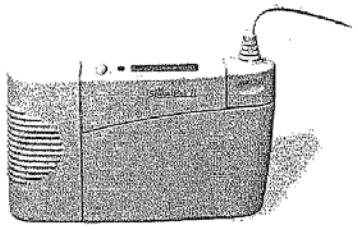
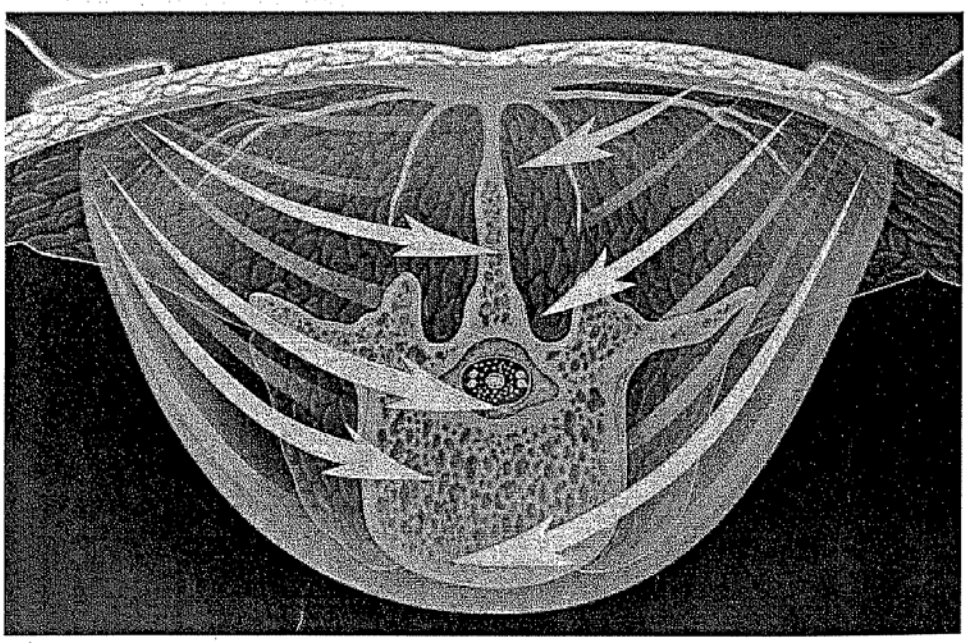
Innovation Meets Evolution...EBI Spine is now:

BIOMET® SPINE

Think of a spine stimulator with the following capabilities...

- *Effective in various cases*
- *A double-blind placebo controlled trial demonstrated radiographic and clinical success rates*
- *Easy to use - the smallest and lightest of its kind*
- *Demonstrated safety profile*





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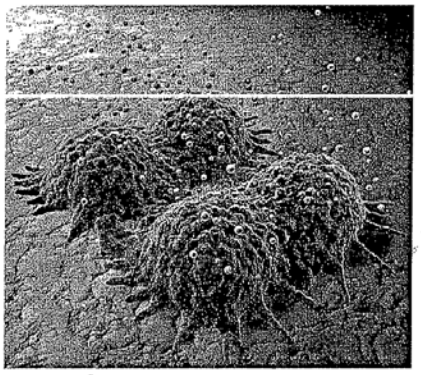
The Biomet SpinalPak® II is all of those and more...

Whether it's clinical success or scientific proof, you can find the answer in the SpinalPak II.

- 84.7% fusion success rate with both clinical and radiographic measures[1]
- The **SpinalPak II** was proven more effective than placebo in smokers, those without instrumentation, and those with multiple level fusions[1]
- Compact and lightweight design allows for enhanced patient compliance and comfort
- The Capacitive Coupling signal that is singular to the EBI **SpinalPak II** has a demonstrated Mechanism of Action[2]



Ask your Biomet sales representative about how the SpinalPak II upregulates BMPs and potentially heals your patients faster.

References:
1 SpinalPak PMA #P850022S9
2 Lorich, Dean G. Biochemical Pathway Mediating the Response of Bone Cells to Capacitive Coupling. Clinical Orthopaedics and Related Research. No. 350, pp. 246-256, 1998.

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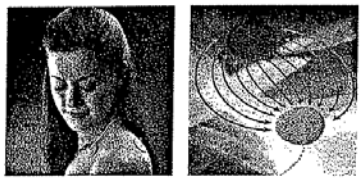
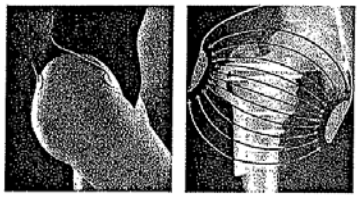
OrthoPak®2 Bone Growth Stimulator



Electrodes adhere directly to skin for easy placement. Red arrows indicate field of influence.



Alternating low-voltage capacitive coupling technology – expands in soft tissue to treat larger areas.

Low profile electrodes allow them to be worn under any type of brace.

- Proprietary capacitive coupling technology offers a large field of influence within area of electrodes for treating even your largest fracture site.
- Flexible electrodes allow for easy placement even in the most difficult-to-reach nonunion fracture sites.
- Capacitive coupling stimulation upregulates naturally occurring BMPs. BMP-2, 4, 5, 6, 7, TGF-ß, FGF-ß, VEGF, and PGE^2
- Continuous 24-hour treatment provides an optimized treatment dose.
- Rechargeable battery system offers convenient easy treatment maximizing patient compliance.

Product Information
OrthoPak2 Bone Growth Stimulator
Item No. 5230
Physician Test Kit 5235 (adapter with meter)

Rx Only - By prescription only

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OSTEOBIOLOGICS
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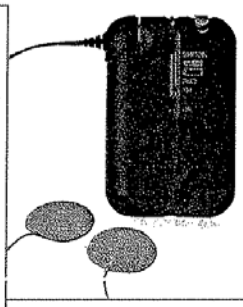
BIOMET® OSTEOBIOLOGICS

