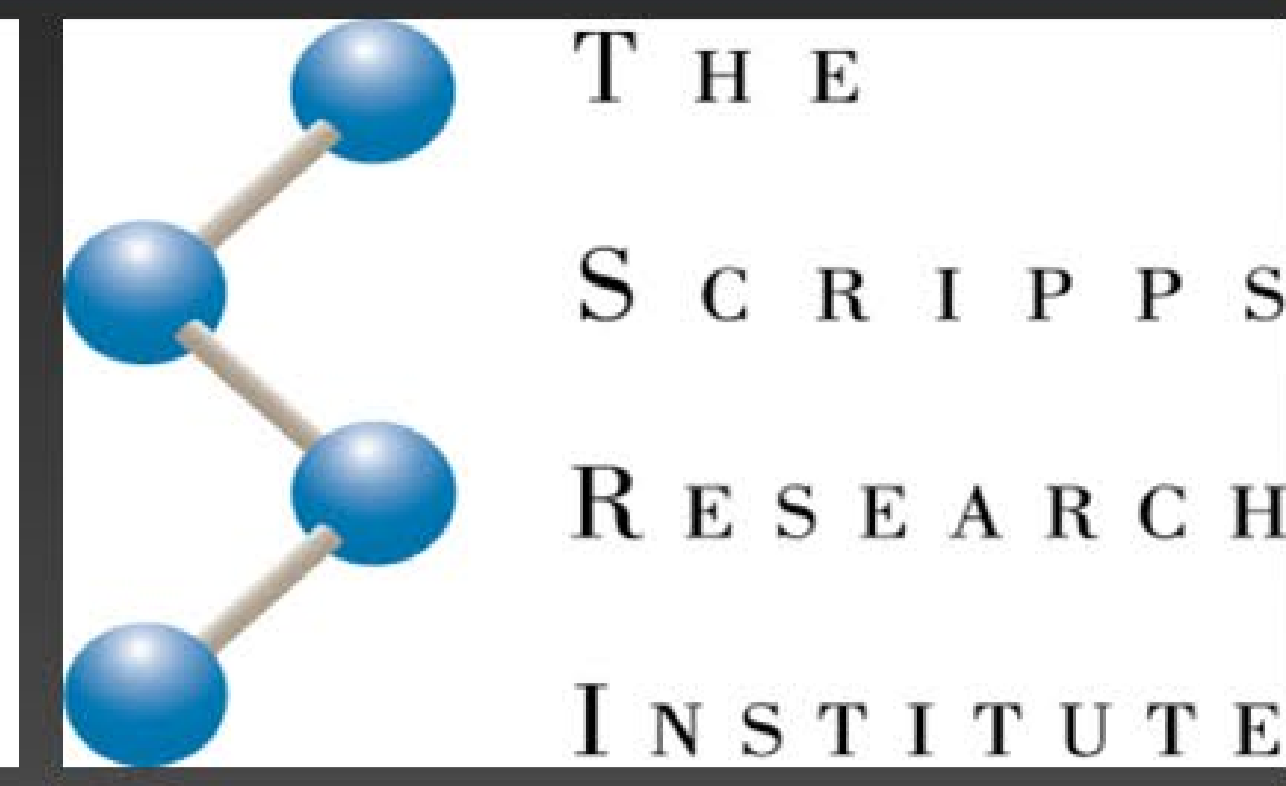


# Acute Hemarthrosis in Hemophilic Mice Results in Expansion of Mesenchymal Progenitor Cells: a Novel Mechanism of Arthropathy

