Scope it Out – Periprosthetic Histopathology in 2020 and Beyond
Implant Section Workshop
2020 Orthopaedic Research Society

Organizers
Pat Campbell, PhD, Professor, UCLA Dept. of Orthopaedic Surgery; Director, Implant Retrieval Laboratory, Orthopaedic Institute for Children, Los Angeles pcampbell@mednet.ucla.edu
Deborah J. Hall, BS, Assistant Professor, Rush University; Director, Implant Retrieval Laboratory, Dept. of Orthopedic Surgery, Rush University Medical Center, Chicago deborah_hall@rush.edu

Significance and Purpose
The purpose of this workshop is to review the role of routine histopathology in the understanding of joint arthroplasty success and failure. The speakers will address this issue and provide a primer on features of routine histopathology samples for both diagnosis and research. The identification and role of the key cells involved in periprosthetic reactions will be presented. Additionally, to address the increasing usage of 3D printed porous components, a presentation of the basic concepts of biological fixation will also be given in light of the technical demands brought about by the newer technological advances in the field of arthroplasty.

Topics for discussion will include the following: If the implantation of any device causes a tissue response, when is a response an anticipated / acceptable local tissue reaction compared with an adverse local tissue reaction responsible for a revision surgery? Is a standard H&E stained paraffin section sufficient to diagnose the reason for revision? Should we be concerned with tissue sampling bias? The workshop will be useful to clinicians and researchers who seek a better understanding of standard orthopaedic tissue histopathology and issues of biological fixation.

Educational Need
Local tissue reactions to wear debris from joint prostheses are unique to the individual patient and can be complex in nature. These cellular responses and the terminology used to describe them are often confusing, especially to the novice clinician / researcher. A better understanding of anticipated or adverse local tissue reactions is needed. This workshop will review basic tissue features and newer methodologies.

Learning Objectives
The attendee will be introduced to the histopathology of the implanted joint as well as histological methods for the assessment of biological fixation. The cellular reactions to failed joint prostheses and methods for their quantitation to improve interpretation of anticipated versus adverse periprosthetic reactions and to facilitate multivariate analyses will be discussed. Lastly, guidelines for the collection and preservation of periprosthetic tissue, and bone-implant interfaces will be provided.
Key Players in Joint Soft Tissue Reactions

1. Resident Cells
   a. Synovial cells – line the innermost joint, make and clean synovial fluid
   b. Fibroblasts – make fibrous tissue fibers and matrix, heal surgical damage
   c. Macrophages – “Big Eaters” (phagocytic cells) of detritus and particulate debris

2. Invited (or Uninvited) Guests
   a. Giant cells – fused macrophages, able to ingest or line larger particles
   b. Neutrophils – hallmark of tissue bacterial infection
   c. Lymphocytes, plasma cells – multiple subtypes of specialized immune responders

Key Features of the Synovial Lining

There are 2 main cell types: fibroblast-like cells produce the hyaluronic acid, and proteins that make up synovial fluid and the macrophage-like phagocytic cells essentially clean the fluid of cell detritus and debris. This is why wear particles are commonly seen in cells along the outer layers of the periprosthetic tissues. The relative position and amount of debris in the synovial tissues can indicate if debris had been produced just before revision surgery (e.g. component fracture, the debris is superficial) or over a more prolonged time (where debris fully infiltrates the tissues). Since the synovium reflects the wear history of the joint, it is important to include this layer in tissue samples for histological analysis.

Key Types of Tissue Reactions around Implants (Anticipated vs Adverse)

1. Healing of the surgical wound and ongoing repair around loose implants (fibrous tissue formation, early but short-lived infiltration by neutrophils (virtually never seen histologically in specimens), then macrophages and giant cells may occupy tissues long-term depending on rate of debris infiltration).

2. Innate response: macrophage dominated, expected response to debris and tissue damage. In severe form, this can lead to massive numbers of cells, thickened tissues, areas of necrosis, osteolysis and / or pseudotumor formation.

