Endocrine Pharmacology

Sustained release of insulin-like growth factor-1 from poly(lactide-co-glycolide) microspheres improves osseointegration of dental implants in type 2 diabetic rats

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A B S T R A C T

Dental implantation is an effective and predictable treatment modality for replacing missing teeth and repairing maxillofacial defects. However, implants in patients with type 2 diabetes mellitus are likely to have a high failure rate and poor initial osseointegration. In the current study, we established an effective drug delivery system designed to improve osseointegration of dental implants in an animal model of type 2 diabetes. Twenty type 2 diabetic rats were divided into two groups: a group receiving recombinant rat insulin-like Growth Factor 1 (rIGF-1) Microsphere Therapy (MST) (10 rats) and a control group (10 rats). The rIGF-1 was encapsulated into poly(lactide-co-glycolide) (PLGA) microspheres to produce a sustained-release effect around titanium (Ti) dental implants in the rIGF-1 MST group. Scanning electron microscopy, confocal laser scanning microscopy, and cumulative-release studies were conducted to verify the release effect of the microspheres as well as rIGF-1 bioactivity. Five rats from each group were sacrificed at weeks 4 and 8 post surgery, and a histological analysis was performed on the rats from both groups. Compared to the control group, rats that received rIGF-1 by PLGA microsphere treatment were observed to have a higher bone–implant contact percentage around the Ti implants at week 4 or week 8 post surgery (P<0.05). This result clearly indicates that sustained release of rIGF-1 through encapsulation by PLGA microspheres positively affects osseointegration of dental implants in type 2 diabetic rats.

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1. Introduction

Over the past decade, the use of dental implants has emerged as a well-accepted treatment modality for the replacement of missing or lost teeth (Pjetursson et al., 2007). However, dental clinicians are facing an increasing number of medically compromised patients who require implant surgery for their oral rehabilitation (Beikler and Fleming, 2003). For example, patients diagnosed with systemic diseases such as osteoporosis and diabetes are considered to be medically compromised for implant therapy (Mombelli and Cionca, 2006). Among various systemic conditions, type 2 diabetes mellitus has become the most common factor contributing to implant failure (Kotsovili et al., 2006). In various retrospective studies, the observed implant failure rates in type 2 diabetic patients ranged from 85.6% to 94.3% (Fiorellini et al., 1999; Morris et al., 2000; Moy et al., 2005), which was lower than in non-diabetic patients (Beikler and Fleming, 2003). Consequently, further research is essential in order to enhance the survival rate of dental implants and to improve their surgical osseointegration in patients with type 2 diabetes.

Growth factors are polypeptides that can either stimulate or inhibit cellular proliferation, differentiation, migration, adhesion, and gene expression (Babense et al., 2000). Recent studies in vitro and in vivo have demonstrated the osteoinductive effects of different growth factors (Lind, 1998). Insulin-like growth factor-1 (IGF-1) has been widely investigated as a potential therapy for the treatment of diabetes due to its metabolic actions, which are similar to those of insulin. In addition, IGF-1 stimulates the replication of osteoblasts and the synthesis of the bone matrix (Daughaday and Rotwein, 1989; Hock et al., 1988). Animal research has revealed that the release of IGF-1 causes cell proliferation and increases matrix apposition rates in bones (Hunziker et al., 1994).

Biodegradable polymers, such as polylactide, polyglycolide, and their copolymer poly(lactide-co-glycolide) (PLGA), are widely used in the pharmaceutical industry as matrices for drug delivery (Hongsen et al., 2008). With their superior biocompatibility, the biodegradable polyesters polylactide and PLGA are the most frequently used biomaterials for the microencapsulation of therapeutics and antigens.
(Smith and Hunneyball, 1986; Anderson and Shive, 1997). In previous studies, IGF-1 encapsulated in PLGA microspheres (Yuksel et al., 2000a,b) and coated with titanium (Ti) (Lynch et al., 1991) had a favourable effect on surrounding cells and tissue. Moreover, the IGF-1 microsphere drug delivery system has been demonstrated to contribute to successful osteoinduction in two different experimental fracture models using large animals (Meinel et al., 2003).

