

Porcine Thermal Injury Rubric User Guide:

The overall goal is to evaluate sections to determine if the epidermis and dermis has been damaged and assess the severity of observed damage. Examples are included to illustrate the various skin associated structures and how they appear when thermally damaged.

1. Two different skin layers will be evaluated for thermal injury
 - A. Epidermis
 - B. Dermis
2. When being asked to grade depth of injury, split the sample up into quarters from superficial to deep and define the **deepest depth** of damage within the dermis (collagen denaturation, vascular blockage, or dermal appendages).
 - A. Grade 0: 0% Normal/Uninjured → there is no observed damage within the dermis
 - B. Grade 1: 1-25% → damage within the dermis was observed within this range
 - C. Grade 2: 25-50% → ""
 - D. Grade 3: 50-75% → ""
 - E. Grade 4: 75-99% → ""
 - F. Grade 5: 100%: → damage was observed throughout the entire dermis (Full-thickness)
3. When being asked to grade thermal injury, you will be also be asked to identify the **intensity** of damage for each metric.
 - A. Grade 0: None → there is no damage to the structure/ECM component
 - B. Grade 1: Mild → there is a chance that the structure/ECM component can recover from the damage
 - C. Grade 2: Moderate → there is a small chance that the structure/ECM component can recover from the damage
 - D. Grade 3: Severe → there is no chance that the structure /ECM component can recover from the damage

Porcine Thermal Injury Rubric User Guide:

4. The rubric consists of 9 questions that assess the:
 - A. Quality of the Section (**Q1**)
 - B. Presence of Thermal Damage (**Q2**)
 - C. Epidermis (**Q3**)
 - D. Dermis (**Q4-Q9**)
 - I. Thermal Damage (**Q4**)
 - II. Collagen Denaturation Depth (**Q5**) and Intensity (**Q6**)
 - III. Depth of Vascular Congestion (**Q7**)
 - IV. Dermal Appendage Depth (**Q8**) and Intensity (**Q9**)

On the following slides you will see:

1. The questions from the rubric
2. Some instructions/explanations
3. Representative examples of how the various structures in damaged skin appear.
4. Images that correspond to the scores to assign.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>1. Do you find the section to be of high enough quality to evaluate? Examples of flaws in the sections that would hinder evaluation include air bubbles, folds or tears, uneven staining, or obvious artifact</p>				N/A

Question 1: Identify if you find the section to be of high enough quality to evaluate. If no, do not proceed. If yes, proceed.

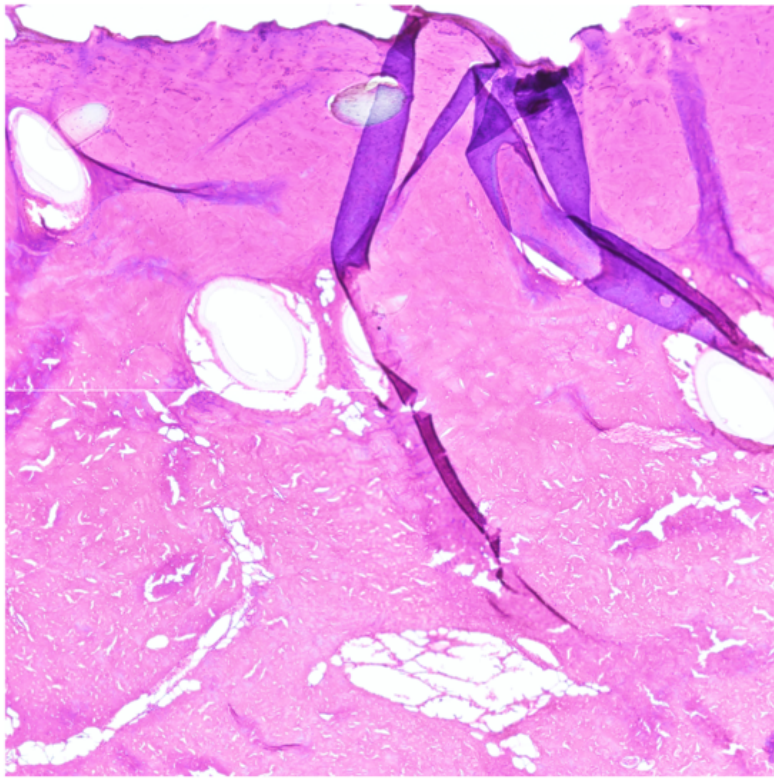


Figure 1. Examples of folds.

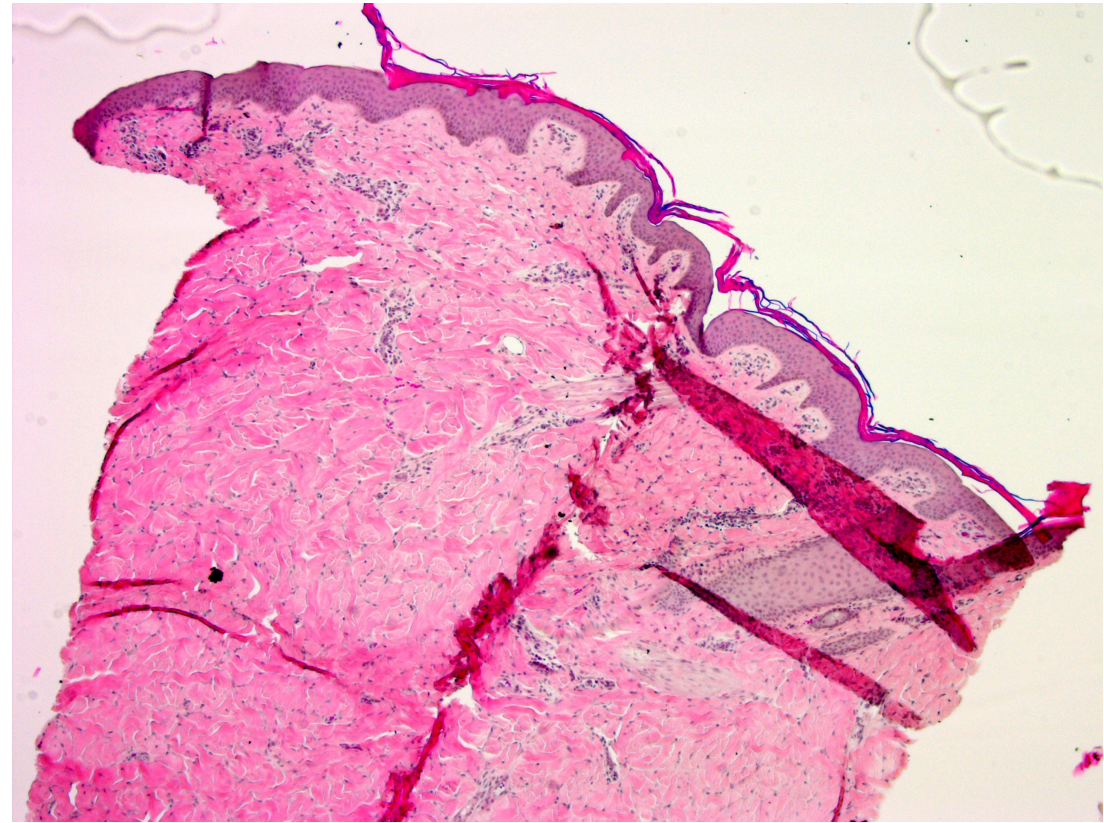


Figure 2. Examples of folds and tears

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
2. Do you perceive there to be thermal damage in this section?				N/A

Question 2: Identify if thermal injury is present at all in the tissue. If no, do not proceed. If yes, proceed.

