



Pre-operative portal vein ligation and MSC injection in a rat model

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Introduction

• What is PVL/PVE?:

Portal Vein Embolization (PVE) or Portal Vein Ligation (PVL) is a technique used to increase the volume of functional liver before surgery. Portal blood flow is redirected to the future liver remnant (FLR), initiating hypertrophy in the FLR.

• Why are MSCs used as an adjunct?

Stem cells have been shown to participate in liver regeneration. The use of MSCs as an adjunct with PVE allows for significantly higher FLR growth than when not in use.

• CT Volumes vs Weights

Two methods were utilized to determine liver size, CT scans to estimate liver volume, and the liver weights taken after the rats are sacrificed.

Methods and Materials

• MSC culture:

Bone-marrow derived Sprague-Dawley rat mesenchymal stem cells (MSCs) labeled with a red fluorescent protein (RFP) were cultured in DMEM with 10% FBS and 1% Pen-Strep. .

• Portal vein ligation/injection:

In a Sprague-Dawley rat model, portal vein branches for the left and middle lobes were ligated. MSCs were then injected into the non- occluded (right) portal vein branches.

• CT imaging for volume estimation:

CT imaging was performed on a 128-slice CT scanner (Somatom Definition Edge, Siemens Healthineers) available at the Dunn Laboratory. Quantification of liver volume was done using MIM7.1.4 software

• Necropsy and histology:

The rats were sacrificed after 2 weeks, and the livers lobes were harvested and weighed. The liver samples were fixed in 10% buffered formalin, paraffin-embedded, and sectioned at 4 μm. The slides were stained for RFP, our MSC marker.

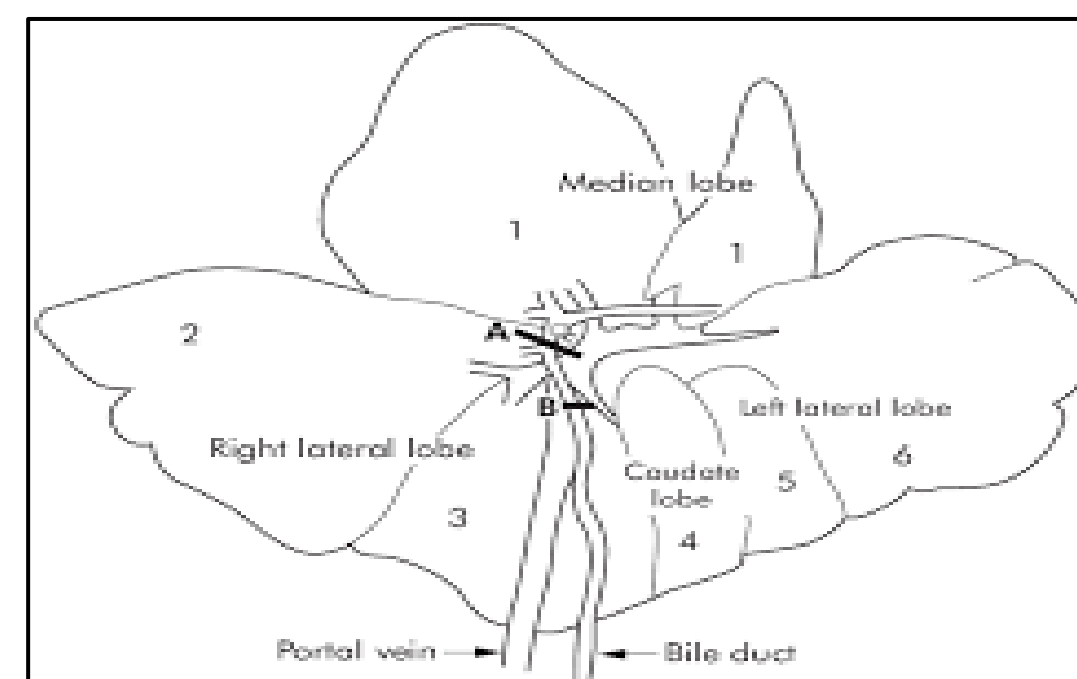


Fig. 1. **Diagram** showing the different lobes of the liver and the branches of the portal vein