On the basis of the above analyses, the present study evaluated a novel strategy for improving osseointegration in a type 2 diabetic rat model. The effects of sustained delivery of IGF-1 on bone healing around dental implants were analyzed in rats with type 2 diabetes using a delivery system in which recombinant rat IGF-1 (rrIGF-1) was physically entrapped within PLGA microspheres. As a result, the polypeptide was gradually released for 30–40 days after implantation.

2. Materials and methods

2.1. Materials

The growth factor rrIGF-1 was kindly supplied by Prospec Corp. (Rehovot, Israel). PLGA (L/G ratio = 50:50) with a molecular weight of approximately 14–20 kDa was purchased from Boehringer Ingelheim (RG502H; Ingelheim, Germany). The emulsifier polyvinyl alcohol (PVA; Mowiol 8-88) was obtained from Clariant (Muttenz, Switzerland). The organic solvents dichloromethane (DCM) and isopropanol were purchased from Sigma (St. Louis, MO). Span 80 and Tween 80 were obtained from Beijing Chemical Corp. (Beijing, China). GENMED Nile red reagent was from USA Scientifics, Inc. (GMS12196 v.A; Ocala, FL), and water was double distilled. All chemicals were used without further purification.

Twenty 9-week-old male specific-pathogen free (SPF)-level Goto-Kakizaki (GK), rats with non-insulin-dependent diabetes mellitus were purchased from the experimental animal centre at the Chinese Academy of Science (Shanghai, China, license no. SCXK 2007-0005). Twenty cylindrical screw-type Ti dental implants were supplied by the Northwest Institute for Nonferrous Metal Research (Xi'an, China). The implant surface was treated by microarc oxidation (Fig. 1), and the implant itself measured 3.3 mm in diameter and 6 mm in length. All dental implants were cleaned ultrasonically in isopropanol and sterilized in an autoclave prior to use.

2.2. Methods

2.2.1. Microsphere preparation

The rrIGF-1–loaded PLGA microspheres were prepared using a so-called water/oil/water (W/O/W) double-emulsion solvent evaporation method (Meinel et al., 2001). In brief, approximately 1 mg of lyophilized rrIGF-1 was dissolved into 0.1 ml of 18 MΩ distilled water (internal aqueous phase, W1). Subsequently, 100 μl of the rrIGF-1 aqueous solution (10 mg/ml) was dispersed into 10 ml of DCM containing 500 mg of PLGA and 140 mg of Span 80 (oil phase, O) using sonication (JY92-II Ultrasonic Cell Crusher, Scientz, China) for 50 s at an energy output of 650 W (working frequency: 22 kHz). This resulted in the formation of a water-in-oil (W1/O) emulsion. During this process, a staining reagent (Nile red) was dissolved in the oil phase before emulsification. The primary emulsion was re-emulsified with the external aqueous phase containing 100 ml of 2% PVA aqueous solution and 150 mg of polysorbate 80 (Tween 80) (re-emulsification solution, W2), using a high-speed digital homogenizer (FJ300-S, Shanghai Specimen Factory, China) at 12,000 rpm for 5 min. In this process, 100 ml of isopropanol was added into the emulsion to accelerate evaporation of DCM. The obtained W1/O/W2 emulsion was poured into 900 ml of 2% PVA aqueous solution. Magnetic stirring at 400 rpm was performed for 8 h to evaporate the organic solvent and to completely solidify the microparticles. These procedures were all performed at room temperature (approximately 25 °C). Subsequently, the so obtained solid microspheres were washed and collected by filtration, sieving, and centrifugation. Finally, the microspheres were stored under dry conditions at −20 °C.
2.2.2. Scanning electron microscopy
The morphology of the rrIGF-1-loaded PLGA microspheres was examined by environmental scanning electron microscopy (Quanta200, FEI, The Netherlands) and field emission scanning electron microscopy (JSM-6700F, JEOL, Japan) after the samples were coated with platinum. The mean diameter of the rrIGF-1-loaded microspheres, as measured by light scattering, was 1.252 ± 0.6436 μm. For each sample, the diameters of 20–30 microspheres were measured and analyzed statistically as shown in Fig. 2A–D.