3. Adaptive: lymphocyte (T-cell) dominated, often with plasma cells, in response to specific antigenic stimuli. In severe form, this response can lead to extensive tissue necrosis.

Key Histological Features of Common Arthroplasty Wear Debris

1. Metallic particles from bearings or loose metal implants appear as dense, dark, grains or tiny flakes making cells look dusty or dark. Identification of the debris requires spectroscopy.

2. Solid corrosion products (from modular junctions) take on a variety of shapes, colors and textures but typically look like irregular plates or shards, probably because they break up during tissue sectioning. Identification of the debris requires spectroscopy.

3. Polymeric debris – can be “invisible” in routine sections or look like voids – polarized light is useful to demonstrate larger debris. Oil Red O stain may also help but FTIR is more reliable.
What is Biologic Fixation of Implants?
Biologic fixation is attachment of the implant to the skeleton by bone and occurs through bone ingrowth and osseointegration.

Bone Ingrowth
1. The process of bone ingrowth resembles fracture healing with the coordinated participation of several cell types.
2. The mechanism of bone formation is intramembranous ossification (mesenchymal cells to bone), not endochondral (mesenchymal tissue to cartilage to bone).
3. **Implant stability is critical** and the mechanically stable environment achieves final bone ingrowth in three phases.
   a. Inflammatory – initial surgical insult stimulates mesenchymal stem cells to differentiate into osteoblasts
   b. Reparative – new (woven) bone can be seen 1 to 2 weeks after implantation
   c. Remodeling – bone matures to lamellar bone, while newer bone is laid down, filling in the pores of the implant coating

Key Histologic Features of Bone Ingrowth
1. Early bone ingrowth consists of proliferative osteoblasts, osteoid, and woven bone (newly formed, immature bone, rich in osteocytes in large lacunae, and randomly arranged bundles of calcified collagen fibers).
2. As the bone matures, the matrix is remodeled as lamellae, consisting of few cells contained in flattened lacunae with the collagen fibers in an ordered, layered arrangement.
3. Bone appositional growth continues, lessening the available space in the coating. The trabeculae thicken and the matrix is seen as concentric lines surrounding osteonic canals. Haversian systems are formed.

Keys to Achieving Good Biological Fixation
1. Pore Size: optimal range between 150 µm and 400 µm
2. Example of a successful coating: c.p. Ti fiber metal mesh: 250 µm diameter, void concentration of 50%, pore size 400 µm
3. Limit interface gaps – initial implant-bone apposition is a prerequisite
4. Micromotion: <28 µm leads to bone ingrowth

Biological Fixation in the Era of 3D Printing
1. The goal of good fixation should be the maintenance or reestablishment of local trabecular morphology within and surrounding the porous coating. The amount of bone required to provide clinical fixation is variable and does not imply the entire coating contains bone.
2. Postmortem studies of well-functioning porous acetabular components had mean extent of bone ingrowth of 33% and mean volume fraction of 13% (Urban, JBJS 2012).
3. Differentiating lack of fixation vs loss of fixation in aseptic loosening
   a. Lack of bone fixation (fibrous tissue formation) can occur in an unstable environment (relative displacements >150 µm), in the presence of interface gaps, inadequate porous coating structure, or in areas with compromised vasculature.
   b. Loss of fixation occurs when bone in the porous coating is lost due to osteolysis. Among the potential causes of periprosthetic osteolysis are wear debris, stress-shielding, infection, and changes in patient biomechanics. This determination incorporates histologic analysis, radiographic and clinical histories along with input from the clinician.
**Scoring Methods for the Interpretation and Reporting of Periprosthetic Tissue Reactions**

The examination of periprosthetic tissues is an important aspect of implant retrieval analysis. Depending on many factors including the type, amount and characteristics of the particulate debris, in addition to the patient specific reaction, the local tissue reaction may range from a minimal amount of inflammation or fibrosis that is unnoticed by the patient to serious clinical effects such as pain, radiographically visible bone resorption or extensive soft tissue necrosis. The latter are referred to as adverse local tissue reactions. To facilitate using histological data in the multivariate analysis of correlations between implant and clinical factors and tissue reactions, the use of a semi-quantitative histological method is recommended. Some examples are included in the table below and other relevant papers are included in the references. It is important to remember that each of these methods ranks features from none/minimal to high/severe and this full spectrum should be used as appropriate.

