5 (13%)	11 (24%)	9 (15%)	13 (23%)	2 (7%)
Bone										
Marked	2 (4%)	12 (22%)[a]	0	21 (40%)[a]	0	0[a]	0	5 (8%)[a]	0	0[b]
Moderate	15 (26%)	28 (51%)	11 (23%)	14 (26%)	0	11 (28%)	0	15 (23%)	5 (8%)	6 (20%)
Mild	23 (40%)	6 (11%)	31 (66%)	11 (21%)	26 (65%)	21 (54%)	28 (51%)	25 (38%)	23 (39%)	16 (53%)
None	17 (30%)	9 (16%)	5 (11%)	7 (13%)	14 (35%)	7 (18%)	27 (49%)	21 (32%)	31 (53%)	8 (27%)

PVDFc, unpoled polyvinylidene fluoride; PVDF, poled polyvinylidene fluoride.
Superscripts indicate bilateral differences in distributions as determined by the chi-square test:
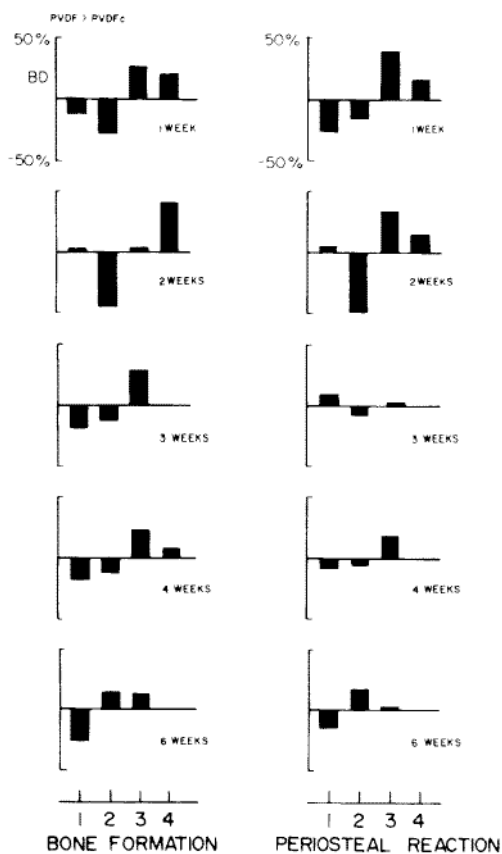[a] $P < 0.001$.
[b] $P < 005$

Fig. 6. Bilateral difference (BD) in the percentage of slides (Groups A and B combined) assigned to the various growth categories. (1) None; (2) Mild; (3) Moderate; (4) Marked.

TABLE 4

The effect of electrical poling of polyvinylidene fluoride on lacunae density in subadjacent cortical bone

Time (weeks)	Lacunae	
	PVDFc	PVDF
3	22±7	28±6
10	19±7	16±8

PVDFc, unpoled polyvinylidene fluoride; PVDF, poled polyvinylidene fluoride; Lacunae, number of lacunae per high-power (microscopic) field.

seen in the vicinity of both PVDF and PVDFc. No histomorphologically recognizable changes in the myocytes or mesenchymal cells in the muscle were observed in association with either film at 3 or 10 weeks postoperatively. The film placed on the femur in the Group C rats elicited no periosteal or bone reaction at either 3 or 10 weeks after surgery. The cell layer in the periosteum adjacent to both PVDF and PVDFc remained 1-2 cell layers thick, and the number of lacunae in the subadjacent bone exhibited no bilateral variation in lacunae density at either 3 or 10 weeks (Table 4). Average lacunae diameter (10-20 µm) was also unaffected by poling.

Discussion

Implantation of PVDF consistently resulted in increased bone growth compared to PVDFc (Table 3, Fig. 6). The largest effects (greatest bilateral differences) were observed at 1 and 2 weeks postoperatively at which time the bilateral differences in periosteal reaction were also significantly different (Table 3, Fig. 6). Since the periosteum is the source of the cells that proliferate and differentiate to form osteoblasts, it is not surprising that bilateral differences in bone growth abated subsequent to the cessation of bilateral differences in periosteal reaction. The bilateral differences in bone formation at 3-6 weeks postoperatively remained statistically significant (presumably because of the large pool of bone cells created by the PVDF during 0-2 weeks postoperatively), but the magnitude of the difference decreased.

The observed differences cannot be attributed to a leachable agent associated with PVDF because such a hypothetical agent would also have been present in the femur implants in the Group C rats, but no effect on the periosteum or bone was observed in these animals. The basic (bilateral) reaction in the Group C bone implants was a walling off of the film by the formation of a fibrous capsule which separated the film from the periosteum and sequestered it from the adjacent muscle tissue. We conclude, therefore, that the observed bilateral differences in growth responses were caused by the quasi-static charge distributions that appeared on the surface of the PVDF which was mechanically loaded via its fixation to the musculoskeletal system (Groups A and B).

Demineralized bone implanted in muscle (and elsewhere) can trigger proliferation and differentiation of local mesenchymal cells, resulting in formation of heterotopic bone [9]. Demineralized bone is piezoelectric, but this property does not account for the bone formation [10], which appears to result from an interaction between the mesenchymal cell and a spatial charge distribution formed by a specific peptide sequence in the matrix [11]. Despite the capability of the mesenchymal cell to respond physiologically to the static charge distribution contained in the matrix, mesenchymal cells lack the capability to respond to the quasi-static charge distribution produced on the surface of PVDF. Thus, the evidence suggests that the osteoprogenitor cell, a more differentiated

cell compared to the mesenchymal cell, was the periosteal cell that actually responded to the PVDF.

The surface polarization of the PVDF is given by $P_3 = d_{31}T_1$, where T_1 is the stress in the plane of the film. Assuming a force of 1 gm, $T_1 = 10^6$ dynes/cm^2. Since $d_{31} = 9$ pC/N $= 9 \times 10^{-17}$ C/dyne, $P_3 = 90$ pC/cm^2. When an intact human femur was subjected to a simulated physiological load, the average of the absolute value of 145 measurements of the polarization was 7 pC/cm^2 (range, 0.3-30 pC/cm^2) [12]. The magnitude and sign of the measured charge densities were theoretically correlated with a self-consistent change in the outline of the bone [4]. Thus the occurrence of an osteogenic response associated with 90 pC/cm^2 is in good agreement with the interpretation of direct measurements of the polarization produced in bone.

The capacitance of the implanted PVDF can be estimated from C= (€A/4πt, where A and t are the surface area and film thickness, respectively, and € is the relative dielectric constant. Assuming the values listed in Table 1, the capacitance of a 3-cm strip of PVDF (4 mm wide) is 540 pF. Since $V_3 = Q_3/C$, where V_3 is the voltage across the PVDF surfaces, and Q_3 is the total charge on each surface, $V_3 = 108$ pC/540 pF $= 0.2$ V. Bimetallic implants have been reported to stimulate regeneration in frogs [13], rats [14], and to promote healing of joint cartilage in rabbits [15]. Silver-platinum couples (apparently the most biologically effective combination) exhibited open-circuit voltages of about 0.3 V [16]. Thus, the piezoelectrically induced voltage associated with the osteogenic response reported here is comparable in magnitude to the electrochemically generated voltages associated with osteogenic responses in other biological studies.

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