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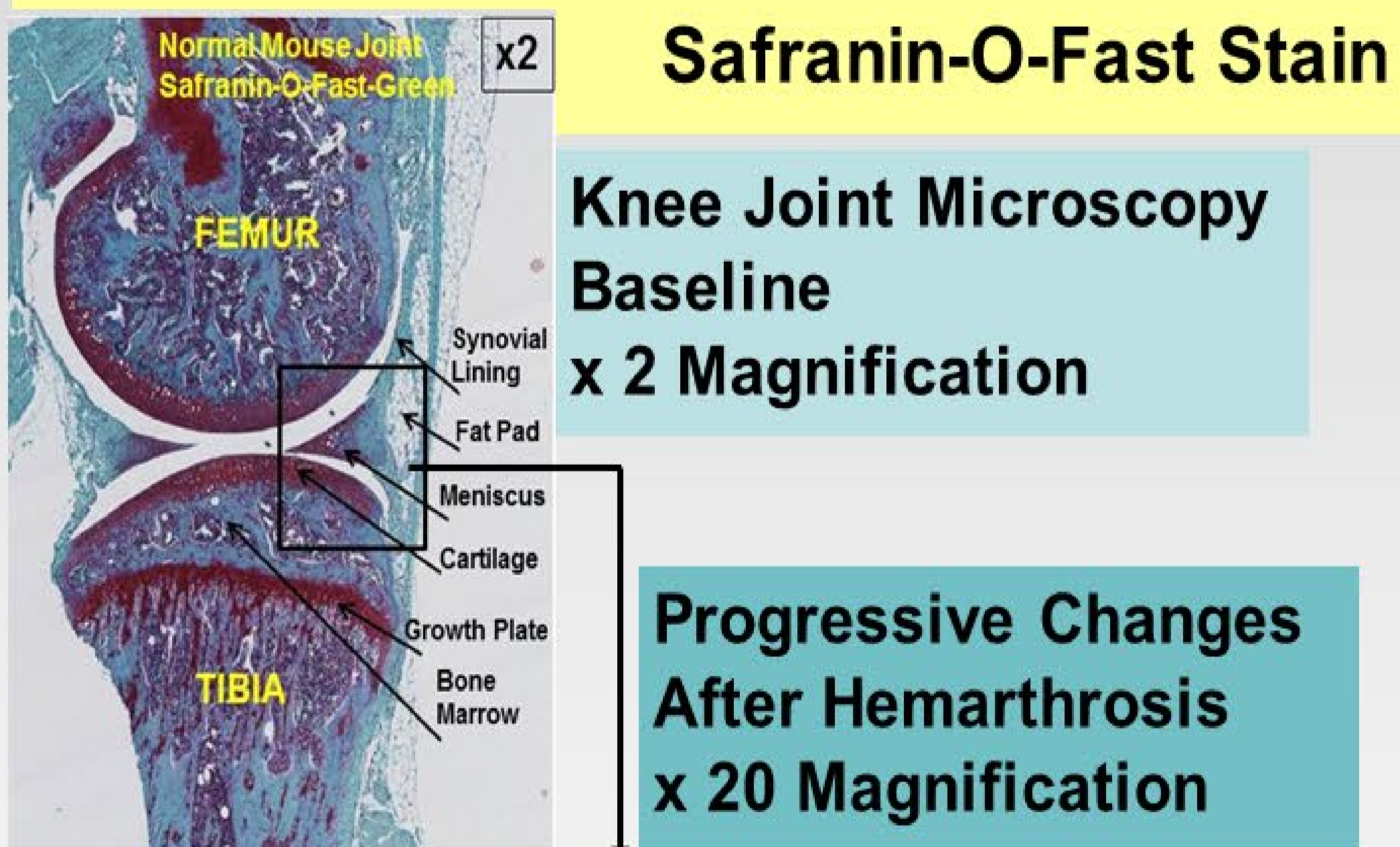
## INTRODUCTION AND OBJECTIVES

The sequence of pathophysiological tissue changes after hemophilic joint bleeding is incompletely understood. To address this, we studied the time course of histopathological and molecular changes in response to acute hemarthrosis in a joint injury model in FVIII-deficient mice.

## METHODS

- ✓ FVIII-deficient mice were subjected to right frontal subpatellar knee needle injury.
- ✓ Bleed induction was ascertained by visual inspection and hematocrit.
- ✓ Histopathological changes were studied at 4 and 7 days, and at 2, 4 and 10 weeks post injury. Findings were compared to the uninjured left knee.
- ✓ Alterations of cartilage, synovium and vascularity were determined by semi-quantitative scoring systems after staining with Safranin-O-Fast-Green.
- ✓ Cell proliferation index and phenotypic characterization of cells were performed by Immunohistochemistry for Proliferating Cell Nuclear Antigen (PCNA) and for mesenchymal/fibroblast progenitor markers ( $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), Stro-1, CD105, CD34).

