Histology-Based Microscale Human Spleen Model for Radiopharmaceutical Therapy Dosimetry

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ABSTRACT

Purpose: For radiopharmaceuticals emitting high linear energy transfer (LET) particles, such as alpha particles, the spleen can receive substantial localized doses due to its reticuloendothelial function. To capture dose heterogeneity, this study aims to develop three-dimensional (3D) mesh-based microscale spleen models for humans, based on the reticulin-stained histology, to enable precise tissue-level dosimetry for radiopharmaceutical applications.

Methods: The microscale human spleen models were developed by segmenting the reticulin-stained human spleen histology slides. Seven regions of interest (ROIs), including four with large blood vessels, were imaged from the histology sections using a Zeiss Axiophot microscope. Among the splenic structures, white pulp (germinal, mantle, marginal zones), trabeculae, and vessels were manually segmented twice by independent observers to reduce segmentation uncertainties arising from human errors. Red pulp sinusoids were segmented based on the intensity thresholding approach. White pulp, trabeculae, and sinusoids were converted to 3D surface meshes using 3D Slicer software, which were then refined to remove defects using Rhinoceros 3D software. To consider the histology-induced blood shrinkage, the mesh models were enlarged to in vivo conditions based on a shrinkage factor, which was derived by measuring the mean diameter of the red blood cells from the images and comparing it with the literature values.

Results: Seven microscale human spleen models were generated in polygonal-mesh format based on histology images. The models capture realistic anatomy of red and white pulp, trabeculae, and blood vessels. These models are watertight, with no intersections or defects. These high-fidelity meshes are ready for Monte Carlo-based dosimetry, enabling tissue-level dose calculations for radiopharmaceutical applications, including high-LET particle therapies.

Conclusions: This study developed seven mesh-based microscale human spleen models that capture the precise splenic tissue anatomy with an approximate volume of 1.2 mm^3. These models will be used to produce radionuclide S values for use in radiopharmaceutical dosimetry.

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INTRODUCTION

Radiopharmaceutical therapy (RPT) is an emerging, novel approach for the treatment of cancer with the goal of high precision tumor cell death through the emission of radiation[1]. This treatment modality provides an advantage over traditional external beam radiotherapy (EBRT) that would otherwise be impractical for the treatment of widespread metastasized cancers, especially those that are rapidly proliferating. Radionuclides are coupled with a carrier system to selectively target characteristics by the tumor microenvironment allowing for DNA-damaging radiation delivery to the region of interest while sparing healthy surrounding tissue. Common carrier molecules coupled with radionuclides include antibodies, peptides, nucleic acids, small molecules, and nanoparticles[2].

Most developed radiolabeled peptides utilize beta emitters such as ^{90}Y and ^{177}Lu. However, due to the significant range of these radionuclides within normal tissue and the resistance to damage by hypoxic tumor tissue, this treatment method can be subject to failure. Alpha particles are a possible alternative given their double positive charge causes them to have a high linear energy transfer (LET). This means that it does more significant damage to DNA, and with their significantly shorter ranges than beta radiation, allows for better selective ablation of tumor cells and dose sparing of healthy tissue[3]. Research and development of hybrid carriers, combining the advantages of different types of radionuclide carriers, is still underway.

Given the systemic nature of radiopharmaceutical administration, the delivery of tumor dose is not the determinant of dose activity, rather the protection of normal organs. To properly estimate tissue dose in different organs and given the short path length of alpha and beta particles, a microscale model is needed to accurately get radiopharmaceutical activity source to target doses (S-values). The objective of this study is to develop a microscale histology-informed dosimetric model of the spleen to support the use of beta- and alpha-particle emitting RPT.

