Optimization of Platelet-Rich Plasma and Its Effects for the Recovery of Erectile Function after Bilateral Cavernous Nerve Injury in Rat Model

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ABSTRACT SUMMARY
The aim of this study was to optimize the Platelet-rich plasma (PRP) preparation method and compare the effects of PRP from different preparation methods in restoring of erectile function in a rat model of bilateral cavernous nerve (CN) injury. Results demonstrated that the optimized PRP prepared by stimulation with chitosan and incubated at -20 °C for 15 days had the largest amount of PDGF-AB and showed a synergistic effect on release (p < 0.05). The functional outcome measurement revealed improvement after bilateral CN injury occurred in the group treated with optimized PRP.

INTRODUCTION
Platelets play pivotal roles in regulating body metabolism and promoting wound-healing and tissue-regeneration functions. When platelets are activated, they release many kinds of growth differentiation factors, and a few were found to facilitate nerve repair and regeneration [1]. PRP is an autologous product and contains high concentrations of growth factors such as PDGF, IGF-I, and VEGF. Over the last decade, PRP has been used to improve clinical outcomes in cardiac, plastic, and periodontal surgical therapies and intrabony defects. Several studies demonstrated high concentrations of growth factors in PRP that may enhance tissue-repair processes [2]. However, those studies used various PRP preparation methods with little or no characterization of the content of the PRP used as therapy.

In the past few years, thrombin and calcium were shown to be potent agents for platelet activation and can increase growth factor release. However, thrombin can cause sensitivity, allergic reactions, and extensive intravascular clotting when injected into large blood vessels of animals [3]. Shen’s study suggested that chitosan might be a potential substitute material for thrombin for preparing PRP [4]. Recent studies also found that different centrifugations, anticoagulants, enrichment factors, and activating factors resulted in different aggregated platelet yields and final growth factor contents [5]. Thus, the purposes of our study were to (1) assess the effects of various anticoagulants, activating factors, reaction temperatures, times, and calcium chloride concentrations on growth factors released by platelets and (2) investigate the effects of PRP prepared by different methods on recovery of erectile function in a rat model of bilateral cavernous nerve (CN) injury.

EXPERIMENTAL METHODS
Preparation of PRP and factors affecting the release of growth factors from PRP
Whole-blood samples were withdrawn and mixed with different types of anticoagulants including EDTA, heparin, ACD-A, and sodium citrate. These mixtures were centrifuged at 25 °C and 400×g for 10 min to obtain platelet-poor plasma (PPP) and at 1500×g for 15 min to obtain PRP. Platelet counts of PRP were made and adjusted to (1~2)×10⁶ platelets/ml. The various PRP solutions prepared using different anticoagulants were measured to evaluate their effects in releasing PDGF-AB. Subsequently the best anticoagulant was selected to prepare PRP. The PRP solutions prepared above were treated under the following conditions to assess their effects on the release of growth factors. A flow chart of the experimental design for the quantitative studies on releasing growth factors from PRP treated under different conditions is shown in Fig. 1.

Measurement of erectile responses
The erectile response was assessed in all rats after 4 weeks by electrostimulation of the CNs and by measuring the intracavernous pressure (ICP) via an MP36 pressure transducer (Biopac Systems). A bipolar stainless steel electrode was used to directly stimulate the CNs. Monophasic rectangular pulses were generated by a computer with a DS3 constant current isolated stimulator (AutoMate Scientific). The stimulus parameters were an amplitude of 1.5 mA; a frequency of 20 Hz; a pulse width of 0.2 ms; and a duration of 60 s. ICPs were recorded and
The effects of an injection of PRP after bilateral CN crushing injury on erectile function are shown in Fig. 3. CN crushing injury consistently resulted in erectile dysfunction (ED). This finding is reflected by the markedly decreased ICP responses to electrostimulation in the vehicle-only group compared to the sham group. Optimized PRP injections resulted in significant increases in the ICP compared to injured controls treated with the vehicle and general PRP.

**RESULTS AND DISCUSSION**

Figure 2 shows the concentration (ng/ml) of PDGF-AB in PRP stimulated with various activating factors (thrombin, chitosan, ADP, collagen, serotonin, and epinephrine) and incubated at -20 °C for 1, 3, 5, 9, and 15 days. The release of PDGF-AB and reaction time appeared to have a strong positive correlation as seen in PRP after adding the various activating factors stored at -20 °C. The amounts of PDGF-AB released from PRP among all groups were > 80 ng/ml at 15 days and showed a statistically significant difference (p < 0.05) compared to other time points. Significantly increased levels of PDGF-AB were released from all PRP (activation by activating factor) groups compared to the control group (non-activated PRP). When comparing the effects of the six kinds of activating factors on the PDGF-AB released from PRP incubated at -20 °C for 15 days, PRP activated by chitosan released a significantly greater amount of PDGF-AB (p < 0.05).

**CONCLUSION**

Our results provide an optimized method for PRP preparation and demonstrated its effect on the recovery of erectile function after bilateral cavernous nerve injury in a rat model. This effect may be even more apparent, depending on the dose, application frequency, and application site which should be determined in further studies.

**REFERENCES**