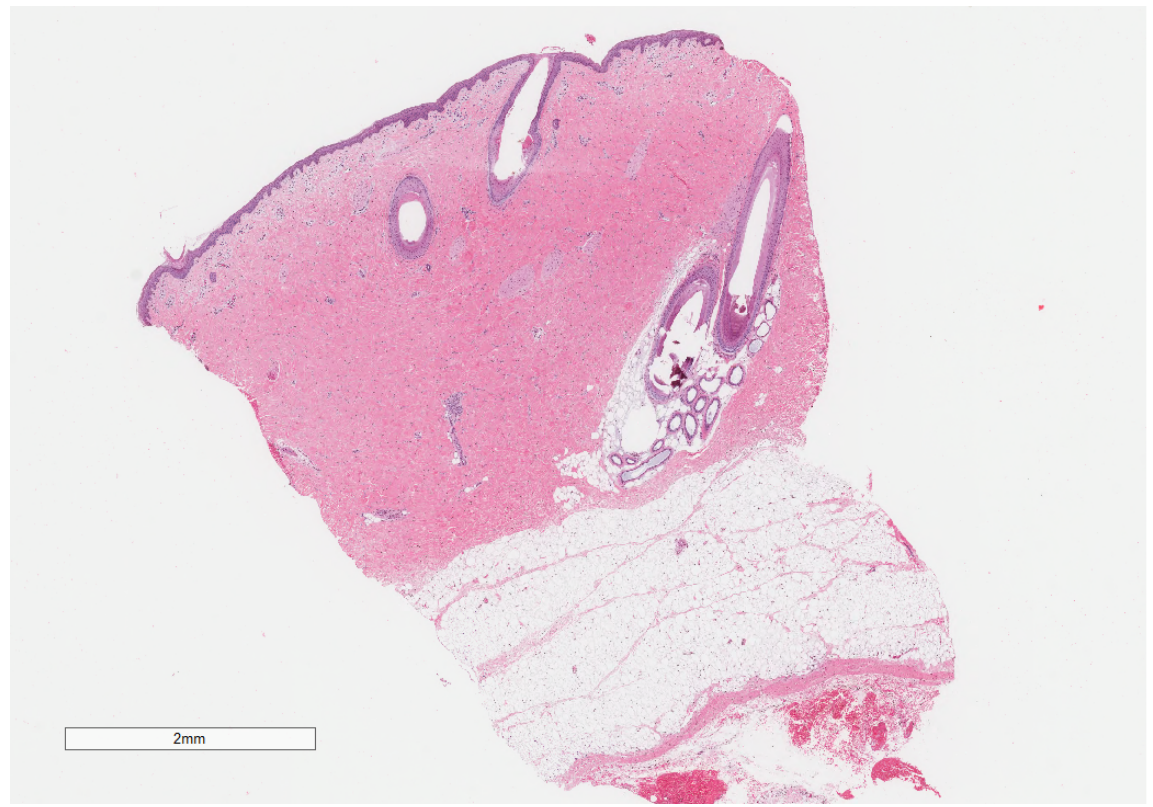


Figure 3. Uninjured skin. 2mm scale bar.

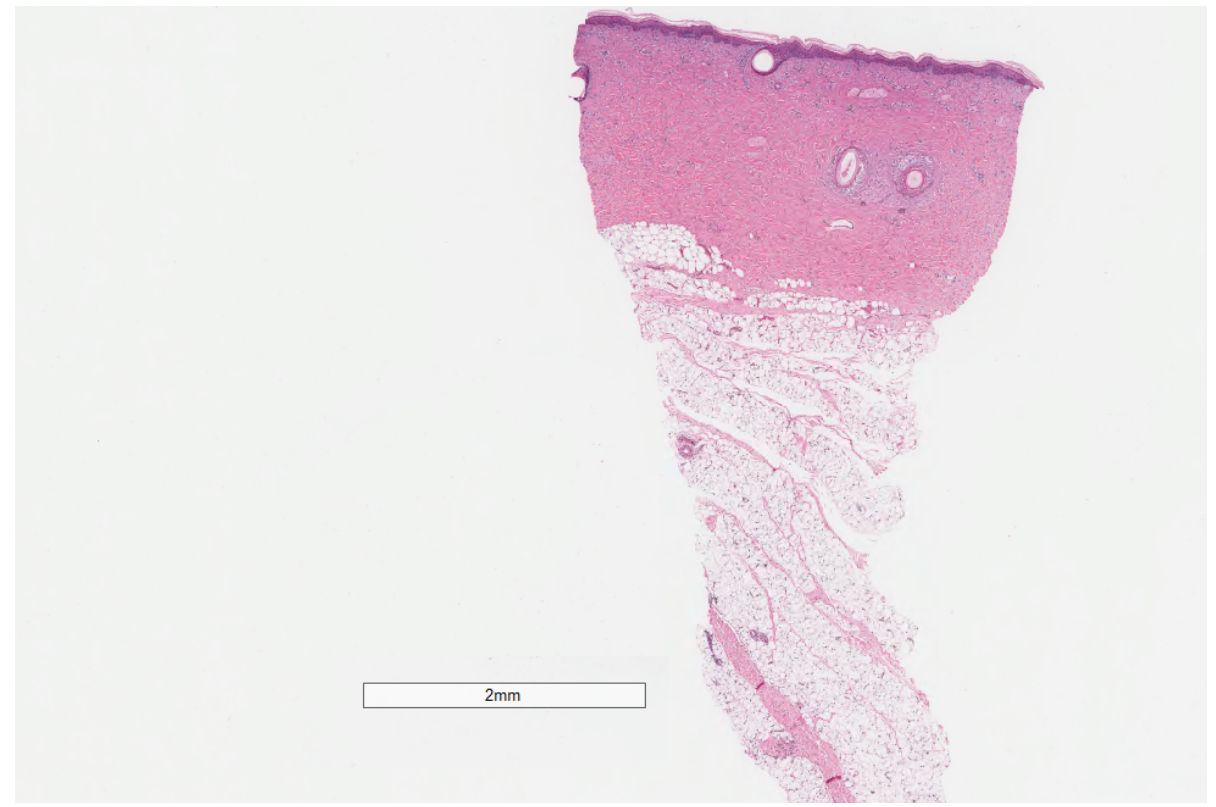


Figure 4. Thermally injured skin. 2 mm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>3. Can you observe any thermal damage to the epidermis and at what intensity?</p> <p>Damage can be defined as elongated string bean nuclei, loss of staining intensity, flattening, disruption, or loss of adherence of epidermal layer.</p>				<p>Reference Figures 5-8</p> <p>0: None</p> <p>1: Mild</p> <p>2: Moderate</p> <p>3: Severe/marked</p>

Question 3: Grade the epidermal damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade.

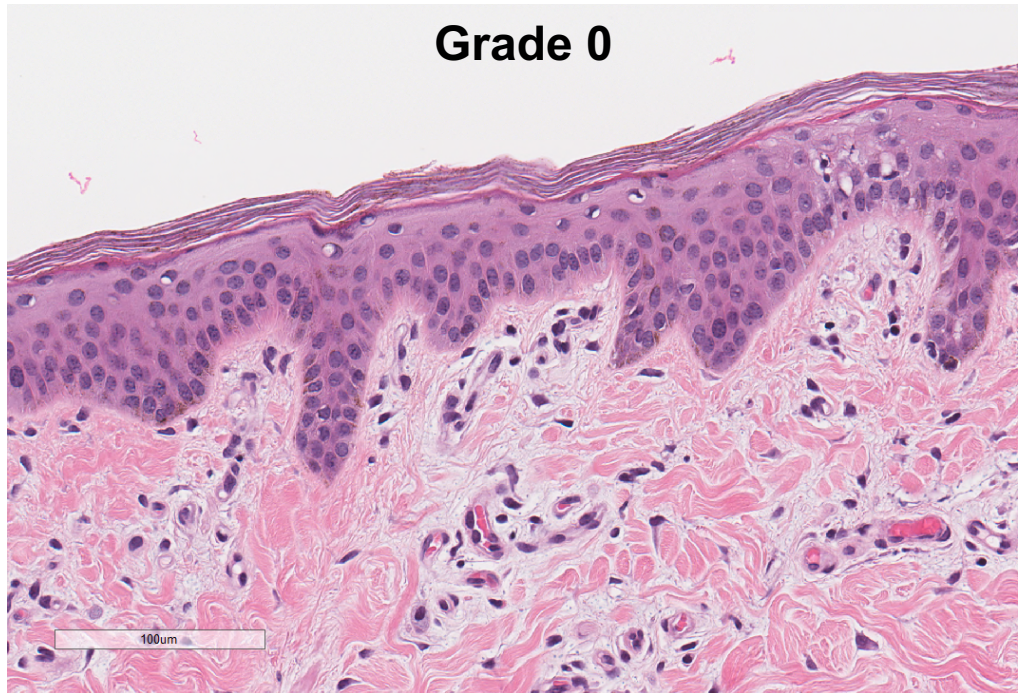


Figure 5. Un-injured skin. 100 µm scale bar.

