

Supplemental Figures and Legends

Supplemental Figure S1.

Macroscopic examination of knee joints. Blood clot formation on the knee joint in trauma group indicates severity and inflammation during joint damage. Note the disappearance of blood clot in PTOA group. (ML: mechanically loaded knee)

Supplemental Figure S2.

Detection of ROS-bioluminescence in knee joints of animals. L-012 is injected intraarticularly in animals after 1st, 4th and 6th mechanical loading session. Note that ROS detection is prominent only after 1st mechanical loading session. The ROS detection was not possible after successive mechanical loading sessions.

Supplemental Figure S3. Overview of DMA

A mouse tibia specimen mounted on the loading machine (Supplement Figure 3A), an example of dynamic mechanical analysis (DMA) to obtain phase angle and dynamic stiffness under oscillatory loading ($-0.3 \pm 0.1\text{N}$) (Supplement Figure 3B).

Supplemental Figure S4. Modified OA Scoring and more histological sections

The knee joints were evaluated by H&E and safranin O staining in at least 2 serial mid-frontal sections. This histopathologic interpretation was conducted by at least three trained scientists (including a veterinary pathologist or an Orthopaedic surgeon) under blinded experiments, and the yielded data was utilized to determine the average value for OA and synovitis.

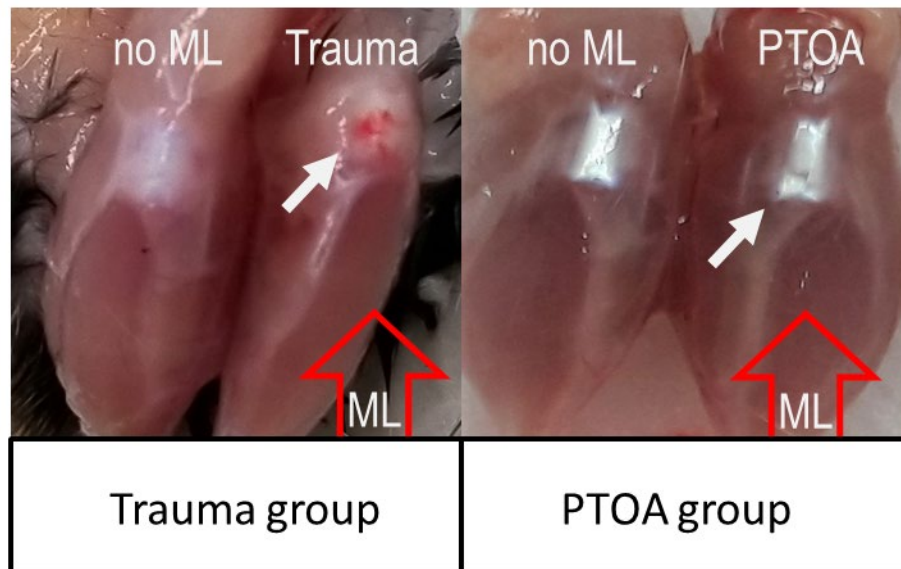
Tissue sections were graded using the osteoarthritis research society international (OARSI) and the Mankin scale with slight modifications. The advantage of these OA scoring system is that it allows scoring of the tissue sections based on the grade and stage of the disease's progression. Thus, a higher score represents a higher level of biological deterioration and disease extent. Therefore, it is a reliable method to differentiate between early- to late-stages of OA.

We further modified the procedure to assess the whole knee joint from an individual animal and sum up the average histological score from each representative group. Furthermore, the whole knee joint was graded by dividing it into four distinct regions as follows: medial femur (MF), medial tibia (MT), lateral femur (LF) and lateral tibia (LT). Each area was graded based on hypocellularity, Safranin-O staining, surface regularity and structure (Supplement Figure 4A). The mean from each area was calculated and summed up with other areas to yield the sum of the average histological score for each group of animals (Supplement Figure 4B and C). The detailed methodology to grade OA score has been outlined in the supplementary figures.

