Effects of Combining Insulin-like Growth Factor 1 and Platelet-derived Growth Factor on Osteogenesis around Dental Implants

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Objective: *To explore the effects of the combination of insulin-like growth factor 1 (IGF-1) and platelet-derived growth factor (PDGF) on bone formation around dental implants.*
Methods: *A total of 24 adult rabbits were included in this experiment. Titanium machine-polished dental implants were placed in the iliac bones to simulate dental implants in the alveolar bone. The rabbits were randomly divided into four groups; a saline treated control group (NS), an IGF-1 treated group, a PDGF-BB treated group, and a combination of IGF-1 and PDGF-BB treated group. The rabbits were sacrificed after 3, 7, and 10 days, and implants and soft tissues around implants were histologically evaluated.*
Results: *All of the rabbits began to recover their appetite, move freely and the operation area started detumescence until after the fourth day. H&E staining showed that the granulation tissue formation, multinucleated giant cells, a small amount of calcium salt deposition and bone tissue were observed in the IGF-1 group and the PDGF-BB group. In the IGF-1 + PDGF-BB group, the granulation tissue had turned into fibrous connective tissue, and calcium salt deposit had formed bone tissue. Masson's trichrome staining showed that the IGF-1 group and the PDGF-BB group had more collagen fibre compared with the NS group. In the IGF-1 + PDGF-BB group, collagen fibre hyperplasia and repairing fibres appeared earlier than in other groups.*
Conclusion: *When applying IGF-1 or PDGF-BB alone, either has the effect of accelerating the wound healing in the short term; while in combination, earlier collagen fibre hyperplasia appeared.*
Key words: *IGF-1, PDGF-BB, implants, osteogenesis*
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How to improve bone integration has been a research focus in oral implant fields. It is generally accepted that titanium endosseous implants can produce good osteointegration interface. Titanium is a metallic element known by several attractive characteristics, such as biocompatibility, corrosion resistance and high mechanical resistance, and is an excellent biomimetic material. But the technique also has problems that need to be solved, such as how to shorten the treatment time of an implant and how to improve integration between the implant and the bone. Some results of experimental studies have established that bone growth factors could play an important role in bone formation and bone repair,
This study was to determine and compare the effect of insulin-like growth factor 1 (IGF-1) and platelet-derived growth factor (PDGF) on bone regeneration around implants. It is hoped that the study may help in some improvements in clinical outcomes over currently available techniques.

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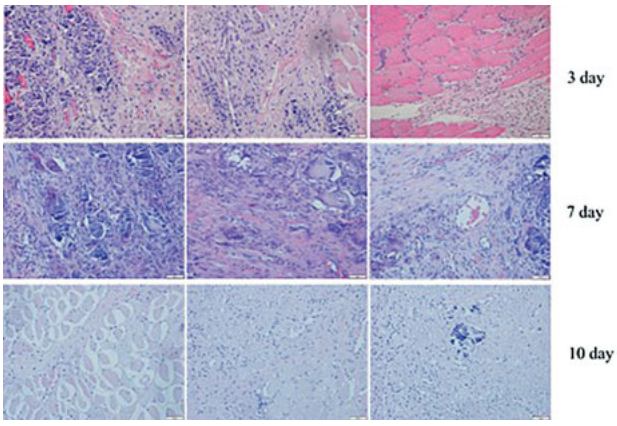

Fig 1 IGF-1 group (HE staining, bar = 50 μm).

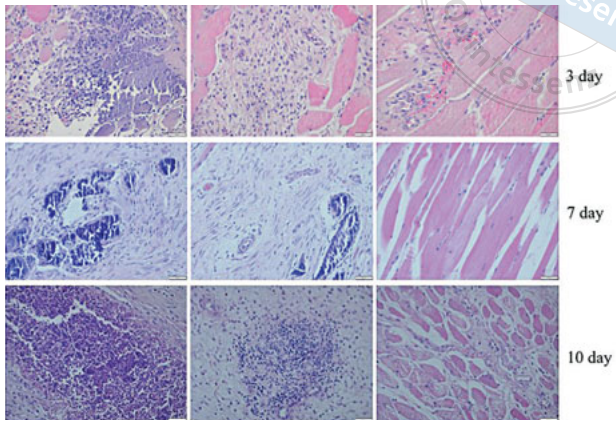

Fig 2 PDGF-BB group (HE staining, bar = 50 μm).