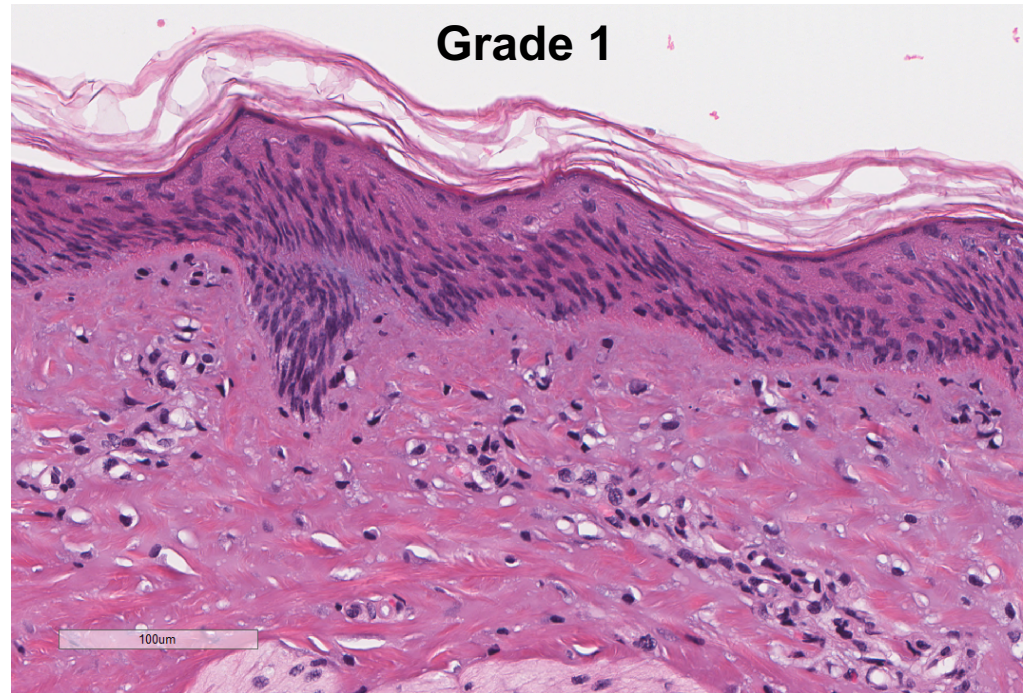


Figure 6. Thermally injured skin. Elongated string bean nuclei make up the vast majority of the cells are present in the epidermis. There is flattening of the dermal-epidermal junction (DE) leading to loss of rete ridges. 100 µm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>3. Can you observe any thermal damage to the epidermis and at what intensity?</p> <p>Damage can be defined as elongated string bean nuclei, loss of staining intensity, flattening, disruption, or loss of adherence of epidermal layer.</p>				<p>Reference Figures 5-8</p> <p>0: None</p> <p>1: Mild</p> <p>2: Moderate</p> <p>3: Severe/marked</p>

Question 3: Grade the epidermal damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade.

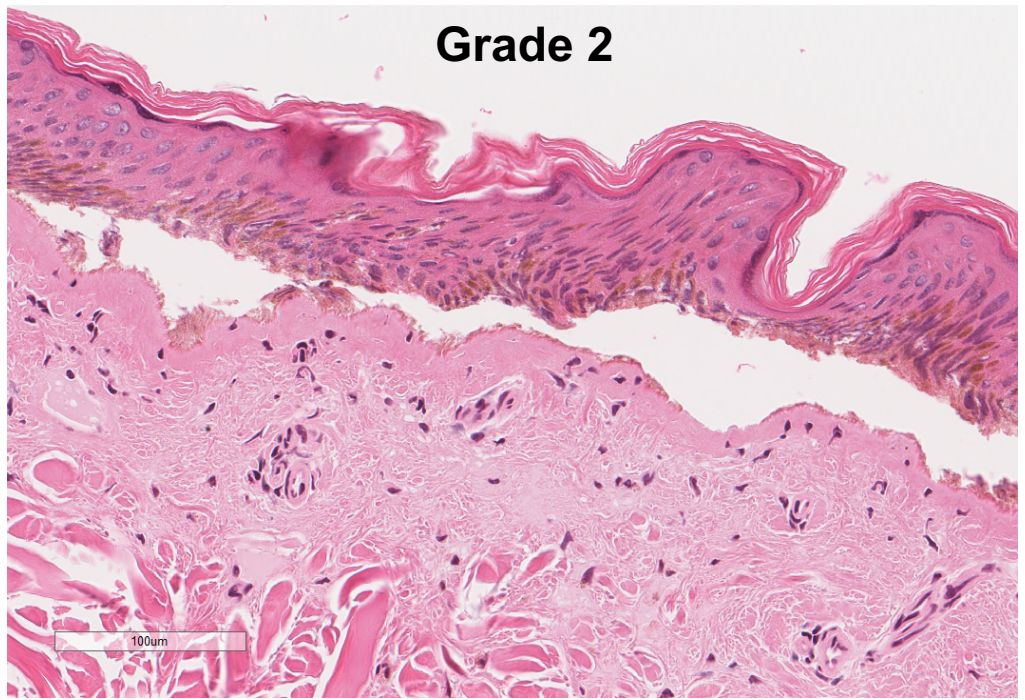


Figure 7. Thermally injured epidermis. Flattening and disruption of DE junction. 100 µm scale bar.

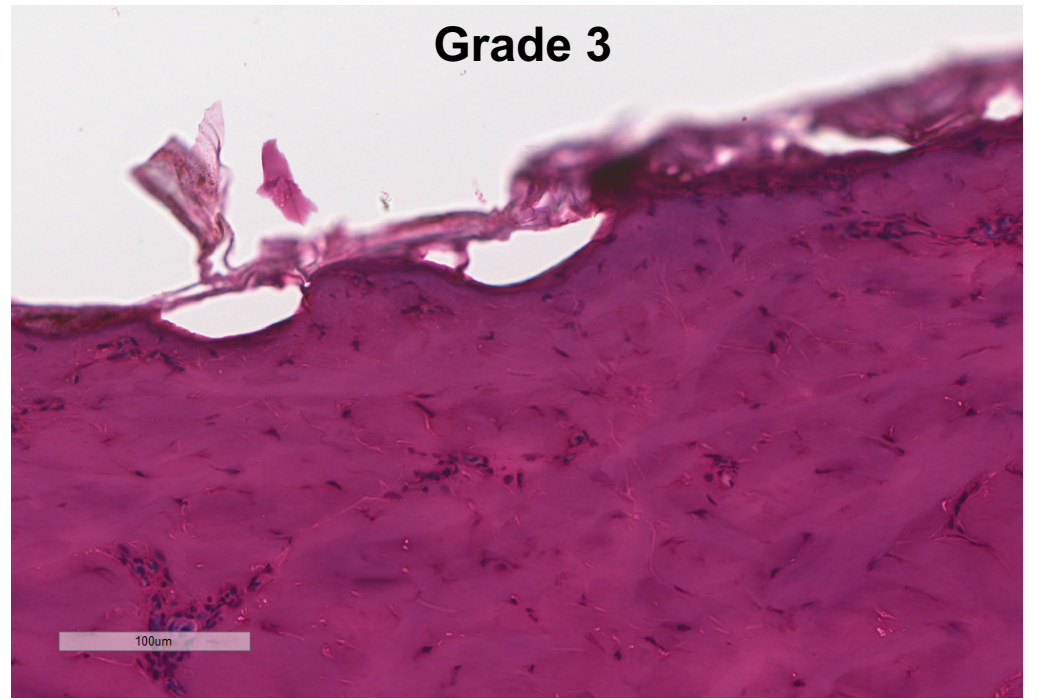


Figure 8. Severely thermally injured skin. The epidermis has lost adherence and is completely destroyed. 100 µm scale bar

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
4. Can you observe any thermal damage to the dermis?				N/A

Question 4: Identify if there is any dermal damage in the section. This question leads into the remaining questions and you may need to examine those examples to become familiar with the features to evaluate dermal damage.

If no, do not proceed. If yes, proceed.

There are 3 metrics by which you can assess dermal damage: collagen denaturation (**Q5 and Q6**), vascular blockage (**Q7**), and dermal appendages (**Q8 and Q9**).

Specifically, you will also be asked to evaluate the **deepest depth** of damage for collagen denaturation (**Q5**), vascular blockage (**Q7**), and dermal appendages (**Q8**).

You will also be asked to evaluate the **intensity** of damage for collagen denaturation (**Q6**) and dermal appendages (**Q9 – 2 sets of examples: 1 with hair follicles (HF) and 1 with HF and glands**).

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>5. Split the sample up into quarters from superficial to deep. What level does the collagen architecture/discoloration- related damage extend to?</p> <p>Damage can be defined as altered or denatured collagen architecture. Collagen fibers are thin, flattened, or absent and can contain fissures.</p>	N/A	N/A		<p>Reference Figures 9-14</p> <p>0: 0%: Normal, uninjured</p> <p>1: 1-25%</p> <p>2: 25-50%</p> <p>3: 50-75%</p> <p>4: 75-99%</p> <p>5: 100%: full thickness (entire dermis damaged)</p>

Question 5: Define the level of collagen denaturation. Below are representative examples of the 50-75% group and a 100% group. It is recommended to zoom in to high magnification and draw a line demarcating the damage.