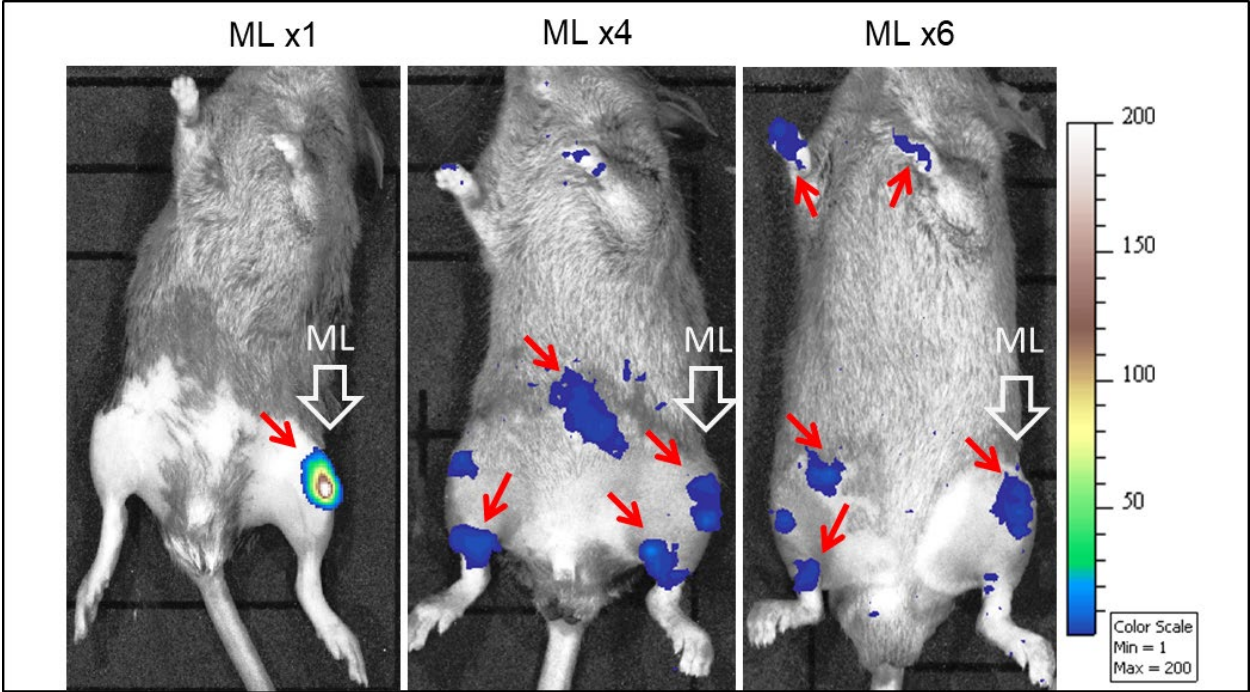
Synovitis in the medial and lateral knee joint was graded as described previously with slight modifications. Briefly, synovitis score was based on the enlargement of synovial lining, density of cells and inflammation. The highest score is 12 from combination of both part of knee joints (Synovitis in medial compartment (6) + lateral compartment (6)).

More histological sections and scoring result in Figure 4D.

Supplemental Figure S1.



Supplemental Figure S2.

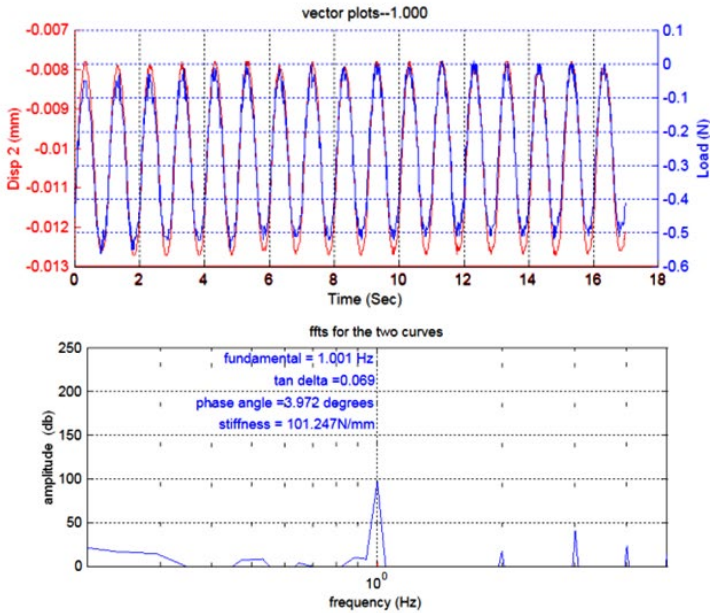


Supplemental Figure S3.

