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Review Article

Preclinical Toxicology Studies of Recombinant Human Platelet-Derived Growth Factor-BB Either Alone or in Combination with Beta-Tricalcium Phosphate and Type I Collagen

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Human platelet-derived growth factor-BB (hPDGF-BB) is a basic polypeptide growth factor released from platelets at the injury site. It is a multifunctional molecule that regulates DNA synthesis and cell division and induces biological effects that are implicated in tissue repair, atherosclerosis, inflammatory responses, and neoplastic diseases. This paper is an overview of the toxicology data generated from a broad testing platform to determine bone, soft tissue, and systemic responses following administration of rhPDGF-BB. Moreover, the systemic and local toxicity of recombinant human PDGF-BB (rhPDGF-BB) in combination with either beta-tricalcium phosphate (β-TCP) or collagen combined with β-TCP was studied to determine dermal sensitization, irritation, intramuscular tissue responses, pyrogenicity, genotoxicity, and hemolytic properties. All data strongly suggest that rhPDGF-BB either alone or in combination with β-TCP or collagen with β-TCP is biocompatible and has neither systemic nor local toxicity, supporting its safe use in enhancing wound healing in patients.

1. Introduction

Human platelet-derived growth factor-BB (hPDGF-BB) is a basic polypeptide growth factor released from platelets [1]. PDGF initiates wound healing and is chemotactic and mitogenic for mesenchymal cells that can differentiate to osteoblasts, chondrocytes and vascular smooth muscle cells [2–5]. Moreover, PDGF-BB is proangiogenic and upregulates vascular endothelial growth factor (VEGF) [4–6]. These properties are profoundly important for homeostasis and regeneration of bone and soft tissues. Consequently, PDGF-BB is a compelling therapeutic to enhance tissue wound healing.

Recombinant human PDGF-BB (rhPDGF-BB) has been combined with biocompatible, osteoconductive β-tricalcium phosphate (β-TCP) to regenerate periodontal tissue [7, 8]. Further, rhPDGF-BB combined with β-TCP is approved by the Food and Drug Administration (FDA) in the United States and Health Canada in Canada for the treatment of periodontal tissues and is available commercially under the trade name *GEM 21S* (Luitpold, NY).

In addition, clinical studies are underway in the United States, Europe, and Canada to establish the safety and efficacy of rhPDGF-BB/β-TCP (*Augment Bone Graft*) for orthopedic applications. A combination of rhPDGF-BB and a soluble bovine type I collagen combined with β-TCP matrix

(*Augment Injectable Bone Graft*) formulated as an injectable paste, is under development. Furthermore, rhPDGF-BB in a topical gel matrix is FDA-approved to treat diabetic skin ulcers (Regranex, Ortho-McNeil/Johnson, and Johnson).

Although the safety of rhPDGF-BB has been established through various clinical applications for dermal and periodontal tissue healing, new combinations of the protein with other materials could lead to unique safety outcomes compared to using the protein alone, or when combined with materials for which there is an established clinical safety profile. Thus, the purpose of this paper is to provide an overview of the preclinical animal toxicology supporting the safe use of rhPDGF-BB in combination with β-TCP and a soluble type I collagen matrix, which represent unique combination products for treating indications involving orthopaedic bone repair.

2. Materials and Methods

Testing was conducted in accordance with guidance provided by the International Organization for Standardization (ISO) and United States Pharmacopeia (USP) for the evaluation of the biocompatibility of medical devices. Moreover, animal work was done at facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) under protocols approved by Institutional Animal Care and Use Committee (IACUC) review boards. All aspects of the studies described below were conducted using Good Laboratory Practices (GLP). A summary of the tests preformed on each type of delivery system for PDGF is included in Table 1.

Recombinant human PDGF-BB (rhPDGF-BB) was provided by BioMimetic Therapeutics, Inc. (Franklin, TN, USA). Tricalcium phosphate (β-TCP) with a particulate size ranging from 1 to 2 mm in diameter was obtained from OrthoVita (Malvern, PA, USA). A matrix consisting of tricalcium phosphate (β-TCP) combined with soluble type I collagen was obtained from Kensey-Nash Corporation (Exton, PA, USA).