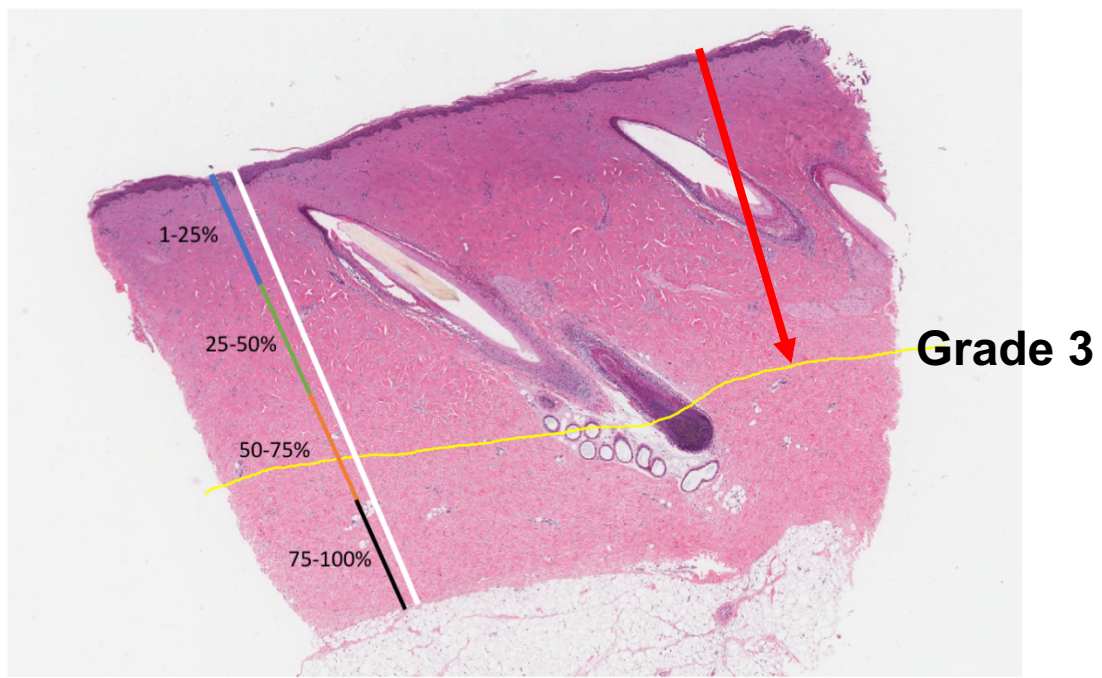


Figure 9. 50-75% Collagen Denaturation

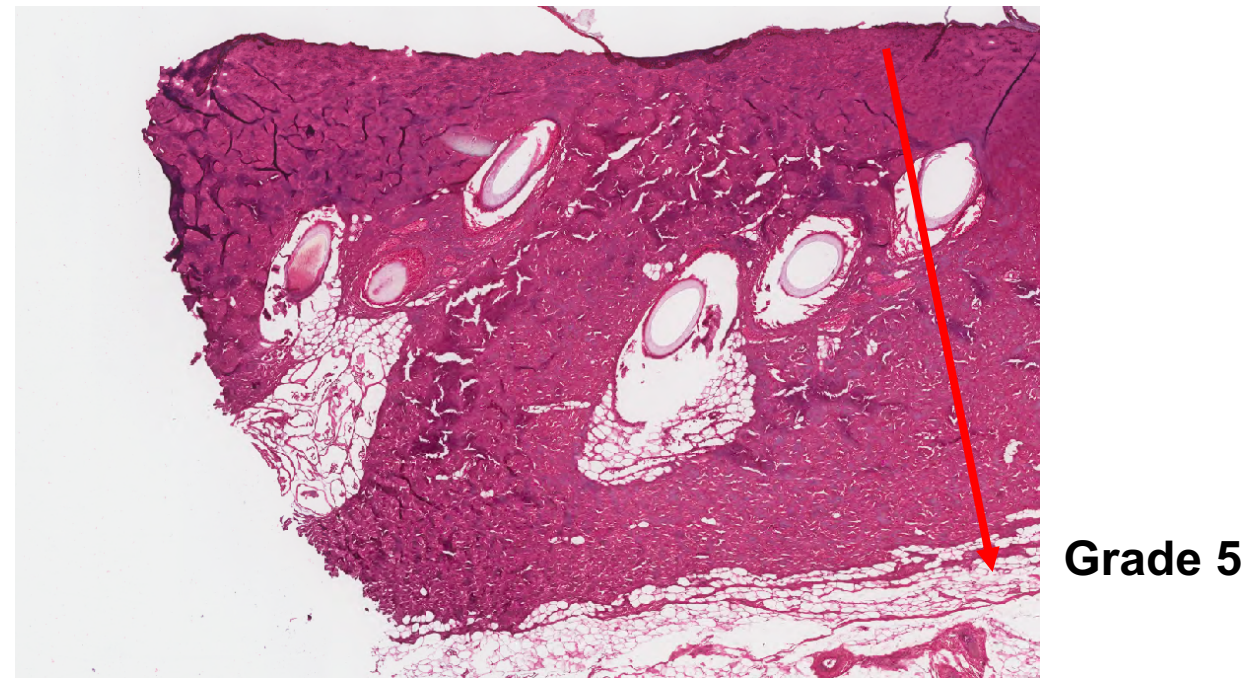


Figure 10. 100% Collagen Denaturation

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
6. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 9-14 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 6: Grade the dermal damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade.

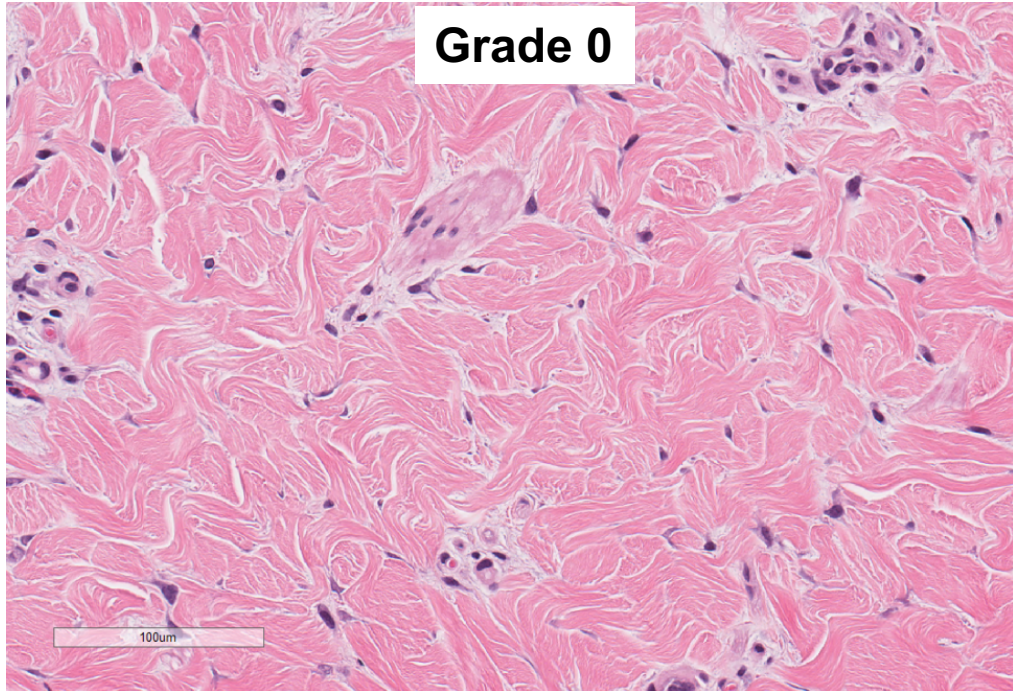


Figure 11. Uninjured skin with normal collagen architecture. 100 μm scale bar.

