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Review Article

Growth/differentiation factor-5: a candidate therapeutic agent for periodontal regeneration? A review of pre-clinical data

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Abstract

Aim: Therapeutic concepts involving the application of matrix, growth and differentiation factors have been advocated in support of periodontal wound healing/regeneration. Growth/differentiation factor-5 (GDF-5), a member of the bone morphogenetic protein family, represents one such factor. The purpose of this review is to provide a background of the therapeutic effects of GDF-5 expressed in various musculoskeletal settings using small and large animal platforms.

Methods: A comprehensive literature search was conducted to identify all reports in the English language evaluating GDF-5 using the PubMed and Google search engines, and a manual search of the reference lists from the electronically retrieved reports. Two reviewers independently screened the titles and abstracts from a total of 69 reports, 22 of which were identified as pre-clinical (in vivo) evaluations of GDF-5. The full-length article of the 22 pre-clinical reports was then reviewed.

Results: Various applications including cranial and craniofacial bone formation, spine fusion, long bone fracture healing, cartilage, and tendon/ligament repair using a variety of small and large animal platforms evaluating GDF-5 as a therapeutic agent were identified. A majority of studies, using biomechanical, radiographic, and histological analysis, demonstrated significant dose-dependent effects of GDF-5. These include increased/enhanced local bone formation, fracture healing/repair, and cartilage and tendon/ligament formation. GDF-5 frequently was shown to accelerate wound maturation. Several studies demonstrated GDF-5 to be a realistic alternative to autograft bone. Studies using pre-clinical models and human histology suggest GDF-5 may also increase/enhance periodontal wound healing/regeneration.

Conclusions: GDF-5 appears a promising therapeutic agent for periodontal wound healing/regeneration as GDF-5 supports/accelerates bone and tendon/ligament formation in several musculoskeletal settings including periodontal tissues.

Key words: bone; GDF-5; growth/differentiation factor-5; periodontal ligament; periodontal regeneration; review; tissue engineering

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Periodontal regenerative therapy aims to predictably restore the tooth supporting structures lost due to periodontal disease or trauma, i.e., the periodontal ligament, cementum, and alveolar bone (Polimeni et al. 2009a). Ideally, periodontal tissue regeneration would result in a replicate, a functional replacement of lost tissues. This would require an orchestrated process involving coordinated migration, proliferation, and differentiation of cells from tissue resources specifically contributing to restoring the periodontal attachment. In the course of wound healing, cells sequestered in the periodontal ligament, including perivascular stem cells, are thought to proliferate, freely migrate into the defect, and differentiate into cementoblasts, periodontal ligament fibroblasts, and osteoblasts (Melcher 1976). What is more frequently observed is periodontal

wound healing resulting from migration and proliferation of cells from all tissue resources circumscribing a periodontal defect, including the gingival connective tissue and epithelium, alveolar bone, and tissue resources sequestered in the periodontal ligament (Wikesjö & Selvig 1999). This stabilizes the site, but does not lead to full functional regeneration.

Traditionally, clinicians deliver periodontal reconstructive surgery using barrier devices, autograft bone, and bone biomaterials in support of periodontal wound healing/regeneration (Polimeni et al. 2006). More recently, the use of combination concepts including matrix, growth and differentiation factors has been introduced (Trombelli & Farina 2008, Kao et al. 2009). The discovery, purification, cloning, and characterization of bone morphogenetic proteins (BMPs) (Urist 1965, Wang et al. 1988, 1990, 1993, Wozney et al. 1988, Celeste et al. 1990, Özkaynak et al. 1990, Sampath et al. 1992, Hötten et al. 1994, 1996) has led to the development of therapeutic agents and protocols for the induction of bone, cartilage, tendons, and ligaments critical to the management of several indications in the axial and appendicular skeleton (Wan & Cao 2005). BMPs have indeed been shown to support tissue formation/reconstruction in a variety of settings in the craniofacial skeleton (Wozney & Wikesjö 2008).

Growth/differentiation factor-5 (GDF-5) is being considered as a candidate therapeutic agent for periodontal indications. GDF-5, also known as cartilage-derived morphogenetic protein-1 (CDMP-1), is a member of the BMP family of proteins, part of the transforming growth factor-β (TGF-β) superfamily (Hötten et al. 1994, 1996). Several studies suggest that GDF-5 (and also related GDF-6 and -7) is essential for the normal development and formation of bone, joints, tendons, and ligaments in the axial and appendicular skeleton (Storm & Kingsley 1996, Erlacher et al. 1998b, Faiyaz-Ul-Haque et al. 2002a, b, Nakase et al. 2002, Settle et al. 2003). In particular, cartilage formation appears to be controlled by GDF-5 (Francis-West et al. 1999, Buxton et al. 2001, Hatakeyama et al. 2004). In situ hybridization and immunostaining of human embryos show expression of GDF-5 at the stage of pre-cartilaginous mesenchymal condensation and throughout the cartilaginous cores of developing long bones, consistent with a role for GDF-5 in chondrocyte differentiation and growth in long bones (Chang et al. 1994). Moreover, GDF-5 is expressed in both foetal and adult normal and osteoarthritic cartilage (Bobacz et al. 2002, Chen et al. 2004). GDF-5 gene mutations have been associated with fibular hypoplasia and complex brachydactyly including phalangeal dysplasia, hip dysplasia, and positional teeth abnormalities (DuPan syndrome) inherited as an autosomal recessive trait as well as in acromesomelic chondrodysplasias, i.e., Hunter–Thompson type and Grebe type, phenotypically related to the DuPan syndrome and other traits (Francis-West et al. 1996, Baur et al. 2000, Faiyaz-Ul-Haque et al. 2002a, b, Archer et al. 2003, Savarirayan et al. 2003, Stelzer et al. 2003, Holder-Espinasse et al. 2004, Schwabe et al. 2004, Dobbs et al. 2005, Szczaluba et al. 2005). GDF-5-deficient mice display structurally weaker tendons than control, mutant Achilles tendons containing 40% less collagen than the control (Mikic et al. 2001). GDF-5 induces chondrogenesis and osteogenesis both in vitro and in vivo (Wolfman et al. 1997, Erlacher et al. 1998a, b, Gruber et al. 2000). GDF-5 also enhances angiogenesis (David et al. 2009). In various cell culture systems it has been shown that GDF-5 initiates chondrogenic differentiation of mesenchymal stem cells, although to a lesser degree in comparison with TGF-β_1 (Bai et al. 2004). Compared with osteogenic protein-1 (OP-1), GDF-5 appears less stimulatory of osteogenic differentiation (Erlacher et al. 1998a, Gruber et al. 2000, 2001).

The factors underlying the process by which mesenchymal stem cells (the target population in vivo) adopt one fate or the other in response to GDF-5, and what governs the predominant tissue outcome, remain to be elucidated. However, the carrier material, in conjunction with other environmental factors, has a significant influence (Shimaoka et al. 2004). In a murine calvarial model, GDF-5 (in conjunction with a recombinant human Type I collagen carrier) induced new bone formation via endochondral ossification (Kadomatsu et al. 2008), but intra-membranous ossification may predominate in other systems (Shimaoka et al. 2004). In the murine calvarial model, GDF-5 induced Sox9 [a transcription factor that plays a major role in endochondral ossification (Akiyama et al. 2005)] in the hypertrophic periosteum and newly formed bone.

As for other BMP proteins, GDF-5 interaction with mesenchymal stem cells leads to hetero-oligomerization of membrane serine/threonine kinase receptors (David et al. 2009) (Fig. 1). BMP type IB (also called activin receptor-like kinase 6, ALK-6) and activin receptor type IIB show the highest affinity for GDF-5 (Heinecke et al. 2009). Receptor activation by ligand binding activates Smad 5 protein (and to a lesser extent, Smad 1 and 8) by phosphorylation, followed by translocation of Smad proteins to the nucleus, where they regulate transcription of various genes both directly and indirectly (Massagué & Wotton 2000, Aoki et al. 2001).

GDF-5 signaling leads to upregulation of expression of genes associated with pre-osteoblast and secretory osteoblast phenotypes, including alkaline phosphatase, bone sialoprotein, and osteopontin (Bessa et al. 2009). GDF-5 activation of the p38 MAPK pathway has been reported to have a role in chondrogenesis (Nakamura et al. 1999, Verheyen 2007), but the role of GDF-5 signaling through the MAPK pathways in osteogenesis remains to be investigated.