A



B

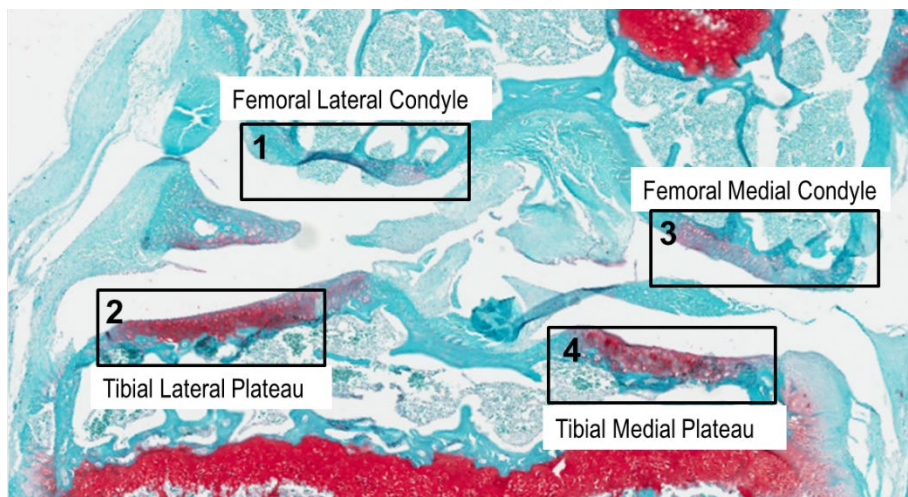


Supplemental Figure S4.

A.

| CATEGORY | | POINTS |
|---------------------|---------------------------------------|--------|
| Hypocellularity | | |
| | Normal <5% | 0 |
| | Small decrease in chondrocytes <25% | 1 |
| | Large decrease in chondrocytes 25-75% | 2 |
| | No cells >75% | 3 |
| Safranin-O staining | | |
| | Normal | 0 |
| | Slightly reduced | 1 |
| | Markedly reduced | 2 |
| | No metachromatin stain | 3 |
| Surface regularity | | |
| | Smooth (>3/4) | 0 |
| | Moderate (1/2 to 3/4) | 1 |
| | Irregular (1/4 to 1/2) | 2 |
| | Severely irregular (<1/4) | 3 |
| Structure | | |
| | Normal & Tidemark | 0 |
| | Deformation | 1 |
| | Fibrillation | 2 |
| | Fissures & Tide mark invaded | 3 |
| | Erosion | 4 |
| | Complete disorganization | 5 |
| Total maximum | | 14 |

B. Determine four areas for histopathological scoring



C. Calculation

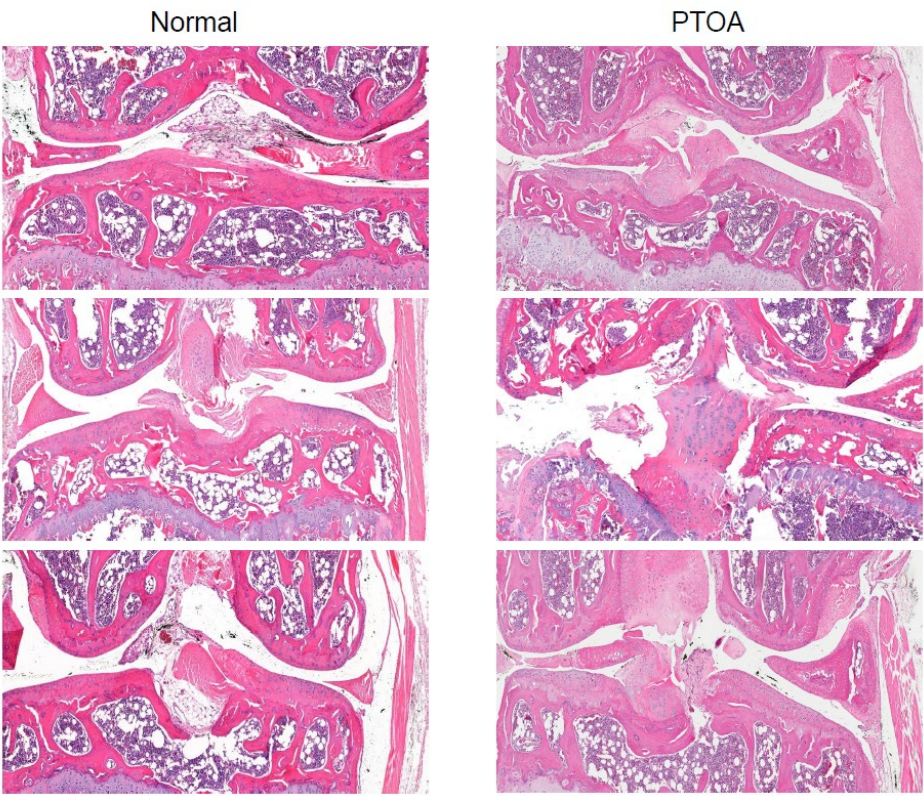
Histological Score Calculation:

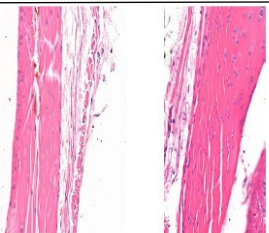
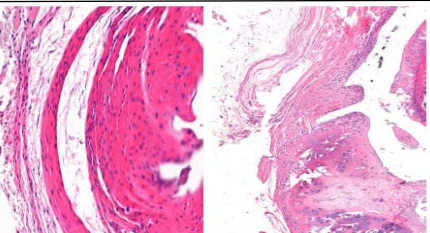
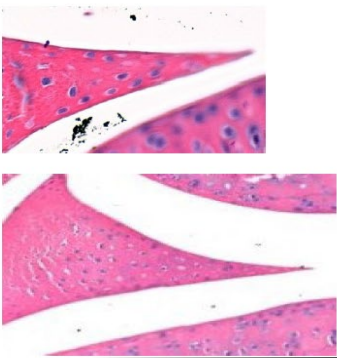
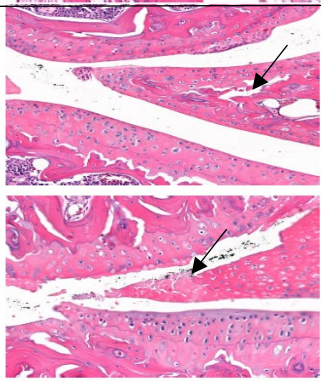
Sum of average histological score from each area per group

$$= \begin{aligned} &\text{average score of area 1 / group} \\ &+ \text{average score of area 2 / group} \\ &+ \text{average score of area 3 / group} \\ &+ \text{average score of area 4 / group} \end{aligned}$$

Highest score = 56 (14x4)
Lowest score = 0 (0x4)

D. more histology sections and blinded scoring result






| | Normal | PTOA |
|-------------------|---|--|
| Synovial membrane |  |  |
| Meniscus |  |  |

Blinded scoring result

Normal
Slide1

| CATEGORY | | POINTS | MT Rvw1 |
|----------------------------|--|-----------|------------|
| Hypocellularity | | | |
| | Normal <5% | 0 | 0 |
| | Small decrease in chondrocytes <25% | 1 | |
| | Large decrease in chondrocytes 25-75% | 2 | |
| | No cells >75% | 3 | |
| Safranin-O staining | | | |
| | Normal | 0 | 0 |
| | Slightly reduced | 1 | |
| | Markedly reduced | 2 | |
| | No metachromatin stain | 3 | |
| Surface regularity | | | |
| | Smooth (>3/4) | 0 | 0 |
| | Moderate (1/2 to 3/4) | 1 | |
| | Irregular (1/4 to 1/2) | 2 | |
| | Severely irregular (<1/4) | 3 | |
| Structure | | | |
| | Normal / Tide mark(intact) | 0 | 0 |
| | Deformation (irregular or unclear tide mark) | 1 | |
| | Fibrillation / surface loss (>1/4) | 2 | |
| | Fissures/ Tide mark(crossed the line) | 3 | |
| | Erosion / loss cartilage (>1/2) | 4 | |
| | Complete disorganization | 5 | |
| Sub Total | | 14 | 0 |
| Total | | 56 | |