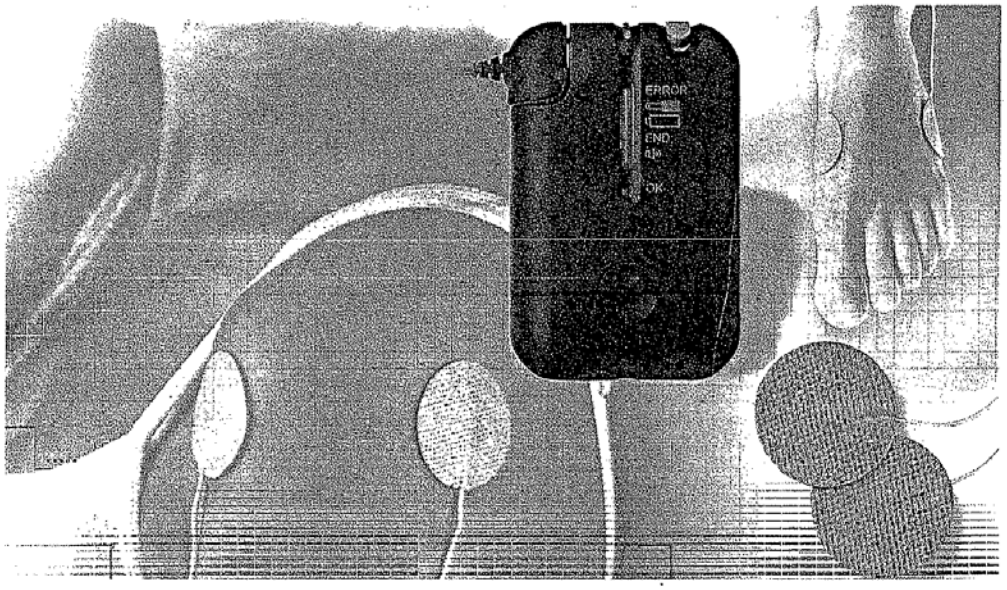
OrthoPak®2 Bone Growth Stimulator

The Lightest And Most Convenient Way To Treat Nonunions

- *Clavicle*
- *Proximal Humerus*
- *Proximal Femur*
- *Metatarsal*

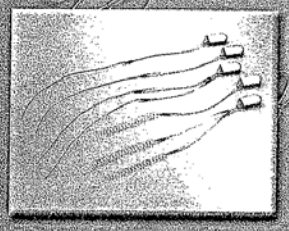


ORTHOPAK®2
Bone Growth Stimulator



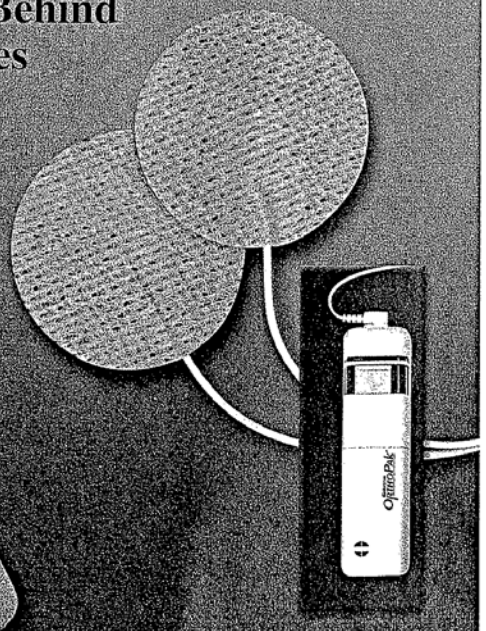
STIMULATING A HEALING MECHANISM OF BONE*

EBI BONE GROWTH TECHNOLOGIES
STIMULATION AND BIOLOGICS



Bone Growth Stimulation Treatment Options for the Appendicular System

The Products Behind the Technologies

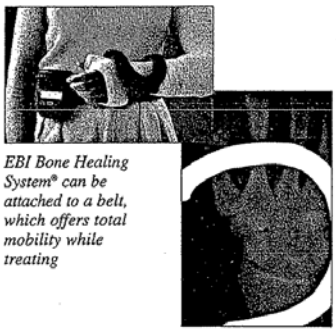


Optimize the Most Appropriate Bone Growth Stimulation Options for Your Patients

EBI® BONE HEALING SYSTEM®

Optimal Potential for Earlier Healing

- 10-hour optimal treatment dose for potential earlier healing[1]
- Only EBI's PEMF offers clinically effective treatment with easy-to-use, lightweight, ultrasoft FLX® flexible treatment coils
- Three approved indications: nonunions, failed fusions and congenital pseudarthrosis in the appendicular system
- Supported by over 250 published clinical studies and over 300 published basic science studies
- Over 22 years of clinical use... prescribed by over 39,000 orthopaedic surgeons for over 290,000 patients



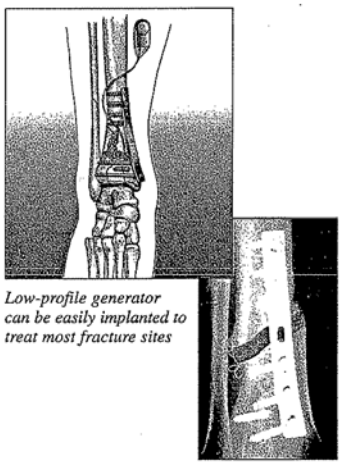
EBI Bone Healing System® can be attached to a belt, which offers total mobility while treating

Red area indicates field of influence

EBI® OsteoGen™
Surgically Implanted Bone Growth Stimulator

Optimal Surgical Adjunct

- Only EBI's direct current technology offers a totally implantable device, so it assures total patient compliance 24 hours/7 days a week
- Available with single or dual leads and with either straight or mesh cathode configurations
- Implantation does not require special instrumentation, and can be used with any surgical technique



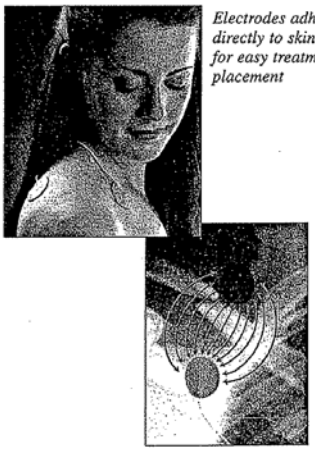
Low-profile generator can be easily implanted to treat most fracture sites

Red area indicates field of influence

Biolectron ORTHOPAK®

Optimal Comfort

- Only EBI's capacitive coupling technology offers the lightest, smallest, most compact, 24-hour noninvasive treatment
- Virtually no weight or system discomfort at fracture site—enhances patient compliance



Electrodes adhere directly to skin for easy treatment placement

Red area indicates field of influence

For more information, contact your EBI representative today or call **1-800-526-2579**

EBI. Turning the Science of Bone Formation Into the Success of Bone Healing

References: 1. EBI PMA P790002/S12

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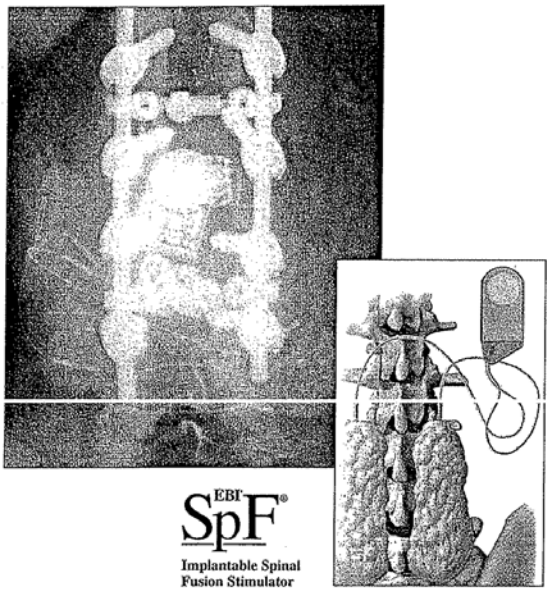
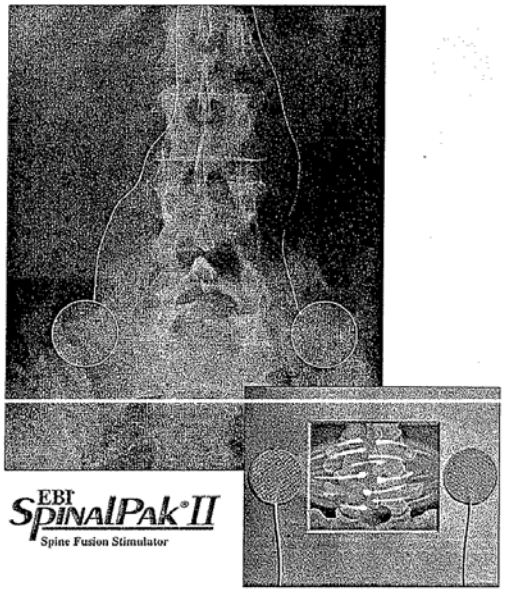
EBI...The Only Company that Can Offer You *Both* Implantable and Noninvasive Spine Fusion Stimulators