METHODS AND MATERIALS

Histology Manipulation

These spleen models were created as accurate to real anatomy as possible. To do this, histology sections of a human spleen were used to create segmentations of the microanatomy of a spleen. The slides used were teaching slides readily available at the University of Florida (UF). Because of the intention of these slides, they were not grouped nor in order, this required sorting through the slides for intact slides of the same group. After a selection of 33 reticulin stained slides were gathered, they were put in order by examining the anatomy that would change predictably from slide to slide. This included large blood vessels and white pulp nodules. 7 regions of interest (ROIs) were selected with generally uniform capture of the spleen microanatomy with 4 of the ROIs containing a large blood vessel to gather dosimetry data adjacent to the large trabecular blood vessels.

The images for each ROI were taken with a Zeiss Axiophot with a 10x objective zoom. Since the histology slides would be made in only roughly the same orientation, they needed to be aligned after imaging for accurate stacking and eventual modeling. This was done by taking each slide and using a combination of landmark and general registration in 3D Slicer. This allowed for each slice to be aligned in the z-axis. Due to the translation and rotation of each slice, some anatomy was missing. Each ROI was cropped so almost all anatomy was imaged with any small missing sections interpolated.

Segmentation

Once each slide was properly prepared, the desired anatomy was segmented. This included the germinal center, mantle zone, marginal zone, and periarteriolar lymphoid sheath (PALS) of the white pulp, sinusoids of the red pulp, trabeculae and the blood vessels. Due to the modeling process only the inside of the blood vessels were needed to segment, and red pulp tissue was not segmented as it was designated as the filler tissue in monte carlo simulations. Each anatomical feature was hand segmented besides the sinusoids as these are complex and therefor segmented using brightness thresholding. The hand segmentation was done twice total by two of us. This allowed for more accurate segmentation and the ability for one person to find any anatomy missed by the other. Figure 1 on the right shows a grey-scale version of a histology slide and the subsequent segmentation labeled.

Modeling

The modeling of the ROIs started with forcing 3D slicer to space out the slices so they could further be manipulated to accomplish the correct z-axis spacing. This spacing was found by taking a germinal center that was entirely captured by one of the ROIs and assuming it would be spherical. The radius at its maximum was taken and using the slides it appeared in, a z-axis spacing of about 10 µm of space between each slide. This value is on the higher side for histology slides but still reasonable and within error considering the incomplete slide set.

Once each slide was correctly spaced the gaps had to be filled to create a valid closed mesh. This was done in Rhino 3D using the Shrink Wrap command. The shrink-wrap closely contours the segmented anatomy while filling in the spaces. This was used to model the white pulp, trabeculae, large blood vessels, and any major junctions in the vessels. Figure 2 shows an example of a germinal center that has been shrink-wrapped.

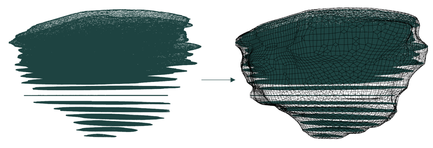

Figure 2: Germinal Center Shrink Wrap before and after

The blood vessels would change readily slide to slide and with how small many of them are, shrink-wrapping was not viable to model them. The modeling of blood vessels involved taking the center of each segmented blood content with additional points based on inference. These points were connected using the curve through points command in Rhino and the line was used a base for the pipe command. The pipe diameters were found using the distance measuring function in 3D slicer and mapped to the correct points in the mesh model, allowing for the creation of cylindrical blood vessels with diameters based off the anatomy. Figure 3 shows an example the blood vessel modeling process.

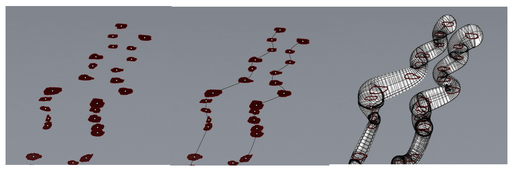

Figure 3: Blood Vessel Modeling process.

The sinusoids were modeled similarly with shrink-wraping but utilizing a shorter edge length to accommodate the fine detail of sinusoids. However, before that was done many of the “sinusoids” had to be culled. Since thresholding was used to segment them there were many tiny artifacts labeled as sinusoids. To have only true sinusoids remain, 112 sinusoids with a preference for lower diameters were measured in 3D slicer and an average diameter was found, then subtracting this by 1.5 times the standard deviation a minimum sinusoid diameter was determined. Anything below this value was highly likely an artifact and was removed from the model.