GDF-5, -6 and -7 messenger RNAs are expressed in developing periodontal tissues in rats as GDF gene expression has been detected in cells associated with periodontal ligament formation, as well as in cells located along the alveolar bone and cementum surfaces, and insertion sites of the periodontal ligament during root formation, whereas GDF expression appears down-regulated upon completion of root formation (Sena et al. 2003). In other experiments, GDF-5, -6, and -7 gene expression has been shown during tooth and periodontal tissue formation in bovine incisor teeth (Morotome et al. 1998). GDF-5 and its receptor have also been demonstrated in human periodontal ligament cells using RT-PCR. Recombinant human GDF-5 (rhGDF-5) dose dependently enhanced human periodontal ligament cell proliferation over 16 days. In contrast, alkaline phosphatase was significantly decreased following addition of rhGDF-5 (Nakamura et al. 2003). Collectively, these studies suggest that GDF-5, and also GDF-6 and -7, may be potent regulatory molecules in the development of the periodontal attachment and may represent powerful candidate technologies for tissue engineering of the periodontium.

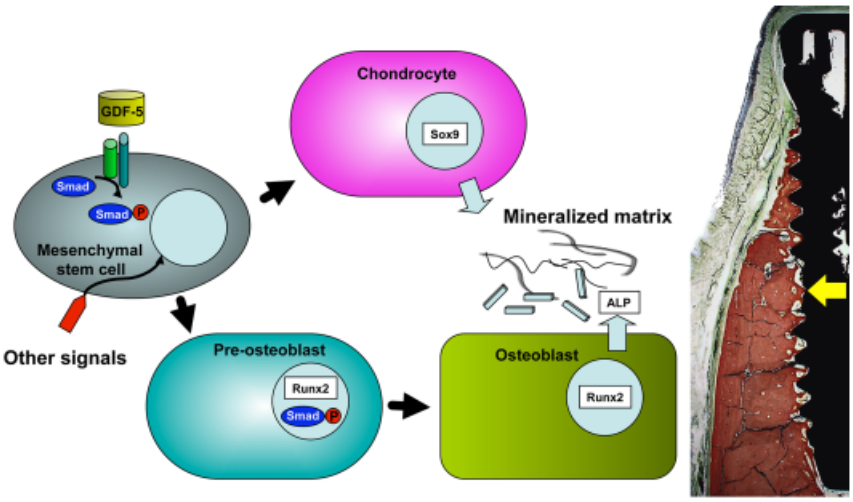

Fig. 1. Biological mechanisms of action of growth/differentiation factor-5 (GDF-5). Binding of GDF-5 to receptors on mesenchymal stem cells (in conjunction with other signaling systems that remain to be characterized) induces differentiation leading to a chondrocyte or osteoblast fate. The Sox9 transcription factor is essential to chondrogenesis, and the Runx2 transcription factor to osteogenesis. GDF-5-induced oligomerization of BMP type IB and activin receptor type IIB (cylinders) on the surface of a mesenchymal stem cell results in phosphorylation of Smad proteins, and their translocation to the nucleus. Phosphorylated Smad proteins regulate gene expression directly, by DNA binding, and indirectly, by associating with other proteins. This can result in differentiation into pre-osteoblasts and secretory osteoblasts, and expression of genes associated with bone formation, such as alkaline phosphatase (ALP). Osteoblasts and chondrocytes secrete extracellular matrices (irregular lines) that can be mineralized (blue needles). In the canine critical-size supraalveolar peri-implant defect model (right), implants coated with 120 μg rhGDF-5 induce significant new bone formation (the yellow arrow delineates the base of the defect), whereas uncoated implants (not shown) produce little new bone (Polimeni et al. 2009b).

The purpose of this review was to provide a background and understanding of GDF-5 as a candidate therapeutic agent. Pre-clinical studies published between 1992 and 2009, reported in the English language, using rodent and large animal platforms evaluating GDF-5 in various settings including cranial, craniofacial, spine, long bone, cartilage, and tendons were subject to a systematic search, and presented in the perspective of GDF-5 as a candidate therapeutic agent for periodontal indications.

Material and Methods

An exhaustive literature search using PubMed and Google search engines was conducted. The following search terms and combinations of search terms were used to identify relevant full-length publications: recombinant human GDF-5, GDF-5, human growth factor, human GDF-5, rhGDF-5, GDF-5, BMPs, BMP-14, cartilage-derived morphogenetic protein-1, cartilage-derived morphogenetic proteins, and CDMP-1. Only reports and reviews published in the English language from 1992 up to 2009 were included. The reference lists of the retrieved reports from the electronic search were screened manually to recover additional reports. Two reviewers (Y.M. and U.M.E.W.) independently screened the titles and abstracts. The full-length of an article was reviewed when the article included animal models.

For the purpose of this report, only full-length articles describing in vivo evaluations of GDF-5 or GDF-5 constructs in pre-clinical models for orthopaedic and craniofacial indications were reviewed. Data were extracted based on the general characteristics (authors and year of publication), study characteristics [type and number of animals; indication(s); intervention strategies; evaluation period; outcome measures; complication(s), methodological characteristics (study design), and conclusion(s)]. Reports from in vitro studies including biochemistry, molecular biology, cell culture, development, and reviews thereof were selectively used for background.

Results and Discussion

A total of 69 full-length references were recovered. Following review of the titles and abstracts, 22 studies were identified as pre-clinical evaluations of GDF-5 using various platforms and applications (Table 1). No clinical evaluations were identified.

Ectopic bone and cartilage formation

Two studies have evaluated the capacity of GDF-5 to drive ectopic bone or cartilage formation. Spiro et al. (2000) used an ectopic rat model to evaluate local bone formation following implantation of rhGDF-5 in a Type I collagen/hydroxyapatite carrier (Healos®II Bone Graft Substitute; DePuy Spine, Raynham, MA, USA) at concentrations of 0.01 and 0.1 mg/ml. rhGDF-5 constructs were implanted subcutaneously in the thoracic region or intra-muscularly in the posterior tibial muscle in 8-week-old male Sprague–Dawley rats. Implants were evaluated following 14- and 21-day healing intervals. The subcutaneous implants showed a dose-dependent increase in alkaline phosphatase activity, reflecting bone metabolic activity.

In the second report, GDF-5 (0, 100, 300, and 500 μg) in Type I collagen disk-shaped sponges was implanted into the calf muscle in 24 6-week-old Wistar rats (Kakudo et al. 2007). Radiographic and histologic analysis followed a 3-week healing interval. Whereas the carrier control and 100 μg dose showed no osteochondral tissue formation, the GDF-5 300 μg dose induced massive cartilage aggregates and some bone formation, and the 500 μg dose induced trabecular bone and marrow without a cartilage component. Both studies using ectopic rat models thus suggest a dose-dependent, and possibly threshold, effect of GDF-5.

Calvarial bone formation

Calvarial bone formation was evaluated in three studies (Kuniyasu et al. 2003, Pöhling et al. 2006, Yoshimoto et al. 2006). Yoshimoto et al. (2006) showed that rhGDF-5 in a collagen carrier supports calvarial bone growth. Briefly, rhGDF-5 in an atelocollagen carrier (20 μg rhGDF-5/site) or a carrier control was injected beneath the scalp along the periosteum in 8-week-old ddY mice. rhGDF-5 implanted ($n = 4$) and control

Table 1. Essential elements of preclinical studies evaluating growth/differentiation factor-5 (GDF-5) for indications in the axial and appendicular skeleton

Study	Platform	Application	Carrier	GDF-5 dose	Healing interval	Bio-mechanical	Radiographs	Histology
Spiro et al. (2000)	Rats	Ectopic	Collagen/HA	0.01, 0.1 mg/ml	2/3 weeks	–	Yes	Yes
Kakudo et al. (2007)	Rats	Ectopic	Collagen	0, 100, 300, 500 μg	3 weeks	–	Yes	Yes
Yoshimoto et al. (2006)	Mice	Calvarial	Collagen	20 μg	3 days, 1/2/3/4 weeks	–	Yes	Yes
Pöhling et al. (2006)	Rats	Calvarial	β-TCP	500 μg	6 weeks	–	–	Yes
Kuniyasu et al. (2003)	Rats	Calvarial	Collagen	1, 10, 100 μg	1/2/3 weeks	–	Yes	Yes
Gruber et al. (2008)	Pigs	Craniofacial	β-TCP	400, 800 μg	4/12 weeks	–	–	Yes
Weng et al. (2009)	Dogs	Craniofacial	β-TCP	–	8 weeks	–	–	Yes
Schwarz et al. (2008)	Dogs	Craniofacial	HA block HA particulate	0.32, 0.57 mg/ml	3/8 weeks	–	–	Yes
Simank et al. (2006)	Rabbits	Craniofacial	Dicalcium phosphate	10 μg	6 weeks	Yes	Yes	Yes
Chujo et al. (2006)	Rabbits	Spine	PBS	10 ng, 1, 100 μg	16 weeks	–	Yes	Yes
Spiro et al. (2001)	Rabbits	Spine	Collagen/HA	0.1, 1.0 mg/ml	–	Yes	–	–
Magit et al. (2006)	Rabbits	Spine	Collagen/HA	0.5, 1.0, 1.5 mg/ml	8 weeks	Yes	Yes	Yes
Jahng et al. (2004)	Sheep	Spine	Collagen/HA	0.5 mg/ml	2/4/6 months	Yes	Yes	Yes
Spiro et al. (2000)	Baboons	Spine	Collagen/HA	0.5, 1.5 mg/ml	20 weeks	–	Yes	Yes
Spiro et al. (2000)	Baboons	Long bone	Collagen/HA	0.022, 0.220, 2.2 mg	21 weeks	–	Yes	Yes
Chhabra et al. (2005)	Mice	Long bone	–	–	4/7/10 days, 2/3/4/5/6weeks	Yes	Yes	Yes
Katayama et al. (2004)	Rabbits	Cartilage	Bone marrow mesenchymal cells	–	2/4/8 weeks	–	–	Yes
Jung et al. (2006)	Pigs	Cartilage	Collagen/Hyaluronan	0.294 mg/site	3/12 months	–	–	Yes
Wolfman et al. (1997)	Rats	Tendon	DBM	–	10/21 days	–	–	Yes
Bolt et al. (2007)	Rats	Tendon	Adenovirus	–	2 weeks	Yes	–	Yes
Dines et al. (2007)	Rats	Tendon	Sutures	25, 55, 556 ng/cm	3/6 weeks	Yes	–	Yes
Forslund et al. (2003)	Rats	Tendon	–	0.4, 2, 10 μg	1/4 weeks	Yes	–	Yes