EBI SpF®
Implantable Spinal Fusion Stimulator

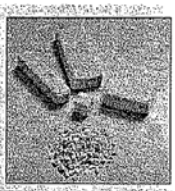


EBI SpinalPak® II
Spine Fusion Stimulator

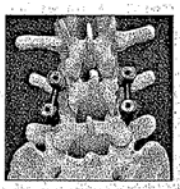
- More efficacious than autograft alone[1, 2]
- **SpF** (Direct Current) and **SpinalPak** (Capacitive Coupling) technologies involve the upregulation of numerous BMPs and osteopromotive growth factors**
- Backed by over 30 published scientific and clinical papers
- Surgeon CPT codes available – reimbursement for implantation and/or explantation

Additional EBI Products for Your Spine Patients.

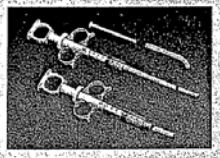
InterGro® DBM



EBI 200R/500R Granules and Blocks



Array™ Spinal System



EBI DBM Putty

For more information, contact your EBI representative today or call **1-800-526-2579**

References:
[1] EBI PMA P850035/PMA P850022/S9
[2] PMA, P850022/S9, Sept. 24, 1999
*EBI's Mechanism of Action involves the upregulation of BMPs and other osteopromotive factors as demonstrated in in vitro and animal in vivo studies.
**As demonstrated in pre-clinical models

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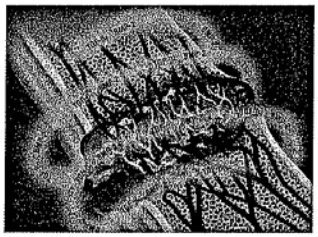
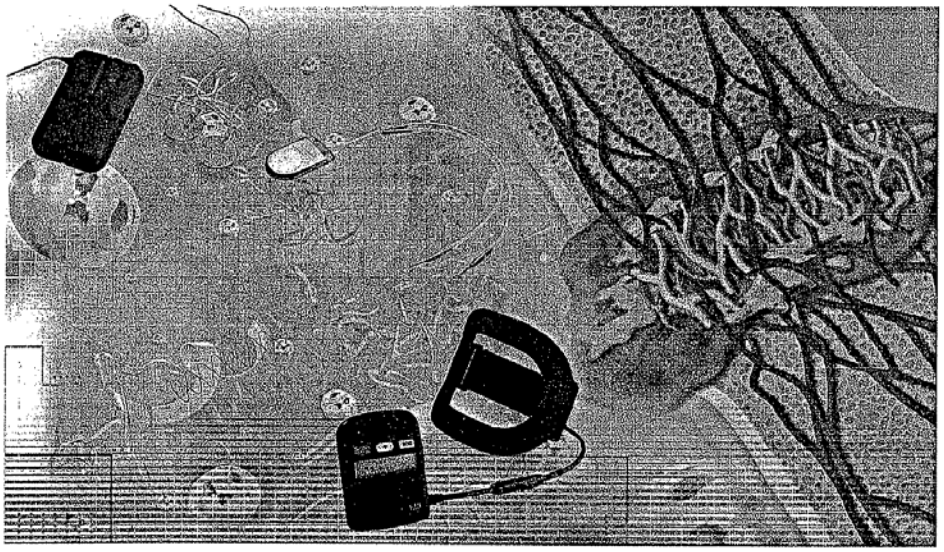
Growth Factor Matrix

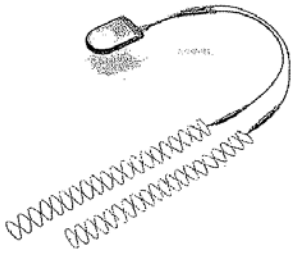


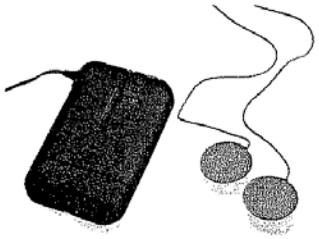
Bone Growth Technologies™
Stimulation And Biologics



The Role Growth Factors Play In Bone Healing

The first several days after a fracture, there is an inflammatory response. Platelets, inflammatory cells, and mesenchymal stem cells enter the treatment area and form a hematoma at the fracture site. Over the next few weeks, chondrogenesis occurs, forming fibrocartilage across the fracture gap. This fibrocartilage becomes the framework for bone calcification.





	Biomet PEMF	Biomet CC	Biomet DC
Stages	✓	✓	✓
BMP-2 **(Bone Morphogenetic Protein-2)** BMP-2 is produced by osteoblasts	✓	✓	✓
BMP-4 **(Bone Morphogenetic Protein-4)** BMP-4 is produced by osteoblasts	✓	✓	
BMP-6 **(Bone Morphogenetic Protein-6)**		✓	✓
BMP-7 **(Bone Morphogenetic Protein-7)** **Or Osteogenic Protein-1 (OP-1)** BMP-7 has been involved in bone healing		✓	✓
TGF-β_1 (Transforming Growth Factor-Beta) TGF-β_1 is found in proliferating mesenchymal stem cells, osteoblasts and in the matrix	✓	✓	
FGF-2 (Fibroblast Growth Factor-2) FGF-2 increases the recruitment of osteoblast and osteoclast precursor cells and stimulates angiogenesis	✓	✓	
VEGF (Vascular Endothelial Growth Factor) VEGF is involved in bone healing		✓	
PGE_2 Prostaglandin E_2	✓	✓	

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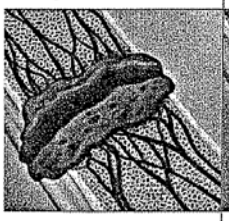
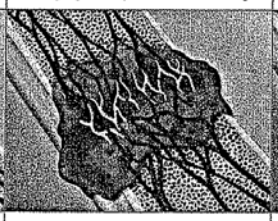
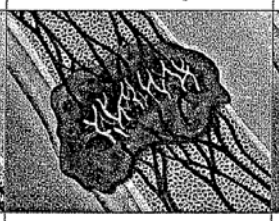
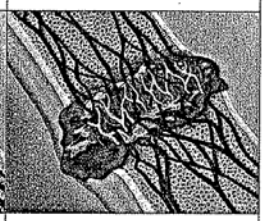
Week 1	Weeks 2 - 4	Weeks 4 - 12	Weeks 12 - 40
Hematoma Formation - Migration Of Cells	**Chondrogenesis -** Bridging Of Gap With Fibrocartilage	**Calcification And Vascularization Of Fibrocartilage**	**Remodeling Into Mature Bone**
			