3. *In Vivo* Testing

3.1. Repeated Administration of rhPDGF-BB and Bone Responses. The experimental design for this study was modeled after that of Knight et al. [9] to evaluate bone responses immediately following repeated injections of rhPDGF-BB. The study was modified to determine bone responses at 24 hours and 6 weeks following the last rhPDGF-BB injection to assess the reversibility of acute tissue responses. The reversibility of bone responses to multiple-dose injections of rhPDGF-BB was evaluated using 80 rats (Fischer 344): 40 females (F) and 40 males (M) were divided into 8 groups consisting of 10 animals each (5 M and 5 F). Dose groups received injections of the negative control 20 mM sodium acetate, pH 6.0, or 10 μg/mL, 30 μg/mL, or 100 μg/mL rhPDGF-BB in 20 mM sodium acetate, pH 6.0 every other day (i.e., days 1, 3, 5, 7, 9, 11, and 13). Injections of 0.1 mL were made between the first and second proximal metatarsi and at the lateral surface of the femur. Four groups of animals (10 rats per group) were sacrificed one day following the last injection and the remaining 4 groups (10 rats per group) were sacrificed 6 weeks after the final injection. The right leg (including femora and metatarsi and a minimum of 5 mm margin of overlying soft tissues) of each rat was recovered at necropsy and fixed in 10% neutral-buffered formalin, decalcified, embedded in paraffin, and sectioned to 10 μm thickness. Transverse tissue sections were stained with hematoxylin and eosin and Masson's trichrome stain. Histopathological assessments of the bone and overlying soft tissues were conducted by a board-certified histopathologist.

3.2. Systemic Toxicology of rhPDGF-BB Alone or Combined with β-TCP or a Collagen/β-TCP Matrix. Toxicology studies were conducted on rhPDGF-BB alone and on two products formulated with rhPDGF-BB that are designed for bone regeneration: rhPDGF-BB combined with β-TCP and rhPDGF-BB combined with collagen/β-TCP. Systemic toxicity was determined for extracts of 1.0 mg/mL rhPDGF-BB combined with particulate β-TCP (1-2 mm mean diameter) or a 20% w/w bovine type I collagen/80% w/w β-TCP (100–300 μm mean diameter). Extracts of the mixed materials were tested for bioreactivity due to leachable substances as described in the following subsections.

3.2.1. Systemic Injection. Based on guidelines provided in the U.S. Pharmacopeia [10], a 1:1 v/v combination of either 1.0 mg/mL rhPDGF-BB with β-TCP or β-TCP alone was extracted with either 0.9% sodium chloride (NaCl), ethanol (diluted 1 : 20 in 0.9% NaCl), polyethylene glycol (PEG), or cotton seed oil (CSO). Naïve male albino Swiss mice (*Mus musculus*) were either injected intravenously with saline or ethanol extracts, or intraperitoneally with PEG and CSO extracts, and observed for signs of toxicity at 0, 4, 24, 48, and 72 hours after injection. The same testing procedure was performed using NaCl and CSO extracts of a 3 : 1 liquid volume to solid mass combination of 1.0 mg/mL rhPDGF-BB and collagen/β-TCP matrix. Unrestricted rhPDGF-BB (0.3 mg/mL in 20 mM sodium acetate, pH 6.5) alone was also injected intraperitoneally, and observations were made as described above, and also at 96, 120, 144, and 168 hours after injection.

3.2.2. Pyrogen Testing. Based on guidelines provided [11], testing for the presence of pyrogens was done on either a combination of 1.0 mg/mL rhPDGF-BB and collagen plus β-TCP matrix or collagen plus β-TCP matrix only extracted in a 0.9% NaCl solution. The extracts were assessed for the presence of chemical pyrogens that can lead to a febrile response upon intravenous (IV) administration. Naïve male and female New Zealand white rabbits were injected IV with the extracts and body temperature was monitored after injection for up to 3 hours.

3.2.3. Allergenic Sensitization. Using guidance provided in ISO 10993-10 [12], extracts of rhPDGF-BB combined with either β-TCP or collagen/β-TCP matrix or extracts of β-TCP

Table 1: Summary of toxicology studies for rhPDGF-BB alone or combined with either β-TCP or collagen together with β-TCP matrix.