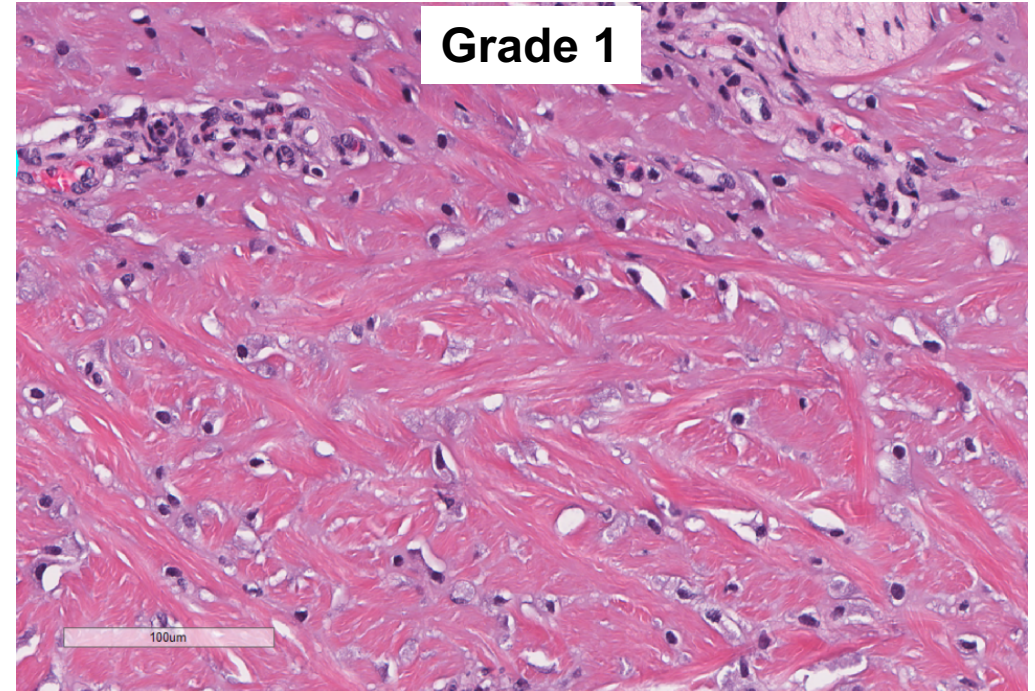


Figure 12. Thermally injured skin with alteration/denaturation of collagen architecture and coloration. 100 μm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
6. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 9-14 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 6: Grade the dermal damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade.

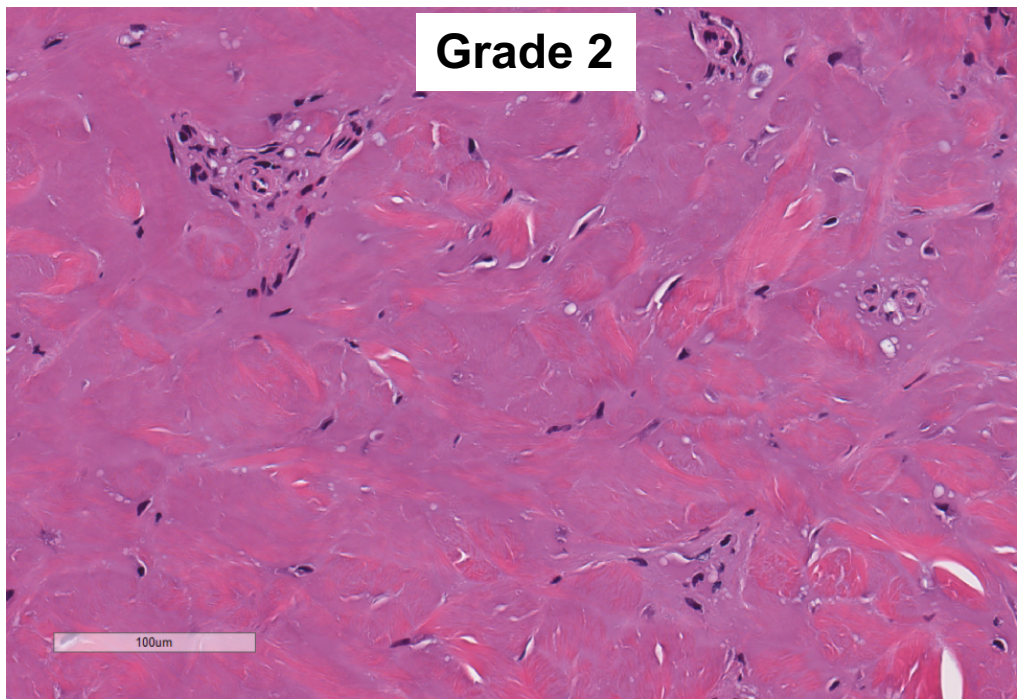


Figure 13. Thermally injured skin with alteration/denaturation of collagen architecture and coloration. 100 μ m scale bar.

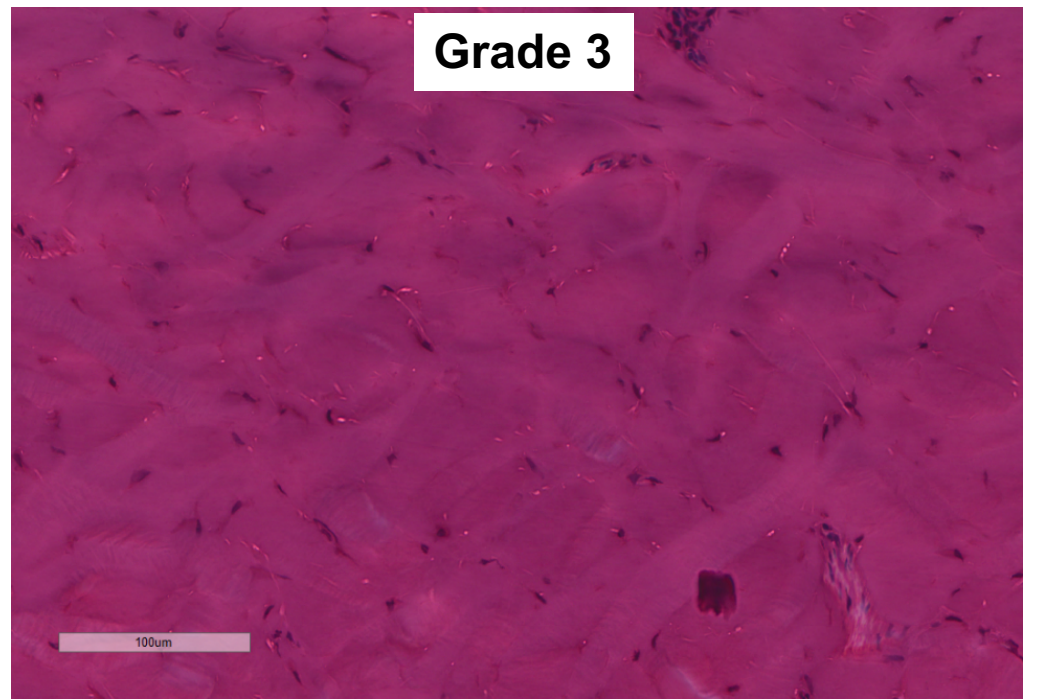


Figure 14. Severely thermally injured skin with severe alteration/denaturation of collagen architecture and coloration. Decreased cellularity. 100 μ m scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>7. Split the sample up into quarters from superficial to deep. What level does the vascular blockage extend to?</p> <p>Vascular blockage can be defined as blocked blood vessels tightly packed with RBCs (vascular congestion/ occlusion).</p>				<p>Reference Figures 15-16</p> <p>0: 0%: Normal, uninjured</p> <p>1: 1-25%</p> <p>2: 25-50%</p> <p>3: 50-75%</p> <p>4: 75-99%</p> <p>5: 100%: full thickness (entire dermis damaged)</p>

Question 7: Identify the deepest level of tissue where there is a vascular blockage at high magnification. Assign a score.