## HISTOPATHOLOGICAL SCORING

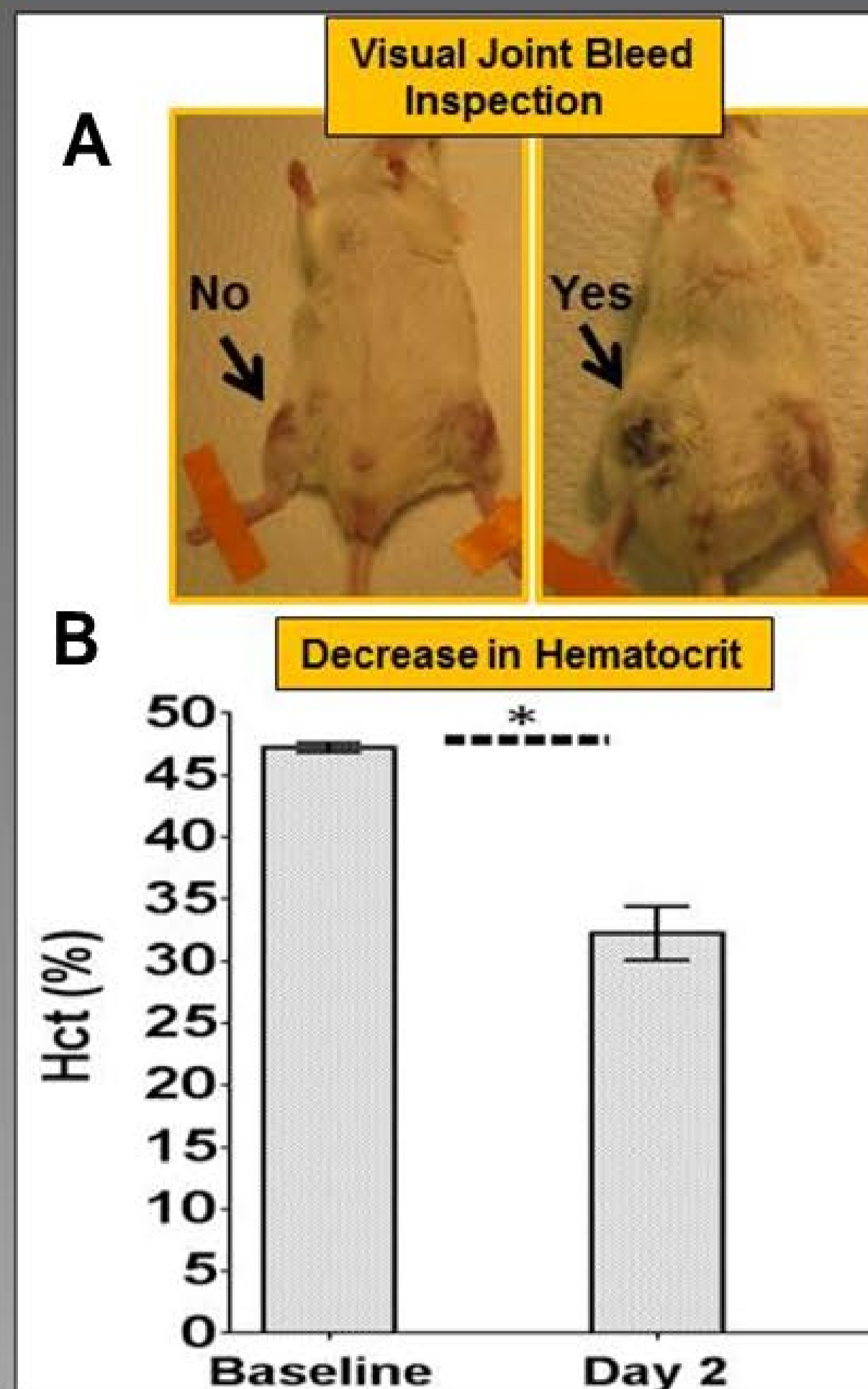


Valentino Score – Synovium				
A				
Proliferation x 20				
Cell Layers	< 4	4-5	6-7	> 7
Score	0	1	2	3
Krenn Score – Stroma				
B				
Proliferation x 10				
Changes	Normal	Mild	Moderate	Severe
Score	0	1	2	3
Glasson Score – Cartilage				
C				
Change x 40				
Changes	Normal	GAG Loss	Fibrillation	Erosion
Score	0	0.5	1	2