2.2.3. Confocal laser scanning microscopy
During the process of loading the PLGA microspheres with rrIGF-1, the fluorescent staining reagent Nile red was dissolved in the oil phase before emulsification. Thus, a confocal laser scanning microscope (PV-1000, Olympus, Japan) was used to confirm that PLGA (stained with Nile red in the external oil layer) had successfully encapsulated rrIGF-1 in the internal aqueous solution. The dried microsphere sample was fixed on a glass slide using a fluorescent fixing agent. The object distance was modified to 10.8 nm and an adapted focus was obtained. The size of the images displayed on the monitor was 1024 × 1024 pixels (Fig. 2E–F).

2.2.4. Encapsulation efficiency
Ten milligrams of freeze-dried microspheres were dissolved in 4 ml of 0.1 M NaOH mixed with 2% (w/v) sodium dodecyl sulphate (SDS) and neutralized with 0.4 N HCl solution. Then, the sample was diluted with 16 ml of distilled water, and its polypeptide content was determined by the Peterson–Lowry method. The rrIGF-1 content was expressed as the percentage of rrIGF-1 per PLGA (wt/wt). The theoretical drug content was calculated under the assumption that the entire amount of drug was encapsulated and that no drug loss occurred at any stage of microsphere preparation.

The encapsulation efficiency was calculated using the following formula:

\[
\text{(Measured polypeptide loading / theoretical polypeptide loading)} \times 100\%
\]

2.2.5. In vitro rrIGF-1 release and rrIGF-1 bioactivity
Lyophilized microspheres (10 ml) were suspended in 1 ml of phosphate-buffered saline (PBS), pH 7.4. The samples were agitated at 37 °C in an incubator shaker at 110 rpm. At desirable time intervals, the microsphere suspensions were centrifuged at 4000 rpm for 10 min. Supernatant (1 ml) was then withdrawn and replaced with 1 ml of fresh PBS. The release of rrIGF-1 was determined by the Lowery–Peterson protein determination method. Release profiles were calculated in terms of cumulative release (%) with incubation time. The bioactivity of released rrIGF-1 was confirmed by an 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) bioassay using quiescent MG-63 cells that are known to show a dose-dependent mitogenic response to rrIGF-1 (Lopaczynski et al., 1993). In brief, cells were grown in minimum essential medium (MEM) containing 8.9% foetal bovine serum (FBS), 1.79 mM-glutamine, 89 U/ml penicillin, and 89 U/ml streptomycin. Cells were split by trypsinization and subsequently stained with MTT solution (2% MTT in PBS for 4 h). After solubilization with 3% SDS and 0.04 M HCl in 2-propanol, plates were incubated for 1.5 h in the dark, and absorbance was read at 570 nm in a plate reader (Varian, Zug, Switzerland). Plates with a standard curve correlation coefficient ≤ 0.96 were discarded.

2.2.6. Animal preparation and induction of type 2 diabetes mellitus
Twenty male SPF-level GK noninsulin-dependent diabetes mellitus rats, age approximately 9 weeks and weight 260 ± 10 g, were purchased from the experimental animal centre at the Chinese Academy of Science. The animal experiments were approved by the Animal Welfare Committee of the Fourth Military Medical University (Xi’an, China) in strict accordance with the policies and principles of the Animal Welfare Act and the recommendations set forth the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, Washington, DC). Animals were housed five per cage and maintained in a 12-h day/night cycle. The rats were fed a forage diet containing high levels of glucose and grease. After 4 weeks, the blood glucose level of all 20 rats was greater than 300 mg/dl (16.7 mmol/l). Thus, 20 male type 2 diabetic rat models were obtained. The rats were divided into two groups: an rrIGF-1 Microsphere Therapy (MST) group (10 rats), which was treated with rrIGF-1-loaded PLGA microspheres, and a control group (10 rats), which was treated with placebo PLGA microspheres.