Test	rhPDGF-BB alone	Orthovita Vitoss β-TCP (250–1000 μm)	Skeletal Kinetics β-TCP (1-2 mm)	CAM Implants β-TCP (1-2 mm)	Kensey-Nash collagen plus β-TCP (100–300 μm)
Bone Toxicity	X				
Systemic Injection	X	X	X	X	X
Pyrogenicity					X
Dermal Irritation	X	X	X	X	X
Intramuscular Implantation-7 days				X	
Intramuscular Implantation-4 weeks		X	X		X
Delayed Hypersensitivity		X	X		X
Cytotoxicity	X	X	X	X	X
Mutagenicity		X	X		X
Hemolytic Properties					X

or collagen/β-TCP matrix alone were assayed for allergenicity using the Kligman maximization test [13]. Hartley guinea pigs were injected intradermally within a 2×4 cm rectangular area over the shoulder with either NaCl or CSO extracts with or without Freund's adjuvant, the NaCl or CSO vehicles alone with or without Freund's adjuvant, or positive control dinitrochlorobenzene (DCNB) with or without Freund's adjuvant on day zero. Seven days after injection, the skin over the injected areas was topically challenged with either the extracts or the appropriate controls, and at twenty-three days after injection, a second topical challenge was applied to the flank of each animal. Following each challenge, the skin reaction was graded according to the Magnusson and Kligman scale for the appearance of erythema and swelling.

3.3. Local Tissue Responses and Dermal Irritation Elicited by Extracts of rhPDGF-BB Combined with β-TCP or a Collagen/β-TCP Matrix

3.3.1. Tissue Responses and Irritation-Intracutaneous Injection. Based on guidance provided in the U.S. Pharmacopeia [10], acute skin irritation due to subcutaneous injection of extracts of 1.0 mg/mL rhPDGF-BB combined with β-TCP or β-TCP matrix alone were evaluated using NaCl, CSO, ethanol, and PEG as extraction solvents. Unextracted rhPDGF-BB (0.3 mg/mL in 20 mM sodium acetate, pH 6.5) alone was also injected subcutaneously for evaluation. Four New Zealand white rabbits received paravertebral injections lateral to the vertebral bodies with extracts of the experimental materials. Skin reactions were scored for erythema, edema, and eschar formation and the data were used to determine responses at the injection sites in each animal. In addition, New Zealand rabbits were used to determine acute skin irritation induced by NaCl and CSO extracts of 1.0 mg/mL rhPDGF-BB combined with collagen/β-TCP matrix or collagen/β-TCP matrix alone.

3.3.2. Intramuscular Responses. Implantation of 1.0 mg/mL rhPDGF-BB combined with β-TCP or β-TCP alone in the dorsolumbar paravertebral muscle was done in New Zealand white rabbits. Control β-TCP alone was combined with 0.9% NaCl solution. Muscle responses were compared to responses elicited by a negative control plastic implanted in the same animal. After seven days, the implantation sites were harvested and processed for histology and a histopathological analysis was conducted.

In addition, 1.0 mg/mL rhPDGF-BB combined with collagen/β-TCP matrix or 20 mM sodium acetate, pH 6.0 combined with collagen/β-TCP matrix were implanted intramuscularly in another set of rabbits and after four weeks, implantation sites were excised and processed for histopathological analysis.

3.4. In Vitro Toxicity Studies

3.4.1. Cytotoxicity. Cytotoxicity assays were conducted according to ISO 10993-5 guidelines [14]. The cytotoxicity of 1.0 mg/mL rhPDGF-BB combined with β-TCP or collagen/β-TCP, and β-TCP or collagen/β-TCP alone were determined by exposing monolayers of L929 mouse fibroblasts to Minimum Essential medium (MEM) tissue culture medium extracts of the test materials for 48 hours at 37°C. The cellular morphology was graded by microscopic examination for signs of cell rounding, reductions in intracellular granularity, cell lysis, and gaps in the cellular monolayer. Moreover, an additional study with L929 mouse fibroblasts was performed using an agar overlay on the cells that included the vital dye, neutral red, to determine cytotoxicity due to diffusible substances released from the MEM extracts or rhPDGF-BB neat soaked into filter papers that were placed on top of the agar. Zones of bioreactivity under the experimental materials were graded according to whether the cells either were stained and therefore viable or were unstained and therefore nonviable. Further,

Table 2: Summary of bone bioreactivity ratings at the rat metatarsus and femur.