($n = 4$) animals were sacrificed at 3, 7, 14, 21, or 28 days, respectively. At 3 and 7 days, thickened tissue containing mesenchymal cells was observed at the rhGDF-5 sites, some of the cells immunoreactive to proliferating cell nuclear antigen, a marker for dividing cells. Controls exhibited no change. At 14 and 21 days, new bone/cartilage-like tissue formation was evident at rhGDF-5-treated sites, also including bone marrow-like structures. These tissues showed immunoreactivity for both Type I and Type II collagen. At 28 days, new bone/cartilage-like tissue formation including osteocyte-like cells and bone marrow-like areas were observed in haematoxylin/eosin-stained sections at rhGDF-5-treated sites. Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (i.e., osteoclasts) were detected in the centre of the marrow-like areas. Smaller, three to five nuclear, and mononuclear TRAP-positive cells adjoined the cell matrix. These tissues were strongly immunostained for Type I collagen, as were areas surrounding the osteocyte-like cells. The newly formed bone/cartilage-like tissue was strongly positive for Type I collagen, the major collagen of bone. In all, these observations suggest the rhGDF-5 in a collagen carrier may effectively induce bone formation using a process similar to endochondral bone formation.

Kuniyasu et al. (2003) demonstrated that rhGDF-5 supports local bone formation in a dose-dependent order following implantation of rhGDF-5 in a Type I collagen matrix onto calvarial bone in 60 4-week-old Wistar rats. rhGDF-5/collagen minidisks containing 0, 1, 10, and 100 μg rhGDF-5 were placed onto the calvarial bone below the periosteum. Animals in groups of five per dose were sacrificed at 1, 2, and 3 weeks post-implantation for radiographic and histometric evaluation. Bone formation gradually increased in a dose-dependent order over the 3-week interval. At 3 weeks, constructs containing 1 μg rhGDF-5 showed new bone formation on the cranial side of the minidisk. Constructs containing 10 μg rhGDF-5 showed bone formation and marrow proceeding to the centre of the construct from both periosteal and cranial aspects. The newly formed bone showed a connected trabecular structure with abundant vascularization. Constructs including 100 μg rhGDF-5 significantly increased bone formation compared with lower dosages. The con-

structs were almost entirely replaced by bone and marrow, and newly formed bone firmly integrated with the resident bone. In contrast, bone formation was not found in the controls.

Bone formation following implantation of rhGDF-5 in a β-tricalcium phosphate (β-TCP) carrier (500 μg rhGDF-5/g β-TCP) was evaluated using routine critical size full-thickness cranial osteotomy defects (Pöhling et al. 2006). Forty-eight adult Sprague–Dawley rats were used. Control animals received bovine bone mineral (Bio-Oss and Bio-Oss Collagen; Geistlich Pharma, Wolhusen, Switzerland and PepGen P-15; Dentsply, Lakewood, CO, USA) and β-TCP (Calciresorb; Ceraver Osteal, Roissy, France; and CeraSorb; Curasan, Kleinostheim, Germany) bone biomaterials, or served as sham-surgery controls. Bone formation following a 6-week healing interval was evaluated using histology. Bone fill following implantation of rhGDF-5 in the β-TCP carrier approximated 50% of the defect area. Defect fill for the sham-surgery control approximated 20% and ranged from 4% to 12% for the bone biomaterials, demonstrating a significant positive influence of rhGDF-5 on local bone formation and a noteworthy negative influence of the bone biomaterials as stand-alone technologies.

Craniofacial and peri-implant bone formation

Craniofacial and related implant applications have been evaluated in four reports (Simank et al. 2006, Gruber et al. 2008, Schwarz et al. 2008, Weng et al. 2009). Simank et al. (2006) used 18 adult female New Zealand White rabbits to evaluate the effect of coating $\varnothing 4.1 \times 5$ mm titanium implants with 10 μg GDF-5, a dicalcium phosphate coating, or 10 μg GDF-5 combined with the dicalcium phosphate coating. Contra-lateral femoral condyles (a trabecular bone model) received uncoated control or coated implants (six animals/experimental condition). The implant sites were evaluated following a 6-week healing interval using micro-CT, non-destructive mechanical testing, and histometric analysis. All surface modifications exhibited a significant positive effect on implant stability compared with control. There was an inverse correlation between bone formation and micro-displacement, and peri-implant soft-tissue formation.

Three reports concerned maxillary sinus and alveolar ridge inlay and onlay indications. rhGDF-5 in a β-TCP carrier and β-TCP as a stand-alone procedure were evaluated for maxillary sinus augmentation using 12 miniature pigs (Gruber et al. 2008). Contra-lateral maxillary sinus sites either received rhGDF-5 at 400 or 800 μg/g β-TCP carrier, or β-TCP alone. Each site also received one dental implant. Histometric analysis of non-decalcified specimens from 4 and 12 weeks showed significantly higher mean bone density at 4 weeks for the 400 μg rhGDF-5 group compared with control (23% *versus* 8%; $p = 0.05$). Bone-implant contact (i.e., osseointegration) was significantly enhanced at 4 weeks for rhGDF-5 (400/800 μg $\sim$ 40%) compared with the β-TCP control (8–16%; $p < 0.05$). There were no significant differences between treatments at 12 weeks suggesting that addition of rhGDF-5 to sinus floor augmentation procedures accelerated local bone formation and osseointegration, but did not produce a net gain over control.

In a second study, rhGDF-5/β-TCP induced bone formation was evaluated in 8 $\times$ 5 mm (height $\times$ width) mandibular through-and-through saddle-type defects using five adult dogs (Weng et al. 2009). A $\varnothing 2 \times 10$ mm screw-type implant was placed in the centre of each defect. Control defects received β-TCP/autograft bone (1:1) or β-TCP combined with guided bone regeneration *versus* rhGDF-5/β-TCP combined with guided bone regeneration. Histological/metric results following an 8-week healing interval suggest that rhGDF-5 significantly enhanced local bone formation in this defect model. All the implants became osseointegrated.

The effect of rhGDF-5 on lateral ridge augmentation has been evaluated in a canine model (Schwarz et al. 2008): maxillary and mandibular edentulated chronic alveolar ridge defects in eight young adult Beagle dogs were used. rhGDF-5 or rhBMP-2 in particulate and block bovine bone mineral matrices, in conjunction with a bovine collagen membrane, were evaluated following 3- and 8-week healing intervals. Control defects received the bovine particulate and block biomaterials and membrane without growth factors. rhGDF-5 and rhBMP-2 both supported local bone formation; their effects highly influenced by the carrier technology.

An additional report has evaluated rhGDF-5-coated titanium porous oxide implants using the critical-size supraalveolar peri-implant defect model (Polimeni et al. 2009). Six dogs received implants coated with 30 or 60 μg rhGDF-5; six dogs implants coated with 120 μg rhGDF-5 or left uncoated (control). The mucoperiosteal flaps were advanced, adapted, and sutured to submerge the implants for primary intention healing. The animals received fluorescent bone markers at week 3, 4, 7, and 8 post-surgery when they were euthanized for histologic evaluation. The histometric evaluation showed a dose-dependent increase in local bone formation for rhGDF-5-coated implants compared with control. Narrow fluorescent zones throughout the newly formed bone indicated relatively slow new bone formation within 3–4 weeks. All treatment groups exhibited relevant osseointegration. Moreover, application of rhGDF-5 appears safe as it was associated with limited, if any, adverse effects.