|  | | | Normal Slide1 |  | | | Normal Slide1 |  | | |
|---|------|-----|------------------|---|------|----------|------------------|---|------|---|
| Rvw2 | Rvw3 | Avg | MF Rvw1 | Rvw2 | Rvw3 | Avg | LT Rvw1 | Rvw2 | Rvw3 | |
| 0 | 0 | | | 0 | 0 | 0 | | 0 | 0 | 0 |
| 0 | 0 | | | 0 | 1 | 0 | | 0 | 0 | 0 |
| 0 | 0 | | | 0 | 0 | 0 | | 0 | 1 | 0 |
| 0 | 0 | | | 0 | 0 | 1 | | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 1 | 0.666667 | 0 | 1 | 0 | |
| stdv | | 0 | | stdv | | 0.57735 | | stdv | | |

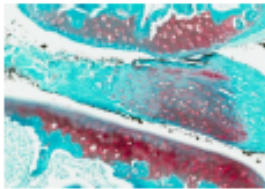
| Normal | | | | | Trauma | | | | |
|----------|------|------|-----------|----------|--------|------|------|----------|------|
| Slide1 | | | | | Slide1 | | | | |
| LF | | | | | MT | | | | |
| MF | | | | | | | | | |
| Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 |
| | | 0 | 0 | 0 | | 0 | 0 | 1 | 0 |
| | | 0 | 1 | 0 | | 1 | 1 | 1 | 1 |
| | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | | 0 | 0 | 1 | | 0 | 0 | 1 | 0 |
| 0.333333 | 0 | 1 | 1 | 0.666667 | 1 | 1 | 3 | 1.666667 | 1 |
| 0.57735 | | | stdv | 0.57735 | | | stdv | 1.154701 | |
| | | | Avg Total | 1.666667 | | | | | |

LT

LF

| Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 |
|-----------|------|----------|------|------|------|----------|------|------|------|
| 0 | 0 | | 0 | 0 | 1 | | 0 | 0 | 0 |
| 1 | 1 | | 1 | 0 | 0 | | 1 | 1 | 2 |
| 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| 0 | 1 | | 0 | 0 | 0 | | 0 | 0 | 1 |
| 1 | 2 | 1.333333 | 1 | 0 | 1 | 0.666667 | 1 | 1 | 3 |
| stdv | | 0.57735 | | stdv | | 0.57735 | | stdv | |
| Avg Total | | | | | | | | | |

[illegible]



PTOA
Slide1

LF

| Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg |
|------|------|----------|------|-----------|------|----------|
| | 1 | | | 1 | 1 | |
| | | 2 | | | | 2 |
| | | | | | | |
| | 2 | 1 | | 2 | 2 | 2 |
| | | | | | | |
| 0 | 0 | | | | 2 | |
| | | | 3 | | 3 | |
| | | | | | | |
| | 0 | | | | | |
| 1 | | | | 2 | 2 | |
| | | | 3 | | | |
| | | | | | | |
| 4 | 3 | 4.333333 | 9 | 7 | 9 | 8.333333 |
| stdv | | 1.527525 | | stdv | | 1.154701 |
| | | | | Avg Total | | 26.33333 |

Normal
Slide2

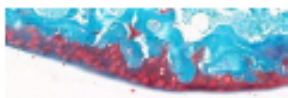


MT

| Rvw1 | Rvw2 | Rvw3 |
|------|------|------|
| | 0 | 0 |
| | | 0 |
| | | |
| | 0 | 0 |
| | | 0 |
| | | |
| | 0 | 0 |
| | | 0 |
| | | |
| | | |
| 0 | 0 | 0 |
| | | |
| | stdv | |



Normal
Slide2

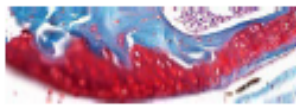


Normal
Slide2



Normal
Slide2

| MF | | | | LT | | | | LF | |
|-----|------|------|------|---------|----------|------|------|-----|------|
| Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 |
| | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | | 1 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 |
| | 0 | 1 | 0 | 0 | 0.333333 | 0 | 0 | 0 | 0 |
| | 0 | | stdv | 0.57735 | | stdv | | 0 | 0 |