[PDGF-BB] (μg/mL)	Average Bone Bioreactivity Rating*-Metatarsus		Average Bone Bioreactivity Rating*-Femur	
	2 weeks	8 weeks	2 weeks	8 weeks
100	2.9	0.0	2.6	0.0
30	1.4	0.0	1.2	0.0
10	0.7	0.0	0.9	0.0
0 (Control)	0.0	0.0	0.0	0.0

* Average Treated Bone scores—Average Control Bone scores. 0–1.5 = No Reaction, >1.5–3.5 = Mild Reaction, >3.5–6.0 = Moderate Reaction, >6.0 = Marked Reaction.

determination was made of changes in cell morphology. The same testing paradigm using L929 mouse fibroblasts was performed with MEM extracts of 1.0 mg/mL rhPDGF-BB combined with collagen/β-TCP matrix or collagen/β-TCP matrix alone.

3.4.2. Mutagenicity. Mutagenicity tests were conducted according to guidelines provided under ISO 10993-3 [15]. Mutagenic properties of NaCl and CSO extracts of 1.0 mg/mL rhPDGF-BB combined with β-TCP, or collagen/β-TCP matrices, or collagen/β-TCP combined with 20 mM sodium acetate solution, pH 6.0 were determined using a reverse mutation assay (Ames test) with mutant strains of *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*) auxotrophic for histidine and tryptophan biosynthesis. Mutant strains of bacteria (his^- and trp^-) were exposed to extracts of the test and control articles for 72 hours. The number of colonies that grew on medium lacking histidine or tryptophan were counted and compared to negative controls (extraction solvents) and positive control mutagenic compounds.

3.4.3. Hemolysis. Hemolysis assays were done according to guidelines described under ISO 10993-4 [16]. The hemolytic properties of 0.9% NaCl extracts of 1.0 mg/mL rhPDGF-BB combined with collagen/β-TCP matrix or collagen/β-TCP matrix alone were determined using blood withdrawn from a New Zealand white rabbit. Blood was added to the experimental extracts and compared to the negative control saline or positive control sterile water spectrophotometrically.

4. Results and Discussion

4.1. In Vivo: Repeated Administration of rhPDGF-BB and Bone Responses. Acute bone and soft tissue responses to repeat dosing of rhPDGF-BB at the metatarsi and femora were determined in rats. Tissue responses were evaluated 24 hours and 6 weeks following the last dose.

Body weights for all animals increased normally during the injection phase of the study (days 1–13), except for one female rat receiving 10 μg/mL doses of rhPDGF-BB, which had ~5% weight loss. Body weights continued to increase normally for all rats over time following the completion of all injections. Swelling was observed at the metatarsal injection sites on day 14 in all 30 rats receiving rhPDGF-BB and no swelling was observed in the 10 control rats. Swelling was temporary and soft tissues overlying the injection sites returned to normal appearance by 8 weeks in all animals. No swelling was observed at the femoral injection sites on day 14.

At day 14, no soft tissue reaction was evident at either the metatarsus or femur for rats receiving either 10 μg/mL or 30 μg/mL rhPDGF-BB based. A mild inflammatory reaction at the metatarsus was observed on day 14 in rats that received 100 μg/mL rhPDGF-BB, while no reaction occurred in the soft tissue at the femur. There was no difference in reactions at the bone for doses $\leq$30 μg/mL of rhPDGF-BB at either the metatarsus or the femur. In day 14 rats that received 100 μg/mL rhPDGF-BB, a mild bone reaction consisting of osteogenesis and fibroplasia in the periosteal cortex was observed. No ectopic bone formation was observed in tissues near either the femur or metatarsus for any animal. By 8 weeks, the bone remodeling observed at 2 weeks with the 100 μg/mL rhPDGF-BB dose had resolved to normal appearance and no osteogenic activity was observed.

The results of multiple injections of rhPDGF-BB in rats led to a mild acute soft tissue inflammatory response and minimal bone formation and fibroplasia in the periosteal cortex at both metatarsal and femoral injection sites. This observation occurred only in rats receiving 100 μg/mL rhPDGF-BB. This result was not unexpected, given that rhPDGF-BB functions to initiate the wound healing process as a potent chemotactic and mitogenic agent for mesenchymally derived cell types. After a recovery period of 6 weeks following the final injection of rhPDGF-BB, soft tissues, and bone were normal (Table 2).