2.2.7. Serum chemistry
To confirm that type 2 diabetic rat models were established, blood was obtained twice by capillary tube insertion into the tunica conjunctiva palpebra under ether anaesthesia: once prior to implantation and again at the time of sacrifice. The serum glucose concentration was determined enzymatically by the glucose oxidase procedure. A glucose concentration of more than 300 mg/dl (16.7 mmol/l) (Takeshita et al., 1997; Bell and Hye, 1983) was defined as indicating diabetes, while a glucose concentration under 200 mg/dl was considered to be normal; animals with glucose levels corresponding to the latter criterion would have been regarded as inappropriate for this study.

2.2.8. Implantation
The implants were cleaned in trichloroethylene (99.5%) for 3 min, rinsed in absolute alcohol thrice, and autoclaved at 120 °C for 30 min to ensure their sterility. The animals were anaesthetized with an intraperitoneal injection of 2% pentobarbitural sodium (bm-007, Merck, Whitehouse Station, NJ). The drugs were administered at a dose of 0.25 ml/100 g body weight. A 3-cm skin incision was made on the medial-proximal surface of the left tibia in the sagittal plane of the head. This was followed by muscular dissection, plane to plane, and detachment of the periosteum, thoroughly exposing the distal part of the femur. The initial pilot osteotomy was made by slow-speed drilling at a distance of 5 mm from the distal edge of the femur. Subsequently, the osteotomy was expanded with 2.2 mm and 2.8 mm trephines activated by a surgical micromotor (4000 rpm) and irrigated with 0.9% sterile saline solution. Then, the dental implant (3.3 mm × 6 mm) was press-fit into position. Microspheres loaded with rrIGF-1 were suspended in 500 μl of blood and left undisturbed until a clot was formed. The implant hole received one of two treatments, randomly distributed among the rats, before implant insertion: (1) placebo PLGA microspheres (control group) in blood clot or (2) 200 μg of rrIGF-1 in PLGA microspheres in blood clot (rrIGF-1 MST group). Finally, the periosteum, muscle, and skin were sutured. After recovery, the rats were allowed to ambulate freely. Post-operative antibiotic treatment consisting of 30,000 IU of penicillin G and 6 mg of gentamycin per kg based on body weight was injected twice daily for 4 days.

2.2.9. Histologic preparation and bone histomorphometry

2.2.9.1. Histologic preparation. Five rats from each group were sacrificed at weeks 4 and 8 after surgery. The bodies were then perfused through the abdominal aorta with a solution of 4% formaldehyde and 2% glutaraldehyde. The femur was then harvested and fixed in 10% buffered formalin for 1 week at 4 °C. Each specimen was dehydrated through a graded series of ethanol solutions and 100% acetone. Subsequently, the bone samples were embedded in polyester resin and mounted in a sawing microtome (Leica SP 1600, Leica Microsystems, Wetzlar, Germany). Thus, we obtained a 30-μm-thick undecalciﬁed section, which was stained with Ponceau Tri-Chrome stain.
2.2.9.2 Bone histomorphometry. Histometric analysis was performed by means of a computer-digitized image analysis system (Leica Imaging System, Cambridge, England) coupled to a light microscope (Olympus BH2 with S Plan FL2 lens, Tokyo, Japan) with a high-resolution video camera (CDC/RGB colour video camera, Sony, Fujisawa, Japan) and a Sony Trinitron monitor (image resolution: 512×512). Histometric analysis included the following variables:

Percentage of bone–implant contact (BIC) — The length of the surface border where the bone tissues directly contacted the implant (B) as well as the circumference of the implant (A) were measured and used to calculate the percentage contact according to the following formula:

\[ \text{Osseointegration rate} = \frac{\text{Bone–implant contact}}{\text{Implant circumference}} \times 100\% \]

Trabecular bone volume (TBV) — Image analysis was carried out in a predefined image window and by colour thresholding representing the ‘actual objects’ in a given window. The percentage of trabecular bone volume was expressed as the amount of bone containing sinusoid spaces filled with connective tissue and red blood cells as a percent of the total area. The percentage of these elements was calculated for each specimen. An average of 20 sections stained with Ponceau Tri-Chrome was used for analysis. Trabecular bone volume was measured in the area from the cortex of the bone to the bone–implant interface. The calcified bone in the stained slides was distinguished from other tissues by its characteristic bluish colour.

2.2.10 Statistical analysis

A total of 20 implants were placed into the femurs of 20 animals. A one-way analysis of variance (ANOVA) with \( P<0.05 \) as the level of significance was applied to determine the effect of the healing time for rrIGF-1-loaded PLGA microsphere therapy on the histomorphometric variables. At each time (4 weeks and 8 weeks after implantation), the variables from the rrIGF-1 MST group and the control group were compared using Student’s paired t-test.

3. Results

3.1 In vitro rrIGF-1 release and encapsulation efficiency

In vitro release of rrIGF-1 from the PLGA microspheres exhibited an initial burst of approximately 55.3% of the total content within the first 2 days, followed by a continuous release totalling 64.8% after 5 days. More than 85% of the encapsulated rrIGF-1 was released from the PLGA microspheres within 20 days (Fig. 3). The remaining 15% was slowly released during days 20–40. Full bioactivity of released rrIGF-1 was confirmed by an MTT mitogenic assay (data not shown).

The encapsulation efficiency of the PLGA 50:50 microspheres used in this study was 78.3±2%, which is close to the results of a previous report (Meinel et al., 2001).

3.2 Experimental animals and serum glucose

All rats had an uneventful post-operative recovery, and their weight remained stable during the experimental period. At sacrifice, no obvious signs of inflammation or adverse tissue reaction were observed. Radiographs taken perpendicular to the long axis of the implant confirmed that all implants were surrounded by trabecular bone. Throughout the experiment, the blood glucose level of the GK rats was maintained at 18.5±1.5 mol/l (332.6 mg/dl±27 mg/dl).

3.3 Histological evaluation

At week 4 post surgery, vertical sections of the femurs of the control group revealed an inconsistent bone reaction around the Ti implants. In some places, bone was loosely organized, and a woven area was seen. There was less new bone formation around the Ti implants as observed using a fluorescence microscope (arrowhead in Fig. 4A). In the rrIGF-1 MST group, there was an increase of bone density and new bone

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Fig. 3. Cumulative release profiles of rrIGF-1 from PLGA microspheres on different time. The data are presented as mean ± standard deviation.

Fig. 4. A. Control group rat 4 weeks after operation. Less new bone formation and poor osseointegration around Ti implants (arrows in A) (vertical section, magnification ×20). B. rrIGF-1MST group 4 weeks after operation. More new bone formation and a thin bone layer encapsulated Ti implants (arrows in B) (vertical section, magnification ×20). C. Control group rat 4 weeks after operation. A large amount of fibrous tissue encapsulated Ti implants (arrows in C). The continuity of bone layer was destroyed (horizontal section, magnification ×20). D. rrIGF-1MST group 4 weeks after operation. An Ti implant was almost encapsulated within a lamellar bone layer (arrows in D). Bone tissue are adherent to the implant surface tightly (horizontal section, magnification ×20).
formation at the borders of the Ti implants. This appeared at week 4 after the operation (arrowhead in Fig. 4B). Furthermore, horizontal sections of the control group showed that the Ti implants were partially circumscribed by a bone layer. There was a large amount of fibrous granulation tissue and some bone resorption around the Ti implants, and the structure of the bone tissue in the control group rats appeared to be less continuous (arrowhead in Fig. 4C). In the rrIGF-1 MST group rats, the lamellar bone around the Ti implants was dense and well organized (arrowhead in Fig. 4D). An extensive area of bone was in direct contact with the implants in the rrIGF-1 group, while in the control group rats, bone was largely intermixed with soft tissue at the implant interface (arrowhead in Fig. 4C).

