

Dear Dr. Mondal,

Thank you for inviting us to share our laboratory techniques with the scientific field. We are now submitting our manuscript entitled “Microsurgical Skills of Establishing Permanent Jugular Vein Cannulation in Rat for Serial Blood Sampling of Orally Administered Drugs”, for publication as a research method paper in *Journal of Visualized Experiments*.

Sequential blood collection from small laboratory animals (e.g., rodents) is necessary for evaluation of the therapeutic index during pharmaceutical lead optimization. Non-surgical approaches to multiple blood sampling, such as orbital sinus puncture and tail snipping, are possible, but the sample volume is small and the procedure can cause harm and stress to the animals, which could potentially affect the experimental results. The surgical cannulation approach, on the other hand, is a preferred alternative to repeated venipuncture because of several advantages of replacing the lost fluid volume, controlling blood volume collection, and reducing animals' pain and stress. Particularly, blood sampling through the jugular vein catheterization (JVC) rat model is a widely applied method for studying drug pharmacokinetics and effects. Despite of its usefulness, successful cannula implantation in jugular vein requires careful practice of microsurgical skills and knowledge of postsurgical care and maintenance.

Therefore, we aim to thoroughly demonstrate how to establish a long-term JVC rat model for blood collection at the laboratory setting and to investigate the physiological status of rats during the postsurgical recovery phase. In the manuscript, we detail the microsurgical procedures for the catheter implantation in the jugular vein of rat with specific focus on the placement and sealing of jugular cannula. We highlight the importance of monitoring postoperative physiological and hematological indicators of the rats to ensure their recovery for use. The established JVC model is applied to determine the time-plasma drug concentration of the orally administered natural phenol antioxidant ellagic acid.

We trust that this study provides informative protocol of establishing and maintaining a long-term blood collection rat model, which will be of great interest to researchers in the biological and medical fields. We appreciate you and reviewers in advance for the kind consideration and valuable comments.

Sincerely Yours,

Rui Xue Zhang, Ph.D., Associate Professor
Northwestern Polytechnical University, Xi'an, Shaanxi, China