MP-2 is induced immediately r fracture, when mesenchymal stem cells are recruited to the site of injury.	BMP-2 promotes bridging of callus, and stimulates mesenchymal cell differentiation to a chondroblastic lineage.	BMP-2 increases overall callus area, accelerates conversion from soft to hard and bony callus and stimulates osteoblast migration and differentiation.	Wolff's law takes effect. New bone appropriately remodels, responding to biomechanical and biological stimuli.
	BMP-4 has been found to play a critical role in the differentiation of mesenchymal cells.	Found in high levels in newly formed bone, BMP-4 levels peak during active osteogenesis, predominantly in osteoblasts lining the calcified cartilage matrix.	
	BMP-6 influences proliferation and differentiation of mesenchymal cells, as well as increasing chondrogenesis and osteogenesis.	BMP-6 initiates osteoblast differentiation.	
em cells are actively recruited from the blood supply and surrounding tissues. BMP-7 ates an environment in which stem cells multiply prior to differentiation.	The cascade of bone healing is supported by the stimulation of other BMP's, TGF-ß, and vascular growth factors to support the bone formation process. The signaled bone-forming cells begin to organize. New vascular ingrowth occurs.	Significant osteoblastic activity is supported by vascular ingrowth. Corollary osteoclasts begin to appear.	
ing early hematoma formation, -ß, is released by platelets and inflammatory cells. TGF-ß, motes blood vessel formation.	TGF-ß, enhances periosteal cell differentiation, chondrogenesis, and osteogenesis.	TGF-ß, promotes blood vessel formation, stimulates proliferation of osteoblasts and chondrocytes, enhances production of extracellular matrix and regulates osteoclastic-osteoblastic interaction.	
uring early bone repair, FGF-2 ncreases cell migration and angiogenesis.	FGF-2 is associated with an increase in mesenchymal cells, and the differentiation of these cells into chondrocytes and osteoblasts. FGF-2 promotes callus formation.	FGF-2 increases the volume and bone mineral content of the callus, enhances osteoblast proliferation, osteoblast activity, and callus remodeling and angiogenesis.	
GF is found in large amounts in fracture hematoma, and promotes angiogenesis.		VEGF induces angiogenesis, regulates vasculogenesis, and is important in the conversion of soft to hard callus[1]. VEGF recruits and activates osteoclasts, and stimulates osteoblast chemotaxis and differentiation, as well as matrix mineralization.	
	PGE_2 stimulates differentiation and proliferation of osteoprogenitor cells and increases callus size.	PGE_2 accelerates fracture remodeling by increasing the number and activity of osteoclasts, stimulating the proliferation of osteoprogenitor cells, and recruiting osteoblasts from their precursors.	

Supplied By Manufacturer

NONUNION DIAGNOSIS:

In a letter from the American Academy of Orthopedic Surgeons to EBI's Medical Director dated April 11, 1988, it was stated:

> "A non-union is that state in the healing of a fracture where the healing process has ceased; the fracture has failed to heal by osseous union and if osseous union is needed, intervention is required. Delayed union is that stage where a fracture has failed to unite with bone in the usual time expected for a given fracture in a given bone, in a given patient being treated by a given method. The classification of the un-united fracture as a delayed union or nonunion is best made by careful assessment of the relevant parameters by an experienced physician."

Supplied By Manufacturer

J Dent Res 73(10):1601-1605, October, 1994

Pulsating Electromagnetic Field Stimulates mRNA Expression of Bone Morphogenetic Protein-2 and -4

M. Nagai and M. Ota[1]

Department of Biochemistry, Iwate Medical University School of Dentistry, Morioka, Iwate 020, Japan; [1]to whom correspondence and reprint requests should be addressed

Abstract. The effects of a pulsating electromagnetic field on mRNA expression of bone morphogenetic protein-2 and -4 in chick embryonic calvaria were examined. From the onset of embryogenesis (Day 0), chick embryos were incubated in a continuously generated pulsating electromagnetic field with a peak of 3.5 milli-Tesla (mean: 2 milli-Tesla) and vibration at 15 Hz. Control chicks were incubated in a normal magnetic field. Northern-blot analysis showed that the mRNAs of bone morphogenetic protein-2 and -4 were expressed in the calvaria. Quantitative analysis of the mRNA expressions was done by means of slot-blot hybridization. The magnetic field enhanced the expressions of both mRNAs. The enhancements were more pronounced in younger chick embryos (Day 15 > Day 17), and no significant change was observed in the 19-day-old embryos. These results indicate that osteo-inductive effects of the magnetic field were mediated at least in part by bone morphogenetic protein-2 and -4.

Key words. Bone and Bones, Extracellular Matrix Proteins, Bone Morphogenetic Protein, Electromagnetic Field.

Received October 25, 1993, Accepted July 21, 1994

Introduction

Over the past two decades, pulsating electromagnetic field (PEMF) has been used extensively for treatment of non-union fractures (Bassett *et al.*, 1974a,b; Connolly *et al.*, 1977; Brighton *et al.*, 1981; Jingushi *et al.*, 1990), and its biological effects on bone tissue have been investigated. Those investigations have revealed that PEMF stimulates all aspects of bone formation: (1) cell proliferation (Rodan *et al.*, 1978; Ashihara *et al.*, 1979; Norton *et al.*, 1979, 1980; Brighton *et al.*, 1984); (2) matrix formation (Brighton *et al.*, 1984; Fitzsimmons *et al.*, 1986); and (3) calcification (Bassett *et al.*, 1979; Colacicco and Pilla, 1984; Norton and Rovetti, 1988; Takano-Yamamoto *et al.*, 1992). However, we do not yet know what translates the electrical stimulation into the initiation signal for bone healing. It is supposed that the initiation signal has bone-inducing ability, because PEMF not only stimulates ongoing bone formation but also initiates *de novo* bone formation in non-union fractures.

Many factors are known to be involved in bone growth and repair, such as fibroblast growth factor (Jingushi *et al.*, 1990), transforming growth factor (Joyce *et al.*, 1990), osteopontin (Ohta *et al.*, 1991), osteocalcin (Ohta *et al.*, 1991), and insulin-like growth factor (Edwall *et al.*, 1992). However, none of them has been shown to induce bone formation by itself. Currently, bone morphogenetic protein (BMP) is the only protein family known to singly induce ectopic bone formation (Wozney *et al.*, 1988; Wang *et al.*, 1990; Ozkaynk *et al.*, 1990). Accumulating *in vitro* evidence indicates that BMP induces various phenotypic expressions of bone cells (Vukicevic *et al.*, 1989, 1990; Katagiri *et al.*, 1990; Takuwa *et al.*, 1991; Yamaguchi *et al.*, 1991; Hiraki *et al.*, 1991; Chen *et al.*, 1991; Thies *et al.*, 1992; Sakano *et al.*, 1993). In addition, Hulth *et al.* reported that BMP may be exuded from the broken ends of bones (Hulth *et al.*, 1988;

Hulth, 1989). Those studies led us to the hypothesis that PEMF-induced bone formation is initiated by BMP expression. In the present study, we examined the effects of PEMF on the expression of BMP mRNA in growing chick embryonic calvaria.

Materials and methods

Electromagnetic stimulator

PEMF was produced by means of a pulse generator and 10 cm x 10 cm Helmholtz coils (Electrobiology, Fairfield, NJ). The generator produced quasi-rectangular, symmetric AC pulses. Each signal had a mean peak amplitude of about 15 mV, a duration of 200 μsec for the main, and 24 μsec for the opposite polarity. The burst duration for the pulse train was 5 msec, and the repetition rate was 15 Hz. The resulting PEMF had a magnetic field of 3.5 mTesla at peak power and an electrical field of 9 mV/m (Goodman *et al*., 1988).