Collectively, these studies evaluating GDF-5 for craniofacial and related indications suggest several beneficial effects: GDF-5 enhances endosseous implant stability in trabecular bone, and GDF-5 accelerates bone formation and osseointegration in the maxillary sinus and in mandibular alveolar defects. These encouraging observations have prompted a prospective, randomized clinical trial on the safety and efficacy of rhGDF-5 coated onto β-TCP for sinus floor augmentation including histologic evaluation of biopsies from the augmented sites presently being completed.

Spine indications

Five reports have evaluated spine fusion and invertebral disc extracellular matrix production (Spiro et al. 2000, 2001, Jahng et al. 2004, Chujo et al. 2006, Magit et al. 2006). A rabbit invertebral disc puncture model was used to evaluate a potential effect of rhGDF-5 on disc repair (Chujo et al. 2006). Two lumbar discs in each of 16 New Zealand White rabbits received anular punctures and 4 weeks later injections of rhGDF-5 (10 ng, 1 and 100 μg) or phosphate-buffered saline into the nucleus pulposus. Animals were evaluated using MRI and histometrics following a 16-week healing interval. Injection of rhGDF-5 resulted in statistically significantly improved healing, including restoration of disc height, improved MRI and histometric scores, suggesting that rhGDF-5

has significant effects on extracellular matrix production.

Bilateral posterolateral lumbar fusion (L5/L6) was evaluated in 99 mature male New Zealand White rabbits (Spiro et al. 2001). Animals in groups of 11 received morselized trabecular bone and marrow from the iliac crest (1 ml/side); a Type I collagen/hydroxyapatite matrix (Healos® II Bone Graft Substitute); the collagen/hydroxyapatite matrix with bone marrow; or rhGDF-5 in three different formulations including soak-loaded onto the collagen/hydroxyapatite matrix; rhGDF-5 soak-loaded onto non-crosslinked mineralized collagen strips and lyophilized; or rhGDF-5 added to a collagen fibre slurry during a mineralization reaction. Mineralized collagen fibres containing rhGDF-5 were then cast into standardized moulds and lyophilized. All rhGDF-5 formulations were prepared at concentrations of 0.1 and 1.0 mg/ml for a total dose of 0.26 and 2.6 mg/side. Fusion rates as high as 80% were observed for the rhGDF-5 formulations. Average biomechanical strength of the most effective rhGDF-5 formulations was 82% greater than that observed for autograft, although this difference was not statistically significant. The fusion mass induced by rhGDF-5 was composed of normal trabecular bone and haematopoietic marrow with a thin outer cortical plate. The results suggest that the rhGDF-5/collagen matrix combination provides an effective alternative to autograft bone.

In a third report, single-level, L5/L6, inter-transverse process fusions in New Zealand White rabbits using rhGDF-5 at 0.5, 1.0, and 1.5 mg/ml in the Type I collagen/hydroxyapatite carrier (Healos® II Bone Graft Substitute) were described (Magit et al. 2006). Control animals received carrier alone or autograft. Thirteen animals were used in each group. The rhGDF-5 treatments all supported a 100% fusion rate evaluated using manual palpation, radiographs, and histology following an 8-week healing interval. The fusion rate for autograft bone was 38% and 0% for the carrier alone. These results suggest rhGDF-5 in the collagen/hydroxyapatite carrier predictably supports inter-transverse process fusion, and could serve as a preferred alternative to autograft bone for spine fusion indications.

The effect of GDF-5 for spine fusion indications has been confirmed using large animal platforms. rhGDF-5 again using the Type I collagen/hydroxyapatite carrier (Healos® II Bone Graft Substitute) was compared with autograft bone to induce and facilitate posterolateral lumbar fusion using an ovine platform and a minimally invasive posterior endoscopic surgical approach (Jahng et al. 2004). Twelve adult sheep received bilateral posterolateral lumbar fusion and pedicle screw fixation, with each animal receiving autograft iliac crest bone or rhGDF-5 inserted into the right and left sides of the spine. Animals in groups of four were euthanized for manual, radiographic, and histologic analysis at 2, 4, and 6 months. At 2 months partial bilateral fusion was observed, with the radiographic evaluation showing greater bone formation at the sites receiving rhGDF-5. Solid bilateral fusion was observed at 4 and 6 months; the autograft and rhGDF-5 fusion sites showing similar histological characteristics without anomalous bone formation. This study suggest that rhGDF-5 in a Type I collagen hydroxyapatite carrier may be used as an effective substitute for autograft bone in conjunction with endoscopic posterolateral lumbar arthrodesis and instrumentation and likely also in other surgical procedures. Importantly, application of rhGDF-5 appeared to accelerate local bone formation.

A non-instrumented, posterolateral lumbar inter-tranverse process fusion of one motion segment (L4/L5) was performed in 36 skeletally mature female baboons using rhGDF-5 and the Type I collagen/hydroxyapatite carrier (Healos® II Bone Graft Substitute) at 0.5 and 1.5 mg/ml for a total dose of 5 and 15 mg/side (Spiro et al. 2000). Control sites received iliac crest autograft (5 ml/side). Lumbar vertebrae were exposed through a posterior approach and the implant site prepared by bilateral transverse process decortication. Two strips of the rhGDF-5 construct/side (5 ml/side) were placed across the L4/L5 transverse processes. Spines were harvested for ex vivo radiographic, computerized tomographic, and qualitative histological evaluation at week 20. The histology revealed new bone formation ranging from complete fusion across the motion segment to limited bone formation. The 0.5 mg/ml concentration showed the most robust bone formation including well-organized fusion masses. Three specimens showed bilateral fusion, two unilateral fusion, and four discontinuous fusion masses. The 1.5 mg/ml concentration showed less bone formation. No bilateral fusions and only one unilateral fusion were observed. No residual matrix was apparent in any of the specimens in both groups. Sites implanted with autograft showed large bone fusion masses consisting of irregular lamellar trabecular bone, haematopoietic marrow, and well-developed cortices.

These studies evaluating GDF-5 technologies for spine fusion indications using rabbit and large animal (goat/baboon) platforms clearly show that GDF-5 in a Type I collagen/hydroxyapatite carrier is an effective candidate treatment and a realistic alternative to autograft bone for spine fusion. Several studies suggested that GDF-5 accelerates spine fusion. Notably, GDF-5 supported posterolateral lumbar inter-tranverse process fusion in the baboon in an inverse dose-dependent order.

Long bone indications

Mouse and baboon models have been employed to elucidate the significance of GDF-5 for fracture healing and bone repair of osteotomy defects (Spiro et al. 2000, Chhabra et al. 2005). Chhabra et al. (2005) used a mouse femoral shaft fracture model to study the significance of GDF-5 on fracture healing. GDF-5 (−/−) brachypodism mice and phenotypically normal hetrozygote (+/−) littermates (control) exposed to femoral closed mid-shaft fractures and stabilized with intra-medullary fixation were evaluated following 4-, 7-, 10-, 14-, 21-, 28-, 35-, and 42-day healing intervals. The results of the biochemical, histologic, and radiographic analysis suggest that GDF-5-deficient mice display significantly delayed short-term (7–14 days) fracture healing. These observations in turn suggest a significant role for GDF-5 in fracture repair by promoting cellular recruitment and chondrocyte differentiation.

Using a non-human primate platform, Spiro et al. (2000) showed that rhGDF-5 supports closure of fibular osteotomy defects in a dose-dependent order. Bilateral 1.5 cm osteotomy defects were created in the fibular mid-diaphysis in 16 skeletally mature male baboons, with the stabilized defects receiving rhGDF-5 at 0.01, 0.1, and 1.0 mg/ml in the Type I collagen/hydroxyapatite carrier (Healos® II Bone Graft Substitute) for a total dose of 0.022, 0.22, and 2.2 mg/defect. Contra-lateral defects served as

carrier controls. Block biopsies of the fibular osteotomy sites were collected at 21 weeks. The qualitative histological evaluation showed 100% union for the 2.2 mg high-dose, 75% for the 0.22 mg mid-dose, and 50% for the 0.022 mg low-dose group. Only 25% of the carrier control sites showed any histological evidence of union. The fibular unions were characterized by mature bone closure with distinct cortices and medullary tissue. Altogether, these studies using long-bone fracture and osteotomy models confirm a significant therapeutic potential for GDF-5 consistent with indications for cranial, craniofacial, and spine settings.

Cartilage formation

Two studies evaluated the effect of GDF-5 on cartilage formation (Katayama et al. 2004, Jung et al. 2006) using rabbit and miniature pig platforms. GDF-5 was investigated for articular cartilage repair using a rabbit knee-joint full-thickness cartilage defect (Katayama et al. 2004). Thirty New Zealand White rabbits received GDF-5 and control gene transfected autologous bone marrow-derived mesenchymal cell constructs into contra-lateral $\varnothing 4 \times 4$ mm osteochondral defects. Sixteen animals served as sham-surgery control. Animals were sacrificed for histologic evaluation at 2, 4, and 8 weeks. Implantation of GDF-5 increased cartilage regeneration compared with controls. The defects filled with hyaline cartilage and the deeper zone showed remodeling to subchondral bone over time. This study suggests that GDF-5 allows enhanced repair and remodeling compatible with hyaline articular cartilage.

