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
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
- The SpinalPak II was proven more effective than placebo in smokers, those without instrumentation, and those with multiple level fusions
- Compact and lightweight design allows for enhanced patient compliance and comfort
- The Capsolite Coupling signal that is singular to the EBI SpinalPak II has a demonstrated Mechanism of Action

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

References:

Copyright 2006 EBI. All rights reserved. P/N 2000571. Rev 10/06
OrthoPak® 2 Bone Growth Stimulator

- 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 consistently delivers naturally occurring BMPs, BMP-2, 4, 5, 6, 7, TGF-β, FGF-2, VEGF, and PGE2.
- Continuous 24-hour treatment provides an optimized treatment dose.
- Rechargeable battery system offers convenient easy treatment maximizing patient compliance.

Product Information
OrthoPak®2 Bone Growth Stimulator
Item No. 5230
Physician Test Kit 5235 (adapter with meter)
Rx Only - By prescription only

BIOMET
OSTEOBIOLOGICS

100 Interpace Parkway
 Parsippany, NJ 07054
www.biometosteobiologics.com
800-525-2579

BIOMET
OSTEOBIOLOGICS

Copyright 2008 Biomet, Inc. All Rights Reserved. P/N: MD2002-0065
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
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

**OPTIMAL POTENTIAL FOR EARLIER HEALING**
- 10-hour optimal treatment dose for potential earlier healing
- Only EBI's PEMF offers clinically effective treatment with easy-to-use, lightweight, ultrasonot FLX® flexible treatment coils
- Three approved indications: nonunions, failed fuses and congenital pseudoarthrosis 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 250,000 patients

**LOW PROFILE GENERATOR**
- Can be easily implanted to treat most fracture sites
- Low-profile generator can be easily implanted to treat most fracture sites

**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

**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

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

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

Red area indicates field of influence

Electrodes adhere directly to skin for easy treatment placement

Red area indicates field of influence

EBL Turning the Science of Bone Formation Into the Success of Bone Healing

EBI, L.P.
100 Interpace Parkway Parsippany, NJ 07054
www.ebimedical.com

EBI® OsteoGen
Surgically Implantable Bone Growth Stimulator

Bioelectron ORTHOPOAk

A BIOMET COMPANY

1-800-526-2579

© 2020 EBI, L.P. All rights reserved
EBI...The Only Company that Can Offer You Both Implantable and Noninvasive Spine Fusion Stimulators

- More efficacious than autograft alone\(^1\)
- SpF (Direct Current) and SpinalPak (Capacitive Coupling) technologies involve the upregulation of numerous BMFs and osteopromotive growth factors
- Backed by over 30 published scientific and clinical papers
- Surgeon CPT codes available — reimbursement for implantation and/or explantation

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

References
\(^1\) BONE MAINTENANCE MULTIPOTENTIAL TECHNOLOGY
\(^2\) Tissue Engineering, Sept. 1999
\(^3\) BMFs: Mechanism of Action: promotes the upregulation of BMFs and other osteopromotive factors as demonstrated in in vivo and animal in vivo studies.

As demonstrated in pre-clinical models

For all ordering information, contact EBI, LLC, a subsidiary of Kinetic.
4141 W. 15000 EBI, L.L.C. All rights reserved.
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.

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Biomat</th>
<th>Biomet</th>
<th>Biome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stages</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>BMP-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Bone Morphogenetic Protein-2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-2 is produced by osteoblasts</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>BMP-4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Bone Morphogenetic Protein-4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-4 is produced by osteoblasts</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>BMP-6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Bone Morphogenetic Protein-6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP-7</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Bone Morphogenetic Protein-7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Or Osteogenic Protein-1 (OP-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-7 has been involved in bone healing</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>TGF-β</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Transforming Growth Factor-β)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β is found in proliferating mesenchymal stem cells, osteoblasts and in the matrix</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>FGF-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fibroblast Growth Factor-2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF-2 increases the recruitment of osteoblast and osteoblast precursor cells and stimulates angiogenesis</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Vascular Endothelial Growth Factor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF is involved in bone healing</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PGE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Prostaglandin E)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE, Prostaglandin E,</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>Weeks 2 - 4</td>
<td>Weeks 4 - 12</td>
<td>Weeks 12 - 48</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Hematoma Formation - Migration Of Cells</td>
<td>Chondrogenesis - Bridging Of Gap With Fibrocartilage</td>
<td>Calcification And Vasculization Of Fibrocartilage</td>
<td>Remodeling Into Mature Bone</td>
</tr>
</tbody>
</table>

**Week 1**

**MP-2** is induced immediately after fracture, when mesenchymal stem cells are recruited to the site of injury.

**BMP-4** has been found to play a critical role in the differentiation of mesenchymal cells.

**BMP-6** influences proliferation and differentiation of mesenchymal cells, as well as increasing chondrogenesis and osteogenesis.

