Objective: We have successfully established steroid-associated osteonecrosis (SAON) animal model in rabbit with a single injection of lipopolysaccharide (LPS) and subsequently three injections of methylprednisolone (MPS). The present study tested an experimental protocol to induce SAON in rats as rat is more suitable animal model to study molecular mechanism of diseases and drugs as well as a cost-effective model to study body metabolism physiologically and pathologically.

Methods: Eight 24-week-old male Sprague-Dawley rats were induced SAON by pulsed injection of one intravenous injection of LPS (1mg/kg) and 24 hours later, three intraperitoneal injections of MPS (100 mg/kg) at a time interval of 24 hours. Animal Ethics Approval was obtained (Ref. No. 15-150-M5). Additional 4 rats were used as normal controls. 2 weeks after induction, bilateral femora and bilateral tibiae were collected for histological examination. Diffused presence of empty lacunae or pyknotic nuclei of osteocytes in the trabeculae, with surrounding necrotic bone marrow were classified as osteonecrotic lesion. Rat with presence of at least one osteonecrotic lesion was considered as ON+ rat.

Results: Four rats died the following day after LPS injection with 50% mortality. The four remaining rats survived after the three injections of MPS and developed osteonecrosis based on histological evaluation with 100% incidence of SAON. Empty lacunae of osteocytes were found in the trabeculae. Edema and fibrous marrow appeared in necrotic bone marrow. In intact bone marrow region of the proximal femur, distal femur and proximal tibia, more newly formed small sized adipocytes were present in ON+ rat, while there were very few adipocytes in these regions in normal control rats. In distal tibia, there were full of large sized adipocytes in bone marrow in normal control rats, while the adipocytes were smaller and more mono-nuclear cells generated in bone marrow of distal tibia in ON+ rat, indicating activated repairing reaction in yellow bone marrow region in distal tibia of ON+ rat.

Conclusion: This study successfully induced SAON in rat model with pulsed injection of LPS and MPS, the pathological changes found in SAON patents and SAON rabbit model, e.g., empty lacunae in osteocytes, edema and fibrous marrow, were also found in our rat model. Further study will be on lower dose of LPS for its potential to decrease the mortality to facilitate translational study using innovative agents for prevention of SAON.

Introduction: Inhibition of sclerostin by systemic administration of a monoclonal antibody (Scl-Ab) significantly increased bone mass and strength at fractured bones in animal models and non-fractured bones in ovariectomized (OVX) rats. In this study, we examined the effects of Scl-Ab in a closed fracture healing model in OVX rats.

Methods: Sixty Sprague-Dawley rats underwent an ovariectomy or a sham operation at 4 months of age, and a closed fracture of the right femur was performed 3 months later. Subcutaneous injections with Scl-Ab (25 mg/kg) or saline were then administered on day 1 after the fracture and twice a week for 8 weeks (n = 20 per group), at which time the fractured femurs were harvested for micro-computed tomography analysis, four-point bending mechanical testing and histomorphometric analysis to examine bone mass, bone strength and dynamic bone formation at the fracture site. The angiogenesis at the fracture site was also examined. Bone marrow stromal cells were also isolated from the fractured bone to perform a colony-forming unit (CFU) assay and an alkaline phosphatase-positive (ALP+) CFU assay.

Results: OVX rats treated with Scl-Ab for 8 weeks had significantly increased bone mineral density and bone volume per trabecular volume compared with OVX rats treated with saline. Similarly, maximum load, energy to maximum load and stiffness in Scl-Ab-treated OVX rats were significantly higher than those in saline controls. The mineralizing surface per bone surface, bone formation rate per bone surface and mineral apposition rate were also significantly increased in Scl-Ab-treated group compared with the saline-treated group. Furthermore, the Scl-Ab-treated group had more CFUs and ALP+ CFUs than the saline-treated group. No significant difference in angiogenesis at the fracture site was found between the groups.

Conclusion: Our study demonstrated that Scl-Ab helped to increase bone mass, bone strength and bone formation at the fracture site in a closed femoral fracture model in OVX rats. Bone marrow stromal cells in OVX rats injected with Scl-Ab also had increased CFUs and ALP+ CFUs.
Results: Comparing the time of surgery (DS: 10.8 min. vs AD: 9.8 min.) and the quality of the defect regarding the diameter and the circularity of the osteotomies, no significant difference could be seen between the two techniques. However, due to the fact that AD disengages automatically the drilling process as soon as it does not encounter anymore resistance, the underlying dura mater integrity was shown to be significantly improved compared to DS (Fig. 1C). Another major advantage was the absence of heat produced during the drilling (no irradiation was used), whereas high temperatures were recorded during DS utilization (up to 46°C) which could potentially damage bone and peripheral tissues (Fig. 1D).

