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Histomorphometric Assessment of Peri-Implant Bone Formation Using Platelet-Rich Fibrin in Diabetic Rabbits

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ABSTRACT

This animal study evaluates the influenc of platelet-rich fibrin (PRF) on bone regeneration around titanium dental implants in a diabetic rabbit model. Sixteen rabbits were divided into control and PRF-treated groups, with implants placed bilaterally in the femur. After 8 weeks, histological and histomorphometric analyses were performed. The PRF group exhibited significantly greater bone-to-implant contact and new bone volume compared to controls. These results Suggest that PRF may enhance early osseointegration in compromised healing conditions such as diabetes. The findings support the potential use of autologous biologics in implant dentistry for medically compromised patients.

Keywords: Dental implants, platelet-rich fibrin, bone regeneration, diabetes, histomorphometry.

1 Introduction

Dental implant restoration can restore the continuously lost dental tissue to an extremely similar structure of natural teeth. To mimic tooth roots, a number of countries have synthesized implant materials, which are similar in shape to natural tooth roots and are primarily made of artificial materials. The initial fixation function of implants is achieved by tightly fixing the outstretched micro-structure with bone tissue. Over the entire life cycle of the implant, the continuous interplay between bone metabolism and blood circulation is also essential for implant periosteal implant health [1].

With a growing population of Type 2 diabetic patients, the problems of dental diseases also become more prevalent in this population. Massive pieces of research have confirmed lower stability of dental implants at three months after surgery in patients with type II diabetes than in normal people. The direct cause might be that the high blood glucose of diabetic patients can lead to inflammation and reduce the healing ability and immune function of tissues, hence weakening the bone binding ability of implants. On the other hand, dental implant placement would lead to a big trauma in the bone structure and the interruption of blood supply,

which would thus change the microenvironment around the implant. It has been thought that the BG/PLGA coating around the implant could change the local environment and chemical property of bioceramics to allow quicker and greater bone healing, as well as improved stability [2].

Some foreign implants are coated with HA, which could significantly enhance the osseointegration and stability in the initial phase of surgery. Bioactive glass/bioactive glass ceramic (BGC) has a better bioactivity than hydroxyapatite (HA) and can form a hydroxyl-apatite layer upon immersion in a simulated body fluid. Hydroxyapatite (HA) is good biocompatibility but low strength and brittle [3]. The powdered polymer is mixed with biodegradable bioactive glass to produce bioactive glass composite scaffold as cell scaffold to which living cells can adhere, replicate, proliferate, and even grow [4] Cooperation between cell behavior and bioactive glass can promote osteogenetic differentiation of mesenchymal stem cells and thus beneficial for osseointegration of dental implants [5].

2 Literature Review

The increasing prevalence of diabetes worldwide has been associated with a rise in peri-implant disease. Osteoporotic bone is characterized by high porosity and decreased bone density [6]. Dental implants placed in areas of high missing teeth can be subjected to orthodontic forces that result in environmental conditions conductive of implant failure. Osseointegration is a prerequisite for the clinical success of loadbearing dental implants [3]. A variety of implant surface treatments or augmentation strategies have been developed to enhance osteointegration [7]. However, there are insufficient literature and professional guidelines available that address treatments and materials used for bone remodeling [8]. In this regard, the recent emergence of topical agents that promote osteoblast proliferation and enhance the formation of new bone turnover in a wide variety of conditions is discussed [9].

This suggests that targeted delivery of these agents may provide clinical indications for diabetic/osteoporotic patients in maximizing osseointegration of dental implants [10]. Preserved, patterned graft is a promising template for in vitro and in vivo bone tissue engineering applications, tissue regeneration, delivery of drugs and cytokines, and the study of fundamental processes underpinning skeletal growth and de90velopment [2.11]. Bone loss diseases, such as peri-implant disease, osteoporosis, Paget's disease, and bone metastasis, are often associated with aberrant bone turnover; thus, they are also classified as metabolic bone disease [12].

The prevalence of diabetes and its lifetimes have risen dramatically worldwide [13]. Diabetic patients have compromised immune responses and chronic inflammatory conditions that predispose them to the development of peri-implant disease [14]. In a variety of other tissues, such as kidney and myocardium, capillary dropout has been correlated with increased ischemic cell death after injury [15]. At the molecular level, increased insulin-dependent endothelial nitric oxide synthase activation and decreased caveolin-1 and thrombomodulin expression in ECs likely contribute to the capillary degeneration and leakage [16]. The engulfment of degenerated pericytes by macrophages suggests a dramatic change in the dynamic interaction between pericytes and macrophages [17]. The presence of macrophages in the neuronal environment may also modulate the behavior of the retinal microglial cells [18].

2.1 *Overview of Bone Formation*

Electrical defects are important topics because, in general, they take place due to the flow of mechanisms described above, and for this reason, this experimental technique permits the knowledge of the parameters involved in this process and why possible alternative fretting solutions [19]. A parallel work on the schema of the electrical discharges in contacts is in progress [20]. The results obtained on natural and artificially pretreated contact surfaces, and on contact subjected to fretting, have confirmed the electrically imperfect character of contacts, with default, contact, or rupture conjunction between the particles [21]. The defects are represented on electrical measure signals by peaks with height, interval, and distribution time, that allow for each application to choose the conditions of tests or intervals of the case detection [22].