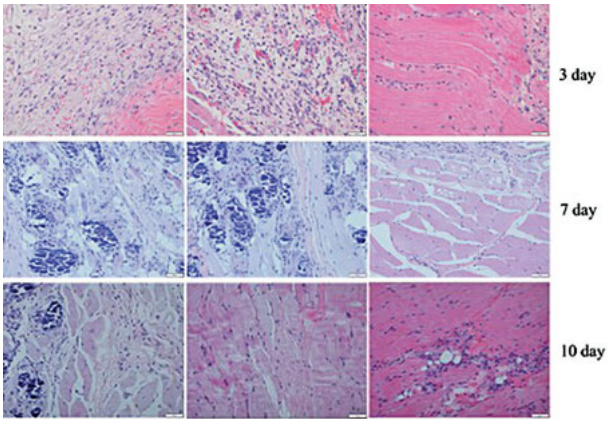

Fig 3 IGF-1 + PDGF-BB group (HE staining, bar = 50 μm).

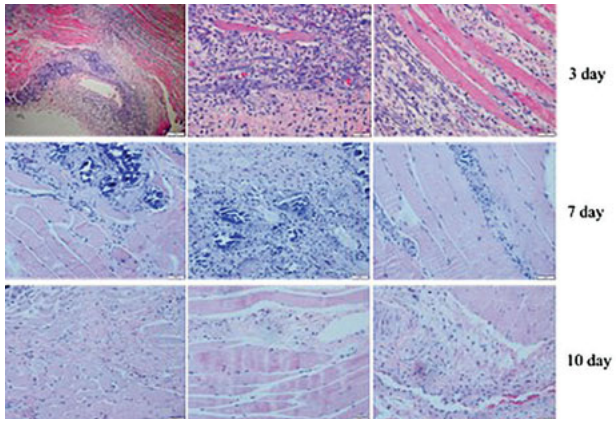

Fig 4 Control group (HE staining, bar = 50 μm).

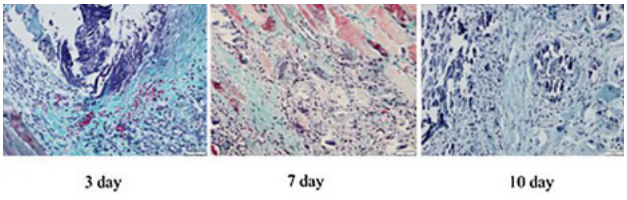

Fig 5 Control group (Masson's trichrome staining bar = 50 μm).

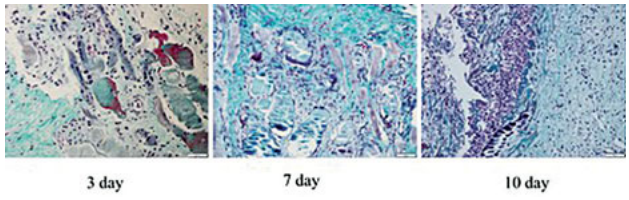

Fig 6 IGF-1 group (Masson's trichrome staining bar = 50 μm).

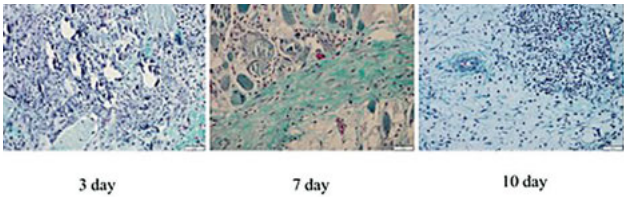

Fig 7 PDGF-BB group (Masson's trichrome staining bar = 50 μm).