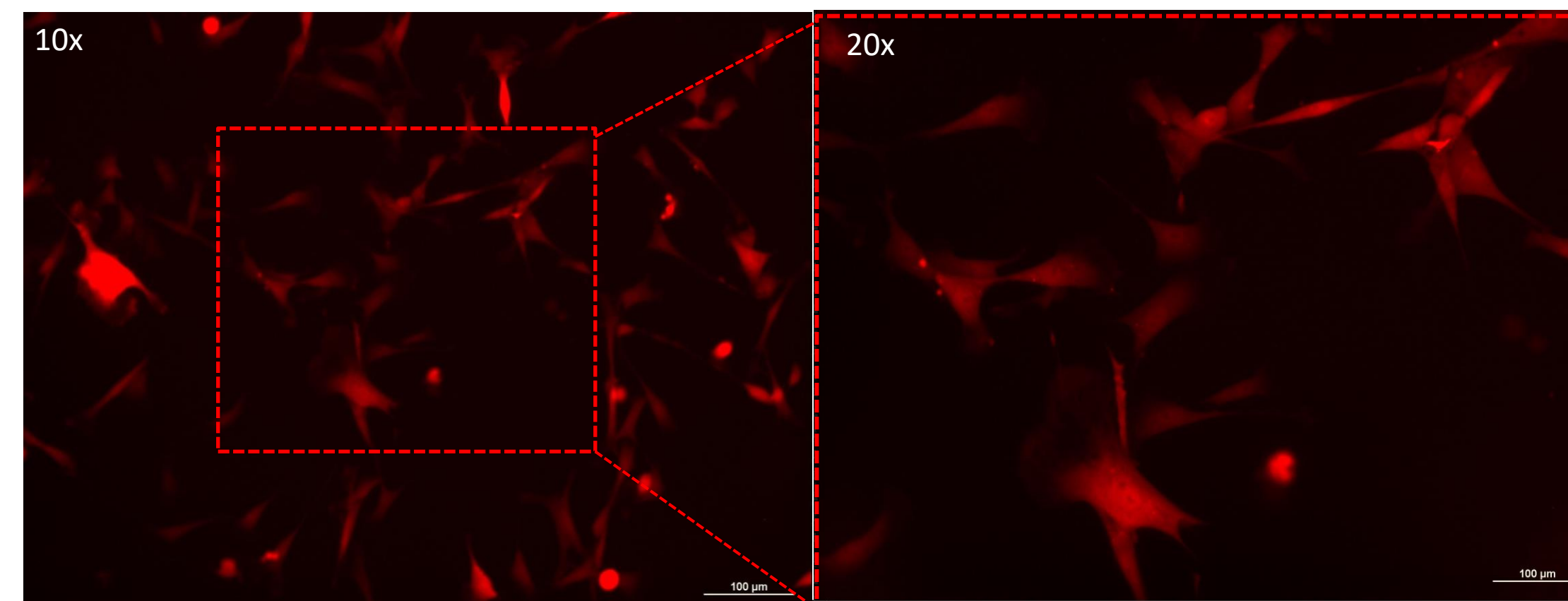


Fig. 2. **MSCs** marked with a red fluorescent protein.