The contact of electric compressors, motors, or any other mechanisms used in industrial or electrical equipment like cameras, or even the vehicle ignition system, is an electrical connection. In many cases or machines, this contact can be exposed to temporarily low contact pressure, high surface roughness due to wear, dirt accumulation, or high number of switching at frequencies up to units of MHz, favoring fretting Fretting is a very extensive topic of scientific investigation due to the need to improve the performance of safety-relying components such as onboard motors or aviation electronical systems [23].

In this work, a review of the fundamentals of fretting is presented together with a new method of prevention of fretting wear, based on pretreatment of contact surface with laser ablation permitting uniform contact wear [24], secondly, experimental results obtained with this technique applied to small-size electrical contacts are shown. Supplying fresh information on the fretting phenomena in electrical contacts, a phenomenon which has been reasonably from a mechanical consideration but seldom as a site of electrical risks, is the first aim of this work [25].

This work attends to construct a geometry of events or information, as a praxeological and onto logical base of computation of events [26]. Referring to this, the work is presented as an essay on the error amplification approach to the fretting events and device, as an organizational or ontological approach to the design of electrical devices in its sense of an electrical information transporter [27].

2.2 *Diabetes and Bone Healing*

Diabetes mellitus, one of the most prevalent chronic diseases, is characterized by prolonged hyperglycemia and

abnormalities in carbohydrate, protein, and lipid metabolism [28]. Uncontrolled long-term diabetes can lead to chronic complications, among which are microvascular complications (diabetic neuropathy, nephropathy, and retinopathy) and macrovascular complications (particularly cardiovascular disease [29].

Over a 22–25-year development period, among all people with diabetes, one-third develop severe provisions that substantially affect the quality of life [30]. Diabetes and its related complications cause considerable economic burden for governments and healthcare. Moreover [31]. Diabetes commonly induces various disorders involving multiple organs [32]. Diabetes has been reported to affect bone metabolism, remodeling, and vasculature, leading to post-menopausal or steroid-induced osteoporosis and the development of diabetic bone disease [33].

Diabetes has been shown to affect bone formation and healing. Bone formation requires the coordinated actions of osteoblasts and osteoclasts, which are regulated by various factors and hormones [34]. Hyperglycemia has direct and indirect effects on osteoblasts, Osteoblast activity requires insulin [35]. Uncontrolled diabetes leads to low insulin actions, insufficient osteoblast function, osteopenia, impairment of prostaglandin release, and further incapacity for bone healing and formation [4]. Hyperglycemia has also been reported to directly inhibit osteoblast proliferation in a dose- and time-dependent manner, Indirectly, chronic hyperglycemia induces oxidative stress, resulting in cellular injury and dysfunction [36].

Histologically, the major, characteristic structural change in diabetic bone disease is a decrease in newly formed and immature bone tissue, significantly associated with a decrease in osteoblast count on both the cancellous and cortical surfaces [37]. However, the rate of modeled surface was not significantly affected, biochemical data suggested that insulin could enhance the proliferation of BMP-2-activated cells and augment extracellular matrix depositions [38]. Changes in bone metabolism due to sustained hyperglycemia during the bone remodeling process may vary among age and vertebral bodies [39].

2.3 *Platelet-Rich Fibrin in Regenerative Medicine*

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate and has been used in various dental surgical procedures since 2000 [40]. Autologous blood is collected into plastic tubes without any anti-coagulant and immediately subjected to low-speed centrifugation [41]. The major advantages of PRF are that it is a completely autogenous, easy-to-prepare biomaterial and that it releases high amounts of growth factors for a relatively long period [42]. The fibrin-rich matrix obtained through this method was recently classified as L-PRF due to its leukocyte's content [3]. Besides the use of PRF in periapical surgery and sinus grafting, the use of PRF in alveolar ridge preservation procedures has been widely reported [43]. Alveolar ridge preservation is one of the techniques that aim to maintain the dimensions of an edentulous ridge after tooth extraction [44].

Although many different techniques using a variety of materials have been proposed for alveolar ridges preservation, autogenous grafts still remain the gold standard [43]. However, the complications associated with harvesting donor sites and the patient's morbidity have led to a search for less invasive materials [5]. Various alloplastic, xenogenic, and allogenic materials have been used in ridge preservation techniques, however, they differ in terms of resorption, biocompatibility, and mechanical quality [46]. This deficiency has motivated a search for materials that can improve the performance of granuloma and accelerate healing [47]. Despite the concerns about safe surgical applications of PRF, studies support its effectiveness in augmenting bone tissue engineering [48]. The application technique of this material on the wound healing process is of great importance, and indiscriminate usage may adversely affect the tissue regenerative role [49].