Discussion and Conclusion: The current state of the art method for cranial osteotomy is a trephine and burr. As shown in this study, the risk of damaging the dura mater using this method is relatively high. Several studies have shown that DM is critical in calvarial re-osseification by providing cellular element such as osteoblasts and growth factors [1], and impairing DM results in altered bone regeneration. Consequently, the use of a new device enables to create reproducible calvarial defects in a satisfying surgical time with minimal effect of the surrounding tissues would be of major interest. In this investigation, we demonstrated that the use of Anspach high speed handheld drill is a safe, efficient, precise and fast method for creating circular defects in rabbit cranium.

References

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Z: During the average follow-up of 3.6 years, according to the Harris hip score, 83.7% of the experiment group and 56.5% of the control group achieved good to excellent score, which is statistically significant (p<0.05). 73.9% of patients in the control group achieved perfect union while this number was 100% in the experiment group (p=0.038).

Discussion and Conclusion: This study shows that the combination of quadratus femoris muscle pedicle and rhBMP-2 in femoral neck fracture can get a better results in union time, union rate and Harris hip scoring, but cannot reduce the incidence of femoral head osteonecrosis. The separation of nonunion and femoral head necrosis, as we found in this study, is puzzling but inspiring. Avascular damage has been suggested to be the explanation for both of two complications. But in our study, not only fracture reduction and internal fixation but also muscular pedicle bone and BMP-2 have been performed in these patients. All of these measures are helpful to both faster union time and restoration of avascular perfusion. Why there were still three patients (14.3%) developed femoral head necrosis even after their fracture line has successfully united? This phenomenon strongly suggests that potential pathology factors, besides avascular damage, stay veiled in development of nonunion and femoral head necrosis. To ultimately manage these two complications, further investigation over their pathology needed. In conclusion, this study demonstrates that combination of quadratus femoris muscle pedicle graft and rhBMP-2 in femoral neck fracture can get a better results in union time, union rate and Harris hip score.

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CHITOSAN/PVA/BIOGLASS MULTILAYER FILMS FOR WOUND DRESSING APPLICATION
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Background: Wound healing is a dynamic process in which a variety of cellular and matrix components act in concert to reestablish the integrity of injured tissue, and it was widely accepted that a moist wound free of infection provides an optimum microenvironment beneficial to continuous tissue repair processes. To create a moist environment for rapid wound healing, a chitosan/PVA/Bioglass multilayer film with sustained antibacterial capacity had been developed by electrosprinning. This triple-layer film consists of a PVA-Bioglass top layer and chitosan sublayer separated by a PVA-chitosan blended layer, which assumed that the sub-layer would contact the wound surface, promote tissue regeneration. The mid-layer, with moisture retention and antimicrobial agents, could prevent bacterial invasion and control water vapor evaporation, and the top-layer with bioglass would release beneficial ions which promote self-healing.

Subjects and Methods: The nano-Bioglass (nBG), with a composition of 80SiO2/16CaO/4P2O5 (mol %), were prepared using a base-catalyzed sol–gel method. The fibrous films were prepared using the electrosprinning technique (Figure 1A). 7 wt % chitosan in TEA and 10 wt% PVA in water were separately prepared under magnetic stirring. 5, 10, 20 and 40 wt% nBG (with respect to polymer weight) were first dispersed in 10 wt% PVA solutions. The prepared composite polymer solutions were then electrospinning.

Results: Chitosan and PVA films have been successfully prepared by electrosprinning. The average fibre diameter is 400–800 nm. The nBG were added at varying contents (5, 10, 20, and 40%) to PVA solution in order to generate top-layer of the multilayer films. The SEM images of the resulting fibers clearly showed that the nBG nanoparticles were well distributed in the polymer matrix (Figure 1B). TG analysis suggested that the PVA organic matrix was completely burnt out below 600°C (Figure 1C). However, the nBG added films showed remnant weights after ~400°C. The remnant weights increased with increasing nBG content, which matched the designed contents. Furthermore, the capacity of the multilayer films to heal full-thickness skin defects was evaluated in a rodent model. The result showed that the wounds of treated groups had almost closed by day 12 whereas the untreated wounds were not, indicating that the multilayer films can significantly promote the wound healing.

Discussion and Conclusion: An ideal wound dressing, therefore, should protect the wound from bacterial infection and maintain a moist healing environment. Hence, an attempt was made to design a three-layered composite film to meet the divergent demands of the healing process. Firstly, chitosan (CS) was selected as the sub-layer because of its extremely biocompatibility and antibacterial properties. The mid-layer contained PVA and chitosan was established by dual-channel mixed electrosprinning. PVA is a hydrophilic biodegradable polymer which could maintain a moist healing environment. For top-layer, bioglass was successfully incorporated in PVA fibers. One of the important characteristics of bioactive glasses is their ability to release beneficial ions, which promote self-healing. In conclusion, the results obtained in this work indicated that the new type of multilayer films has the potential for wound dressing application.