In the second study, rhGDF-5 in a Type I collagen/hyaluronan sponge carrier was used to study osteochondral repair in a medial femoral condyle defect using a miniature pig platform (Jung et al. 2006). Bilateral $\varnothing 6.3 \times 10$ mm cylindrical osteochondral defects received rhGDF-5, carrier control, or served as sham-surgery controls. Ten animals received rhGDF-5 *versus* sham-surgery and 10 animals the collagen/hyaluronan carrier *versus* sham-surgery. Five animals from each group were sacrificed for histological evaluation at 3 and 12 months. rhGDF-5 and carrier control increased localized bone formation at 3 months compared with the sham-surgery control ($p = 0.05$). The increased amount of bone with collagen/hyaluronan carrier alone did not reach statistical significance. By 12 months, there were no longer significant differences in bone formation, nor were there any improvements in cartilage formation at any observation interval. Thus, in this model system, GDF-5 again appeared to accelerate tissue maturation, but did not influence the final amount formed.

Tendon/ligament formation and repair

Tendon formation and repair was examined in four reports, all using rat models (Wolfman et al. 1997, Forslund et al. 2003, Bolt et al. 2007, Dines et al. 2007). In the earliest study, GDF-5, -6, or -7 in a demineralized bone matrix carrier were implanted into subcutaneous and intra-muscular sites to evaluate *de novo* tissue formation (Wolfman et al. 1997): 165 implants were placed into subcutaneous sites and 18 implants into the quadriceps muscle in 4-week-old rats. Implants were subsequently evaluated using light and electron microscopy. Ten-day GDF implants were highly cellular while 21-day implants had undergone remodeling to become mainly acellular. Maturing implants consisted of densely packed connective tissue composed of collagen fibres that under polarized light showed an intensity of birefringence and regular periodicity characteristic of tendons and ligaments. In contrast, control BMP-2 implants induced endochondral bone formation. Aggrecan mRNA was present in GDF-5, -6, or -7 induced tissue consistent with the ability of cells resident in tendons and ligaments to produce a fibrocartilaginous extracellular matrix. Matrix proteins known to be abundant in adult tendon/ligament were present in the GDF implants with elastin, Type I collagen, and decorin showing high levels of expression.

In a later study, GDF-5, -6, and -7 were evaluated at 0 (control), 0.4, 2, and 10 μg to investigate wound healing in a rat Achilles tendon model (Forslund et al. 2003). Differences in osteogenesis between GDF-5, -6, and -7 at the various doses were also evaluated. GDF-5, -6, and -7 were injected into the Achilles tendon injury sites 6 h post-operatively. Biomechanical evaluation at 8 days showed a significant dose-related inzcrease in Achilles tendon strength and stiffness for all three proteins, without significant differences among them. Comparing the high GDF-5, -6, and -7 dose to OP-1 with respect to cartilage and bone formation at 4 weeks showed cartilage formation in all groups, without remarkable differences between them. Bone formation also occurred in all groups except the control, again without notable differences among the groups. The GDF-5, GDF-7 and OP-1 groups contained significantly more calcium than controls. The results suggest that GDF-5, -6, and -7 are suitable candidate therapeutic agents for tendon repair.

Two more recent reports corroborate these early studies on the effects of local application of GDF-5 on tendon repair. Bolt et al. (2007) used a Sprague–Dawley rat Achilles tendon laceration model to examine tendon repair following application of adenovirus-mediated GDF-5. Adenovirus expressing either the gene for green fluorescent protein (AdGFP) or the gene for human GDF-5 and green fluorescent protein (AdGDF-5) were incorporated in the right Achilles tendon in conjunction with surgical repair. Additional animals served as sham-surgery controls. Severed Achilles tendons were examined histologically and biochemically 2 weeks post-treatment. Sites transduced with GDF-5 showed minimal gapping, tensile strength greater than 70%, and a greater number of neotenocytes compared with sites receiving AdGFP or sham-controls. Ectopic bone or cartilage was not observed in tendons transduced with GDF-5. Moreover, the adenoviral vector did not elicit an appreciable immune response. These observations suggest that GDF-5 enhances Achilles tendon repair in rats and may indicate its potential use in tendon repair elsewhere in the musculoskeletal system.

Dines et al. (2007) evaluated the potential of rhGDF-5 to influence tendon repair. Sutures soak-loaded with rhGDF-5 at 0 (control), 24, 55, and 556 ng/cm were applied in a rat tendon repair model. Histologic and biomechanical parameters showed that rhGDF-5 accelerated tendon repair compared with control at 3 weeks whereas there were no notable differences between rhGDF-5 and control sites at 6 weeks. At 3 weeks, rhGDF-5-coated sutures displayed higher tensile load and stiffness, pronounced tendon hypertrophy, increased healing rate, and minimal isolated cartilage formation compared with control. In all, this study suggests that rhGDF-5 significantly accelerates tendon repair.

These reports suggest that GDF-5, and also GDF-6 and -7, may have therapeutic effects beyond bone and cartilage formation. Tendon repair must be confirmed, where possible, using large animal platforms before clinical use. The successful development of this candidate therapy would have several significant uses, one of them being sports medicine.

Periodontal perspectives

The pre-clinical studies above using small and large animal platforms clearly demonstrate favourable effects of GDF-5 on ligament, tendon, cartilage, and bone formation, making GDF-5 also a potential candidate for periodontal indications; cementum, ligament and bone formation being critical components of periodontal wound healing/regeneration. The temporal and spatial association of GDF-5 in development of the periodontal attachment (Morotome et al. 1998, Sena et al. 2003) further supports the candidacy of GDF-5 as therapeutic agent for periodontal wound healing/regeneration. Moreover, in vitro assays demonstrate that rhGDF-5 dose dependently enhances human periodontal ligament cell proliferation and matrix formation (Nakamura et al. 2003). A battery of preliminary reports have described the evaluation of rhGDF-5 combined with various carrier technologies in periodontal settings (Bennett et al. 2009, Kim et al. 2009, Kwon et al. 2009a, b, Lee et al. 2009, Sculean et al. 2009, Stavropoulos et al. 2009, Wikesjö et al. 2009, Windisch et al. 2009). rhGDF-5 at 20 μg in a β-TCP carrier or at 1, 20, 100 μg in an absorbable Type I collagen sponge carrier was implanted into mandibular pre-molar, one-wall, intra-bony periodontal defects in dogs (Kim et al. 2009, Lee et al. 2009). Controls received β-TCP, the collagen sponge, or sham-surgery. rhGDF-5 groups exhibited significantly enhanced bone and cementum formation including a functionally oriented periodontal ligament compared with controls at the end of the 8-week study; the rhGDF-5/β-TCP combination obviously being the most effective treatment. Limited, if any, adverse reactions were observed. Other studies evaluated injectable poly(D,L-lactide-co-glycolide) carriers for ease of use and minimally invasive protocols (Bennett et al. 2009). Still others evaluated a mouldable composite poly(D,L-lactide-co-glycolide) carrier for onlay indications (Kwon et al. 2009a). An FDA-approved recombinant human platelet derived growth factor (rhPDGF) in a β-TCP carrier preparation (GEM-21S; BioMimetic Therapeutics, Franklin, TN, USA) was used to benchmark the competitive value of the rhGDF-5/β-TCP treatment for periodontal wound healing/regeneration (Kwon et al. 2009b). Mandibular premolar, one-wall, intra-bony periodontal defects in dogs received rhGDF-5/β-TCP and rhPDGF/β-TCP in contra-lateral jaw quadrants. rhGDF-5/β-TCP promoted significantly greater (2–2.5 $\times$) bone and cementum formation compared with the rhPDGF/β-TCP construct. These promising results warranted follow-up clinical investigations.

A phase IIa randomized, controlled, clinical and histological parallel group design pilot study in 20 patients was conducted to evaluate the effect of rhGDF-5/β-TCP *versus* that following sham-surgery control in severely advanced intra-bony periodontal defects at teeth treatment planned for extraction (Sculean et al. 2009, Stavropoulos et al. 2009, Windisch et al. 2009). The results following a 6-month healing interval suggest that rhGDF-5/β-TCP can be considered safe; no adverse reactions were observed. Less gingival recession and almost two times greater clinical attachment gain were observed. The histologic evaluation suggests that rhGDF-5/β-TCP favourably affects bone formation, and may substantially support periodontal regeneration (Stavropoulos et al. 2009).

Summary

The pre-clinical studies reviewed herein evaluating GDF-5 for musculoskeletal indications point to the importance of a variable and sequenced approach to study the effect of therapeutic agents in pre-clinical settings prior to clinical evaluation. Observations in small animal screening models must be confirmed in well-characterized dentoalveolar models using large animal platforms. Not only variable approaches need to be considered as here for bone, tendon/ligament, and cartilage repair, but also dose, release kinetics, healing intervals, as well as delivery/carrier technology. Thus far, pre-clinical studies evaluating GDF-5 in periodontal settings corroborated by a human histologic pilot study point to a sound rationale for GDF-5 as a therapeutic agent for periodontal wound healing/regeneration.