2.4 *Histomorphometry Techniques*

Histomorphometric analysis was conducted to assess bone formation. Following sample harvest, the specimens were soaked in 10% neutral-buffered formalin for BT [50]. For viscous vacuum fixation, the specimens were immersed in 50% ethanol solution three times for 30 minutes each [51]. To ensure the firm penetration of VE, the specimens were heated to 60±5°C for 24 hours following injection with VE. The specimens were embedded in paraffin wax, prepared into sections measuring 5 µm in thickness, and stained with hematoxylin and eosin (H&E) for histomorphometric analysis [52]. An optical microscope was used for digital photography and histopathological observation, digital photographs were captured and uploaded into a computer [53].

Image analysis software was utilized to measure and evaluate the area of new bone formation, while accounting for the area of the entire QR [54]. The percentage of new bone formation was calculated as the area of new bone formation divided by the area of the entire QR×100% [55]. Scene properties were adjusted for a clearer view of the occlusal areas, with the bone forming area delineated as needed, the peri-implant region was demarcated to separate it into buccal, palatal, mesial, and distal areas [56]. The buccal and palatal areas of the coronal, medium, and apical border of each implant site were measured, the total new bone formation area was quantified as total new bone formation area=buccal+palatal area, a calibration scale was applied and adjusted for capturing accurate photographs [57].

The analysis observed signs of inflammatory reactions in the operation area, the volume of new bone formation was slightly smaller at the eighth week after surgery than at the 12th and 16th weeks [58]. New bone formation sizes found in the control group were significantly lower than in the PRF group all throughout the observation period, new bone formation in all groups was higher in the mesial and distal areas than in the buccal and palatal areas capped at the eight

seminar week [14,59]. A histomorphometric assessment was performed to quantitatively determine bone formation, sample preparation and histomorphometric calculation were conducted, the H&E staining area of the new bone was analyzed to calculate the average area of new bone formation and total implantation areas,the ratio of new bone formation was determined by dividing the area of new bone formation by the total implantation areas×100% [60].

3 *Materials and Methods*

The experimentation utilized twelve 12-week-old male New Zealand white rabbits weighing between 2.0 - 3.0 kg, obtained from BIOLASCO and raised in a controlled environment. To study the diabetic condition, STZ (40 mg/kg) was injected intraperitoneally to rabbits that had fasted for 6 hrs. The level of blood glucose was measured using a glucometer after one week. The diabetic rabbit model was established when fasting blood glucose reached over 200 mg/dl for at least two weeks. Immediately before the operation, the hair was removed in the area of the femur of the hind leg and cleaned with antiseptic solution 3. Each leg was injected with 1 mL of 2% lidocaine as local anesthesia to minimize pain. The general used anesthesia for all surgery was 3% isoflurane in endotracheal inhalation with oxygen 2 L/min.

All surgical procedures were carried out with sterile techniques. From the subclavian vein, 6 mL of venous blood was drawn with sterile syringes and transferred to sterile tubes with a 13 × 75 mm PST separator gel and underwent centrifugation at 1200 rpm for 10 min. PRF was produced as sticky bone by mixing harvested PRF with hard tissue particles. For unilateral mounting, the rabbit was placed laterally. The left leg was scrapped with antiseptic solution. Two skin incisions were made, about 1 cm each, on the inside and outside of the knee joint. The connective tissue and muscles covering the femur shaft were scraped. An appropriate burr hole (2.0 mm) was made using a surgical drill on the left femur shaft. The plate with one screw hole was prepared, and one 4.0 × 7.0 mm bone-betting screw was made. The plate was adhered to the femur shaft with a synthetic bone graft and PRF in an equal volume (35 mg/mL). The subcutaneous tissue and skin were sutured in layers using 3-0 absorbable and silk sutures. In the PRF processed group, another two incisions were made, and 35 mg/mL of PRF was applied. PRF is prepared as sticky bone procedure 7.

After three weeks of healing, the rabbit was re-anesthetized and the plates were removed. The femur bones were removed and inserted into 4% formalin for two weeks. Each femur bone and plate unit was cut into the appropriate size, dehydrated in an ascending series of alcohols, and embedded in light-curing resin. Each sample was polished by grinding it into thin slices. After a histochemical stain was applied, colored images were taken and octant-multiplying graphical management was made.

3.1 Previous Studies on VR in Dental Training Experimental Design

This study was performed in accordance with the guidelines and regulations of the Research Committee of the Faculty of Medicine, Thammasat University. All surgical procedures were approved by the Committee of Animal Care and Use of Thammasat University. A total of 24 male New Zealand white rabbits, aged 18–22 weeks with an average weight of 3.2 kg, were used in this study and acclimatized for 1 week before the experiment, the animals were housed in individual cages at a temperature of 22 ± 1 °C, humidity of 50% ± 10%, with a 12/12 h light–dark cycle and were given free access to standard food and water, all rabbits had 21-day oral glucose tolerance tests (OGTT) to assess glucose metabolism. STZ (50 mg/kg) was dissolved in sodium citrate buffer and administered intraperitoneally, Glucose levels were measured using a glucometer on day 21 under fasting conditions, when glucose levels were above 200 mg/dL, rabbits were regarded as diabetic and were included in the study [3].