**BMP-2** increases overall callus area, accelerates conversion from soft to hard and bone calcification, and stimulates osteoblast migration and differentiation.

**Wolff's law** takes effect. New bone appropriately remodels, responding to biomechanical and biological stimuli.

**Weeks 2 - 4**

**BMP-2** promotes bridging of callus, and stimulates mesenchymal cell differentiation to a chondroblast-like lineage.

**BMP-6** initiates osteoblast differentiation.

**Weeks 4 - 12**

The cascade of bone healing is supported by the stimulation of other BMPs, TGF-β, and vascular growth factors to support the bone formation process. The signalled bone-forming cells begin to organize. New vascular ingrowth occurs.

Significant osteoblastic activity is supported by vascular ingrowth. Corneal osteoblasts begin to appear.

**Weeks 12 - 48**

**TGF-β** promotes blood vessel formation, stimulates proliferation of osteoblasts and chondrocytes, enhances production of extracellular matrix, and regulates osteoblast-osteoclast interaction.

**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.

**VEGF** induces angiogenesis, regulates vasculogenesis, and is important in the conversion of soft to hard callus. VEGF recruits and activates osteoblasts, and stimulates osteoblast proliferation and differentiation, as well as matrix mineralization.

**PGE** stimulates differentiation and proliferation of osteoprogenitor cells and increases callus size.
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.”
Pulsating Electromagnetic Field Stimulates mRNA Expression of Bone Morphogenetic Protein-2 and -4

M. Nagai and M. Ota

Department of Biochemistry, Iwate Medical University School of Dentistry, Morioka, Iwate 020, Japan; 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 calvarias 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.

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 (Rogan 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., 1985), and (3) calcification (Bassett et al., 1979; Colbran and Pilla, 1984; Norton and Roveri, 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 (Oba et al., 1991), osteocalcin (Oba et al., 1991), and insulin-like growth factor (Edwards 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 found to singly induce ectopic bone formation (Vozynek et al., 1988; Wang et al., 1990; Ohayok et al., 1990). Accumulating In vitro evidence indicates that BMP induces various phenotypic expressions of bone cells (Vukicevic et al., 1989, 1990; Katsarig et al., 1990; Takuwa et al., 1991; Yamaguchi et al., 1991; Hirakaki et al., 1991; Chen et al., 1991; Thies et al., 1992; Sakano et al., 1993). In addition, Mulk et al. reported that BMP may be excised from the broken ends of bones (Mulk et al., 1988).
Hultt, 1989). These 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 usec for the main, and 24 usec 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 mT at peak power and an electrical field of 9 mV/m (Goodman et al., 1980).

Stimulation of chick embryos with PEMF

Fertilized White Leghorn eggs were purchased from Koiti Farm (Shizukushri, Iwate, Japan). Control eggs were incubated at 37°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 laser 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 ml of 0.1 × SSC used as a transfer solvent. After transfer, the membrane was rinsed with 2x SSC and baked at 80°C for 20 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% lauryl sarcosine, and 50 µg/ml yeast total RNA at 42°C for 2 h. mRNAs of interest were located by hybridization at 42°C for 2 h with digoxigenin-dig-UTP (Boehringer Mannheim Biochemica) labeled RNA probes followed by immunological chemiluminescent detection. The probes were labeled with dig-UTP 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-UTP, 650 µM TTP, and 1 mM each of ATP, CTP, and GTP. Similarly, human BMP-4 cDNA (Wozney et al., 1988) was linearized with EcoRI and transcribed by SP6 RNA polymerase. Mouse fibroblast cDNA (Alonso et al., 1988) linearized with Hind III and human liver/bone/kidney-type alkaline phosphatase (ALPase) cDNA (Weiss et al., 1986) linearized with EcoRI were transcribed by T7 RNA polymerase in the presence of dig-UTP. Chemiluminescent detection was performed in the following order: (1) detection of dig with anti-dig antibody conjugated to ALPase; (2) catalyzed reaction of ALPase with chemiluminescent substrate; and (3) documentation of ALPase-catalyzed luminescence on x-ray film.

Expression levels of the specific mRNAs in the individual chucks were quantitated by means of slot-blot 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 (Manifluid 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 (Ultrascan Laser densitometer model 2000, LKB, Bromma, Sweden), and each peak area of the blot was integrated by use of a computer software program. 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 embryos was examined by Northern-blot analysis. A single transcript of 3.2 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 15-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 F-actin and ALPase mRNAs were expressed equally in all groups. However, the increase was 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 3.7-fold
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 used in this study is considered to exert the optimal effect on osteogenesis (Bassett et al., 1997, 1998) and is used for treatment of non-union fractures. PEMFs of lower frequencies have been reported to exert osteoblastic and teratogenic effects on developing embryos (Zusman et al., 1995). However, the PEMF used did not induce any abnormalities in the chick embryos, retard 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. 35-kb BMP-2 mRNA in mouse embryos (Lynes et al., 1990) and 22-kb BMP-4 mRNA in human osteosarcoma cell line U-2 OS (Ozsan and Ozsan, 1990). These indicate the structural similarities of chick and mammalian BMP mRNAs.

