Dear reviewers:

We appreciated your hard works for reviewing our works and giving us so many valuable suggestions. We will try our best to answer the questions and revise it properly. The followings are point-to-point responses for the reviewers comments.

Reviewer 1:

This is a well-written manuscript that demonstrates dosing of platelet-rich plasma to enhance angiogenesis using adipose-derived stem cells. Work is done both in vitro and in an in vivo model of rodent ischemic limb. All experiments done are straightforward. This is a particularly important communication where pretreatment of human adipose-derived stem cells with platelet rich plasma will allow for their ability to express angiogenic genes such as Hif1, CD31, and VEGF and thus when applied to the surgical wound in the ischemic limb model, enhances new blood vessel formation. There is potential clinical utility of this approach using autologous platelet rich plasma.There are minor queries regarding

1) if infiltration of inflammatory cells such as macrophages were found in the ischemic limb as macrophages can contribute to release of angiogenic factors

   Response: Macrophage is activated and increased in response to tissue injury. Previous studies have presented macrophages have the capability to influence the angiogenic process by release of proteases, growth factors (bFGF, GM-CSF, TGF-alpha, IGF-I, PDGF, VEGF/VPF, TGF-beta). However, the macrophages usually occurred in early inflammation stage of injury. In our experiment, mice were sacrifice at day 28 so that we did not use it as an indicator for angiogenesis in our experiment since it was already late stage of injury.

   also, the authors mention that other studies have shown that tube formation is seen at lower platelet-rich plasma concentrations; the explanation of the discrepancy between these other studies and their study where tube formation was seen in only

the higher concentrations of platelet rich plasma could have been more fully discussed.

Response: The other studies showed the lower PRP can have better tube formation which is different from our outcome is because the target cell for tube formation is HUVECs in their study and ADSCs in our study. To our knowledge, no study evaluate the dose-dependent effect of PRP on endothelial differentiation of ADSCs especially in tube formation assay. Hence, our study just showed the optimal PRP concentrations for the tube formation of ADSCs.

2) with longer incubation of platelet rich plasma with the adipose-derived stem cells, did these stem cells not demonstrate differentiation into chondrogenic or osteogenic cells that can also make angiogenic factors such as VEGF? It would be good if the authors could look at markers (e.g sox 9, type II collagen for cartilage or osterix for osteoblasts, or runx2 which would mark chondro-osseous precursors) of such other committed cell types at the later treatment time with platelet rich plasma;

Response: Yes, we truly did the job to verify the osteogenic, chondrogenic and adipogenic marker of adipose derived stem cells at PRP treatment without differentiation medium. In our preliminary, unpublished finding, the bone maker will increase and the cartilage and fat marker will decrease and still can make VEGF. It will be our next study and paper. Thanks for reviewer's reminding.

4) Figure 7b should perhaps be modified to have the capillary density as capillary numbers/mm^2 as mentioned in the figure legend rather that % of "interestion" area. "Interestion" is not a word; being consistent with what is mentioned in the figure legend and what the figure shows would be helpful.

Response: Thanks for your instruction. We had put a new figure which is made based on our histological section quantification results and consistent to our figure legend.

Reviewer 2:

The manuscript is about the effects of PRP and ADSCs on neovascularization in mouse injury model. The study is well-design and written. The points should be considered by the authors are:

1- the name of muscle that injections were done, has not mentioned

Response: In our group, ADSCs were not injected into a single muscle. Instead, ADSCs were injected into medial and lateral muscle groups of ischemic hindlimbs. We observed the angiogenesis and neovascularization of the ischemic hindlimbs.

2- In the discussion, the authors did not explain enough why some PRP concentrations had a better effect than others.

Response: One of the goals in our study was to determine the optimal concentration of PRP to enhance angiogenesis of ADSCs. However, our results revealed that angiogenic effect is not proportion to PRP concentration. There were some other reports had similar findings. Higher concentration of PRP lowered proliferation rate of ADSCs and further reduced endothelial differentiation. Pro-angiogenic and anti-angiogenic molecules are released from plasma during tissue injury. Balance between local concentration of proangiogenic factors and anti-angiogenic factors(like thrombospondin family and endostatin) accounts for the net biological effect on injured tissue. Thus, certain PRP concentrations had a better angiogenic effect than others on ADSCs.

Above content was also included into our modified discussion sections. The best dose of PRP for cell proliferation of ADSC varies from study to study may be due to different ADSC and PRP preparation methods. Lack of standardized protocols for ADSC and PRP collection and made it difficult to compare results between studies.