**A word on Infection:** Note that tissues from joints removed for infection are typically not included in studies because the histology can be significantly changed. However, it is important to note that the diagnosis of infection is not always straightforward and there may not be neutrophils in the tissues. The introduction of the Musculoskeletal Infection Society (MSIS) criteria for periprosthetic joint infection (PJI) in 2011 resulted in improvements in diagnostic confidence and research collaboration. The emergence of new diagnostic tests and the lessons learned from the past 7 years using the MSIS definition, prompted Parvizi et al (2014) to develop an evidence-based and validated updated version of the criteria. The AAOS Guideline on the Diagnosis of Periprosthetic Joint Infections of the Hip and Knee provides a useful review of the reliability of diagnostic tests for joint infections.


**Table summarizing the tissue features rated using histological scoring methods**

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A Glossary of Commonly used (And Abused) Terms

**ALTR (Adverse Local Tissue Reaction)**
The term is loosely and broadly applied to describe a spectrum of histological (e.g. necrosis, macrophages with debris, lymphocyte infiltration) and clinical (e.g. pseudotumor, cyst, metal staining, metal allergy, unexplained pain) features presumed to be the result of exposure to metal wear and corrosion products from total joints.

**ARMD (Adverse Reaction to Metal Debris)**
The term is loosely and broadly applied to describe a spectrum of histological (necrosis, macrophages with debris, lymphocyte infiltration, lymphoid neogenesis, vasculitis, fibrin formation) and clinical (pseudotumor, metal staining, metal allergy, synovitis, unexplained pain) features presumed to be the result of exposure to metal wear debris.

**Antigen**
A foreign or “non-self” macromolecule that triggers an immune response.

**ALVAL (Aseptic Lymphocyte-Dominated Vasculitis-Associated Lesions)**
As coined by Prof Hans Willert et al in 2005, this term covers a number of histological features including diffuse and perivascular lymphocytes, high endothelial venules, fibrin exudation, necrosis and macrophages with “drop-like inclusions” that were associated with a hypersensitivity reaction to metal wear products. It has come to loosely mean metal sensitivity.

**Bone Ingrowth**
New bone formation directly into the porous structure of an implant. Accurately demonstrating ingrowth requires that the interface be sectioned for direct examination.

**Bone Ongrowth**
New bone formation directly onto the surface but does not penetrate the porous structure of an implant. It is important to recognize that the appearance of bone on an implant may hide a layer of fibrous tissue between the bone and the biological ingrowth surface.

**Exudate**
A protein-rich fluid which oozes out of damaged blood vessel, typically due to inflammation.

**Fibrin**
Fibrin is an insoluble clotting protein that helps stem leakage following damage to a blood vessel, by setting up into a void and eventually being reorganized and removed. It is not tissue and does not contain collagen fibers. Fibrin should be differentiated from necrotic / acellular tissue by careful microscopy and the use of polarized light to confirm the presence or absence of collagen.

**Fibroblasts**
Fibroblasts make the fibers (e.g. collagen, elastin) and matrix that together form fibrous connective tissue – the stuff that holds us all together. Fibroblasts are important for tissue repair but may be sparse in adult tissues that are not remodeling.

**Giant cells**
When particles exceed about 10 microns, macrophages will fuse into giant cells, which can become quite large, contain many nuclei and take on interesting shapes. Giant cells can ingest particles or may line up along larger particles.