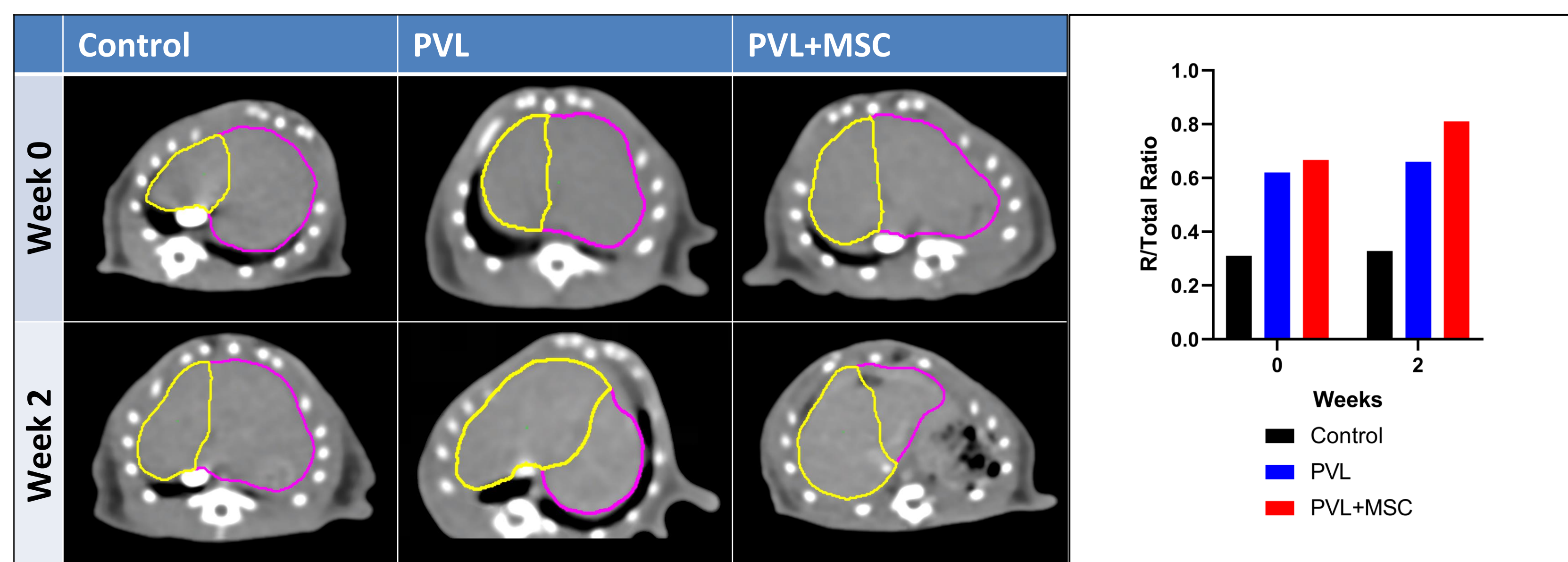


Fig. 3. **CT imaging of the rat model livers.** The scans indicate a significant increase in volume proportion of the non-ligated lobes when MSCs are in use.

Table 1. **Liver volume** estimations calculated using MIM7.1.4

Week 0	Control	PVL	PVL+ MSC	Week 2	Control	PVL	PVL+ MSC
	Whole	3.15 ml	4.31 ml		4.96 ml	Whole	4.34 ml
Left	2.10 ml	2.96 ml	3.15 ml	Left	2.99 ml	1.17 ml	1.54 ml
Right	1.05 ml	1.35 ml	1.81 ml	Right	1.35 ml	1.91 ml	3.08 ml
FLR/Total Ratio	0.33	0.31	0.36	FLR/Total Ratio	0.31	0.62	0.67