Data at 14 days following repeated injections near the metatarsi and femora of rats were consistent with data previously reported for rhPDGF-BB (Becaplermin/Regranex) [9]. Moreover, the outcome of rhPDGF-BB on soft and hard tissues at 2 weeks at both sites was completely reversed by 6 weeks. This underscores the reversibility of the tissue response to PDGF when its administration is discontinued. Lastly, no ectopic bone formation was observed.

4.2. Systemic Toxicology of rhPDGF-BB Alone or in Combination with β-TCP or a Collagen/β-TCP Matrix. Systemic injections of rhPDGF-BB alone, NaCl, CSO, ethanol, and PEG extracts of rhPDGF-BB plus β-TCP or NaCl and CSO extracts of rhPDGF-BB plus collagen combined with β-TCP matrix, or similar extracts of control materials showed no signs of toxicity in any animal. All animals gained weight

Table 3: Summary of systemic toxicity studies for rhPDGF-BB combined with β-TCP or collagen/β-TCP.

Strain/Species	Route of Administration*	Test Article	Control Article(s)	Solvent for Extraction†	Number per Dose	Summary of Results
Swiss albino/mouse	IV IP	1.0 mg/mL rhPDGF-BB + β-TCP	β-TCP alone and plastic	NaCl Ethanol CSO PEG	5 males per extract	(i) No systemic toxicity (ii) Body weights increased in all animals
Swiss albino/mouse	IV IP	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collagen/β-TCP alone	NaCl CSO	10 animals per extract	(i) No systemic toxicity (ii) Body weights increased in all animals
New Zealand white/rabbit	IV	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collagen/ β-TCP alone and NaCl vehicle	NaCl	3 animals per extract, 1 animal for NaCl control	(i) No significant febrile reaction in any animal (ii) No extractable pyrogens present
Hartley guinea pigs	ID	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collagen/β-TCP alone, NaCl and CSO vehicles, DNCB positive control	NaCl CSO	20 animals per test extract 10 animals for negative controls 5 animals for positive controls	(i) Body weights increased (ii) No systemic toxicity (iii) No erythema or swelling observed after topical challenges (iv) No allergic sensitization to test article

* Abbreviations: IV: intravenous, IP: intraperitoneal, ID: intradermal.
† Abbreviations: NaCl: 0.9% sodium chloride, CSO: cotton seed oil, PEG: polyethylene glycol, DNCB: dinitrochlorobenzene.

during the studies. Details of the studies are provided in the sections below.

4.2.1. Pyrogen Testing. Rabbits injected with extracts of rhPDGF-BB combined with either β-TCP or collagen/β-TCP were evaluated for increases in body temperature due to the presence of pyrogens. There was no evidence of a febrile reaction in any of the animals receiving extracts of any of the test articles. All test and control materials were determined to be nonpyrogenic.

4.2.2. Allergenic Sensitization. All of the animals gained weight over the duration of the study and there were no clinical signs of systemic toxicity in either treated or control animals. None of the animals treated with NaCl or CSO extracts of β-TCP combined with rhPDGF-BB, the collagen with β-TCP matrix combined with rhPDGF-BB, β-TCP matrix alone, collagen with β-TCP matrix alone, or extraction vehicle alone showed signs of sensitization or developed an allergic response. In comparison, extracts of the positive control dinitrochlorobenzene (DNCB) elicited a strong allergic response with erythema and swelling at each topical challenge site.

In summary, in mice and rabbits, NaCl or CSO extracts of rhPDGF-BB combined with β-TCP or collagen/β-TCP matrix did not lead to acute or delayed hypersensitivities. Further, the β-TCP and collagen/β-TCP without rhPDGF-BB did not lead to hypersensitivity. Moreover, extracts of 1.0 mg/mL rhPDGF-BB combined with a collagen/β-TCP matrix or collagen/β-TCP matrix alone in NaCl or CSO did not elicit an allergic response after an induction phase in guinea pigs.

Study designs and data for systemic toxicity testing are summarized in Table 3.