## RESULTS

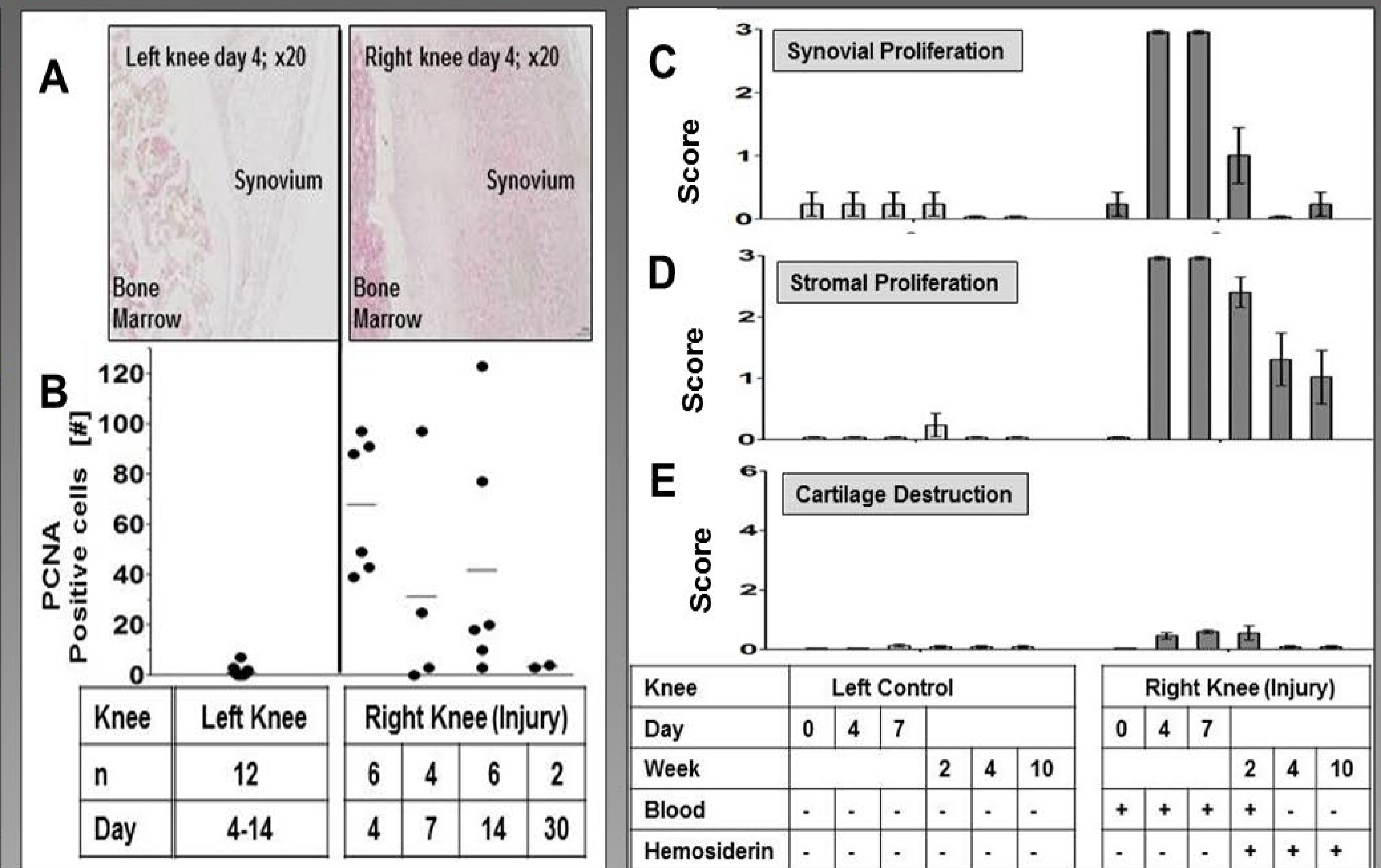
### 1. Joint bleeding

- A:  $\uparrow$  Hematoma
- B:  $\downarrow$  Hematocrit