Stimulation of chick embryos with PEMF

Fertilized White Leghorn eggs were purchased from Koiwai Farm (Shizukuishi, Iwate, Japan). Control eggs were incubated at 37.6°C in a humidified incubator from the onset of embryogenesis (Day 0) to Day 15, 17, or 19. The experimental eggs were placed vertically at the center of the pair of Helmholtz coils, 15 cm apart, set in a separate incubator under the same conditions.

RNA isolation and analysis

After each incubation period, calvaria were dissected, immediately frozen in liquid nitrogen, and stored at -80°C until later isolation of RNA could be performed. Total RNA was isolated from calvaria by the acid guanidine phenol chloroform method (Chomczynski and Sacchi, 1987). For subsequent Northern hybridization, 20 μg of pooled total RNA of 10 calvarial halves was resolved on a denaturing agarose/formaldehyde gel. The size-fractionated RNA was transferred onto a nylon membrane (Zeta probe GT membrane, BIO-RAD, Richmond, CA) with 50 mM of NaOH used as a transfer solvent. After transfer, the membrane was rinsed with 2X SSC and baked at 80°C for 30 min. Then the membrane was pre-hybridized in a solution of 7% SDS, 50% formamide, 5X SSC, 50 mM sodium phosphate buffer, 2% blocking reagent (Boehringer Mannheim Biochemica, Germany), 0.1% lauroylsarcosine, and 50 μg/mL yeast total RNA at 42°C for 2 h. mRNAs of interest were located by hybridization at 42°C for 6 h with digoxigenin(dig)-11-UTP (Boehringer Mannheim Biochemica) labeled RNA probes followed by immunological chemiluminescent detection. The probes were labeled with dig-11-dUTP by use of a dig RNA labeling kit (Boehringer Mannheim Biochemica). Briefly, to make the antisense probe, human BMP-2 cDNA (Wozney *et al*., 1988) was linearized with Hind III and transcribed *in vitro* by T7 RNA polymerase with NT labeling mixture containing 350 μM dig-11-UTP, 650 μl TTP, and 1 mM each of ATP, CTP, and GTP. Similarl human BMP-4 cDNA (Wozney *et al*., 1988) was linearize with EcoRI and transcribed by SP6 RNA polymerase. Mous β-actin cDNA (Alonso *et al*., 1989) linearized with Hind I and human liver/bone/kidney-type alkaline phosphatas (ALPase) cDNA (Weiss *et al*., 1986) linearized with EcoR were transcribed by T7 RNA polymerase in the presence o dig-11-UTP. Chemiluminescent detection was performed i the following order: (1) detection of dig with anti-di antibody conjugated to ALPase; (2) catalyzing reaction o ALPase with chemiluminescent substrate; and (3 documentation of ALPase-catalyzed luminescence on x-ra film.

Expression levels of the specific mRNAs in the individual chicks were quantitated by means of slot-blo hybridization. Sixteen-microgram total RNA from the individual calvaria were dissolved in 200 μL of 50 mM NaOH and blotted onto a filter by use of a slot manifold (Manifold II, Schleicher and Schuell, Dassel, Germany). Then, hybridization and detection were performed as above. The signal intensities were determined by densitometry. Several exposures were made for the same blot to ensure that band intensities were within an appropriate range for densitometric analysis. Slot-blot signals of BMP-2 and -4 developed on x-ray films were scanned with a laser densitometer (Ultroscan Laser Densitometer model 2202, LKB, Bromma, Sweden), and each peak area of the blot was integrated by use of a computer software program, NIH image (Wayne Rasband, National Institutes of Health). The slot-blot analysis was carried out in triplicate, and the mean values and standard deviations of the three results in each group were computed. The statistical analysis was performed by Student's *t* test; values for control and PEMF-stimulated embryos were compared ($^{*}P < 0.05$; $^{**}P < 0.01$).

Results

At first, the expression of BMP-2 and -4 mRNA in chick embryonic calvaria was examined by Northern-blot analysis. A single transcript of 3.3 kb for BMP-2 and 2.2 kb for BMP-4 was observed (Fig. 1). In the control embryos, these expressions were stronger in the 19-day-old embryo than in the 15-day-old embryo. Interestingly, in the 15-day-old embryo, the mRNA expressions of both BMP-2 and -4 appeared to be enhanced by PEMF, while β-actin and ALPase mRNAs were expressed equally in all groups. However, the increases were not very impressive, and the analysis was performed on a pool of RNA from 5 embryos per group. Therefore, slot-blot analysis for BMP-2 and -4 was performed on the individual embryos in order to make a statistical evaluation (Fig. 2). As a result, BMP-2 mRNA expression was significantly increased by PEMF; a 2.7-fold

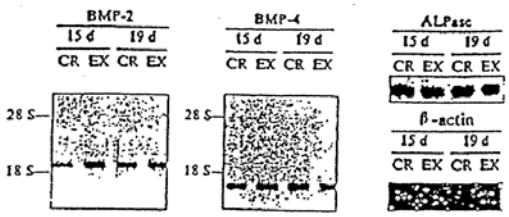


Figure 1. Northern-blot analysis of total RNA in chick embryonic calvaria. Total RNA was extracted from five embryos per group and pooled. Control chick embryos (CR) were incubated in a normal magnetic field. Experimental chick embryos (EX) were incubated in the continuously generated PEMF. Twenty micrograms of the pooled RNA was laid on each lane for Northern blot. Blots were hybridized with probes for BMP-2 and -4, ALPase, and β-actin.

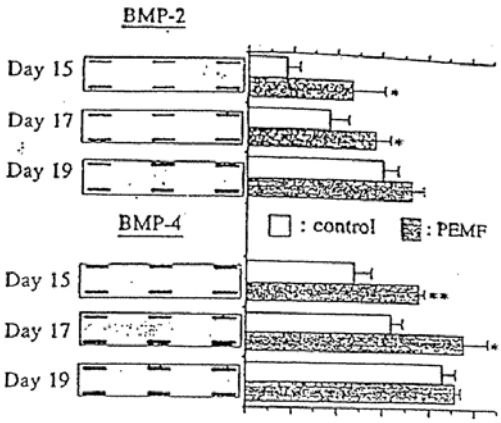


Figure 2. Slot-blot analysis of total RNA in chick embryonic calvaria. Total RNA was extracted from 15-, 17-, and 19-day-old embryos. Control chick embryos (CR) were incubated in a normal magnetic field. Experimental chick embryos (EX) were incubated in continuously generated PEMF. Sixteen micrograms of total RNA were blotted onto a nylon membrane and hybridized with probes for BMP-2 and -4. The slot-blot analysis was carried out in triplicate for each group, and quantitation of the signal intensities on each blot was performed by use of a laser densitometer (Ultroscan Laser Densitometer model 2202, LKB, Bromma, Sweden). Peak area of each blot was integrated by use of a computer software program, NIH image (Wayne Rasband, National Institutes of Health). Mean values and standard deviations of the three results in each group were computed and shown in the right bar graph. The statistical analysis was performed by Student's *t* test; values for control and PEMF-stimulated embryos were compared (*$P < 0.05$; **$P < 0.01$).

increase in Day 15 and a 1.6-fold increase in Day 17. Although the mRNA expression of BMP-2 increased with the age of the embryo, there was no significant difference between the control and the PEMF-stimulated calvaria in the 19-day-old embryo. A similar increase in BMP-4 mRNA expression was induced by PEMF; 1.6-fold and 1.5-fold increases were observed in Days 15 and 17, respectively. At Day 19, no significant difference was observed.