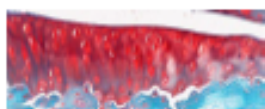
Trauma
Slide2



Trauma
Slide2

| MT | | | MF | | |
|-----------|------|----------|------|------|------|
| Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 |
| 0 | 0 | | 0 | 1 | 0 |
| | | | | | |
| | | | | | |
| 0 | 1 | | 0 | 1 | 1 |
| | | | | | |
| 0 | 0 | | 0 | 0 | 0 |
| | | | | | |
| 0 | 0 | | 0 | 0 | 0 |
| | | | | | |
| 0 | 1 | 0.333333 | 0 | 2 | 1 |
| stdv | | 0.57735 | | stdv | |
| Avg Total | | 0.666667 | | | |

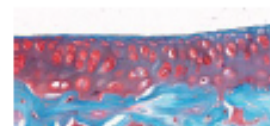
| Trauma | | | | Trauma | | | | PTOA | |
|----------|------|------|------|----------|------|-----------|------|----------|------|
| Slide2 | | | | Slide2 | | | | Slide2 | |
| LT | | | | LF | | | | MT | |
| Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 |
| | 0 | | 1 | 1 | | 1 | 1 | 1 | 1 |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | 0 |
| | 1 | | 2 | 2 | | 1 | 1 | 1 | |
| | | | | | | | | | |
| | 0 | 0 | 0 | | | | | | 0 |
| | | | | | | 1 | | | |
| | | | | | 2 | | 2 | | |
| | | 0 | 0 | | | | | | 0 |
| | 1 | | | | 1 | 1 | 1 | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 4.666667 | 2 | 3 | 3 | 2.666667 | 5 | 4 | 5 | 4.666667 | 1 |
| 0.57735 | | stdv | | 0.57735 | | stdv | | 0.57735 | |
| | | | | | | Avg Total | | 13 | |



PTOA
Slide2



PTOA
Slide2



| | | | MF | | | | LT | | | |
|------|------|----------|------|------|------|----------|------|------|------|---|
| Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | |
| | | 0 | | | | | | | | |
| 1 | | | | 2 | | 2 | | 1 | 1 | 1 |
| | | | | | 3 | | | | | |
| 0 | | | | | | | | | | |
| | 1 | | | 2 | 2 | 2 | | 1 | 1 | 2 |
| 0 | 0 | | | | | | | 0 | 0 | 0 |
| | | | | 2 | 2 | | | | | |
| | | | | | | 3 | | | | |
| 0 | | | | | | | | 0 | | |
| | 1 | | | | | | | | 1 | 1 |
| | | | | 4 | 4 | 4 | | | | |
| 1 | 2 | 1.333333 | 10 | 11 | 11 | 10.66667 | 2 | 3 | 4 | |
| stdv | | 0.57735 | | stdv | | 0.57735 | | stdv | | |



PTOA
Slide2



Normal
Slide3



| PTOA | | | | | Normal | | | | |
|--------|------|------|-----------|----------|--------|------|------|---------|--|
| Slide2 | | | | | Slide3 | | | | |
| LF | | | | | MT | | | | |
| Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | |
| | | | | | | | | | |
| | | 2 | 2 | 3 | 0 | 0 | 0 | | |
| | | | | | 0 | 0 | 0 | | |
| | | 2 | 2 | 2 | | | | | |
| | | | 1 | | 0 | 0 | 0 | | |
| | | 2 | | 2 | | | | | |
| | | | | | 0 | | | | |
| | | | | | | 1 | 1 | | |
| | | 4 | 4 | 5 | | | | | |
| 3 | 10 | 9 | 12 | 10.33333 | 0 | 1 | 1 | 0.66667 | |
| 1 | | | stdv | 1.527525 | | stdv | | 0.57735 | |
| | | | Avg Total | 25.33333 | | | | | |