References

Akiyama, H., Kim, J. E., Nakashima, K., Balmes, G., Iwai, N., Deng, J. M., Zhang, Z., Martin, J. F., Behringer, R. R., Nakamura, T. & de Crombrugghe, B. (2005) Osteochondroprogenitor cells are derived from Sox9 expressing precursors. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14665–14670.

Aoki, H., Fujii, M., Imamura, T., Yagi, K., Takehara, K., Kato, M. & Miyazono, K. (2001) Synergistic effects of different bone morphogenetic protein type I receptors on alkaline phosphatase induction. *Journal of Cell Science* **114** (Part 8), 1483–1489.

Archer, C. W., Dowthwaite, G. P. & Francis-West, P. (2003) Development of synovial joints. *Birth Defects Research. Part C, Embryo Today* **69**, 144–155.

Bai, X., Xiao, Z., Pan, Y., Hu, J., Pohl, J., Wen, J. & Li, L. (2004) Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. *Biochemical and Biophysical Research Communications* **325**, 453–460.

Baur, S. T., Mai, J. J. & Dymecki, S. M. (2000) Combinatorial signaling through BMP receptor IB and GDF5: shaping of the distal mouse limb and the genetics of distal limb diversity. *Development* **127**, 605–619.

Bennett, W., Kwon, D., Rodriguez, N. A., Pippig, S., Bastone, P., Susin, C. & Wikesjö, U. M. (2009) An injectable rhGDF-5 composite for minimally invasive periodontal regenerative procedures. *Journal of Dental Research* **88** (Special Issue A), IADR-abstract 2192.

Bessa, P. C., Cerqueira, M. T., Rada, T., Gomes, M. E., Neves, N. M., Nobre, A., Reis, R. L. & Casal, M. (2009) Expression, purification and osteogenic bioactivity of recombinant human BMP-4, -9, -10, -11 and -14. *Protein Expression and Purification* **63**, 89–94.

Bobacz, K., Gruber, R., Soleiman, A., Graninger, W. B., Luyten, F. P. & Erlacher, L. (2002) Cartilage-derived morphogenetic protein-1 and -2 are endogenously expressed in healthy and osteoarthritic human articular chondrocytes and stimulate matrix synthesis. *Osteoarthritis and Cartilage/OARS, Osteoarthritis Research Society* **10**, 394–401.

Bolt, P., Clerk, A. N., Luu, H. H., Kang, Q., Kummer, J. L., Deng, Z. L., Olson, K., Primus, F., Montag, A. G., He, T. V., Haydon, R. C. & Toolan, B. C. (2007) BMP-14 gene therapy increases tendon tensile strength in a rat model of Achilles tendon injury. *The Journal of Bone and Joint Surgery. American Volume* **89**, 1315–1320.

Buxton, P., Edwards, C., Archer, C. W. & Francis-West, P. (2001) Growth/differentiation factor-5 (GDF-5) and skeletal develop-

ment. *The Journal of Bone and Joint Surgery. American Volume* **83-A** (Suppl. 1), S23–S30.

Celeste, A. J., Iannazzi, J. A., Taylor, R. C., Hewick, R. M., Rosen, V., Wang, E. A. & Wozney, J. M. (1990) Identification of transforming growth factor β family members present in bone-inductive protein purified from bovine bone. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 9843–9847.

Chang, S. C., Hoang, B., Thomas, J. T., Vukicevic, S., Luyten, F. P., Ryba, N. J., Kozak, C. A., Reddi, A. H. & Moos, M. Jr. (1994) Cartilage-derived morphogenetic proteins. New members of the transforming growth factor-beta superfamily predominantly expressed in long bones during human embryonic development. *The Journal of Biological Chemistry* **269**, 28227–28234.

Chen, A. L., Fang, C., Liu, C., Leslie, M. P., Chang, E. & Di Cesare, P. E. (2004) Expression of bone morphogenetic proteins, receptors, and tissue inhibitors in human fetal, adult, and osteoarthritic articular cartilage. *Journal of Orthopaedic Research* **22**, 1188–1192.

Chhabra, A., Zijerdi, D., Zhang, J., Kline, A., Balian, G. & Hurwitz, S. (2005) BMP-14 deficiency inhibits long bone fracture healing: a biochemical, histologic, and radiographic assessment. *Journal of Orthopaedic Trauma* **19**, 629–634.

Chujo, T., An, H. S., Akeda, K., Miyamoto, K., Muehleman, C., Attawia, M., Andersson, G. & Masuda, K. (2006) Effects of growth differentiation factor-5 on the intervertebral disc – in vitro bovine study and in vivo rabbit disc degeneration model study. *Spine* **31**, 2909–2917.

David, L., Feige, J. J. & Bailly, S. (2009) Emerging role of bone morphogenetic proteins in angiogenesis. *Cytokine Growth Factor Reviews* **20**, 203–212.

Dines, J. C., Weber, L., Razzano, P., Prajapati, R., Timmer, M., Bowman, S., Bonasser, L., Dines, D. M. & Grande, D. P. (2007) The effect of growth differentiation factor-5-coated sutures on tendon repair in a rat model. *Journal of Shoulder and Elbow Surgery* **16** (Suppl. 5), S215–S221.

Dobbs, M. B., Gurnett, C. A., Robarge, J., Gordon, J. E., Morcuende, J. A. & Bowcock, A. M. (2005) Variable hand and foot abnormalities in family with congenital vertical talus and CDMP-1 gene mutation. *Journal of Orthopaedic Research* **23**, 1490–1494.

Erlacher, L., McCartney, J., Piek, E., ten Dijke, P., Yanagishita, M., Oppermann, H. & Luyten, F.P (1998a) Cartilage-derived morphogenetic proteins and osteogenic protein-1 differentially regulate osteogenesis. *Journal of Bone and Mineral Research* **13**, 383–392.

Erlacher, L., Ng, C. K., Ullrich, R., Krieger, S. & Luyten, F. P. (1998b) Presence of cartilage-derived morphogenetic proteins in articular cartilage and enhancement of matrix replacement in vitro. *Arthritis and Rheumatism* **41**, 263–273.

Faiyaz-Ul-Haque, M., Ahmad, W., Wahab, A., Haque, S., Azim, A. C., Zaidi, S. H., Teebi, A. S., Ahmad, M., Cohn, D. H., Siddique, T. & Tsui, L. C. (2002a) Frameshift mutation in the cartilage-derived morphogenetic protein 1 (CDMP1) gene and severe acromesomelic chondrodysplasia resembling Grebe-type chondrodysplasia. *American Journal of Medical Genetics* **111**, 31–37.

Faiyaz-Ul-Haque, M., Ahmad, W., Zaidi, S. H., Haque, S., Teebi, A. S., Ahmad, M., Cohn, D. H. & Tsui, L. C. (2002b) Mutation in the cartilage-derived morphogenetic protein-1 (CDMP1) gene in a kindred affected with fibular hypoplasia and complex brachydactyly (DuPan syndrome). *Clinical Genetics* **61**, 454–458.

Forslund, C., Rueger, D. & Aspenberg, P. (2003) A comparative dose–response study of cartilage-derived morphogenetic protein (CDMP)-1, -2 and -3 for tendon healing in rats. *Journal of Orthopaedic Research* **21**, 617–621.

Francis-West, P. H., Abdelfattah, A., Chen, P., Allen, C., Parish, J., Ladher, R., Allen, S., MacPherson, S., Luyten, F. P. & Archer, C. W. (1999) Mechanisms of GDF-5 action during skeletal development. *Development* **126**, 1305–1315.

Francis-West, P. H., Richardson, M. K., Bell, E., Chen, P., Luyten, F., Abdelfattah, A., Barlow, A. J., Brickell, P. M., Wolpert, L. & Archer, C. W. (1996) The effect of overexpression of BMPs and GDF-5 on the development of chick limb skeletal elements. *Annals of the New York Academy of Sciences* **785**, 254–255.

Gruber, R., Mayer, C., Bobacz, K., Krauth, M. T., Graninger, W., Luyten, F. P. & Erlacher, L. (2001) Effects of cartilage-derived morphogenetic proteins and osteogenic protein-1 on osteochondrogenic differentiation of periosteum-derived cells. *Endocrinology* **142**, 2087–2094.

Gruber, R., Mayer, C., Schulz, W., Graninger, W., Peterlik, M., Watzek, G., Luyten, F. P. & Erlacher, L. (2000) Stimulatory effects of cartilage-derived morphogenetic proteins 1 and 2 on osteogenic differentiation on bone marrow stromal cells. *Cytokine* **12**, 1630–1638.