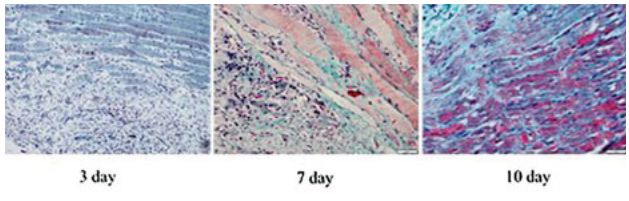

Fig 8 IGF-1 + PDGF-BB group (Masson's trichrome staining bar = 50 μm).

Materials and methods

Animals

The animals were obtained from the animal experiment centre at the Bethune Medical School at Jilin University (Changchun, China) and the Laboratory Animal Management Regulations and Measures approved the experimental protocol for the administration of experimental animal license. The 24 healthy, male and female rabbits, each weighing between 2 and 2.5 kg, were kept at room temperature and given water freely. Implant surgeries were performed after the rabbits were allowed to acclimatise for 1 week.

Experimental procedures

Animal surgery
The rabbits were divided into four groups of six. Each rabbit received general anaesthesia with 4% Chloral hydrate (Shanghai Chemical Reagent Co Ltd, Shanghai, China) intravenously and were intubated, then underwent the implant surgery. An incision was made along the edge of the ilium and the tissues separated step by step until the ilium was fully exposed. As in clinical practice the cavity was prepared and the implants (Leiden Company, Beijing, China), were placed in the ilium. Each rabbit received two implants – 3 mm in diameter and 8.0 mm in length – in the unilateral ilium. The soft tissue was then sutured. Immediately after surgery, they were given a local subperiosteal injuection of 1 mg IGF-1 (Peprotech Inc, Rocky Hill, USA), PDGF-BB (Peprotech), PDGF-BB+IGF-1, and normal saline (Shijiazhuang SiYao Co Ltd, Shijazhuang, China) respectively, for the four different groups. The postoperative rabbits received no other treatment or special diet.

Specimen processing

The rabbits were sacrificed after 3, 7 and 10 days respectively post-surgery, two at a time in the four groups. The implants were removed and put into the neutral buffered formalin to be fixed for 1 week and then embedded and sectioned in conventional paraffin. HE staining and Masson's trichrome staining were performed. The histological specimens were observed and photographed with OLYMPUS BX52, OLYMPUS SP610 and the related micrography system (Olympus Corporation, Tokyo, Japan), provided by Bethune Medical Laboratory Equipment Center of Jilin University.

Results

General observation of samples

Within the first 3 days after their operations, the rabbits were inactive and ate less food. They preferred to lie still, were swollen in the operated area and could not stretch their limbs. After 4 days all the rabbits began to recover their appetite, moved more freely and the operation site began detumescence. The rabbits showed no inflammatory necrosis tissue; also there was no implant detachment and the implants remained solidly connected with the surrounding tissue at the time each rabbit was sacrificed in all of the groups.

Histological observation

HE staining:
For the IGF-1 group, the granulation tissue formation was observed; the existence of multinucleated giant cells and a small amount of calcium salt deposition and bone tissue were found (Fig 1). For the PDGF-BB group, the granulation tissue, the formation of bone tissue and the calcium salt deposit were also observed (Fig 2). For the IGF-1 + PDGF-BB group, the granulation tissue had been turned into fibrous connective tissue, and calcium salt deposit had formed bone tissue (Fig 3). There were local tissue necrosis and inflammatory cell infiltration and no obvious granulation tissue formation in the normal saline group (Fig 4).