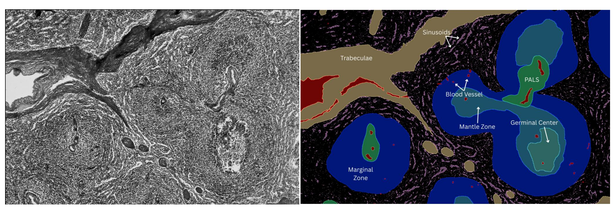

Figure 1: ROI5 segmentation histology slide and segmentation.

ALRADS *Advanced Laboratory for Radiation Dosimetry Studies*

Final Adjustments

To make the model accurate to in vivo conditions, two scaling factors were required. One scaling factor was to account for 3D slicer assigning its own dimensions to the images. This was solved by taking an image of a B&L micrometer with the same parameters as the histology slides. The distance in 3D slicer was measured and a slicer distance to real distance ratio was found to be about 0.48.

The next scaling factor required was to account for the histology slide creation process. When the sample prepared for slicing, the sample will shrink. To make the model match in vivo conditions, this shrinkage factor has to be found. This was accomplished by looking at the average red blood cell diameter in the histology slides and comparing that to the known value of average RBC diameter. The shrinkage factor was found to be 42.37%, meaning the models had to be scaled to rectify this discrepancy.

The models at that point are done and scaled properly. However, for tetrahedralization to work for PHITS inputs, there must be no intersections between models and within the same model. This was rectified using many different methods in both Blender and Rhino 3D. Once this was done, the models were tetrahedralized using POLY2TET and prepared for PHITS runs.

RESULTS

The models of all 7 ROIs were finished while preserving anatomical accuracy to the imaged histology slides. Figure 4 shows the example of ROI 7 with the sinusoids included and removed, as the sinusoids block the view of much of the anatomy.

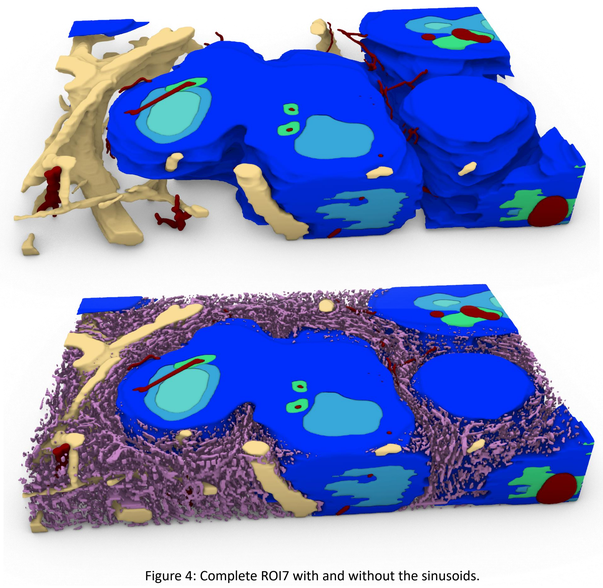

Figure 4: Complete ROI7 with and without the sinusoids.

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REFERENCES

1. Shi M, Jakobsson V, Greifenstein L, et al. Alpha-peptide receptor radionuclide therapy using actinium-225 labeled somatostatin receptor agonists and antagonists. *Front Med*. 2022;9:1034315. doi:10.3389/fmed.2022.1034315
2. Zhang T, Lei H, Chen X, et al. Carrier systems of radiopharmaceuticals and the application in cancer therapy. *Cell Death Discov*. 2024;10(1):16. doi:10.1038/s41420-023-01778-3
3. Koh TT, Bezak E, Chan D, Cehic G. Targeted alpha-particle therapy in neuroendocrine neoplasms: A systematic review. *World J Nucl Med*. 2021;20(4):329-335. doi:10.4103/wjnm.wjnm_160_20