At week 8 post surgery, both groups revealed trends similar to those seen at week 4 after the operation. During week 8, in the control group, there was still no bone–implant contact in some places, and many fibrous tissues circumscribed the implant. Although the Ti implants in the control rats were osseointegrated, the bony architecture was located a distance away from the implant surface and appeared to be more immature and woven in nature (Fig. 5A). The Ti implants in the rrIGF-1 MST group specimens were osseointegrated, appeared to be more mature, and exhibited extensive bone formation (Fig. 5B). Under high magnification of the bone–implant contact area by scanning electron microscopy, there was still a narrow gap between the bone and the Ti implants in the control group, which appeared to demonstrate no osseointegration (arrowhead in Fig. 5C). In contrast, several osteoblast-like cells appeared to have integrated with the Ti implants at the implant surface in the rrIGF-1–MST group (arrowhead in Fig. 5D).

3.4. Histomorphometric outcome

The results of the histomorphometrical measures are listed in Table 1. BIC percentages showed a general increase with healing time (one-way ANOVA; P<0.05) in rats treated with rrIGF-1–loaded PLGA microspheres. At week 4 post surgery, BIC was 56±5% in the rrIGF-1 MST group and 39±4% in the control group. At week 8 post surgery, BIC was 70±7% in the rrIGF-1 MST group and 53±11% in the control group. Student’s t-test showed a significant difference in the BIC percentage between the rrIGF-1 MST group and the control group at weeks 4 and 8 after the operation (P<0.05).

Trabecular bone volume showed an increasing trend with a longer healing time (one-way ANOVA; P<0.05) in the rrIGF-1–loaded PLGA MST group. At week 4 post surgery, the trabecular bone volume was 39±3% for the rrIGF-1 MST group and 36±8% for the control group. At week 8 post surgery, trabecular bone volume was found to be 58±6% in the rrIGF-1 MST group and 47±7% in the control group. Student’s paired t-test found no significant difference at week 4 after the operation between these two groups (P>0.05). However, at week 8 post surgery, there was a significant difference between the two groups (P<0.05).

4. Discussion

Worldwide prevalence of diabetes is extremely high (20.1% of individuals over 65 years of age) and has increased drastically over the past decades (Powers, 2005). In 1980, only 1% of Chinese patients were diagnosed with diabetes, whereas today more than 50 million Chinese patients have been diagnosed with diabetes, and they account for about one-fifth of the world’s diabetic population. Many specialists forecast that the morbidity of diabetes mellitus in China will increase to 14% in the next decade (Guang-Wei, 2006). Type 2 diabetes represents about 90% of the total number of diabetic cases. This type of diabetes is associated with a wide range of systemic complications, such as retinopathy, nephropathy, neuropathy, microvascular and macrovascular diseases, and altered wound healing (Eastman and Vinicor, 1997). Moreover, diabetes-associated alterations in microvascularization lead to a diminished immune response and a reduction in bone remodelling (Olson et al., 2000). All of the above systemic changes can impact the insertion and osseointegration of dental implants, and dental implants in type 2 diabetic patients frequently have a high failure rate and poor initial osseous healing. Therefore, improvement in the osseointegration of dental implants in type 2 diabetic patients is a challenge to clinicians.