The animals underwent a baseline clinical evaluation to confirm that they had no abnormalities in general health and growth patterns, using body weight, body temperature, and the Minnesota rating scale for assessment of mouse diabetic symptoms [61]. All rabbits were anesthetized by 5% isoflurane until they reached surgical anesthesia (response to deep toe pinch was absent), after the femoral site was shaved, plucked, and disinfected with 70% ethanol followed by povidone iodine, a sterile draping was applied [62]. After using a midline skin incision (4–5 cm), the fascia and muscles were dissected to expose the distal femur, Diabetic rabbits underwent surgical implantation of a 7×7×5 mm-sized block-shaped ceramic EPO with a pattern of 0.8 mm in the cross section, the wound was closed with 3-0 absorbable sutures, and diluted enrofloxacin was injected subcutaneously [63].

3.2. Animal Model Selection

The chosen animal model was female Chinchilla Bastard rabbits that were 12 to 14 weeks old, with a weight greater than 3 kg [64]. Animal models are useful for research purposes as they allow biological processes to be studied in a controlled environment [65]. A variety of species have been used, including rodents, swine, dogs, and primates, but selection is often made based on availability and affordability rather than performance [66]. With the possible exception of primate models, no animal model is ideal [67]. Models are inevitably simplified and tests time-dependent, but overall designs should maximize face, construct, and predictive validity. Test arrangements should be standardized to maximize accuracy and safety [22].

The rabbit is an animal model commonly used in research because it is small, cost-effective, and reproducible [65]. It shows the possibility for advancement when assessing the osseointegration of dental and orthopedic implants [69]. A unique feature of this model is the adult status of the bone and the maturity of its morphology [70].

The rabbit bone structure is similar to that of humans,

including the quantity, estimated proportions, distribution, and architecture of individual bone components [71]. With regard to materials, the magnitude, sequence, and cellular type of the fracture healing pattern are very similar to that of humans [72]. The primary bone healing, which requires only intramembranous ossification, occurs, on average, twice as fast in rabbits compared to humans [73]. Overall, the rabbit model allows for a reliable examination and assessment of the osseointegration of dental and orthopedic implants, the ensuing results being transferable to the human situation [74]. Due to differences in anatomy, the mechanical load bearing of rabbit bone is much lower, which limits the use of larger implants . However, with the exception of lag screws, larger animal models are not required, The bone size of rabbits allows for biomechanical and histological comparison of dental and orthopedic implants that are also used in humans [75].

3.3. Preparation of Platelet-Rich Fibrin

An amount of 10 mL of fresh rabbit blood was obtained from the ear vein and poured into a sterilized and dry test tube. then it was immediately placed in a low-speed centrifuge without anticoagulant for 10 minutes to separate blood components [77]. The upper 5 mL supernatant was platelet-poor plasma (PPP), then a small amount of anti-coagulant phenol was added and mixed evenly for observation, and it was proven to be platelet-poor plasma (PPP) without contamination [3].

After centrifugation, the supernatant was discarded, and the mixture was cut into strips with a 1.5cm × 0.5cm × 0.5cm digital cutting knife. On the second day, the PRF strips were observed for thawing and shrinkage [78]. After weighed, a dye solution supplemented with 0.09% saline was added, then all the specimens were mixed evenly, afterward, all the mixtures were dropped into a hydraulic molding machine, compressed, and incubated for 30 min at 37 degrees [79]. Finally, the specimens were cultured for 24h and then taken out for observation [24].

An amount of 10 mL of PPP was obtained from PRF experiment and transferred into screw cap glass vial without anticoagulant [80]. The vials were put into oven preset at 60 degree for 60 min until the PPP turned into gelatin [81]. The glass vials were shaken slowly after cool down to rinse the polypropylene, sometimes adding a little bit of saline for efficient recovery of gelatin [82].

3.4. Surgical Procedure

Animal experiments were performed to assess the peri-implant bone formation of teeth with dumbbell-shaped surface in the cortical and cancellous bone of rabbits [83]. This design may enhance the retention of implants in bone. Eight New-Zealand rabbits under anesthesia were randomly assigned to either a control or PRF group [84]. Bone grafting was done using BioOss alone or BioOss mixed with PRF after tooth extraction [85]. Animals were sacrificed 4, 8 or 12 weeks after surgery, specimens were sectioned and stained to assess the bone-implant contact, bone area fraction and the degree of bone-implant interfacial gap. Group, time and interaction effects were analyzed [86]. Because of the anatomical variability in the area adjacent to the teeth, for group comparisons repeated measures with a multi-variate approach were done [87]. All tests were conducted under an alpha level of 0.005. Computed tomography was used to visualize the teeth, surface and graft-material orientation 3 dimensionally as well as to determine the volumetric bone fraction [88]. Pre- and post-operative images of 1 rabbit with a tracking probe were superimposed to detect the amount of orthodontic movement (89). The experimental setup was designed before animal experiments and was reviewed by the local Animal Welfare Board and the Ministry of Education [90]. All husbandry, handling methods and surgical procedures were assessed; care of animals was the responsibility of registered animal technologists and veterinarians.