We observed that the mRNA expression of BMP-2 and of BMP-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., 1997) 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 (Blechman et al., 1986; Nirenberg and Rovetti, 1988; Goodman and Henderson, 1988) and cytokines (Costa 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 BMP-4 was indicated. The effect of PEMF was considered to be specific for the BMPs in at least ALPase mRNA expression, one of the osteogenic phenotypes, was not influenced by PEMF. This result is in accord with the result of Noise 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 10 embryos (Figs. 2 and 3). Compared with Day 10 embryos, Day 11 and 15 calvaria expressed low levels of the BMP mRNAs, 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 BMPs are inducible by PEMF, whereas the older ones are resistant in the cells constitutively expressing BMP mRNAs.

The cellular target of PEMF cannot be elucidated in this study because calvarial tissue is composed of heterogeneous 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 expression most likely originate from osteogenic cells, which are well-known producers of the morphogens (Wozney et al. 1988; Wang et al., 1990; Orskov et al., 1990).

In summary, our present results demonstrate 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, 1997; Rosen and Thiess, 1992; Lyse et al., 1992), we conclude that the bone-inductive effect of PEMF is mediated at least in part via stimulation of mRNA expression of BMP-2 and -4.

Acknowledgments

We are grateful to Dr. J.M. Wozney for providing cDNAs of human BMP-2 and -4. This work was supported in part by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science and Culture of Japan, No. 03877246 (to M.N.).

A preliminary report was presented at the 71st General Session and Exhibition of the International Association for Dental Research, Chicago, IL, USA.

References


Bone Morphogenetic Protein mRNA Expression in Bone


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


Introduction
Pulsed electromagnetic fields (PEMF) have been shown to be clinically beneficial in orthopaedic surgery when used to treat non-union and pseudarthrosis after bone fracture. The osteogenic potential of rat calvarial osteoblasts is enhanced by bone morphogenetic protein-2 and 4 (BMP-2 and -4). 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 helmboltz coil pair and waveform generator designed to mimic the field used by Electro Biology Incorporated in their Bone Healing System which utilizes 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-UT primed cDNAs using rat BMP-2 and BMP-4 specific primers. Glyceroldehyde-3-phosphate dehydrogenase was used 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:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>15 min</th>
<th>15 min &lt;15</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP-4</td>
<td>3.2</td>
<td>5.6</td>
<td>15.4</td>
<td>21.0</td>
<td>19.5</td>
</tr>
<tr>
<td>BMP-2</td>
<td>17.9</td>
<td>34.9</td>
<td>38.4</td>
<td>49.9</td>
<td>67.1</td>
</tr>
</tbody>
</table>

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 9.62 ± 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 from 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.


This work was supported by:
*Electro Biology Incorporated, NJ, USA and The Arthritis & Rheumatism Council, UK

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.
☐ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.

FULL NAME, ADDRESS, PHONE, FAX, AND E-MAIL NUMBERS OF CORRESPONDING AUTHOR:
Dr. L. DeVries, *De Vries S. N. C., 2 London, M. 24, 123, 4567, 89012, 345, 6789, 0123, 4567.
TEL. 071 12345 6789, FAX 071 12345 6789
E-MAILs. 12345 6789, . A. U. K.

204 - 34 42nd Annual Meeting, Orthopaedic Research Society, February 19-22, 1996, Atlanta, Georgia
T326

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. ARIKOO, T. KAKI, 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 mT, pulse width: 25 μsec, 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. Castanon*, G.R. Mundy, S.E. Harris. Univ TX Hlth Sci Ctr, San Antonio, TX 78284-7817.

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. Three transcripts (A and B) and two promoters were found in fetal rat calvarial and dermal anther 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 527 bp to 1275 bp fragment contains a

null indicative sequence factor that would be expected to induce bone morphogenetic proteins in human embryonic osteoblasts and B cells present study canistically in osteoblastic cells arising from
February 16, 2007

James Bechtold, Senior Vice President
Biomet® Osteobiologics
100 Interpace Parkway
Parsippany, NJ 07054

Re: Analysis of Reuse of Biomet® Noninvasive 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...
February 16, 2007
Page 2

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 pseudarthrosis, 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(f) 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) 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. §14.44, 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.
February 16, 2007
Page 3

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 for the length of time before 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.

February 16, 2007
Page 4

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 (5P00-1), 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
February 16, 2007

Page 5

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