Masson's trichrome staining:
The NS control group showed collagen fibre (Fig 5). The IGF-1 group showed obvious fibrous repair among the muscle tissues compared with the NS group. A large part of this granulation tissue with the fibrous repair appeared after 10 days (Fig 6). The PDGF-BB group showed more collagen fibre in comparison to the NS group. After 10 days, granulation tissue hyperplasia and fibre could be observed (Fig 7). For the IGF-1 + PDGF-BB group, after 3 days, collagen fibre hyperplasia and repairing fibres appeared earlier, and granulation tissue grew obvious fibroblasts. After 7 days and 10 days, muscle tissues surrounding the implants had obvious fibrous tissues growing (Fig 8).

Discussion

The therapeutic management of new bone formation remains one of the key issues in periodontology and dental implantology. Bone repair is a complex process involving a variety of stem cell differentiation, prolifera-

tion, extra-cellular matrix, signal molecular recognition, related growth factor expression, new bone maturation and a series of chain processes, which are affected by many factors and the bone growth factor plays an important role in this process. The growth factors may regulate osteoblast behaviour and bone formation locally and huge advances have been made in the understanding of cellular and molecular control of bone formation. Bone growth factor for promoting osteointegration has two meanings: (1) Reducing osteointegration time; (2) Improving bone-implant contact rate. Sometimes the two effects are not separated.

Within the periodontal environment, some growth factors found in bone, cementum and healing tissues include transforming growth factor-3, basic fibroblast growth factor, insulin-like growth factors (IGF), platelet-derived growth factor (PDGF) and bone morphogenetic proteins (BMP).

PDGF is thought to be an important, vulnerable agent for tissue repair processes in vivo. PDGF is secreted by platelets, and has also has been found in various tissues, especially in bone, and has various isoforms (AA, AB, BB, CC and DD) which signal through two distinct dimerized receptors.

In this process, PDGF is one of these important molecules to take on new significance in the vasculature-pericyte-MSC-osteoblast bidirectional interactions[1-4]. PDGF stimulates cell growth of normal osteoblastic (HOB) cells[5-6] and MC3T3-E1 cells[7]. PDGF had been shown to stimulate bone formation in vivo[8-9]. In our research, the granulation tissue and the calcium salt deposit were observed in the PDGF-BB group and more collagen fibres were formed compared with the NS group, indicating that PDGF-BB promoted bone repair around implants.

Many studies have shown that IGF-1 is one of the key factors during osteogenic differentiation and presents a beneficial effect on bone development[10-12], An experiment indicated that insulin-like growth factor-1 (IGF-1) could stimulate the proliferation of osteoblasts and bone matrix formation In vitro[13]. Our results showed that the granulation tissue formation, multinucleated? giant cells, a small amount of calcium salt deposition and bone tissue appeared in the IGF-1 group.

Masson's trichrome staining showed obvious fibrous repair among the muscle tissues compared with the NS group, which indicated that IGF-1 promoted bone repair around the pure titanium implants.

A number of studies have tested the potential for tissue regeneration stimulated by PDGF and also combined with IGF-1 or dexamethasone[14-20]. In general terms, these studies (carried out on animals) have demonstrated an enhanced rate and an increased total amount of regenerated tissue compared with controls[21]. In this study, in the IGF-1 and PDGF-BB combined group the granulation tissue had been turned into fibrous connective tissue, and calcium salt deposit had formed bone tissue. After 3 days, collagen fibre hyperplasia and repairing fibres appeared earlier, and granulation tissue grew obvious fibroblasts. After 7 days and 10 days, muscle tissues surrounding the implants saw an obvious growth of fibrous tissues, which indicated the healing effect was accelerated compared with both the NS group and the single factor groups. The results demonstrated that the combination of IGF and PDGF-BB has the best effect in this study.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Dr Wan Lin ZHOU performed the experiments, collected and analysed the results and prepared the manuscript; Dr Lin Lin LI and Dr Qi AN carried out implant surgery; Dr Xin Ru QIU prepared and revised the manuscript; Dr Mei Hua LI designed and supervised the study and finally revised the manuscript.

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