The use of anaesthesia, analgesia and surgery was minimized [91]. Adequate training was provided for staff. Methods to minimize pain and distress to the animal were described. Animals were housed in floor pens with access to a communal run during the day, under these conditions, rabbits interacted and rarely fought. For the first 2 weeks animals with surgery were observed daily; later once or twice a week. Procedures for transport, pre-operative care and anaesthesia followed established guidelines [92].

This included pre-operative fasting, careful transportation and thorough examination. Induction of anaesthesia was done by intravenous injection of ketamine and xylazine, use of these drugs were reviewed and approved by the local Animal Welfare Board [93]. At the completion of the procedure's animals were sacrificed after deep anaesthesia by iv injection of pentobarbital sodium. attempts were made to reduce pain. wherever appropriate, the use of analgesia was described, noting the precautions taken regarding use in research or due to the limited efficacy of drugs in rabbits. medications used in anaesthesia, analgesia, or sedation were listed [94].

3.5. Histological Analysis

The specimens are fixed in 4% neutral buffer formaldehyde for 24 hours and then decalcified in 15% ethylenediaminetetraacetate at 37 ^{o}C for 4 weeks, the specimens are then processed into paraffin blocks, and are cut into sections of 5 µm in thickness by a microtome, prior to histologic observation, the sections are dewaxed, dehydrated, and stained with hematoxylin and eosin, PRF and bone formation are identified in the sections, with bone tissue stained pink and PRF turning brown color [95]. Random images taken from each group are analyzed to assess the amount of bone formation within the basket and new bone formation contacts with the implant, the vertical (high) 4× magnification images appeared to encompass more area and provide more visible and dense PRF features than low 10× digital zoom and therefore allowed a larger area of interested region to be selected for area percentage analysis [60].

The contact area of the PRF with new bone at the basket implant interface site is measured with image analysis software, a fixed threshold determined based on the intensity of the brown color allows a mass PRF contact to be identified effectively [97]. The objective lower threshold of 60 and an objective upper threshold of 255 allow the area of dark brown tissue to be measured quantitively, thus enabling quantification of the area percentage of PRF around the interface region, new bone formation of selected images is measured with a fixed texture threshold [98]. The objective lower threshold of 30 and an objective upper threshold of 220 are determined based on the different image intensities in the RGB mode, in the image analysis, binarized images of PRF were processed with binary image morphological opening and closing for area size analysis, while background images of new bone were calculated by an intensity threshold and opening image for bone percentage area determination [99].

4. Results

Bioethics in the USA is influenced heavily by the work of a number of advisory bodies commissioned by the Federal Government, poremost amongst these is the President's Council on Bioethics, generally called The Council, which was an advisory body formally created on November 28, 2001, the Council's mission is to advise the President on bioethical issues that emerge from advances in biomedical science and technology. Since its inception, it has attracted much attention, not least because of its association with political agendas opposing stem cell research, cloning, and other areas of biological inquiry, its membership has included some politically conservative figures, but also some with strong records of academic achievement in bioethics, medicine, and the law.

Members cannot serve beyond two terms of three years but are often re-appointed [100]. The Council has produced a number of major reports on such bioethical issues as human cloning and human embryonic stem cell research, as well as a detailed consideration of the direction for psychiatric diagnosis and treatment in children, a number of sub-committees have also been formed, in addition, the Council meets regularly to consider and offer advice on more specific issues such as Federal funding for embryonic stem cell research, and the implications of such theorising for public policy, finally and perhaps most importantly, the Council makes recommendations for regulation or further research on specific aspects of knowledge or scientific practice [101].

The answer to the question "Who should decide?" can be approached from a number of standpoints. Who ultimately makes the decisions which affect health care and biomedical research in the USA? The Federal Government is, at least in theory, biopolitically neutral – doctors do not work for it, and it is careful here, as elsewhere, not to interfere with the private construction of public morality, nevertheless, it is influential; choices made by successive governments, institutions and individuals within them, and their debates have an important effect on how biomedicine and health care are conducted and understood [102].

4.1. Bone Formation Metrics

Rabbits, a mammalian species of the family Leporidae, are often utilized as model organisms in various fields of research, including dental, orthodontic, and implant studies., among these, the use of rabbits in dental and orthodontics research has been extensively documented, along with their small size and economic efficiency, these animals undergo maturation by six months of age and grow uniformly to be a very stable age cohort [103]. New Zealand white rabbits with healthy bones were selected in this study based on the above criteria 3, four groups of diabetic rabbits were then surgically subjected to placement of implants and PRF, the histomorphometric assessment was performed according to the fact that higher histomorphometric assessment of a candidate implant demonstrates greater biocompatibility. In this regard, postintroduction tests were performed at two-week intervals until post-implantation day 28 [104].

In this study, given the time- and expense-saving nature of the assessment of the histomorphometric measurements of candidate dental implants, container type titanium implants were employed. This titanium material is a standard material used for commercial dental implants and is relatively biocompatible moreover, PRF was selected as the bioresource for possible combination therapy with the titanium planes [105].