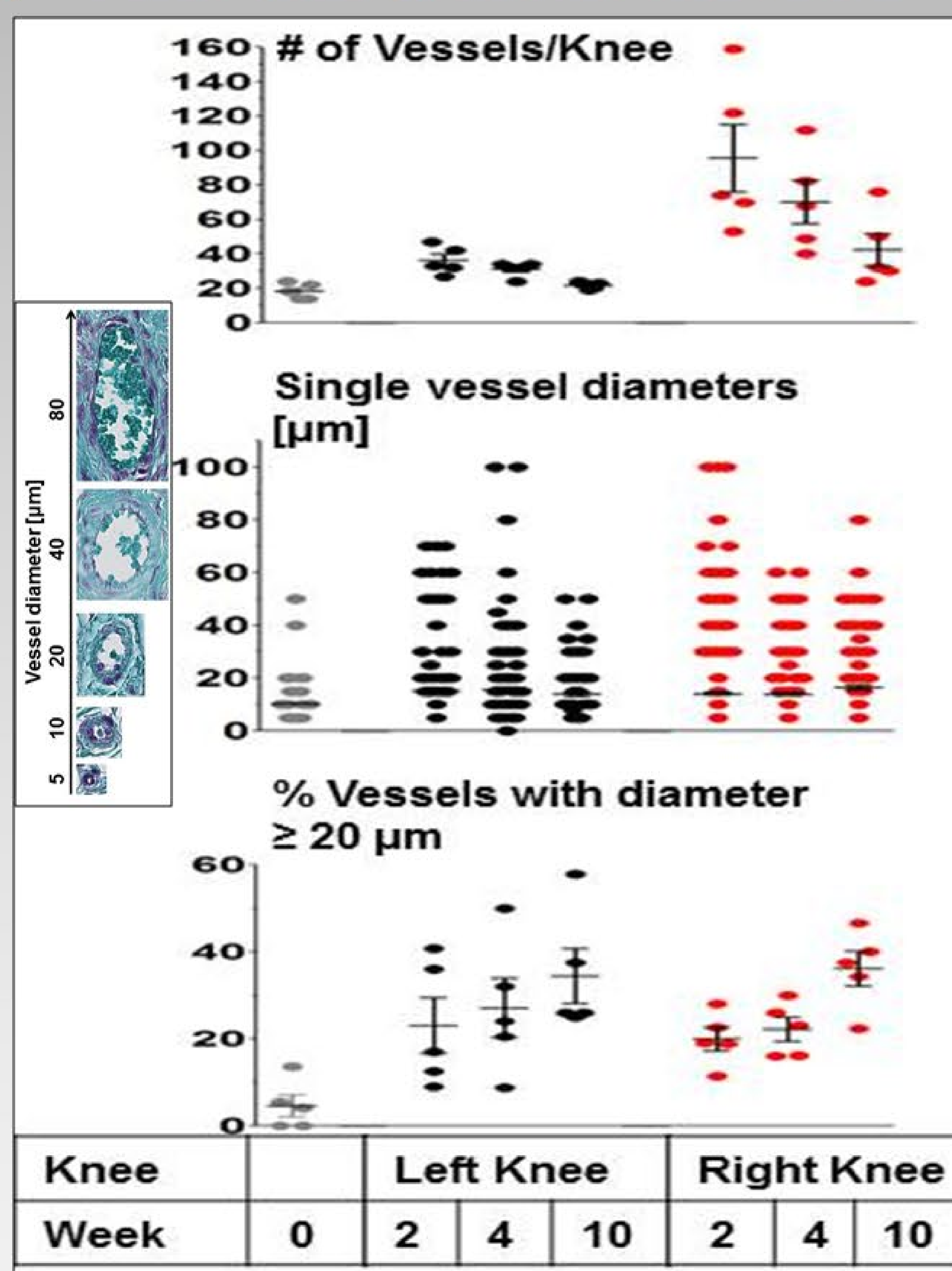
### 2. Tissue Changes were transient and most pronounced Day 4-14