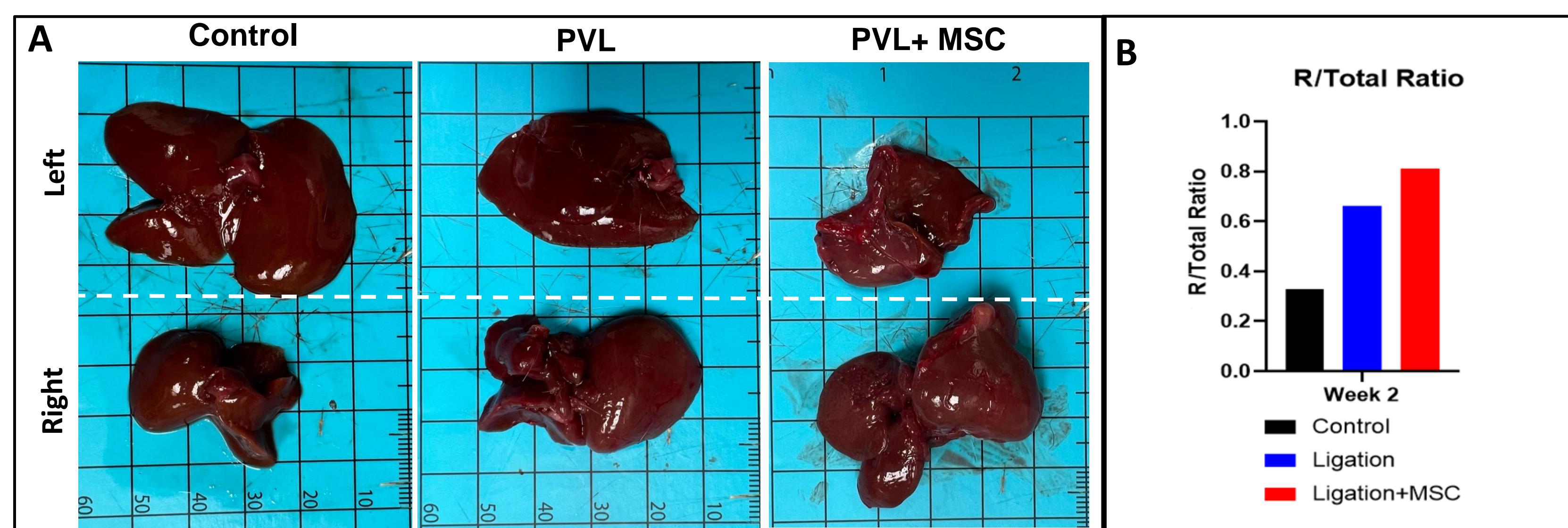


Figure 4. **Necropsy** of the ligated (left & middle) and non-ligated (right & caudate) liver lobes. (A) Photographs of the collected liver samples. (B) Quantification of the showed that the ligation + MSC-treated group had the highest FLR (right & caudate) to total weight ratio.

Table 2. **Liver weights** of the three rats at 2 weeks

Liver	Control	PVL	PVL+MSC
Left	5.2120 g	2.2039 g	1.3277 g
Right	2.5480 g	4.2783 g	5.6780 g
Total	7.7600 g	6.4822 g	7.0057 g
FLR/Total Ratio	0.33	0.66	0.81

Results and Discussion

- The data from our experiment clearly shows that MSCs are effective in increasing the FLR/Total weight ratio ($\approx 70\%$ increase) when used in conjunction with PVL, compared to the PVL only group ($\approx 40\%$ increase), as shown in Table 2.
- The correlation between the right to total liver ratios for the liver volumes estimated through the CT scans, and the liver weights, shows that CT scans are a viable option for determining the size of a patient's liver