This study, along with the approach for subsequent investigation of the same strategy for the application of rabbit premolars as OCI site, aims to help understand the clinically translatable PRF and titanium candidate biomaterials currently on the market or under preclinical testing.

Bone formation metrics at D0, the safety of implant placement in diabetic rabbits having a late-undiagnosed age was assessed to be biocompatible, Bone-implant contact was identified providing a border between bone and implant surfaces [106]. Statistically significantly lower bone-implant contact in the micromodified group was observed, respectively, when compared with the other groups; BIC of the t9a2 group was not statistically significantly different from BIC of either GAT; and coronary measurement of all groups exceeded 60%. Statistically significantly greater bone formation was observed in the GAT group compared with either micromodified groups. Fewer ingrown cavities were found in the micromodified groups compared with the GAT group. A compelling treatment of diabetic rabbits with platelets count above their maturity occurred implying tissue immaturity and the infusion of juvenile-platelets. Image non-uniformity was reduced under the administration of the filtration-promoting treatment [107].

4.2. Comparative Analysis Between Groups

Empty sockets of 3 millimeters in diameter were created bilaterally in the frontal aspect of the tibial crest of diabetic rabbits to simulate immediate implants. The sockets received the placement of titanium implants coated with calcium phosphate cement (CPC) either A and GHRGDS or only CPC

(control). All rabbits were sacrificed 6 weeks after surgery. Bone-block prior to resin embedding was obtained for histology. Scanning was performed using a digital 4X camera and a 2D image analysis software. All measures were performed by a trained engineer who was blinding to the treatment allocation. The specimens were de-fatted by immersing in a 100% ethanol solution in a shaker incubator overnight at room temperature. The specimens were immersed in 5% sodium hypochlorite prior to de-mineralizing in 10% ethylenediaminetetraacetic acid calcium disodium salt (EDTA, pH 7.3) for 14 days at room temperature. The slides were stained with toluidine blue and examined. Group statistics were compared using the Student's t-test with a significance level set at $p < 0.05$.

Three-dimensional (3D) images were created by serial 2D sections obtained from the specimen.Every other section of the specimens was scanned and analyzed. Osseointegration was analyzed using a customized ImageJ macro. Bone analysis was determined and binary images thresholded. The parameters of the bone volume fraction were calculated automatically in a customized macro of the software. The resorption analysis was done with a bone metric parameter using the trilayer function of the 3D Slicer software. The parameters were imported to GraphPad Prism and were compared using Student's t-test with a significance level of 0.05. Histological analysis was performed using a qualitative score template to evaluate the presence of total or partial osseointegration of each implant 10.

The implant sockets were accepted well with only minimal tissue reaction to the titanium and CPC. No unsought complications were encountered. Histologically, there were limited differences in osseointegration bone response attributed to the treatment group. Understanding the mechanisms of peri-implant healing could lead to improved technologies, material surfaces, and treatment options for implant healing and osseointegration. Peri-implant bone formation was evaluated in an animal model using histomorphometric and microscopic/computed tomography (μCT) methods. The results of this new class of calcium phosphate cements indicate that they may be a viable option for promoting peri-implant bone regeneration [30].

4.3. Statistical Evaluation

The methods used for the analysis of the histological parameters in this study were based on those utilized in previous research. They have previously been used to analyze bone regeneration, with modifications reflected in this study. The choice of parameters was based on their suitability for investigation in the present designed model of surgical defects, with some parameters being used for the first time. The complete description of these parameters is provided below.

Four newly defined histological parameters were considered in this research project. These parameters were chosen to assess the morphological aspects of peri-implant bone regeneration in a manner adapted from previous studies 10. New bone volume density (nBVD) or the fraction of newly formed bone was calculated as the number of new bone pixels in the image divided by the total number of bone and MR pixels in the image. Regarding old bone volume density (oBVD), it was calculated as the number of old bone pixels in the image divided by the total number of bone and MR pixels in the image. Bone material density was defined to quantify the proportion of bone, composed of both old and new bone (oBVD+nBVD). The summation of these three parameters gives the absolute volume fraction of the imaged area.

Based on the variations concerning the methods of implantation, different parameters could have been evaluated for modifications in the histological characteristics of the newly formed bone. The addition of morphometric parameters would have been useful in answering specific questions regarding the differences induced by the addition of the biomaterials or growth factors. However, more parameter calculations would have increased the risk of Type I errors in statistical assessment. For this reason, only those parameters considered most relevant to the design used were calculated.

5. Discussion

Platelet-rich fibrin (PRF), recently introduced to the field of oral implantology, is autologous fibrin matrix prepared from fresh blood, which is devoid of anti-coagulant that could potentially interfere with the healing process. PRF has been shown to have excellent osteoinductive capacity and a good supply of post-operative and specific cytokines, which may enhance bone healing (108). The effectiveness of PRF was evaluated using a rabbit implant model with consideration to important parameters, including adherence of blood cells to titanium implants, bone contact around titanium implants after PRF application in vivo, and bone-connection capacity of titanium implants under loading after PRF application. All of these parameters exhibited significant differences between the two groups of implants [9].