Our results suggest that local sustained delivery of IGF-1 from PLGA microspheres can significantly promote the osseointegration of dental implants in type 2 diabetic animal models. More new bone formation and deposition around the interface of Ti implants were observed in type 2 diabetic rats treated with PLGA microspheres loaded with 200 μg of rrIGF-1 after only 4 weeks (Fig. 4B). Furthermore, the structure of the
bone tissue was continuous, intact, and contained less fibrous soft tissue (Fig. 4D). The osseointegration around dental implants showed a general increase with more rapid healing time in the rrIGF-1 MST group. At week 8 post surgery, the rrIGF-1 MST group exhibited more osteoblast-like cells adhering to the surface of dental implants (Fig. 5D). The bone healing and maturation around the dental implants in the rrIGF-1 MST group was better than in the control group, and these phenomena were ultimately demonstrated by histological measurement and morphometric analyses (Table 1). We used a novel strategy to promote the osseointegration of dental implants in a type 2 diabetic animal model. Previous studies in type 2 diabetic patients have focused mainly on controlling blood glucose (i.e., treatment with insulin and calcitonin) to improve implant osseointegration (McCracken et al., 2006; Flavio et al., 2005). Here, we attempted to establish an effective drug delivery system that sustained a local release of therapeutics to stimulate new bone formation around the dental implant.

We believe that the beneficial effects of IGF-1 in the bone microenvironment (as shown in Table 1) are attributable to two main sources: an anabolic effect that stimulates osteoblast progenitor cells and the anti-inflammatory response of IGF-1 that prevents bone resorption and bacterial infection around dental implants in the diabetic rat's tibia. IGF-1 initiates its growth-promoting function on bone via both endocrine and autocrine/paracrine actions (Rosen et al., 1997). IGF-1 was previously reported to play a role in bone formation and maintenance (Clemens and Chernausek, 2004; Niu and Rosen, 2005). Circulating levels of IGF-1 also play a major role in bone remodelling and directly impact linear bone growth (Kupfer et al., 1993; Borst et al., 2001). The potential mechanisms of IGF-1’s beneficial effects on implantation may include several important factors. First, recent study suggests that osteoblast-secreted IGF-1 plays a role in osteoblast recruitment and that it is regulated via a phosphatidyl inositol 3 kinase (PI3K) signalling pathway. IGF-1 has been shown to activate PI3K and extracellular signal-related kinase (ERK) signalling pathways in osteoblasts (Nakasaki et al., 2008). Second, IGF-1 has also been shown to affect nitric oxide synthesis, which is important for osteoblast proliferation (Lagumdzija et al., 2004). Third, IGF-1 influences inflammation, at least partially, via the nuclear factor-kappaB (NF-κB) pathway, which plays an important role in inflammation regulation (Balaram et al., 1999; Hec et al., 1999).

Microencapsulation of recombinant proteins for controlled release has been successfully performed with human growth hormone, interferon-γ, and interleukin-2 (Johnson et al., 1996; Yang and Cleland, 1997; Hora et al., 1990). Moreover, IGF-1 sustained-release systems have recently been successfully applied in many related fields. Thus, a localized IGF-1 delivery system may have considerable therapeutic potential in the treatment of bone fractures and bone defects provided it can be combined with a suitable three-dimensional matrix that affords mechanical stability (Meinel et al., 2003). Encapsulated recombinant human insulin-like growth factor 1 (rhIGF-1) using PLGA microspheres to produce a sustained-release system is considered an effective therapy for the treatment of diabetes and was found to be particularly suitable for type 2 diabetic patients who become insensitive to insulin treatment (Xanthe et al., 2000). Locally controlled delivery of IGF-1 from dextranso-gelatin microspheres promotes the enhancement of periodontal tissue regeneration (Chen et al., 2006). However, the properties of the rrIGF-1-loaded microspheres in the current study are different from those of previous studies in several respects. The volume-mean diameter of 1.2525 ± 0.6436 μm of the rrIGF-1-loaded microspheres used in our study was considerably smaller than that used in previous reports (Meinel et al., 2001). Several factors resulted in this phenomenon: (1) in the process of W1/O emulsion formation, sonication was applied at a higher energy output power (650 W, 22 kHz) compared with previous studies; (2) when the W1/O emulsion was re-emulsified, our homogenizer reached a faster speed (12000 rpm) than was used in previous IGF-1–loaded microsphere preparations (8000 rpm); and (3) Span 80 and Tween 80 surfactants were used to stabilize the globules in our microsphere preparation because surfactant type and concentration have been determined to be the primary factors controlling globule stability, whereas the effects of pH and ionic strength are minor (Hou and Kyriakos, 1997). The morphology of PLGA microspheres loaded with rhIGF-1 (Fig. 2A–F) was similar to that of prior reports (Meinel et al., 2003, 2001), but the surface of the microspheres was smoother and glossier.