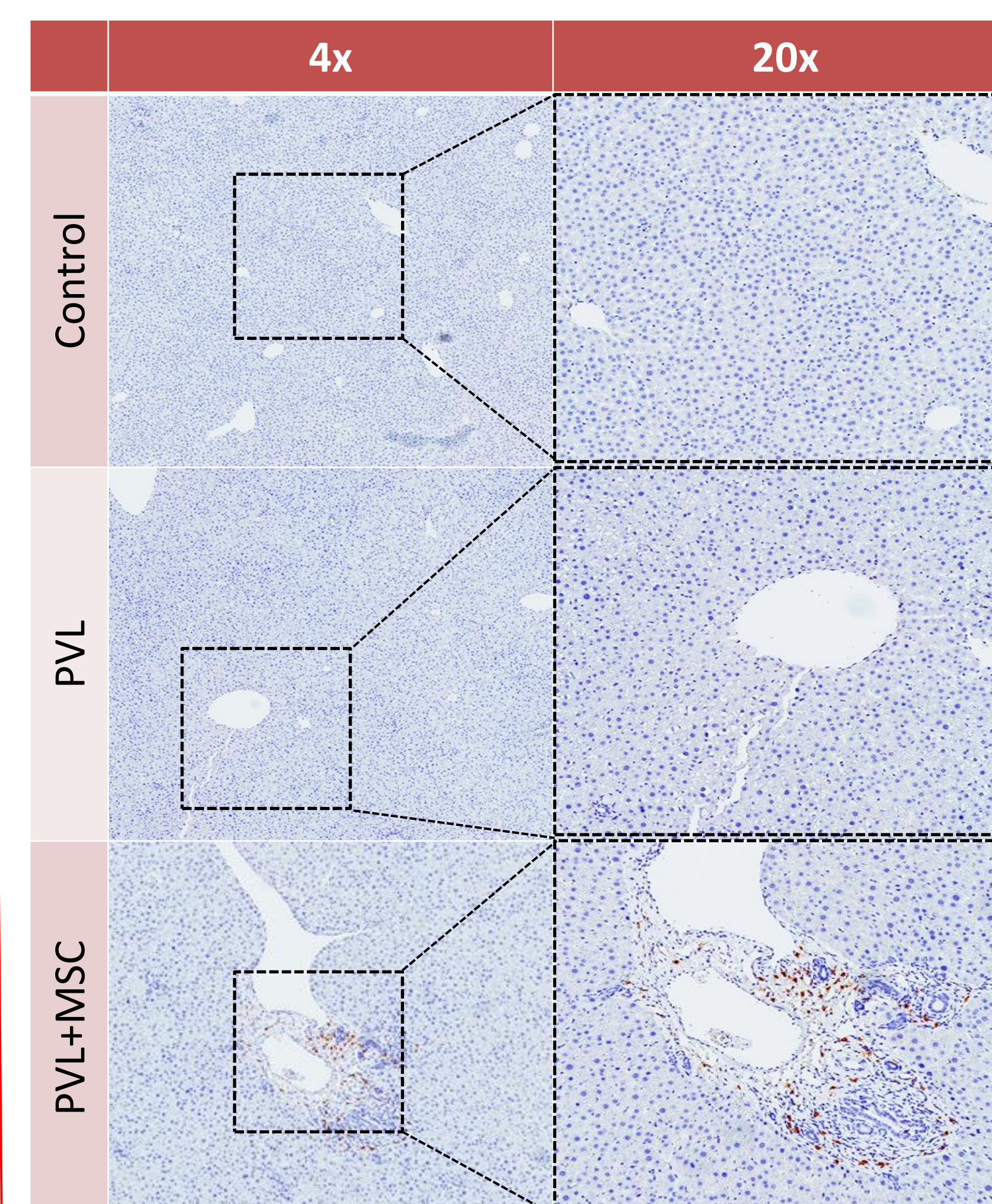


Fig.5. **Histology.** Liver samples were stained for the RFP and showed the presence of MSCs within the liver of the PVL+MSC rat.

Conclusion

The increase in liver growth with MSCs is significant due to the baseline FLR size needed for life saving surgery to be performed. Some patients may suffer from conditions such as mild cirrhosis or have had major chemotherapy exposure, causing the percentage of liver remnant volume needed for surgery to increase drastically. The increased liver growth caused by MSCs allow for a safe surgery and recovery post operation.

This experiment sets up baseline data for future experiments on liver growth and regeneration.

References

- Vyas et al. Portal vein embolization and ligation for extended hepatectomy. *Indian J Surg Oncol.* 2014 Mar;5(1):30-42. doi: 10.1007/s13193-013-0279-y.