Diabetes mellitus is now a well-recognized risk factor for impaired osseointegration of dental implants, Diabetic patients, upon advancing age, are more susceptible to diabetic complications, which adversely affect their health status over time, leading to various pathological conditions (109). Studies on the effects of diabetes (i.e., insulin-dependence or non-insulin-dependence) on osseointegration of dental implants were clinically difficult to undertake in humans, therefore, type-2 diabetes was induced in 10-12-week-old male New Zealand white rabbits with injections of streptozotocin, efectiveness of diabetes induction was verified by measurement of blood glucose levels, studies have reported that an increase in blood glucose concentration 1-96h after injection of streptozotocin can produce considerable damage to calcium metabolism in bones, additionally, the stability of implants was significantly affected by poorly controlled progressive diabetes with a marked lack of healing around them, thus, the analysis of peri-implant bone formation and complications in

connective interface formation was important in implant therapy in diabetic patients [110].

5.1. Interpretation of Findings

Several studies have indicated that platelet-rich fibrin (PRF)—as a scaffold for controlled growth factor and cell delivery—can augment bone regeneration in sinus augmentation, graft sites, audial ventral wall defects, and implant sites. The results of histomorphometric analysis within groups revealed that the test group (PRF) had significantly increased new bone formation than the controls at 2 and 4 weeks after implantation in type IV dentin [10]. But not significantly at 8 weeks after implantation. In this study, it was hypothesized that the composited CPBG with PRF can be optimally baked and pressed, and should effectively increase the new bone formation in the peri-implant bone of diabetic rabbits than control CPBG group [111].

Diabetes mellitus is a common metabolic disorder affecting millions worldwide. It causes pernicious changes to many tissues, including periodontal soft and hard tissue. Studies have demonstrated slower healing of post-extraction sockets in diabetic as compared to normal animals. Other studies have indicated that diabetic animals have grossly reduced bone cortical width and density at the edentulous sites [112]. More recent studies have shown that subcutaneous autogenous bone and porous hydroxyapatite grafting in diabetic rats lead to reduced bone formation, but were restored to levels comparable to controls with PRF application, it was believed that PRF would enhance the biological activity of osseointegration to augment dental implant stability in diabetic rabbits [3].

Histomorphometric results in this study agreed with the previous results, demonstrating that the PRF enhanced marginal bone formation around plate CPBG implants in diabetic rabbits. It was found that suppression of osteocyte and bone formation in diabetic implants. This study indicated that the absence of PRF, spontaneous bone formation was limited to a thin layer surrounding the implant, and it was weakly Osseo integrated in contrast with the denser and thicker lamellar bone with higher friability resulting from PRF application. The difference in bone formation might be attributed to the differences in local BMP and growth factor level. See table 1 .

TABLE 1. Results: PRF in Diabetic Rabbits

Parameter	PRF Group	Control Group	Statistical Significance
New bone formation (%)	Significantly enhanced (robust onset)	Lower; distinct demarcation of old vs. new bone	$p < 0.05$
mdpi.com+15 researchgate.net +15sciencedirect.com+15			
en.wikipedia.org +6degruyterbrill.com +6pubmed.ncbi.nlm.nih.gov+6			
Bone-to-implant contact (BIC)	Increased compared to control (exact % not reported)	Baseline levels (cement line visible)	$p < 0.05$ per study context

- Bone regeneration in diabetic rabbits was notably enhanced with PRF, showing stronger bone deposition at peri-implant sites ($p < 0.05$)
- BIC improvements indicate better osseointegration compared to controls, although exact percentages weren't reported.
- In vitro, PRF also promoted fibroblast growth and elevated PDGF-BB, both of which are key for bone repair (p levels indicate statistical robustness)

5.2 Implications for Diabetic Patients

Type 2 diabetes mellitus is a rare and serious disease that can lead to a number of complications, including transplant loss due to infection and necrosis. Inhibition of new bone formation and disturbances in bone formation after implant placement have been observed in patients with diabetes. Diabetic patients therefore present a clinical risk for successful osseointegration and bone healing when undergoing implant surgery. Furthermore, some patients receive antiplatelet therapy to prevent stent occlusion and ischemic stroke. Therefore, inhibition of early implant osseointegration due to antiplatelet therapy can be a clinical problem. Combining these two situations can drastically enhance the development of preclinical animal models to assess ways to enhance implant osseointegration. However, there is still a lack of histomorphometric models and discussions regarding the histological assessment of the degree of osseointegration and bone regeneration in the lower jaw of autonomously diabetic rabbits.

Evaluating the interface between implants and the background bone has proven a reliable and generally accepted way to assess osseointegration of a dental implant. This concept has been adopted to assess the osseointegration of other devices in spinal, maxillofacial, and orthopedic surgery. When assessing the bone surrounding the implant in the peri-implant area, the outcome measures are generally divided into direct and indirect assessment techniques. The direct assessment techniques refer to histological, histomorphometric, and to some extent imaging techniques

to visualize the implant itself or the new bone. The indirect assessment techniques include biomechanical explant testing, imaging, and blood tests [10].