- A/B: Marked increase in cell proliferation (PCNA)
- C/D: Marked increase in synovial and stromal cellularity
- E: Mild cartilage changes; mostly loss of glycosaminoglycans



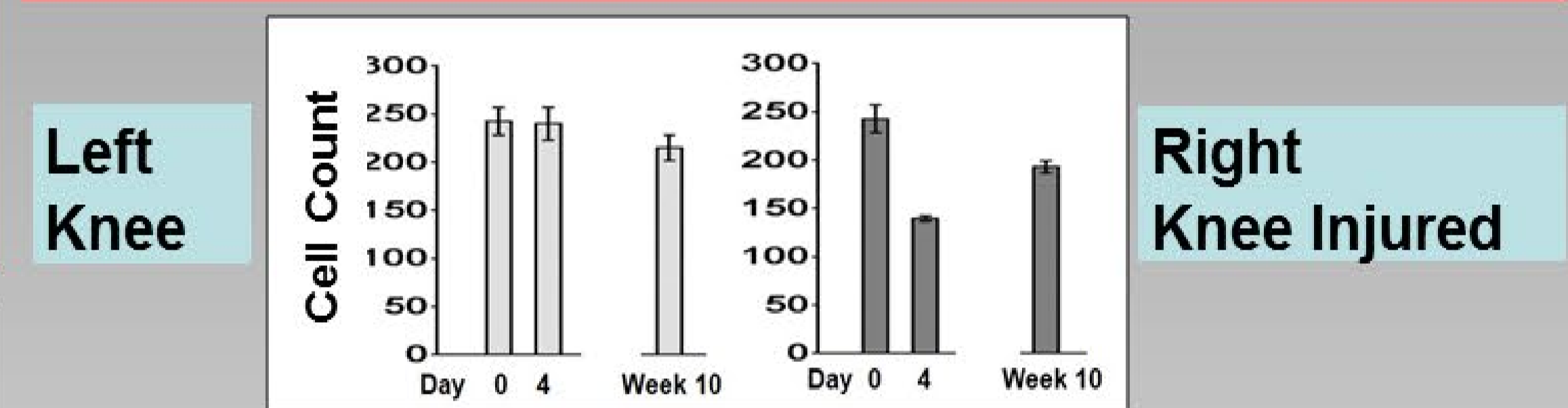
### 3. Vascularity

- Transient**  
~ 3-fold  $\uparrow$  of vessels injured knee only
- Persistent**  
~ 2-fold  $\uparrow$  of large vessels both knees