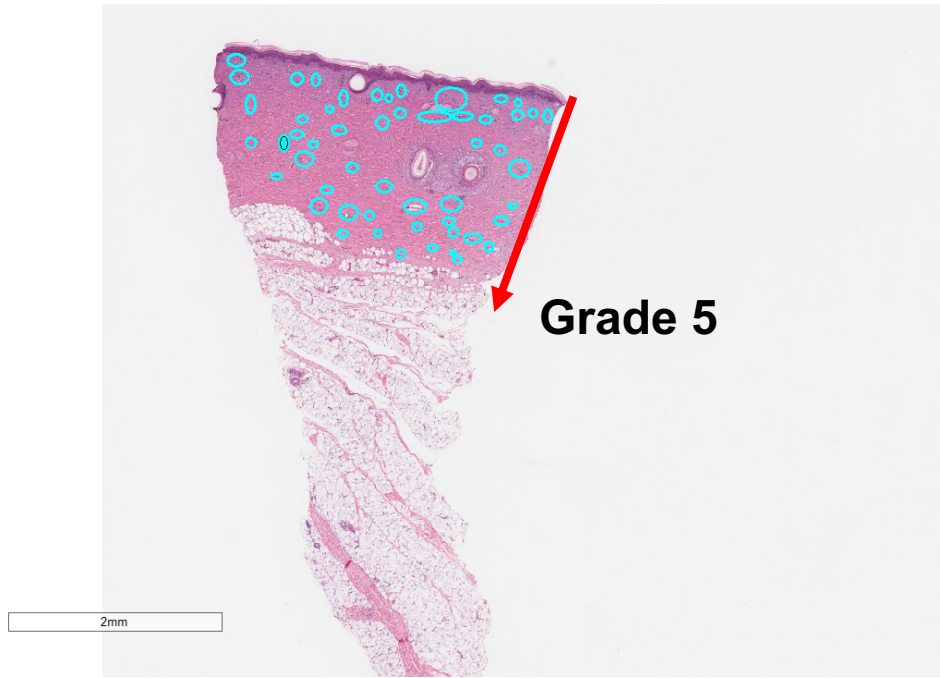


Figure 15. Thermally injured skin. Blue circles=blood vessels. 2 mm scale bar.

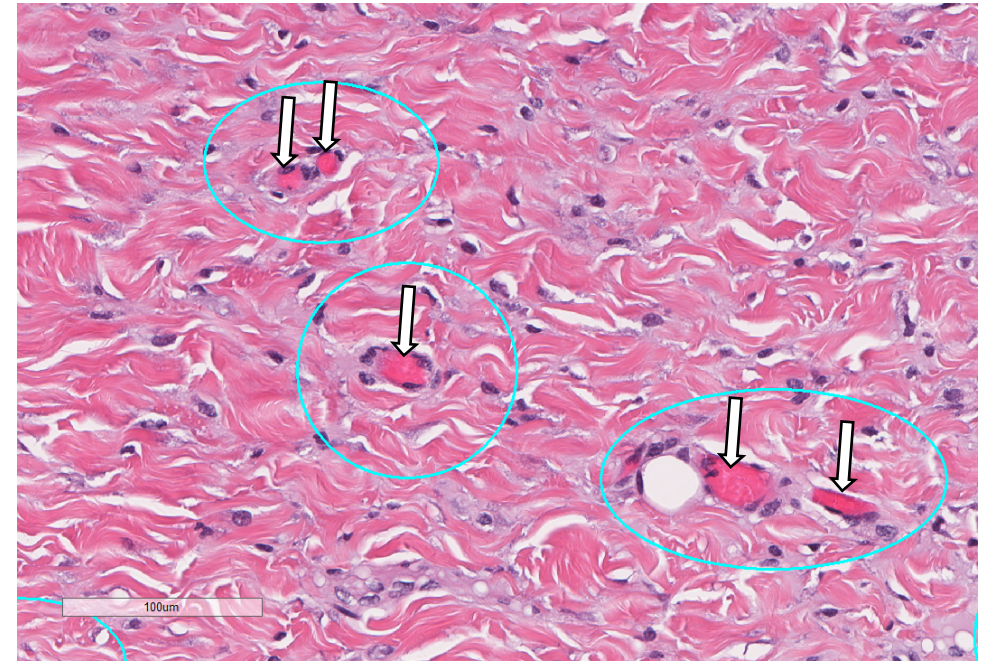


Figure 16. Thermally injured skin. Blue circles=blood vessels. White arrows=occlusion. 100 µm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
<p>8. Split the sample up into quarters from superficial to deep. What level does the damage extend to when evaluating the dermal appendages, i.e. epithelial cells in hair follicles, apocrine sebaceous glands, endothelial cells in vessels and vessel lumens.</p> <p>Damage can be defined as vacuolization, elongated string bean nuclei, congested vessels, condensed chromatin, or cells that are broken apart or destroyed.</p>				<p>Reference Figures 17-26</p> <p>0: 0%: Normal, uninjured</p> <p>1: 1-25%</p> <p>2: 25-50%</p> <p>3: 50-75%</p> <p>4: 75-99%</p> <p>5: 100%: full thickness (entire dermis damaged)</p>

Question 8: Define the level of appendage damage. Below are representative examples of the 50-75% group and a 100% group. It is recommended to zoom in to high magnification and draw a line demarcating the damage.



Figure 17. 50-75% Dermal appendage damage. Red circle = undamaged, blue circle=damaged, yellow line = line of damage indicators. 1 mm scale bar.

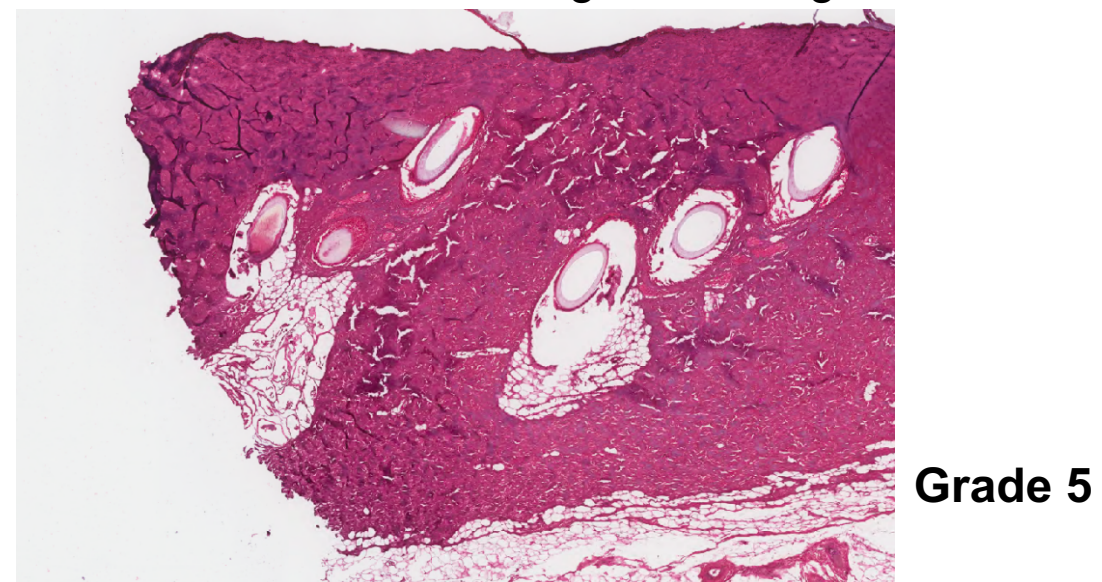
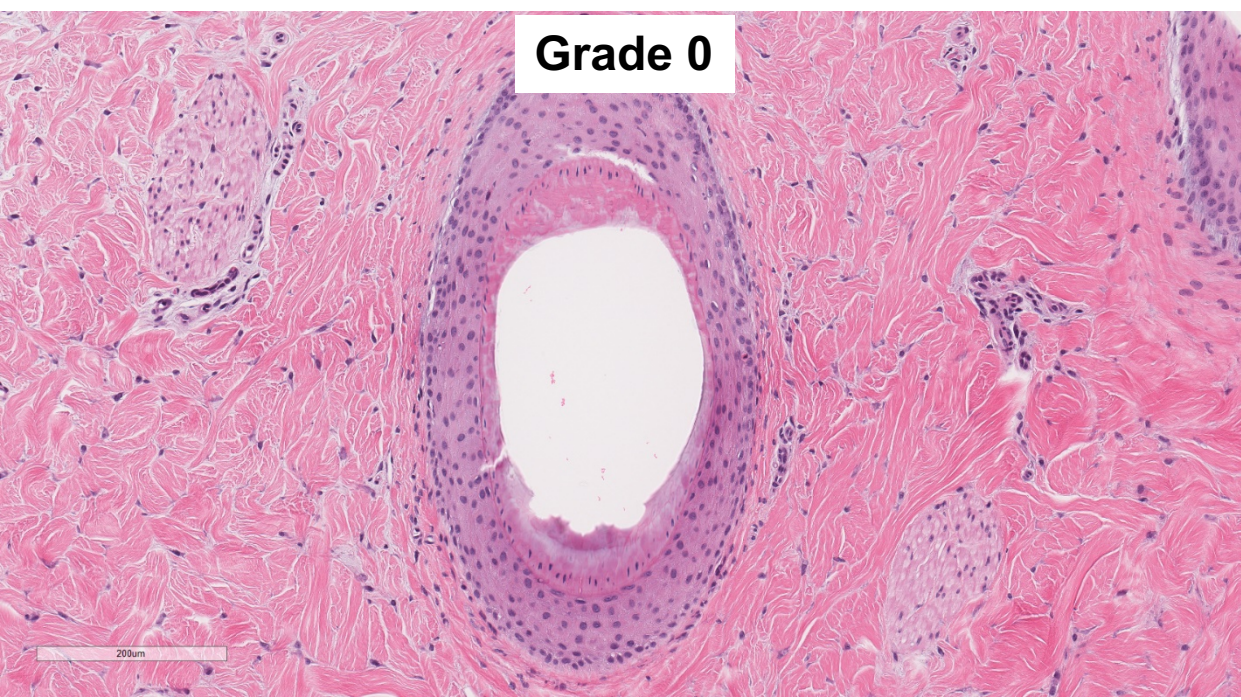


Figure 18. 100% Dermal appendage damage. 1 mm scale bar.