Gruber, R. M., Ludwig, A., Merten, H. A., Achilles, M., Pöhling, S. & Schliephake, H. (2008) Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a histological and histomorphometric study in the Göttingen miniature pig. *Clinical Oral Implants Research* **19**, 522–529.

Hatakeyama, Y., Tuan, R. S. & Shum, L. (2004) Distinct functions of BMP4 and GDF5 in the regulation of chondrogenesis. *Journal of Cellular Biochemistry* **91**, 1204–1217.

Heinecke, K., Seher, A., Schmitz, W., Müller, T. D., Sebald, W. & Nickel, J. (2009) Receptor oligomerization and beyond: a case study in bone morphogenetic proteins. *BMC Biology* **7**, 59.

Holder-Espinasse, M., Escande, F., Mayrargue, E., Dieux-Coeslier, A., Fron, D., Doual-Bisser, A., Boute-Benejean, O., Robert, Y., Porchet, N. & Manouvrier-Hanu, S. (2004) Angel shaped phalangeal dysplasia, hip dysplasia, and positional teeth abnormalities are part of the brachydactyly C spectrum associated with CDMP-1 mutations. *Journal of Medical Genetics* **41**, e78.

Hötten, G., Neidhardt, H., Jacobowsky, B. & Pohl, J. (1994) Cloning and expression of recombinant human growth/differentiation factor 5. *Biochemical and Biophysical Research Communications* **204**, 646–652.

Hötten, G. C., Matsumoto, T., Kimura, M., Bechtold, R. F., Kron, R., Ohara, T., Tanaka, H., Satoh, Y., Okazaki, M., Shirai, T., Pan, H., Kawai, S., Pohl, J. S. & Kudo, A. (1996) Recombinant human growth/differentiation factor 5 stimulates mesenchyme aggregation and chondrogenesis responsible for the skeletal development of limbs. *Growth Factors* **13**, 65–74.

Jahng, T. A., Fu, T. S., Cunningham, B. W., Dmitriev, A. E. & Kim, D. H. (2004) Endoscopic instrumented posterolateral lumbar fusion with Healos and recombinant human growth/differentiation factor-5. *Neurosurgery* **54**, 171–180.

Jung, M., Tuischer, J. S., Sergi, C., Gotterbarm, T., Pohl, J., Richter, W. & Simank, H. G. (2006) Local application of a collagen type I/hyaluronate matrix and growth and differentiation factor 5 influences the closure of osteochondral defects in a minipig model by endochondral ossification. *Growth Factors* **24**, 225–232.

Kadomatsu, H., Matsuyama, T., Yoshimoto, T., Negishi, Y., Sekiya, H., Yamamoto, M. & Izumi, Y. (2008) Injectable growth/differentiation factor-5-recombinant human collagen composite induces endochondral ossification via Sry-related HMG box 9 (Sox9)expression and angiogenesis in murine calvariae. *Journal of Periodontal Research* **43**, 483–489.

Kakudo, N., Wang, Y. B., Miyake, S., Kushida, S. & Kusumotok. (2007) Analysis of osteochondro-induction using growth and differentiation factor-5 in rat muscle. *Life Science* **81**, 137–143.

Kao, R. T., Murakami, S. & Beirne, O. R. (2009) The use of biologic mediators and tissue engineering in dentistry. *Periodontology 2000* **50**, 127–153.

Katayama, R., Wakitani, S., Tsumaki, N., Morita, Y., Matsushita, I., Gejo, R. & Kimura, T. (2004) Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. *Rheumatology (Oxford, UK)* **43**, 980–985.

Kim, T-G., Wikesjö, U. M., Cho, K-S., Chai, J-K., Pippig, S., Siedler, M. & Kim, C-K. (2009) Periodontal regeneration following implantation of rhGDF-5 in a collagen carrier. *Journal of Dental Research* **88**, (Special Issue A), IADR-abstract 2193.

Kuniyasu, H., Hirose, Y., Ochi, M., Yajima, A., Sakaguchi, K., Murato, M. & Pohl, J. (2003) Bone augmentation using rhGDF-5-collagen composite. *Clinical Oral Implants Research* **14**, 490–499.

Kwon, D., Bisch, F., Herold, R., Bastone, P., Herberg, S., Lewis, J., Rodriguez, N., Susin, C.

& Wikesjö, U. (2009a) Effect of rhGDF-5 in a novel carrier on periodontal regeneration. *Journal of Dental Research* **88**, (Special Issue A), IADR-abstract 1593.

Kwon, H-R., Wikesjö, U. M., Jung, U-W., Kim, Y-T., Bastone, P., Pippig, S. & Kim, C-K. (2009b) Periodontal regeneration following implantation of rhGDF-5 vs. rhPDGF in dogs. *Journal of Dental Research* **88**, (Special Issue A), IADR-abstract 1711.

Lee, J-S., Wikesjö, U. M., Kim, Y-T., Choi, S-H., Pippig, S., Siedler, M. & Kim, C-K. (2009) rhGDF-5 in a β-tricalcium phosphate carrier significantly supports periodontal regeneration. *Journal of Dental Research* **88**, (Special Issue A), IADR-abstract 2185.

Magit, D. P., Maak, T., Trioano, N., Raphael, B., Hamouria, Q., Polzhofer, G., Drespe, I., Albert, T. J. & Grauer, J. N. (2006) Healos/recombinant human growth and differentiation factor-5 induces posterolateral lumbar fusion in a New Zealand white rabbit model. *Spine* **31**, 2180–2188.

Massagué, J. & Wotton, D. (2000) Transcriptional control by the TGF-b/Smad signaling system. *EMBO Journal* **19**, 1745–1754.

Melcher, A. H. (1976) On the repair potential of periodontal tissues. *Journal of Periodontology* **47**, 256–260.

Mikic, B., Schalet, B. J., Clark, R. T., Gaschen, V. & Hunziker, E. B. (2001) GDF-5 deficiency in mice alters the ultrastructure, mechanical properties and composition of the Achilles tendon. *Journal of Orthopaedic Research* **19**, 365–371.

Morotome, Y., Goseki-Sone, M., Ishikawa, I. & Oida, S. (1998) Gene expression of growth and differentiation factors-5, -6, and -7 in developing bovine tooth at the root forming stage. *Biochemical and Biophysical Research Communications* **244**, 85–90.

Nakamura, K., Shirai, T., Morishita, S., Uchida, S., Saeki-Miura, K. & Makishima, F. (1999) p38 mitogen-activated protein kinase functionally contributes to chondrogenesis induced by growth/differentiation factor-5 in ATDC5 cells. *Experimental Cell Research* **250**, 351–363.

Nakamura, T., Yamamoto, M., Tamura, M. & Izumi, Y. (2003) Effects of growth/differentiation factor-5 on human periodontal ligament cells. *Journal of Periodontal Research* **38**, 597–605.

Nakase, T., Sugamoto, K., Miyamoto, T., Tsumaki, N., Luyten, F. P., Inui, H., Myoui, A., Tomita, T. & Yoshikawa, H. (2002) Activation of cartilage-derived morphogenetic protein-1 in torn rotator cuff. *Clinical Orthopaedics and Related Research* **399**, 140–145.

Özkaynak, E., Rueger, D. C., Drier, E. A., Corbett, C., Ridge, R. J., Sampath, T. K. & Oppermann, H. (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-β family. *The EMBO Journal* **9**, 2085–2093.

Pöhling, S., Pippig, S. D., Hellerbrand, K., Siedler, M., Schütz, A. & Dony, C. (2006) Superior effect of MD05, beta-tricalcium phosphate coated with recombinant human growth/differentiation factor-5, compared to conventional bone substitutes in the rat calvarial defect model. *Journal of Periodontology* **77**, 1582–1590.

Polimeni, G., Susin, C. & Wikesjö, U. M. E. (2009a) Regenerative potential and healing dynamics of the periodontium: a critical-size supra-alveolar periodontal defect study. *Journal of Clinical Periodontology* **36**, 258–264.

Polimeni, G., Xiropaidis, A. V. & Wikesjö, U. M. E. (2006) Biology and principles of periodontal wound healing/regeneration. *Periodontology 2000* **41**, 30–47.

Polimeni, G., Wikesjö, U. M. E., Susin, C., Qahash, M., Shanaman, R. H., Rohrer, M. D. & Hall, J. (2009) Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor-5 (rhGDF-5). Histologic observations. *Journal of Clinical Periodontology*, under review.

Sampath, T. K., Maliakal, J. C., Hauschka, P. V., Jones, W. K., Sasak, H., Tucker, R. F., White, K. H., Coughlin, J. E., Tucker, M. M., Pang, R. H., Corbett, C., Özkaynak, E., Oppermann, H. & Rueger, D. (1992) Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *The Journal of Biological Chemistry* **267**, 20352–20362.