Normal
Slide3

Normal
Slide3



Normal
Slide3



MF

LT

LF

Rvw1

Rvw2

Rvw3

Avg

Rvw1

Rvw2

Rvw3

Avg

Rvw1

Rvw2

0

0

0

0

0

0

0

1

0

0

1

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0

0

0

0

0

0

0

0

0

0

0

0

0

1

1

0

0

0

0

1

0

1

stdv

2

1

1

0

0

stdv

0

0

0

0

2



Trauma
Slide3



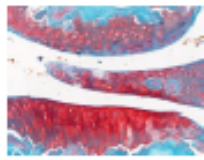
Trauma
Slide3



| MT | | | MF | | |
|-----------|----------|------|------|------|------|
| Rvw3 | Avg | | Rvw1 | Rvw2 | Rvw3 |
| 1 | | 0 | 1 | 1 | 2 |
| 1 | | 1 | 1 | 2 | 2 |
| 0 | | 0 | 0 | 1 | 2 |
| 1 | | 1 | 1 | 2 | 3 |
| 3 | 1.666667 | 2 | 3 | 4 | 8 |
| stdv | 1.527525 | stdv | 1 | stdv | 9 |
| Avg Total | 3.333333 | | | | |



Trauma
Slide3

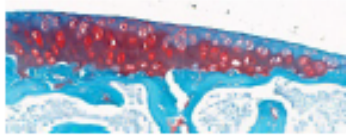


Trauma
Slide3



PTOA
Slide3

| LT | | | | LF | | | | MT | |
|----------|------|------|------|----------|------|-----------|------|----------|------|
| Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 |
| | 0 | 1 | 0 | | 0 | 1 | 0 | | 1 |
| | 0 | 0 | 0 | | 1 | 1 | 1 | | 1 |
| | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 |
| | 0 | 1 | 0 | | 1 | 1 | 1 | | 0 |
| 7.666667 | 0 | 2 | 0 | 0.666667 | 2 | 3 | 2 | 2.333333 | 2 |
| 1.527525 | | stdv | | 1.154701 | | stdv | | 0.57735 | |
| | | | | | | Avg Total | | 13.66667 | |



PTOA
Slide3



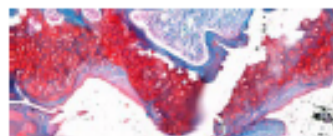
PTOA
Slide3



| | | | MF | | | | LT | | | |
|------|------|----------|------|------|------|----------|------|------|------|---|
| Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | Avg | Rvw1 | Rvw2 | Rvw3 | |
| 1 | 1 | | | 2 | | | | 2 | 2 | 2 |
| | | | | 3 | 3 | | | | | |
| 1 | | | | | | | | | | |
| | 2 | | 2 | 2 | | | | 2 | 2 | 2 |
| | | | | | 3 | | | | | |
| 0 | 0 | | | | | | | | | |
| | | | 2 | | | | | | | |
| | | | | 3 | 3 | | | 3 | 3 | 3 |
| 0 | | | | | | | | | | |
| | 1 | | | | | | | | | |
| | | | | 4 | 4 | 4 | | 4 | | 4 |
| | | | | | | | | 5 | | |
| 2 | 4 | 2.666667 | 10 | 12 | 13 | 11.66667 | 11 | 12 | 11 | |
| stdv | | 1.154701 | | stdv | | 1.527525 | | stdv | | |



PTOA
Slide3



LF

| Avg | Rvw1 | Rvw2 | Rvw3 | Avg |
|----------|------|-----------|------|----------|
| | 2 | 2 | 2 | |
| | 1 | 2 | 2 | |
| | 2 | 3 | 2 | |
| | 4 | 5 | 5 | |
| 11.33333 | 9 | 12 | 11 | 10.66667 |
| 0.57735 | | stdv | | 1.527525 |
| | | Avg Total | | 36.33333 |

| | slide1 | slide2 | slide3 | Avg | stdv | p value | | |
|--------|----------|----------|----------|----------|----------|------------|------------|---------------|
| | | | | | | t N v Trau | t N v PTOA | t Trau v PTOA |
| Normal | 1.666667 | 0.666667 | 3.333333 | 1.888889 | 1.347151 | 0.039357 | 0.005045 | 0.014102 |
| Trauma | 5.333333 | 13 | 13.66667 | 10.66667 | 4.630815 | | | |
| PTOA | 26.33333 | 25.33333 | 36.33333 | 29.33333 | 6.082763 | | | |