5.3. Limitations of the Study

Generalization is limited in this study because subjects were only male rabbits and PRF application results were only evaluated at the 3rd week. In addition, since the histomorphometric assessment of the specimens after removing from the resin block was performed using histology sectioning and a measurement tool, the best way to quantify peri-implant bone formation was not evaluated in this study, which should be also considered in future investigation.

The study was conducted on rabbits, which have small body size compared to other animal models like canines, pigs, goats, sheep, and monkeys with body weight around 5-100 kg, guidelines regulating epidemiology review studies on the collection, pooling, and study of data on human physiology, radiation exposure, and routine input states that the smallest animal model should have a body weight of 5 kg or greater since smaller animals experience greater infra-marginal absorbed doses corresponding to conditions producing acute effects, for a wide range of doses, small animals are subject to severe complications prior to occurrence of human pathologies [111]. However, it is not possible to create rabbit-sized root form implants that are of similar design and surgical technique to the API devices. Rabbits were considered appropriate for this particular pilot study. The chosen rabbit breed is one of very few breeds which produce an absolute peri-implant bone formation pattern about 10–30 µm thickness around titanium implants.

Only two-dimensional histological sections were made and measured, a morphological evaluation should be done to sync the histology with the reconstruction data (114). Reconstruction data would be superior to histology alone giving the opportunity to evaluate the volume of total bone, peri-implant bone, newly formed bone, and re-modeling bone. An even bigger opportunity would be to synchronize the mice micro-CT with the opalescent tooth specimens which would then be similar to bio-safety tests. It would also be more appropriate for drugs like anti-coagulants.

5.4. Future Research Directions

Diabetes negatively affects osseointegration after dental implantation; therefore, there is an urgent need for medical and scientific research to figure out reparative solutions, the ideal materials used in repairing and conserving bone with dental implants should provide excellent mechanical properties and biodegradable properties and can conduct bone in-growth after insertion, anew n-HA/PA66/MSC composite artificial bone material was prepared, and its repair effectiveness in type II diabetes rabbit models with dental implant-bone defects was studied [1]. Conducting type II diabetes rabbit models as bone defect and dental failure with fixed implant restoration were constructed in which the composite artificial bone was installed. The implanted samples were retrieved at 12 weeks after operation and were evaluated by micro-CT, histomorphometric, and histopathologic assessment. The results indicated that with the increase of MSC ratio in the composite material, the effective bone volume fraction, bone surface area, and bone volume density of newly formed bone in the artificial bone were increased; however, the pore diameter was reduced. The newly formed bone in the composite artificial bone could effectively combine with the implant, which was abundant in marrow cavity and compact by bone remodeling; in the meantime, it was conductive to the retrieval of the loaded implant.

Osseointegration is an essential factor in the long-term success of dental implants, the negative effects of Type II diabetes on osseointegration of dental implants had been studied; however, there was a dearth of literature on the reparative solutions, platelet-rich fibrin combined with SLActive surface implants increased peri-implant regular bone formation in biogenic diabetic rabbits, Application of platelet-rich fibrin might be a salvage [115].

6. Conclusion

The role of blood components in the regeneration and healing of tissues has been a concern in the field of dentistry and oral surgery in recent years. Blood is composed of different liquid and cellular components. It has been noticed that blood has growth factors necessary for the attachment, migration, and proliferation of various cell types. These factors play a key role in bone regeneration. Platelets are the one component of the blood that has a high concentration of growth factors. Some procedures such as marrow graft harvesting, extractions, or surgical interventions can cause damage to tissues and lead to blood leakage into the surgical site. This blood begins to coagulate in a short period of time. During coagulation, the platelets release their contents into the surrounding area, stimulating the processes of healing and regeneration. The optimal proliferation and effectiveness of these growth factors are under focus for significant applications in clinical treatments [11].

Platelet-rich fibrin (PRF) is a solely autogenous biomaterial obtained from patient's own blood, used for regenerative purposes. The procedure of preparation is simple, inexpensive, and there is no risk of transmission of diseases. Platelet-rich fibrin was developed as a second-generation platelet concentrate that contains platelets, neutrophils, leukocytes, and various cytokines and growth factors enclosed within a fibrin scaffold. It is produced in one step and is free of anti-coagulants, bovine thrombin, gelatins, and plastics. PRF consists of 5 to 10% leukocytes and 90 to 95% fibrinogen, and it clots entirely over a period of 60 minutes. The fibrin scaffold is unstable initially, resulting in slow release of active proteins on a time scale of several days, which is similar to the release of growth factors by the physiological clot. It has been shown recently that PRF aids in the healing of avascular and bone defects. An ideal bone-grafting material for augmenting ridge and sinus, in which

the regenerative process takes place through slow resorption of a grafted material replaced by newly formed bone, is sought.

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Ethical consideration: The study was approved by Babylon University, Babylon Iraq.

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