Savarirayan, R., White, S. M., Goodman, F. R., Graham, J. M. Jr., Delatycki, M. B., Lachman, R. S., Rimoin, D. L., Everman, D. B. & Warman, M. L. (2003) Broad phenotypic spectrum caused by an identical heterozygous CDMP-1 mutation in three unrelated families. *American Journal of Medical Genetics. Part A* **117A**, 136–142.

Schwabe, G. C., Türkmen, S., Leschik, G., Palanduz, S., Stöver, B., Goecke, T. O. & Mundlos, S. (2004) Brachydactyly type C caused by a homozygous missense mutation in the prodomain of CDMP1. *American Journal of Medical Genetics. Part A* **124A**, 356–363.

Schwarz, F., Rothamel, D., Herten, M., Ferrari, D., Sager, M. & Becker, J. (2008) Lateral ridge augmentation using particulated or block bone substitutes biocoated with rhGDF-5 and rhBMP-2: an immunohistochemical study in dogs. *Clinical Oral Implants Research* **19**, 642–652.

Sculean, A., Windisch, P., Szendröi Kiss, D., Horvath, A., Molnar, B., Syilágyi, E., Rosta, P., Tihanyi, D., Stavropoulos, A., Dony, C., Capsius, B. & Wikesjö, U. M. (2009) Protocol and preliminary data evaluating rhGDF-5/β-TCP for periodontal wound healing/regeneration. *Journal of Dental Research* **88**, (Special Issue A), IADR-abstract 1712.

Sena, K., Morotome, Y., Baba, O., Terashima, T., Takano, Y. & Ishikawa, I. (2003) Gene expression of growth differentiation factors in the developing periodontium of rat molars. *Journal of Dental Research* **82**, 166–171.

Settle, S. H. Jr., Rountree, R. B., Sinha, A., Thacker, A., Higgins, K. & Kingsley, D. M. (2003) Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse GDF6 and GDF5 genes. *Developmental Biology* **254**, 116–130.

Shimaoka, H., Dohi, Y., Ohgushi, H., Ikeuchi, M., Okamoto, M., Kudo, A., Kirita, T. & Yonemasu, K. (2004) Recombinant growth/differentiation factor-5 (GDF-5) stimulates osteogenic differentiation of marrow mesenchymal stem cells in porous hydroxyapatite ceramic. *Journal of Biomedical Materials Research Part A* **68**, 168–176.

Simank, H. G., Stuber, M., Frahm, R., Helbig, L., van Lenthe, H. & Müller, R. (2006) The influence of surface coatings of dicalcium phosphate (DCPD) and growth and differentiation factor-5 (GDF-5) on the stability of titanium implants in vivo. *Biomaterials* **27**, 3988–3994.

Spiro, R. C., Liu, L., Heidaran, M. A., Thompson, A. Y., Ng, C. K., Pohl, J. & Poser, J. W. (2000) Inductive activity of recombinant human growth and differentiation factor-5. *Biochemical Society Transactions* **28**, 362–368.

Spiro, R. C., Thompson, A. Y. & Poser, J. W. (2001) Spinal fusion with recombinant human growth and differentiation factor-5 combined with a mineralized collagen matrix. *The Anatomical Record* **263**, 388–395.

Stavropoulos, A., Sculean, A., Windisch, P., Gera, I., Capsius, B. & Wikesjö, U. M. E. (2009) A phase IIa randomized, controlled, clinical and histological pilot study evaluating rhGDF-5/β-TCP for periodontal regeneration. Histological results. *Journal of Clinical Periodontology* **36** (Suppl. 9), EUABS064243, 11.

Stelzer, C., Winterpacht, A., Spranger, J. & Zabel, B. (2003) Grebe dysplasia and the spectrum of CDMP1 mutations. *Pediatric Pathology & Molecular Medicine* **22**, 77–85.

Storm, E. E. & Kingsley, D. M. (1996) Joint patterning defects caused by single and double mutations in members of the bone morphogenetic protein (BMP) family. *Development* **122**, 3969–3979.

Szczaluba, K., Hilbert, K., Obersztyn, E., Zabel, B., Mazurczak, T. & Kozlowski, K. (2005) Du Pan syndrome phenotype caused by heterozygous pathogenic mutations in CDMP1 gene. *American Journal of Medical Genetics. Part A* **138**, 379–383.

Trombelli, L. & Farina, R. (2008) Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *Journal of Clinical Periodontology* **35** (Suppl. 8), 117–135.

Urist, M. R. (1965) Bone: formation by autoinduction. *Science* **150**, 893–899.

Verheyen, E. M. (2007) Opposing effects of Wnt and MAPK on BMP/Smad signal duration. *Developmental Cell* **13**, 755–756.

Wan, M. & Cao, X. (2005) BMP signaling in skeletal development. *Biochemical and Biophysical Research Communications* **328**, 651–657.

Wang, E. A., Israel, D. I., Kelly, S. & Luxenburg, D. P. (1993) Bone morphogenetic protein-2 causes commitment and differentiation

in C3H10T1/2 and 3T3 cells. *Growth Factors* **9**, 57–71.

Wang, E. A., Rosen, V., Cordes, P., Hewick, R. M., Kriz, M. J., Luxenberg, D. P., Sibley, B. S. & Wozney, J. M. (1988) Purification and characterization of other distinct bone-inducing factors. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 9484–9488.

Wang, E. A., Rosen, V., D'Alessandro, J. S., Bauduy, M., Cordes, P., Harada, T., Israel, D. I., Hewick, R. M., Kerns, K. M., LaPan, P., Luxenburg, D. P., McQuaid, D., Moutsatsos, I. K., Nove, J. & Wozney, J. M. (1990) Recombinant human bone morphogenetic protein induces bone formation. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 2220–2224.

Weng, D., Pöhling, S., Pippig, S., Bell, M., Richter, E-J., Zuhr, O. & Hürzeler, M. (2009) The effects of recombinant human growth/differentiation factor-5 (rhGDF-5) on bone regeneration around titanium dental implants in barrier membrane-protected defects: a pilot study in the mandible of Beagle dogs. *The International Journal of Oral & Maxillofacial Implants* **24**, 31–37.

Wikesjö, U. M. E., Lee, J-S., Kwon, H-R., Jung, U-W., Kim, Y-T., Siedler, M., Bastone, P., Pippig, S. & Kim, C-K. (2009) rhGDF-5 in a β-tricalcium phosphate carrier significantly supports periodontal wound healing/regeneration. *Journal of Clinical Periodontology* **36** (Suppl. 9), EUABS064349, 11.

Wikesjö, U. M. E. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

Windisch, P., Stavropoulos, A., Szendröi-Kiss, D., Szilágyi, E., Rosta, P., Tihany, D., Capsius, B., Wikesjö, U. M. E. & Sculean, A. (2009) A phase IIa randomized, controlled, clinical and histological pilot study evaluating rhGDF-5/β-TCP for periodontal regeneration. Clinical results. *Journal of Clinical Periodontology* **36** (Suppl. 9), EUABS065945, 12.

Wolfman, N. M., Hattersley, G., Cox, K., Celeste, A. J., Nelson, R., Yamaji, N., Dube, J. L., DiBlasio-Smith, E., Nove, J., Song, J. J., Wozney, J. M. & Rosen, V. (1997) Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6, and 7, members of the TGF-beta gene family. *Journal of Clinical Investigation* **100**, 321–330.

Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. & Wang, E. A. (1988) Novel regulators of bone formation: molecular clones and activities. *Science* **242**, 1528–1534.

Wozney, J. M. & Wikesjö, U. M. E. (2008) rhBMP-2: biology and applications in oral and maxillofacial surgery and periodontics. In: Lynch, S. E., Wisner-Lynch, L. A., Nevins, M. & Marx, R. E. (eds). *Tissue Engineering: Applications in Oral and Maxillofacial Surgery and Periodontics*, 2nd edition, pp. 159–177. Chicago: Quintessence Publishing Company.

Yoshimoto, T., Yamamoto, M., Kadoatsu, H., Sakoda, K., Yonamine, Y. & Izumi, Y. (2006) Recombinant human growth/differentiation factor-5 (rhGDF-5) induced bone formation in murine calvariae. *Journal of Periodontal Research* **41**, 140–147.

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Clinical Relevance

Scientific rationale: GDF-5 is being evaluated as a candidate therapy for periodontal wound healing/regeneration. The objective of this text was to, in perspective, review the potential utility of GDF-5 as a therapeutic agent in musculoskeletal settings throughout the axial and appendicular skeleton.

Principal findings: Application of GDF-5 in a variety of carrier systems increased/accelerated local bone formation, fracture healing/repair, cartilage and tendon/ligament formation, and periodontal wound healing/regeneration as evaluated using biomechanical, radiographic, and histological parameters. Significant dose-dependent effects were observed.

Practical implications: GDF-5 appears a promising technology in support of periodontal wound healing/regeneration. Clinical evaluation for several musculoskeletal indications including periodontal wound healing/regeneration appears motivated.