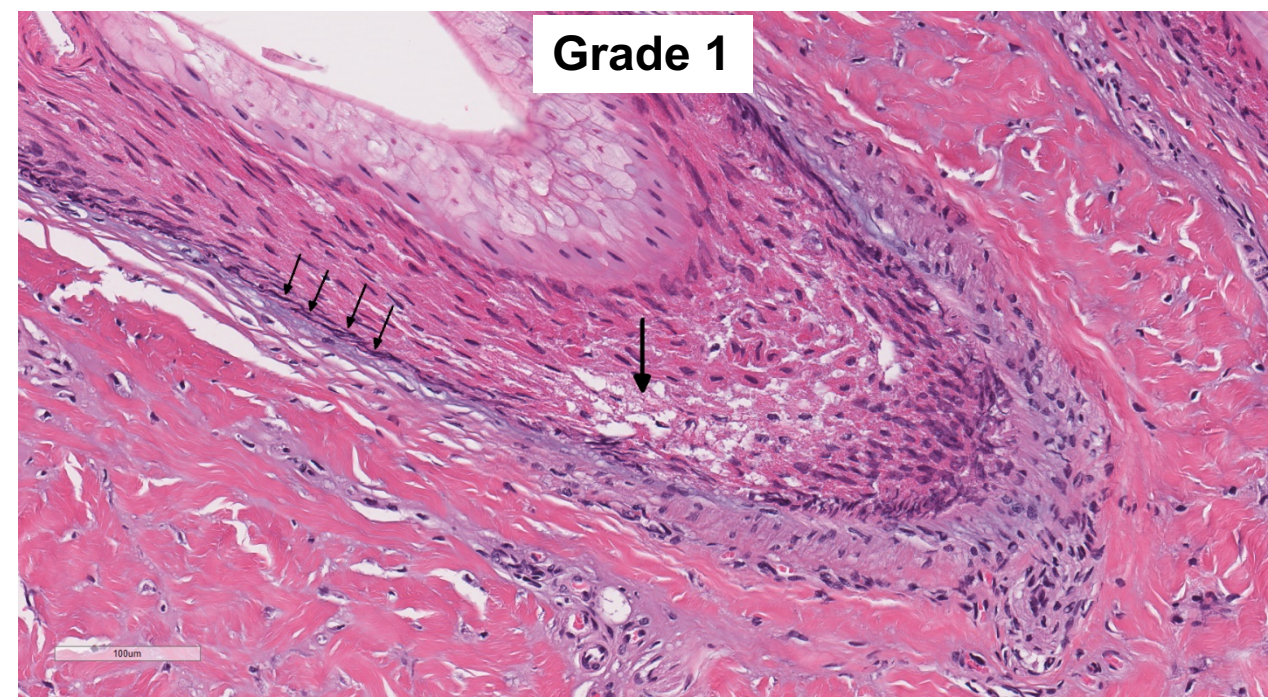
Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
9. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 19-26 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 9: Grade the dermal appendage damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade for the **HAIR FOLLICLE**.



Grade 0

Figure 19. Uninjured skin with normal collagen architecture. 200 μm scale bar.

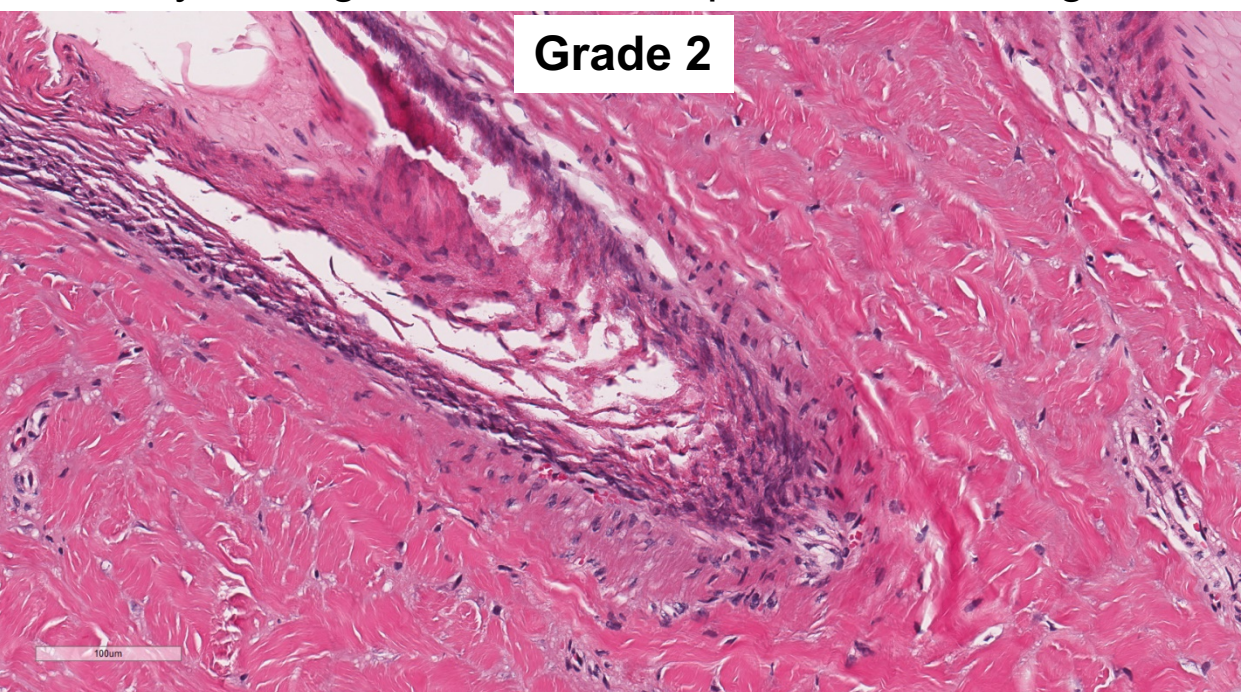


Grade 1

Figure 20. Thermally injured skin. Elongated string bean nuclei are present. The cells at the base of the hair follicle are damaged. 100 μm scale bar.

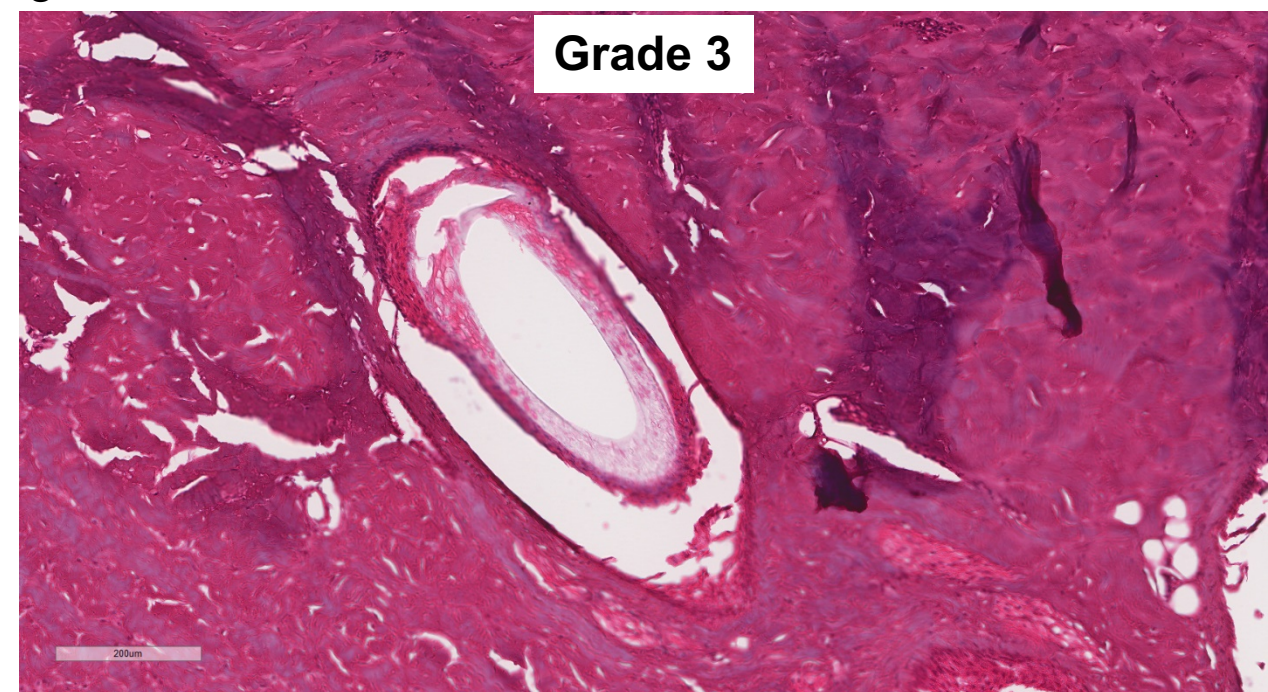
Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
9. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 19-26 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 9: Grade the dermal appendage damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade for the **HAIR FOLLICLE**.