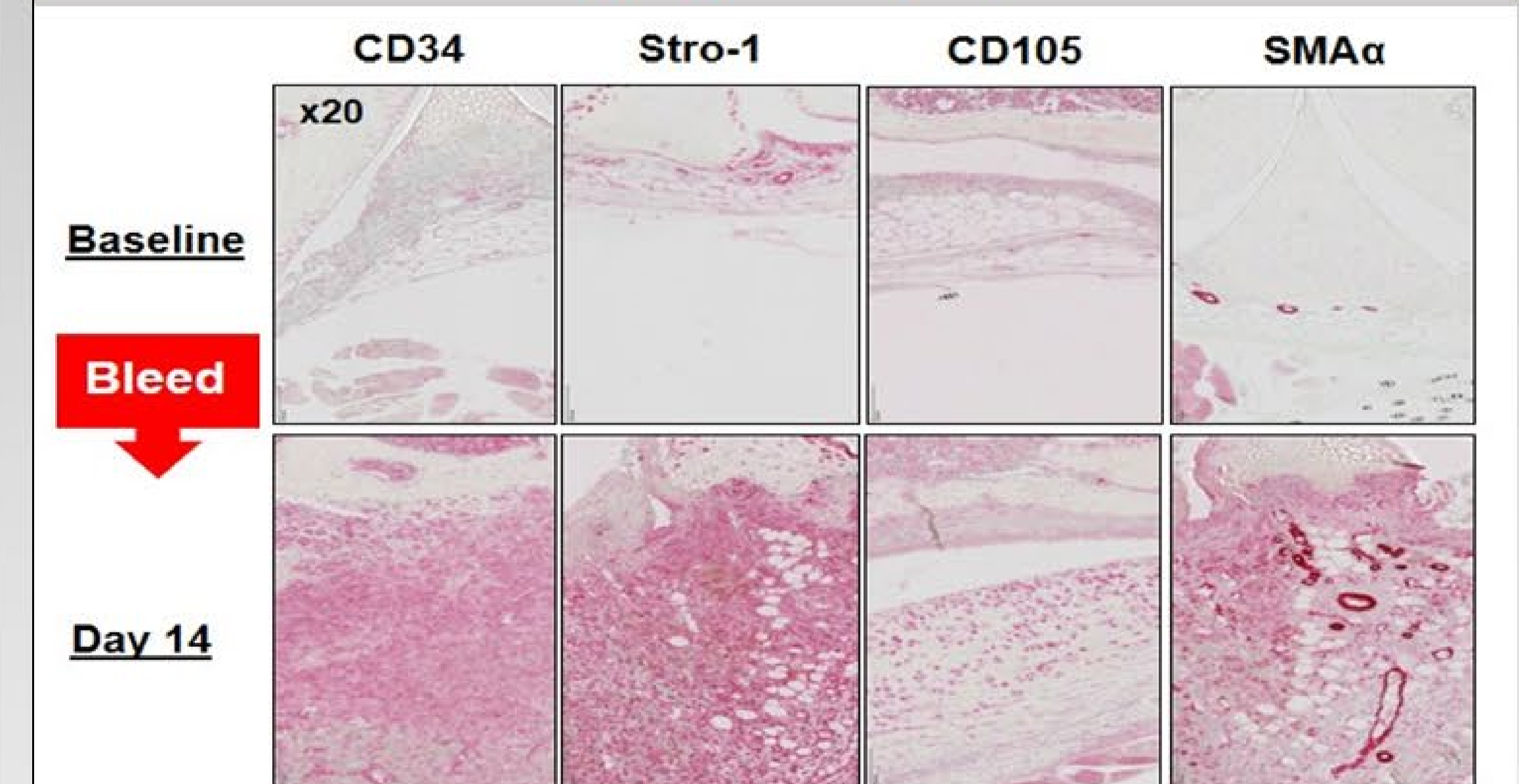
### 4. Chondrocytes

- $\downarrow$  ~ 50% Day 4  $\rightarrow$  near recovery Week 10



### 5. Mesenchymal progenitor cell markers

- Pronounced expression in proliferating tissue 4 to 14 days post injury



### Mesenchymal stem cell-like markers

- SMA $\alpha$ : Myofibroblastic, contractile elements
- CD105: Endoglin, vascular cell, neoangiogenesis
- Stro-1: Mesenchymal stem lineage marker
- CD34: Fibroblastic and hematopoietic progenitors

## CONCLUSIONS

- ✓ Acute hemophilic joint bleeding induced strong, but transient, synovial, stromal and vascular proliferation with mild cartilage changes between days 4-14.
- ✓ While increased vascularity in the injured knee was transient, abnormally large vessels persisted and were also present in the contralateral joints suggesting systemic effects of vascular remodeling.
- ✓ Cells expressing mesenchymal stem cell-like markers were strongly involved in the acute tissue reactions suggesting a unique mesenchymal mechanism for tissue repair following hemophilic joint bleeding.
- ✓ This mesenchymal response to joint bleeding may provide a new avenue for targeted intervention.

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