**Lymphocytes**
These specialized cells are commonly known as “immune cells” and include many subsets with specific roles in the innate and adaptive immune responses. Histologically, lymphocytes appear as small, dense purple nuclei and have very little visible cytoplasm. Lymphocytes can be diffusely distributed or gather around blood vessels (perivascular) often in very large numbers.
Macrophages (The cleaners)
These are the “cleaners” of the joint tissues and the name macrophage literally means “Big Eater”. They are the primary innate immune cell and are easily seen in tissues when they have ingested visible debris such as metal. Histologically they typically have a prominent nucleus and a large amount of cytoplasm around them containing particles or granules.

Neutrophils
Neutrophils are “white blood cells” that are associated with an innate and rapid response to bacterial invasion and tissue damage. Recent research is showing that these cells can participate in tissue repair as well. It is important to note that the histological criteria for infection based on the number of neutrophils per field vary from center to center (typically 5 to 10 cells per high power field).

Osseointegration
The direct structural and functional connection between ordered living bone and the surface of a load-carrying implant. Although surgeons may use this term to indicate the radiographic appearance of bone associated with a porous-coated implant, true osseointegration is a histologic term implying “bonding” with an implant, such that no gap between the bone and implant can be seen at the highest magnification.

Osteoblasts (The Builders)
Large, polygonal (columnar) cells with eccentric nuclei that lay down bone matrix.

Osteoclasts (The Remodelers)
Multinucleated, non-dividing cells that remove (resorb) bone matrix from sites where bone is deteriorating, remodeling or not needed.

Osteocytes
Mature bone cells that keep organic matrix in good repair by secreting enzymes to maintain mineral content and coordinate bone resorption and formation. The appearance of osteocyte nuclei in bone is a generally accepted sign of bone viability but the absence of nuclei may not necessarily indicate dead bone as it can be the effect of poor tissue preservation.

Osteolysis
The textbook definition is the pathological destruction of bone tissue. As hypothesized by Prof. Hans Willert in 1976, periprosthetic osteolysis is the result of an abundance of debris-stuffed macrophages signaling to osteoclasts to remove bone to make room to store the masses of cells and their debris.

PJI (Periprosthetic Joint Infection)
The demonstration and diagnosis of infection is not always clear, even with the newer tests available today. See the helpful guidelines on the AAOS website. [https://www.aaos.org/research/guidelines/PJIsummary.pdf](https://www.aaos.org/research/guidelines/PJIsummary.pdf)

Plasma cells
Plasma cells are a form of B lymphocyte that is specialized to produce antibodies. Histologically they typically have a distinct, clear halo around an eccentric nucleus which sometimes has a checkerboard (or clock-face) pattern. They are often mixed in with aggregates of lymphocytes.

Synovial Cells
The synovium is the innermost lining of the joint capsule. There are 2 main cell types of the synovial lining: 1) fibroblast-like cells produce the hyaluronic acid, & proteins that make up synovial fluid and 2) the macrophage-like phagocytic cells essentially clean the fluid of debris.

Woven Bone
Woven bone is newly formed, immature bone, also called primary bone. Its distinguishing features are abundant osteocytes contained in large lacunae and randomly arranged bundles of calcified bone matrix. Osteoclasts and osteoblasts are numerous in the surrounding endosteum.
Each step in tissue preparation is interdependent. Failure in one step will directly affect both ease of sectioning and the quality of the sections.

1. **Fixation**
   The purpose of fixation is to preserve tissue from degradation and maintain the structure of cells and sub-cellular components. This is accomplished by irreversibly cross-linking the proteins. The most common fixative for light microscopy is **10% neutral buffered formalin** (4% formaldehyde in phosphate buffered saline). Formalin is also an acceptable method of decontamination.

   *Fixation is the most important step! Without good fixation, histological evaluation of the tissues/cells is impossible.*

   *Tissues need to be placed in fixative solution as soon as possible once removed from the patient. For proper fixation, the ratio of formalin to specimen should be >10:1. For large specimens, fixative solutions should be replaced with fresh solution during fixation.*

2. **Dehydration**
   Water is immiscible with most embedding media and therefore needs to be removed from tissues so it can be replaced with the embedding medium. Dehydration is performed by transferring the tissue through baths of progressively more concentrated ethanol.