Grade 2

Figure 21. Thermally injured skin. Elongated string bean nuclei are predominant. The cells at the base of the hair follicle are damaged and broken apart. 100 μm scale bar.



Grade 3

Figure 22. Thermally injured skin. The hair follicle and all cells are destroyed. 200 μm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
9. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 19-26 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 9: Grade the dermal appendage damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade for the **HAIR FOLLICLE and GLANDS**.

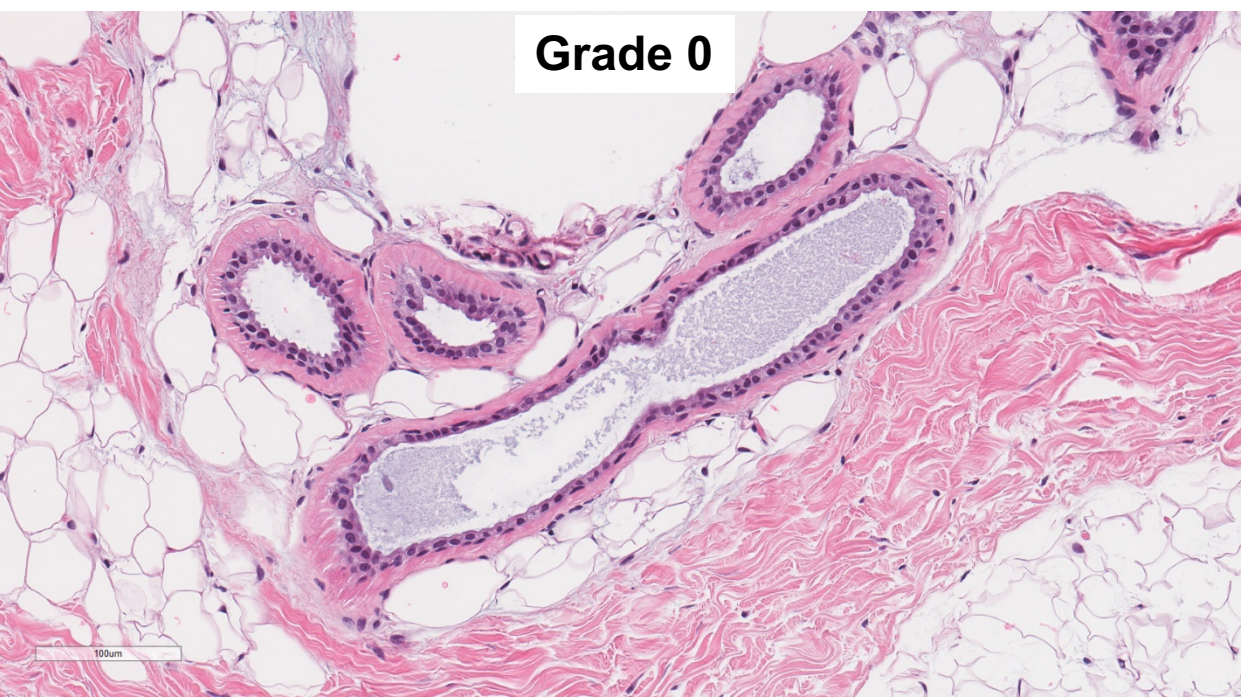


Figure 23. Uninjured skin. 100 μm scale bar.

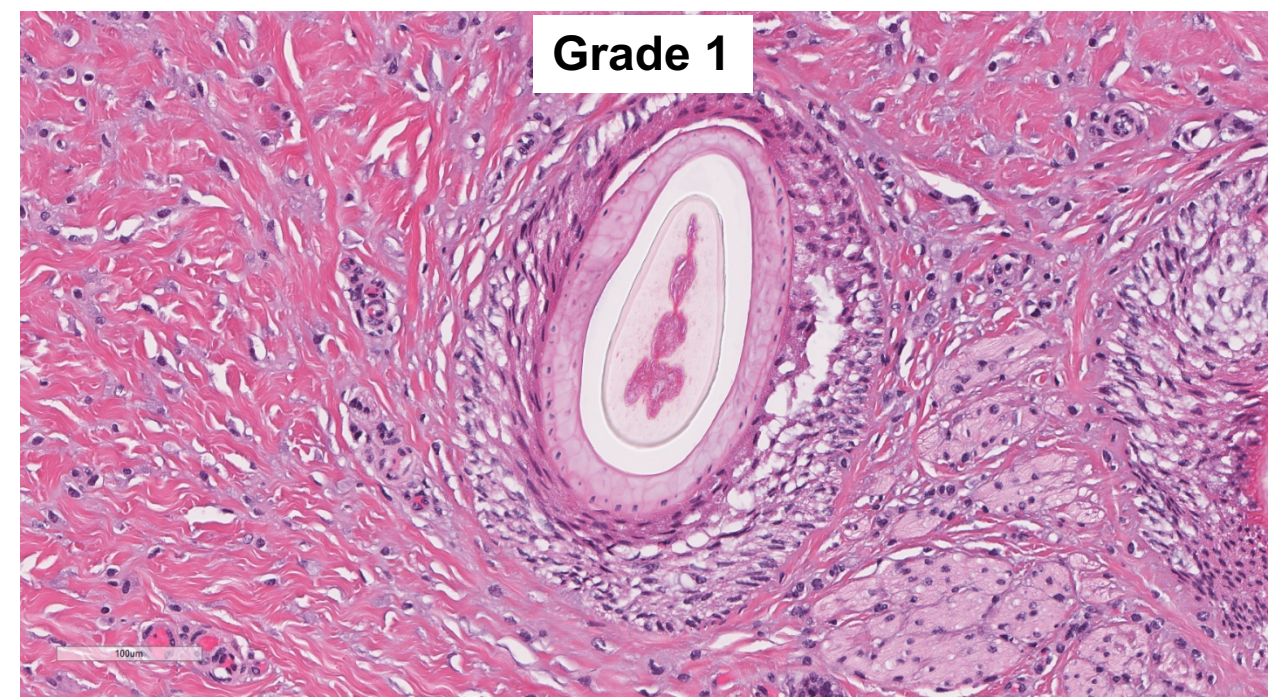


Figure 24. Thermally injured skin. Elongated string bean nuclei are present. The cells within the hair follicle are damaged. There are some congested vessels beside the follicle. The glands still look normal. 100 μm scale bar.

Question	Yes	No	Comments	Score/Intensity of Damage: Circle One
9. What is the intensity of damage at the level of the deepest dermal damage?	N/A	N/A		Reference Figures 19-26 0: None 1: Mild 2: Moderate 3: Severe/marked

Question 9: Grade the dermal appendage damage on a scale of 0-3 with 0 being no damage and 3 being the most severely damaged. Below are representative images of each grade for the **HAIR FOLLICLE and GLANDS**.



Grade 2

Figure 25. Thermally injured skin. Elongated string bean nuclei are present in the hair follicle, with condensed chromatin in cell nuclei within the follicle, surrounding dermal tissue and glands. The cells within the hair follicle are damaged. The glandular structure is destroyed. 100 μm scale bar.



Grade 3

Figure 26. Thermally injured skin. Elongated string bean nuclei are present in the hair follicle, with condensed chromatin in cell nuclei within the follicle, surrounding dermal tissue and glands. The cells within the hair follicle are damaged. The glandular structure is destroyed. 100 μm scale bar.