Discussion

The PEMF tested in this study is considered to exert the optimal effect on osteogenesis (Bassett *et al.*, 1977, 1981) and is used for treatment of non-union fractures. PEMFs of lower frequencies have been reported to exert embryotoxic and teratogenic effects on developing embryos (Zusman *et al.*, 1990). However, the PEMF we used did not induce any abnormalities in the chick embryos; retarded growth or development and significant changes in body weight or size were not observed.

The present study showed that the sizes of BMP mRNAs (BMP-2, 3.3 kb; BMP-4, 2.2 kb) expressed in chick embryonic calvaria were similar to those reported for other tissues or cells: 3.5-kb BMP-2 mRNA in mouse embryos (Lyons *et al.*, 1990) and 2.2-kb BMP-4 mRNA in human osteosarcoma cell line U-2 OS (Özkaynk *et al.*, 1990). These indicate the structural similarities of chick and mammalian BMP mRNAs.

We observed that the mRNA expression of BMP-2 and of -4 in the calvaria increased with the age of the chick embryo. However, the studies on Xenopus embryos (Nishimatsu *et al.*, 1992) and mouse embryonic limbs (Bassett *et al.*, 1979) showed these expressions to be more pronounced in earlier developmental stages. This discrepancy is probably due to the difference in animal species or in tissues. For example, there is a difference in cellular composition between calvaria and limb, because osteoblastic lineage cells mainly act in calvaria, while chondroblastic lineage cells act first in the limbs.

Many reports have been published on the PEMF-induced alteration of levels of proteins (Brighton *et al.*, 1984; Norton and Rovetti, 1988; Goodman and Henderson, 1988) and cytokines (Cossarizza *et al.*, 1989, 1993), whereas little is known concerning the effects of PEMF on gene expression. In the present study, a stimulatory effect of PEMF on the gene expression of BMP-2 and -4 was indicated. The effect of PEMF was considered to be specific for the BMPs—at least ALPase mRNA expression, one of the osteogenic phenotypes, was not influenced by PEMF. This result is in accord with the result of Mose and Martin (1993). ALPase level was not affected by PEMF in chick embryos.

PEMF-mediated stimulation of expression of both BMP mRNAs was observed in Day 11 and 15 embryos but not in Day 19 embryos (Figs. 2 and 3). Compared with Day 19 embryos, Day 11 and 15 calvaria expressed low levels of the BMP mRNA, but responded well to PEMF to increase the levels of these mRNAs. This is probably caused by the difference in the number of cells induced to express BMP

mRNA expression by PEMF, i.e., the younger embryonic calvaria are rich in cells whose mRNA expressions of BMP's are inducible by PEMF, whereas the older ones are predominant in the cells constitutively expressing BMP mRNAs.

The cellular target of PEMF cannot be elucidated in this study, because calvarial tissue is composed of heterologous cell populations, including osteogenic cells and hematopoietic cells. However, since no reports of BMP mRNA expression in hematopoietic cells have been published to date, the PEMF-stimulated BMP mRNA expressions most likely originate from osteogenic cells, which are well-known producers of the morphogens (Wozney *et al.*, 1988; Wang *et al.*, 1990; Özkaynk *et al.*, 1990).

In summary, our present result demonstrates that PEMF augments mRNA expression of BMP-2 and -4. Taking into account the fact that BMP is involved in bone growth and repair (Wozney, 1992; Rosen and Thies, 1992; Luyten *et al.*, 1992), we conclude that the bone-inducible effect of PEMF is mediated at least in part *via* stimulation of mRNA expression of BMP-2 and -4.

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PULSED ELECTROMAGNETIC FIELDS INDUCE OSTEOGENESIS AND UPREGULATE BONE MORPHOGENETIC PROTEIN-2 AND 4 mRNA IN RAT OSTEOBLASTS *IN VITRO*.

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Introduction

Pulsed electromagnetic fields (PEMF) have been shown to be clinically beneficial in orthopaedic surgery when used to treat non-union and pseudoarthrosis after bone fracture. The osteogenic potential of rat calvarial osteoblasts is enhanced by bone morphogenetic protein-2 and 4 (BMP-2 and -4)[1]. To investigate the clinical implications of this with respect to PEMF treatment, a model system was designed to examine PEMF effect on 1) mRNA expression of BMP-2 and -4, and 2) formation of mineralised bone-like nodules by neonatal rat calvarial osteoblasts.

Materials and methods

PEMF were produced by a helmholtz coil pair and waveform generator designed to mimic the field used by Electro Biology Incorporated in their Bone Healing System which utilises a sawtooth waveform consisting of 4.5ms bursts of pulses repeating at 15 Hz, with the magnetic field rising to 18 gauss in 200μs during each pulse. Rat calvarial osteoblast cultures were exposed for 15, 30 and 60 minutes and RNA extracted immediately after treatment, and in one case 15 minutes after a 15 minute exposure. Levels of mRNA in control and exposed cells were assayed by Northern blotting and semi-quantitative reverse transcriptase/polymerase chain reaction (RT-PCR™). Northern blots were hybridised with biotin-labelled probes. PCR were performed on oligo-dT primed cDNAs using rat BMP-2 and BMP-4 specific primers. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal standard. Bone nodule formation in long-term culture was assessed by image analysis of osteoblast monolayers stained with Alizarin red after PEMF exposure.

Results

Results of duplicate experiments showed that PEMF exposure dramatically increased both BMP 2 and 4 mRNA (given as % GAPDH mRNA) with as little as 15 minutes PEMF exposure as follows:

	Control	15 min	15 min +15	30 min	60 min
BMP-4	3.2	5.6	15.4	21.0	19.5
BMP-2	17.9	34.9	38.4	49.9	67.1

One 24 hour exposure of PEMF to cultures of rat calvarial osteoblasts resulted in a twofold increase in the area of nodules formed (% of total culture area) over subsequent 3 week incubation (control mean 9.67 +/- 1.53%; exposed mean 23.00 +/- 1.73%, n=5, $p < 0.001$ by analysis of variance). Longer exposures of PEMF, up to 6 days gave similar increases in nodule formation but were not significantly different form the increase achieved by 24 hours exposure.

Discussion

We have established a reproducible osteogenic effect of PEMF in our *in vitro* model system. We believe that this effect may be, at least in part, due to the transcriptional upregulation of BMPs in osteoblasts by PEMF and speculate that this may contribute to the mechanism of action of clinically applied PEMF.

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EFFECTS OF PULSING ELECTROMAGNETIC FIELDS ON GENE EXPRESSION OF BONE MORPHOGENETIC PROTEINS IN HUMAN OSTEOBLASTIC CELL LINE *IN VITRO*. A. YAJIMA, M. OCHI, Y. HIROSE, O. NAKADE, Y.ABIKO, T. KAKU and K. SAKAGUCHI. Health Sciences Univ. of Hokkaido, Sch. of Dent., Hokkaido, Japan.