4.3. Local Tissue Responses and Dermal Irritation Elicited by Extracts of rhPDGF-BB Combined with Either β-TCP or a Collagen/β-TCP Matrix

4.3.1. Tissue Responses and Irritation-Intracutaneous Injection. In studies with NaCl, ethanol, CSO, or PEG extracts of rhPDGF-BB combined with β-TCP, or NaCl or CSO extracts of rhPDGF-BB combined with collagen/β-TCP, there were no signs of skin irritation such as erythema, edema, or eschar formation in any animal injected intracutaneously. Intracutaneous injection of rhPDGF-BB leads to slight irritation of the skin in rabbits compared to saline controls. This is an expected response given that rhPDGF-BB is a chemotactic and mitogenic agent for mesenchymally derived cells and cells involved in the inflammatory cascade that function to initiate normal wound healing.

4.3.2. Intramuscular Implantation. Intramuscular implantation of extracts of rhPDGF-BB combined with either β-TCP or collagen/β-TCP matrices was performed in rabbits to evaluate acute soft tissue responses to the test articles. Each test article elicited a mild inflammatory response involving small numbers of polymorphonuclear leukocytes, macrophages, giant cells, and fibroblasts associated with the test article implant. RhPDGF-BB is a chemoattractant for macrophages, monocytes, and fibroblasts, consequently, this observation was expected given the known biological properties of PDGF. However, the tissue response elicited by the experimental material was mild and similar to that of the plastic implant used as a negative control, and thus,

Table 4: Summary of local irritation and implantation studies for rhPDGF-BB combined with β-TCP or collagen/β-TCP.

Strain/Species	Route of Administration*	Test Article	Control Article(s)	Solvent for Extraction†	Number per Dose	Summary of Results
New Zealand white/rabbits	SC	1.0 mg/mL rhPDGF-BB + β-TCP	β-TCP alone and vehicle	NaCl Ethanol CSO PEG	4 animals, 5 injections per test article extract	No signs of erythema, edema, or eschar formation
New Zealand white/rabbits	SC	1.0 mg/mL rhPDGF-BB + collagen/ β-TCP	collagen/ β-TCP alone and plastic	NaCl CSO	2 animals, 5 injections per test article extract	No signs of erythema, edema, or eschar formation
New Zealand white/rabbits	IM	1.0 mg/mL rhPDGF-BB + β-TCP	β-TCP alone and plastic	None-test and controls implanted neat	2 animals, 5 paravertebral implants per animal Endpoint 7 days	(i) Mild cellular infiltration including PMNs, macrophages, giant cells, and fibroblasts (ii) No significant tissue response over negative control plastic
New Zealand white/rabbits	IM	1.0 mg/mL rhPDGF-BB + collagen/ β-TCP	collagen/ β-TCP alone and plastic	None-test and controls implanted *neat*	3 animals, 5 paravertebral test implants per animal Endpoint 28 days	(i) Mild cellular infiltration including PMNs, macrophages, giant cells, and fibroblasts (ii) No significant tissue response over negative control plastic

* Abbreviations: SC: subcutaneous, IM: intramuscular.
† Abbreviations: NaCl: 0.9% sodium chloride, CSO: cotton seed oil, PEG: polyethylene glycol.

none of the test article extracts elicited a significant biological response following intramuscular implantation.

In summary, extracts of 1.0 mg/mL rhPDGF-BB combined with either β-TCP or a collagen/β-TCP matrix, or β-TCP or collagen/β-TCP matrix alone did not cause acute skin irritation following subcutaneous injection in rabbits. In addition, intramuscular implantation of 1.0 mg/mL rhPDGF-BB combined with either β-TCP or collagen/β-TCP or either of these materials alone did not elicit any significant tissue responses when compared to implanted negative controls. Study designs and results are summarized in Table 4.

4.4. In Vitro Toxicity Studies: Cytotoxicity, Mutagenicity, and Hemolysis

4.4.1. Cytotoxicity. After 48 hours, no signs of cytotoxicity were noted for MEM tissue culture medium extracts of rhPDGF-BB combined with β-TCP, β-TCP alone or the plastic used as negative control. In distinction, severe cytotoxicity occurred with extracts from the natural rubber used as positive control. For the second test using the agar overlay method, no zones of bioreactivity were seen after 48 hours of incubation with either experimental material or negative control plastic extracted in MEM tissue culture medium absorbed into the filter paper.

