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Effect of chronic kidney disease on the healing of titanium implants

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ABSTRACT

Chronic kidney disease (CKD) has become a worldwide public health problem. However, its effect on osseointegration of dental implants is largely unknown. The aim of this study is to investigate whether CKD impairs the quality of the osseointegration of titanium implants. Uremia was induced by 5/6 nephrectomy in mice, and serum levels of BUN, FGF23, PTH and ALP were significantly increased. For *in vitro* tests, bone marrow mesenchymal stem cells (BMMSCs) were obtained and cultured on titanium discs. There was no significant difference in term of expression of osteogenic marker genes including Osx, Col-1, Ocn, and Opn, as quantified by qPCR. Moreover, Alizarin Red S staining showed comparable mineralized nodules formation. Histomorphometrical analysis of experimental implants inserted in the femurs of CKD mice revealed a trend of decreased BIC ratio at 2-week healing. The strength of bone-implant integration, as measured by a push-in method, was significantly lower for the CKD group at 2 weeks, although a comparable level was reached at 4 weeks. These results demonstrated that CKD only negatively affects the osseointegration of titanium implants at the early stage.

Introduction

Chronic kidney disease (CKD) has become a worldwide public health problem, with growing prevalence, high cost and severe complications [1–3]. Studies have shown that the prevalence of CKD in USA and Norway was 13.0% and 10.2%, respectively [4,5]. More recently, a cross-sectional survey of a nationally representative sample of Chinese adults revealed that the overall prevalence of CKD was 10.8% [6]. The number of patients with CKD in this rapid developing country was estimated to be 119.5 million, the highest prevalence in the world.

In CKD patients, the normal physiological mechanisms regulating blood levels of calcium, phosphate, vitamin D, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) are disturbed, which subsequently impact on the bone structural integrity [7,8] and lead to chronic kidney disease-mineral and bone disorders (CKD-MBD) [9]. In CKD patients, histological evidence of bone disease affected 84% of all subjects (32% osteitis fibrosa, 20% mixed bone disease, 8% osteomalacia, 6% mild disease and 18% adynamic bone disease) [10]. More severely, only 2% of dialysis patients have normal histomorphometric analyses of bone biopsy. A recent study examined the characterization of the mandibular bone in a mouse model of chronic kidney disease, and the results showed a significant reduction in cortical bone thickness [11].

The chronic kidney disease is also regarded as a risk factor of periodontitis [12–15]. Borawski reported that the loss of clinical attachment level of the CKD patients was significantly higher than that of general population subjects, indicating a high severity of periodontitis in the renal failure patients [16].

Although chronic kidney disease has been considered as a worldwide public health problem, its effect on dental implant treatment is largely unknown. A quite common opinion in both the oral and nephrological literature suggests that osseous periodontal surgical procedures such as bone grafting or dental implants may be contraindicated in patients with significant renal osteodystrophy [14,17]. Others, however, investigated the quantity and quality of the alveolar bone of dialysis patients, which showed that the residual bone volumes were adequate for implant insertion, suggesting this type of treatment is applicable to CKD patients [18].

The aim of this study is to investigate the effect of CKD on osteogenic differentiation of bone marrow mesenchymal stem cells (BMMSCs) and peri-implant bone formation in a CKD mouse model, so as to determine to what extent CKD impairs the osseointegration of titanium implants.

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Materials and methods

Animals

Female C57BL mice at age of 9-week-old were purchased from Charles River Laboratories International Inc. (Wilmington, MA). The animals were kept under climate-controlled conditions and fed with standard diet. All studies were approved by the Institutional Animal Care and Use Committee at the Harvard Medical School (Boston, MA).

Surgical procedure to induce uremia

The CKD mice were established by a two-step 5/6 nephrectomy to induce uremia as described previously [19]. Briefly, the first procedure involves electrocautery of the left kidney. It was approached through a 2-cm-long lumber incision and exposed by fine dissection of the surrounding tissues including the peri-renal fat and adrenal gland. The entire cortex of the right kidney was cauterized except for a 2-mm area around the hilum. The kidney was then returned to the renal fossa, and the subcutaneous tissues were sutured with 6–0 silk. The skin was closed with surgical clips. After 1 week, a total nephrectomy of the right kidney was performed by ligation of the renal hilum with a 5–0 silk suture and surgical excision of the kidney. The wound was closed as the first surgery. Sham surgery consisted of anesthetic, flank incision exposing the kidney, and closure of the abdominal wall. The illustration of the work flow is shown in Fig. 1A.