We have previously demonstrated that pulsing electromagnetic fields (PEMFs) has osteogenic action in *vitro* and *in vivo*. It has also been shown that PEMFs stimulated mRNA expression of bone morphogenetic protein (BMP) -2 and -4 in chick embryonic calvaria. However, the effects of PEMFs on the gene expression of other BMPs are still unclear. The current study was undertaken to examine the effects of PEMFs on the gene expression of BMPs (BMP-1 to -7) in SV-40 large T antigen-immortalized human osteoblastic cells (SV-HFO cells) by reverse transcription-polymerase chain reaction (RT-PCR). When the cells were 75 % confluent, the cells were continuously exposed by the osteogenic PEMFs (intensity; 0.3 mTesla, pulse width; 25 μ sec, frequency; 100 Hz) for 12, 24 and 48 hrs, respectively. We found that while SV-HFO cells, under basal condition, expressed significant amounts of mRNA for BMP-1,-2,-4,-5 and -7, these cells did not express detectable amounts of mRNA for BMP-3 and -6. Quantitative analysis of PCR products using a laser densitometer demonstrated that PEMFs reproducibly and markedly increased the mRNA for BMP-4,-5 and -7 but not that for other BMPs in SV-HFO cells. The stimulation was time-dependent with maximal increase seen after 24-hr treatment. These results indicate that the osteo-inductive effects of PEMFs may be, in part, mediated by bone morphogenetic protein-4,-5 and -7 in human osteoblastic cells *in vitro*.

T328

TRANSCRIPTIONAL REGULATION OF A BMP 4-LUCIFERASE GENE IN TRANSGENIC MICE DURING DEVELOPMENT. J.Q. Feng, M. Feng, M.S. Castano*, G.R. Mundy, S.E. Harris. Univ TX Hlth Sci Ctr, San Antonio, TX 78284-7877.

Bone morphogenetic protein 4 (BMP 4) is mediator of inductive tissue interactions required for the establishment of a variety of organ systems, such as bone, skin and teeth etc. BMP 4 gene regulation is complex. Two transcripts (A and B) and two promoters were found in fetal rat calvarial and deer antler tissue. Quantitative RT-PCR indicates that the A transcript is 10 times more abundant than the B transcript in fetal rat calvarial (FRC) cells in vitro. By an in vitro promoter study, we have shown that the -2372 to -1268 region of the

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Supplied By Manufacturer

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Re: Analysis of Reuse of Biomet® Nonivasive Bone Growth Stimulators

Dear Mr. Bechtold:

You have requested that we provide our opinion on the Food and Drug Administration ("FDA" or "the agency") regulatory implications of the reuse of Biomet® Osteobiologics' ("Biomet" or "the company") noninvasive bone growth stimulators ("noninvasive bone growth stimulators" or "the device"), as is implicit in the occasional inquiries of third party payers as to why the device is not available on a rental basis. We have provided this regulatory analysis in this letter.

I. Overview

Noninvasive bone growth stimulators are considered class III medical devices by FDA. Such devices are subject to a premarket approval ("PMA") process under which a sponsor must provide valid clinical data establishing the device's reasonable safety and effectiveness. Importantly, the labeling of a PMA-approved device is carefully reviewed and approved by FDA, ensuring that this labeling accurately reflects the clinical data which supports its summary of safety and effectiveness and is the basis for approval.

The clinical studies supporting the approval of the company's noninvasive bone growth stimulators utilized a new device on each patient. Reflecting these clinical studies, the FDA-approved labeling does not describe the use of the devices on multiple patients, nor does it include specific instructions with regard to disinfection and device life that are required for products used on multiple patients. In order for Biomet to legally market any of its noninvasive bone growth stimulators for use on multiple patients, the company would have to submit a PMA Supplement. This PMA Supplement would require data demonstrating that the safety and effectiveness of the devices are maintained despite the change in the devices themselves or in their labeling to allow reuse. Should a third party wish to reprocess a used Biomet noninvasive bone growth stimulator for reuse, that reprocessor also would be subject to the premarket

requirements of the Federal Food, Drug, and Cosmetic Act ("FDCA"). These provisions require that a third party gain PMA approval in order to legally market a reprocessed single-use, class III device such as the Biomet noninvasive bone growth stimulators.

II. Regulatory Status of Noninvasive Bone Growth Stimulators and Implications for Reuse

A. Bone Growth Stimulators as Class III Devices

Noninvasive bone growth stimulators are medical devices that use electromagnetic fields to stimulate bone growth. Currently, these products are FDA-approved for a variety of intended uses, including treatment of traumatic nonunion and congenital pseudoarthrosis, and as an adjunct to lumbar spinal fusion. Such devices are currently classified as class III devices in accordance with Section 513(f) of the FDCA.

FDA regulation of medical devices is based on a tiered, risk-based classification system that includes classes I, II, and III. Class III represents the highest level of such regulation. Under Section 513(a)(1)(C) of the FDCA, class III is reserved for products that:

> (i)(I) cannot be classified as a class I device because insufficient information exists to determine that the application of general controls are sufficient to provide reasonable assurance of the safety and effectiveness of the device, and (II) cannot be classified as a class II device because insufficient information exists to determine that the special controls [for class II devices] would provide reasonable assurance of its safety and effectiveness, and
>
> (ii)(I) is purported or represented to be for a use in supporting or sustaining human life or for a use which is of substantial importance in preventing impairment of human health, or (II) presents a potential unreasonable risk of illness or injury,

The classification of a medical device is made by FDA, often with the advice of an expert Advisory Panel. While there are provisions to downclassify or otherwise alter the class of a legally marketed product under Sections 513(e) and 513(f) of the FDCA, downclassification is a complicated process that is infrequently used.

Class III devices such as noninvasive bone growth stimulators are typically subject to premarket approval as outlined under Section 515(d) of the FDCA. Under these provisions, such devices may only be approved for market upon a demonstration of reasonable safety and effectiveness. In practice, FDA requires valid scientific evidence, including clinical data, to establish reasonable assurance of safety and effectiveness. As part of the PMA application process under 21 C.F.R. 814.44, FDA also reviews and approves the sponsor's proposed device labeling to ensure that these materials are consistent with the device's safe and effective operation. The labeling for PMA-pathway devices is thus specifically approved by the agency as part of the product's overall marketing approval and reflects the valid scientific data on which that approval was based. As outlined below, the agency considers use of a device in a manner inconsistent with this labeling as constituting "off-label" use for which the safety and effectiveness of the product has not been established. A manufacturer cannot legally market a class III device for an off-label use.

B. General Labeling Considerations for Reusable Medical Devices

In order for a medical device to be safely and effectively used on multiple patients, that product must contain labeling that supports reuse. Such reuse, at a minimum, requires that the labeling address minimizing the transmission of infectious disease between patients and a product's durability, specifically the amount of use that the device can tolerate without degradation of its therapeutic effect or diagnostic ability. For body-worn devices such as noninvasive bone growth stimulators, there is the possibility that the devices may easily come into contact with a patient's potentially infected secretions and/or bodily fluids. To minimize the possibility of the transmission of infection from such exposure, body-worn devices typically require disinfection, a chemical process whereby recognized pathogenic microorganisms are inactivated and/or physically removed from the device's surface, between users. 1/ In the case of a device that contacts intact patient skin, at least low-level disinfection is typically required by FDA to support the use of the product on multiple patients. However, noninvasive bone growth stimulators are often used in post-operative patients in the vicinity of damaged skin. Where a medical device could contact damaged skin, the agency typically requires that the product undergo a higher level of disinfection between patients. In either instance, disinfection would require cleaning of the device and/or the use of chemical germicidal agents to achieve the requisite level of disinfection.