MEM tissue culture medium extracts of rhPDGF-BB combined with collagen/β-TCP matrix or collagen/β-TCP matrix alone, after 48 hours, had slight bioreactivity consisting of loosely attached, rounded cells lacking intracytoplasmic granules and, rarely, lysed cells. No bioreactivity was observed for extracts of the plastic used as negative control compared with severe bioreactivity for the natural rubber used as positive control. The cellular response to the test materials was not unexpected. The bovine type I collagen was modified to be water soluble and consequently may compete with the tissue culture plastic for cellular adhesion binding sites on the cell surfaces. This effect may cause cells to become more loosely attached to the tissue culture plastic. There were no cytotoxic outcomes elicited by the experimental materials. The data are summarized in Table 5.

4.4.2. Mutagenicity. The mutagenic properties of NaCl and CSO extracts of rhPDGF-BB combined with collagen/β-TCP or collagen/β-TCP alone were evaluated using the Ames test with strains of *E. coli* and *Salmonella typhimurium* that were auxotrophic for histidine or tryptophan. There were no statistically significant ($P \leq .05$) increases in the number of revertant colonies following exposure to the test article extracts compared to negative controls. Thus, extracts of rhPDGF-BB combined with collagen/β-TCP matrix or collagen/β-TCP matrix were nonmutagenic in the bacterial assays. The data are summarized in Table 5.

Table 5: Summary of *in vitro* toxicity studies for rhPDGF-BB combined with β-TCP or collagen/β-TCP.

Cell Line/Strain	Route of Administration	Test Article	Control Articles	Solvent for Extraction†	Number per Dose	Summary of Results
L929 mouse fibroblasts	Direct Contact or Agar Diffusion	1.0 mg/mL rhPDGF-BB + β-TCP	β-TCP alone and vehicle	MEM tissue culture medium	3 flasks per extract	(i) No signs of cytotoxicity (ii) No zones of bioreactivity
L929 mouse fibroblasts	Direct Contact	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collage/β-TCP + sodium acetate solution	MEM tissue culture medium	3 flasks per extract	(i) Few loosely attached cells or lysed cells with or without rhPDGF-BB (ii) No significant cytotoxicity
S. typhimurium strains TA98, TA100, TA1535, TA1537 *his*$^-$ *E. coli* strain WP2 *trp*$^-$	Top agar overlay, +/− microsomal metabolic activation	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collage/β-TCP + sodium acetate solution	NaCl and CSO	3 plates per extract	(i) No significant increase in revertant colonies for test or control article (ii) Nonmutagenic
Blood from New Zealand white rabbit	Direct Contact	1.0 mg/mL rhPDGF-BB + collagen/β-TCP	collage/β-TCP + sodium acetate solution	NaCl	3 tests per extract	(i) 2.9% hemolysis compared to positive control for test article (ii) 2.5% hemolysis compared to positive control for control article (iii) Test and control article nonhemolytic

† Abbreviations: MEM: Modified Eagle medium, NaCl: 0.9% sodium chloride, CSO: cotton seed oil.

4.4.3. Hemolysis. NaCl extracts of rhPDGF-BB combined with collagen/β-TCP or collagen/β-TCP alone, resulted in 2.9% hemolysis compared to positive controls. The extracts of the matrix alone resulted in 2.5% hemolysis. Both materials were considered nonhemolytic compared to controls.

5. Conclusion

The safety and biocompatibility of rhPDGF-BB alone or in combination with either β-TCP, or a bovine type I collagen/β-TCP matrix were demonstrated following extensive *in vivo* and *in vitro* toxicological and biocompatibility testing. The experimental materials were not mutagenic, hemolytic, cytotoxic, pyrogenic, or allergenic. There was evidence of neither local nor systemic toxicity and all combinations of rhPDGF-BB with the materials tested were biocompatible following intramuscular implantation. Repeated administration of rhPDGF-BB delivered by intramuscular injection near the metatarsi and femora of rats at a dose of 100 μg/mL led to elevated bone remodeling and soft tissue responses. However, 6 weeks after the last injection, bone remodeling and soft tissues returned to a normal baseline state. This outcome underscores the normal, reversible effect of rhPDGF-BB on bone and overlying soft tissues, and further underscores the safety of the products evaluated in this paper which intended clinical use is as a single-dose, locally implanted materials for periodontal and orthopedic tissue repair.

Conflict of Interests

Some authors declare employment financial interests. Gino Bradica declares employment financial interests: an author who is current employees of a medical device company (Kensey-Nash).

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