Serum biochemical assays

Eight weeks after the secondary surgery, blood of the mice was collected by cheek pouch. Serum biochemistry was performed using commercially available kits: Blood urea nitrogen (BUN) (Roche Diagnostics, Indianapolis, IN); FGF23 (Immutopics, San Clemente, CA), PTH (Immutopics, San Clemente, CA); 1,25$(OH)_2$D (Immunodiagnostic Systems Ltd., Fountain Hills, AZ); Calcium and Phosphate (Stanbio Laboratory, Boerne, TX). For the assay of serum ALP activity, the serum was diluted 25 times and measured using a SensoLyte® *p*NPP Alkaline Phosphatase Assay Kit (AnaSpec Inc., Fremont, CA).

Primary culture of BMMSCs on titanium disks

Eight weeks after the secondary surgery, five mice from each group were sacrificed and the femurs were dissected. The bone marrow of the femurs was flushed, pooled together, and cultured with alpha-modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotic-antimycotic solution. At about 80% confluence, cells were detached and seeded onto SLA titanium disks at a density of 3×10^4 cells/cm^2, with osteogenic medium, which contains 50 ug/ml ascorbic acid and 10 mM Na-β-glycerophosphate. The culture medium was renewed every other day.

Alkaline phosphatase staining

After 10 days, cultured BMMSCs were washed twice with saline, and fixed in citrate buffered acetone for 30 seconds, followed by gently rinse in deionized water for 1 min. Cells were incubated with 120 mM Tris buffer (pH 8.4) containing 0.9 mM naphthol AS-MX phosphate and 1.8 mM fast blue RR covered from light at room temperature. After 30 min, the cells were rinsed thoroughly in deionized water.

Alizarin Red S staining

After 21 days, cells were washed three times with PBS and fixed in 10% neutral formalin for 5 min. Cells were then incubated in 2 wt%

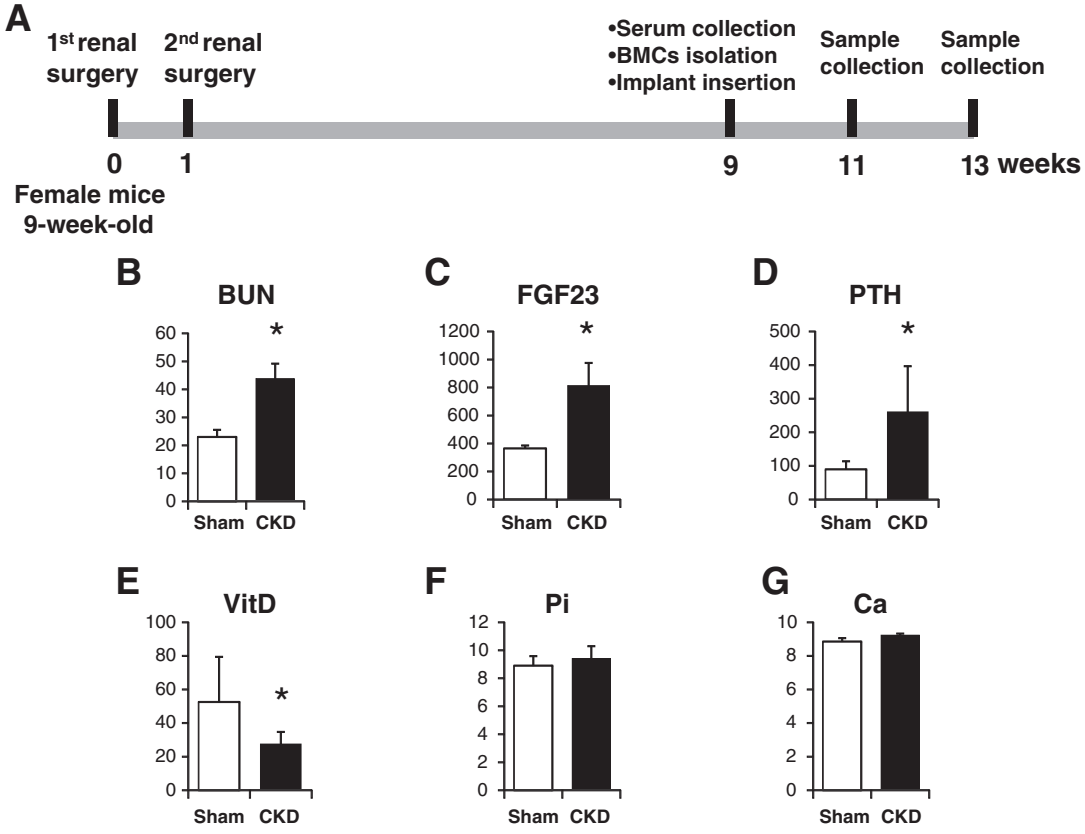

Fig. 1. Illustration of the work flow and serum biochemical measurements. (A) Illustration of the work flow. (B) Serum BUN. (C) Serum FGF23. (D) Serum PTH. (E) Serum vitamin D. (F) Serum phosphate. (G) Serum calcium. *: $p < 0.05$.