This study may provide reliable and valuable biological data on the effects of type 2 diabetes mellitus on osseointegration of dental implants. The GK rat genetic animal model of type 2 diabetes mellitus was created by selective breeding of an outbred colony of Wistar rats, with selection for high glucose levels in an oral glucose tolerance test (Goto et al., 1975). Type 2 diabetes mellitus in the GK rat appears to be polygenic, with at least three different loci involved in the disease (Howarth et al., 2007). This rat model bears a close resemblance to human type 2 diabetes, which represents the majority of adult-onset diabetes mellitus cases. The present study employed a very rough Ti surface created by a microarc oxidation treatment. There is consensus that micron-scale roughness promotes osteoblastic activity, leading to faster and firmer osseointegration, compared to a relatively smooth machined surface (Cooper et al., 1998; Masuda et al., 1998). Therefore, the effect of type 2 diabetes on osseointegration around such micro-roughened implants should be of great and immediate interest. In the present study, an important finding was that many dental implants in type 2 diabetic rats without loaded rrIGF-1 microsphere treatment demonstrated more fibrous tissue in close contact with the implant surface (Fig. 4C). In healing, fibrous tissue around dental implants replaced new bone formation. This may be the primary reason that type 2 diabetic patients have poor osseointegration and a high failure rate.

Although we tried to control factors influencing the experiment, such as age, genetic por-tet, and living environment, some limitations must be considered when interpreting our results. The low number of animals, mainly in the dental implant insertion study (only 10 Ti implants per group), was chosen to satisfy ethical concerns. Furthermore, the administration of PLGA microspheres presented a practical problem. The microspheres were suspended in fresh blood and administered into the tissue surrounding the dental implant upon coagulation. During dental implant insertion, some of the implanted microspheres might have drifted away. Therefore, a better carrier matrix for microspheres would have been preferred, especially if such matrix possessed an osteoconductive effect. Finally, when interpreting and comparing the data from animal and human studies, the following should be noted: First, type 2 diabetes is under control in most human studies; second, biological responses sometimes differ among animal models as well as between animals and humans; and third, in human studies, the research design and inclusion criteria for patients are sometimes not well established in terms of the type and duration of diabetes and the levels and treatment of pathogenesis (Kotsovilis et al., 2006). Consequently, we need to perform an experiment that evaluates sustained release of rhIGF-1 from PLGA microspheres into type 2 diabetic patients’ alveolar bone cells in vitro to determine whether this produces a positive effect. Currently, our laboratory has collected alveolar bone segments from many type 2 diabetic patients during clinical procedures. Research leading to human application will therefore be initiated in the near future.

5. Conclusions

Within the limitations of this study, it can be concluded that sustained release of IGF-1 has a positive influence on the osseointegration of dental implants in type 2 diabetes during the early post-implantation healing phase. Furthermore, this study has yielded important evidence on new bone formation around dental implants in type 2 diabetic rats treated with locally sustained release of IGF-1 from PLGA microspheres. Thus, our combined findings strongly suggest that a
slow and stable release of IGF-1 may be effective for osseointegration of dental implants in type 2 diabetes.

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