FDA expects that any disinfection techniques described in a device's labeling be validated for use with that product. There are two components to this validation. Initially, the technique must demonstrate suitable elimination of pathogenic microorganisms so as to minimize the risk of disease transmission. In addition to demonstrating the effectiveness of the disinfection process in minimizing the possibility of disease transmission, it must be shown that the technique does not damage the medical device to the degree where its performance is impacted. Specifically, disinfection processes often involve the use of harsh germicidal chemicals that may attack a device's parts, including its electronic components, plastic insulation and conducting surfaces, all of which may degrade effectiveness or compromise patient safety.

Product durability is also a key consideration in medical devices that are labeled for reuse. Many products, both diagnostic and therapeutic, have a defined useful life, after which performance degrades. Accordingly, devices that are intended to be reused often specify how the product should be evaluated after disinfection but prior to reuse so as to determine that performance remains acceptable. Explicit limits as to how long a device may be used, in terms of length of treatment or treatment cycles, may also be provided. Any such instructions must take into account the effects of disinfection, which typically result in cumulative device damage.

For any reusable medical device, FDA typically assesses the adequacy of the disinfection process described in the product's labeling and the ability of the product to perform as intended following that process. In the case of class III medical devices subject to the PMA process, labeling, which describes cleaning, disinfection, and/or reuse, must be specifically approved by FDA.

1/ Sterilization or Disinfection of Medical Devices. Centers for Disease Control and Prevention. Available online at http://www.cdc.gov/ncidod/dhqp/bp_sterilization_medDevices.html (accessed December 7, 2006).

C. Present Labeling of Biomet Noninvasive Bone Growth Stimulators and Reuse

Biomet's noninvasive bone growth stimulators are class III devices that have been approved via the PMA process in applications supported by clinical data. As part of this approval process, the devices' labeling has been reviewed and approved by the agency. Accordingly, this labeling represents the conditions and circumstances under which FDA believes that the product is reasonably safe and effective. As described below, this labeling does not support the reuse of the Biomet noninvasive bone growth stimulators. Thus, Biomet cannot legally market or promote these devices for such reuse.

The approved labeling of the Biomet noninvasive bone growth stimulators does not explicitly describe reprocessing or reuse of these devices. In addition, this labeling lacks any information on product disinfection that is ordinarily required in instances where an externally worn medical device is used by more than one person. In fact, the labeling specifically instructs users to use only water and mild soap for device cleaning, and cautions against the use of solvents or other cleaning agents. This effectively prevents any use of germicidal disinfection agents that are medically necessary for use of the devices on multiple patients.

III. FDA-Approved Reuse of Biomet Noninvasive Bone Growth Stimulators

Biomet's noninvasive bone growth stimulators are approved, class III devices that cannot be legally marketed for reuse under their current labeling. As with any medical device, the labeling of noninvasive bone growth stimulators and the devices themselves could be modified, in this case to allow their FDA-approved reuse in multiple patients. Although the regulatory mechanism for expanding the use of these devices differs depending on whether that expanded use is being sought by Biomet or a third party, the regulatory standard for FDA's approval of that expanded use is the same, namely whether there is reasonable assurance that the reusable device is safe and effective. The regulatory mechanisms for obtaining FDA approval for reuse are described below.

A. Modification for Reuse of the Device by Biomet

The modification of any approved, class III product such as the Biomet noninvasive bone growth stimulators, is tightly controlled by FDA. Under 21 C.F.R. 814.39, any change to an approved medical device that affects the product's safety or effectiveness must be reviewed and approved by the agency under a PMA Supplement, unless FDA has advised a sponsor that an alternative submission is permitted. Current agency policy, as fully described in FDA's guidance document, *When PMA Supplements are Required (#P90-1)*, holds that changes to a product's indications for use, labeling, and/or changes in device design all typically require a PMA Supplement. Moreover, any significant changes that affect the safety or effectiveness of a device require the highest level of PMA Supplement submission, the 180 Day Supplement, under 21 C.F.R. 814.39(a).

Biomet's modification of its noninvasive bone growth stimulators to explicitly incorporate device reuse would almost certainly be seen by FDA as affecting the device's safety or effectiveness, and thus require a PMA Supplement. Such a change may be seen by the agency as modifying the devices' indications for use. Explicitly incorporating reuse would involve labeling changes, and could involve physical alterations to the product that necessitate a change

in design or materials. To support the reasonable safety and effectiveness of these changes, the agency would almost certainly require performance data to: (1) validate the disinfection method used between patients; and (2) demonstrate that the devices' effectiveness and safety are maintained when used for prolonged periods on multiple patients.

Validation of product disinfection so as to achieve the desired reduction in pathogenic microorganisms is subject to established testing methods that are typically accomplished with bench testing. A more difficult issue is ensuring that device performance in terms of safety and effectiveness remains stable after disinfection between users and for extended periods of time. As previously noted, the clinical data on which the approvals of Biomet noninvasive bone growth stimulators were based utilized new devices that were used on only a single patient for relatively limited periods of time. Thus, FDA approval of the reuse of these devices would require either so-called "bridging" data that relates the original, single-use data to the reusable product, or a completely new clinical study using reusable devices. Notably, FDA could easily require a clinical study even for bridging data, and would certainly require substantial clinical data if bridging data were insufficient to establish reasonable safety and effectiveness.

Apart from FDA's regulatory requirements, a number of important considerations underlie any decision to seek a PMA Supplement for a reusable bone growth stimulator. Initially, the currently approved devices are safe and effective in promoting bone growth for their respective indications. The modifications to the devices and the labeling necessary to achieve adequate disinfection may significantly alter the products to the point where a major redesign is necessary to maintain performance. There is also the possibility that the materials necessary to fabricate the devices, as well as the finished products themselves, will not tolerate repeated reuse. This is the case with many reusable products, which often have a finite number of uses. Given these uncertainties, it is unreasonable to expect Biomet to pursue approval of a reusable device, particularly given the proven clinical performance of the existing single-use product.

B. Modification for Reuse of a Biomet Device by a Third Party

Reprocessing and reuse of single-use medical devices is a common practice in the US. It is so common that an entire industry has developed to take used, single-use products and reprocess them into devices that may be reused. As this practice has grown, so has FDA's concern that this reprocessing and reuse may impact the device's safety and effectiveness. The agency's response has been to institute a clear policy on the reprocessing and reuse of medical devices, a policy which is articulated in FDA's guidance document, *Enforcement Priorities for Single-Use Devices Reprocessed by Third Parties and Hospitals* (August 14, 2000).

A key feature of FDA's policy on the reprocessing and reuse of single-use devices is that reprocessors are held to a number of regulatory requirements that also govern the original manufacturers of medical devices. These include the premarket requirements found at Sections 513 and 515 of the FDCA, as well as their implementing regulation at 21 C.F.R. Parts 807 and 814. Which specific provisions of the premarket requirements apply to a particular reprocessed device depends on the original classification of the single-use product before it was reprocessed. Thus, if a third party reprocesses a PMA-approved single-use, class III device for use in another patient, that reprocessed device is considered a class III device and is subject to the PMA process under Section 515 of the FDCA. As such, that third party is required to provide valid scientific