3. **Clearing**
   Once completely dehydrated, the tissue is “cleared” of alcohol (dehydration fluid) and fats with the use of a hydrophobic clearing agent that is miscible with alcohol and the embedding media. Xylene is one such clearing agent.

4. **Infiltration**
   Good infiltration of the embedding media is key to having high quality histologic sections that are properly stained and with little artifact.

5. **Embedding**
   The embedding media is chosen based on the hardness of the samples. Soft tissues are embedded in paraffin. Hard tissues (i.e. undecalcified bone) are embedded in harder media such as polymethylmethacrylate.
## Common Stains*

<table>
<thead>
<tr>
<th>Stain</th>
<th>Common Use</th>
<th>Nucleus</th>
<th>Cytoplasms</th>
<th>Red blood Cell (RBC)</th>
<th>Collagen Fibers</th>
<th>Specifically Stains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin</td>
<td>General staining with eosin (i.e. H&amp;E)</td>
<td>Orange, Cyan, Blue or Green</td>
<td>Blue/Brown/Black</td>
<td>N/A</td>
<td>N/A</td>
<td>Nucleic acids – blue, endoplasmic reticulum – blue</td>
</tr>
<tr>
<td>Eosin</td>
<td>General staining with hematoxylin (i.e. H&amp;E)</td>
<td>N/A</td>
<td>Pink</td>
<td>Orange/red</td>
<td>Pink</td>
<td>Elastic, fibers – pink, Collagen fibers – pink, Reticular fibers – pink</td>
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<tr>
<td>Toluidine Blue</td>
<td>General staining Bone and Cartilage</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Mast cells granules – purple, Old bone is paler than new bone in plastic embedded bone sections</td>
</tr>
<tr>
<td>Masson's Trichrome</td>
<td>Connective tissue</td>
<td>Black</td>
<td>Red/pink</td>
<td>Red</td>
<td>Blue/green</td>
<td>Cartilage – blue/green, Muscle fibers – red</td>
</tr>
<tr>
<td>Mallory's Trichrome</td>
<td>Connective tissue</td>
<td>Red</td>
<td>Pale red</td>
<td>Orange</td>
<td>Deep blue</td>
<td>Keratin – orange, Cartilage – blue, Bone matrix – deep blue, Muscle fibers – red</td>
</tr>
<tr>
<td>Weigert's Elastic</td>
<td>Elastic fibers</td>
<td>Blue/black</td>
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<td>N/A</td>
<td>N/A</td>
<td>Elastic fibers – blue/black</td>
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<td>Distinguishing mineralized bone from osteoid</td>
<td>Blue</td>
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<td>N/A</td>
<td>Blue</td>
<td>Mineralized bone – pink, Osteoid – blue, Soft tissue – blue</td>
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<tr>
<td>Basic Fuchsin/Toluidine Blue</td>
<td>General staining for undecalcified plastic embedded bone</td>
<td>Blue/black</td>
<td>Pink</td>
<td>Red/pink</td>
<td>Deep purple</td>
<td>Mineralized bone – purple/pink, Old bone is paler than new bone, Osteoid – light pink</td>
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<tr>
<td>Oil Red O</td>
<td>Lipids, also can demonstrate polyethylene</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Lipid – pink/red, Polyethylene – pink/red</td>
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<tr>
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<td>N/A</td>
<td>N/A</td>
<td>Pink</td>
<td>Glycogen and other carbohydrates – magenta</td>
</tr>
</tbody>
</table>

*From Ross MH, Pawlina W, Histology: A Text and Atlas. Lippincott Williams & Wilkins, Hagerstown, MD, 2006
Useful References

7. Doorn PF, Mirra JM, Campbell PA, Amstutz HC. Tissue reaction to metal on metal total hip prostheses. Clin Orthop 1996;329:


