Assessing Human Articular Cartilage Transcriptome Layers with RNA Sequencing

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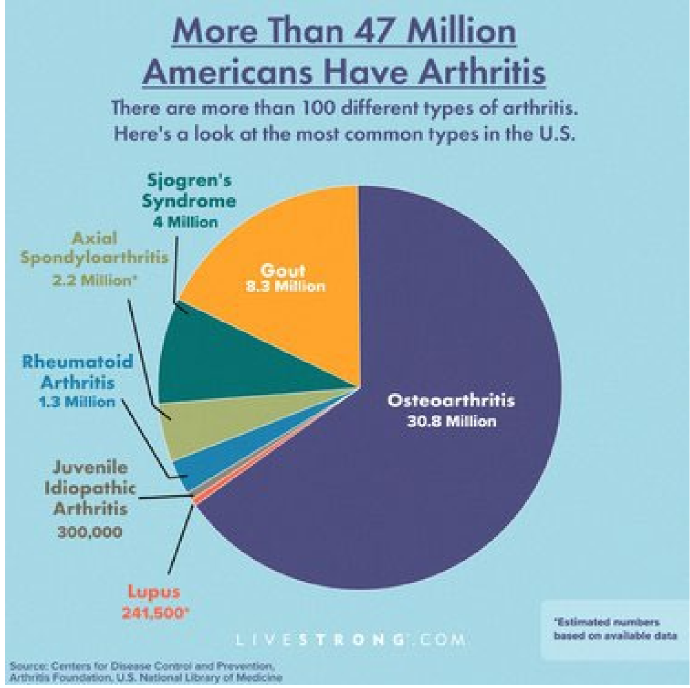
WASHINGTON STATE UNIVERSITY & CORNELL UNIVERSITY
Reproducible Cells and Organoids via Directed-Differentiation Encoding (RECODE)

2024 AIChE Annual Meeting
Medical Devices Session
October 29, 2024

WASHINGTON STATE UNIVERSITY
NIH Protein Biotechnology Training Program

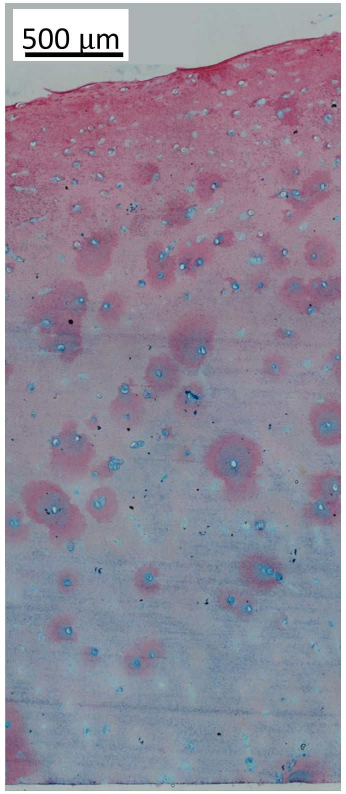
Overview

A

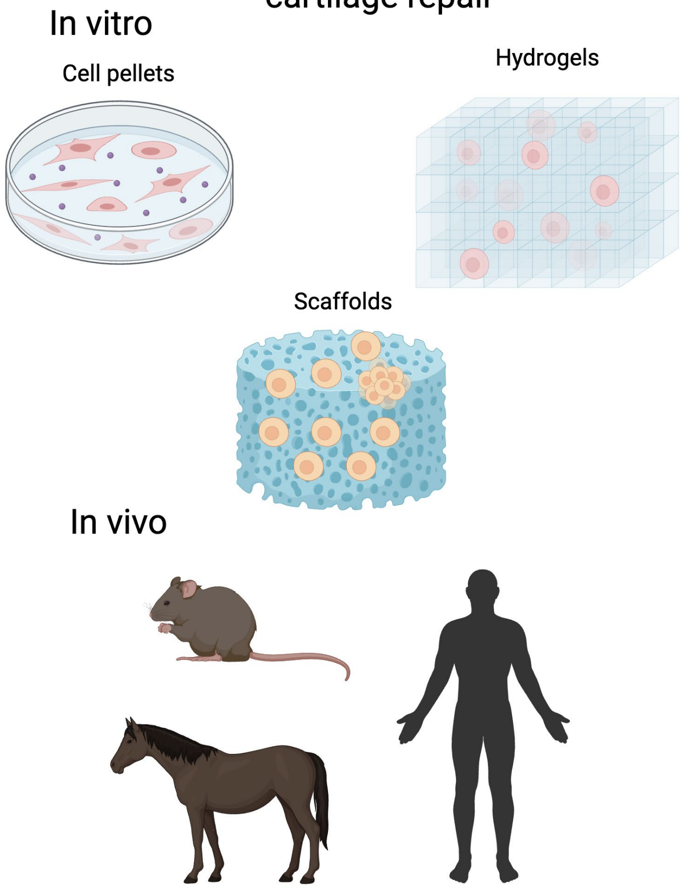


B

Histological Staining Human Articular Cartilage



Applications of articular cartilage repair



Highlight

- 32.5 M U.S. adults OA (2023)
- No reasonable cure; typical option: total knee replacement.

Objectives:

Sequence RNA of tri-layer articular cartilage:

- Normal articular cartilage (nHAC)
- Osteoarthritis articular cartilage (OA)
- Mesenchymal Stromal Cells (MSCs)
- MSC-derived (MSCs-Bioreactor)

Figure 1. A) Osteoarthritis statistic (U.S.) [1], B) Articular cartilage staining & hydrogel application [2]

Outline

- Objectives
- Background
- Methodology
- Results
- Summary & Acknowledgements
- Future work

Motivation and Problem Statement

Figure 2. Graphic of human articular cartilage to microscale [3]

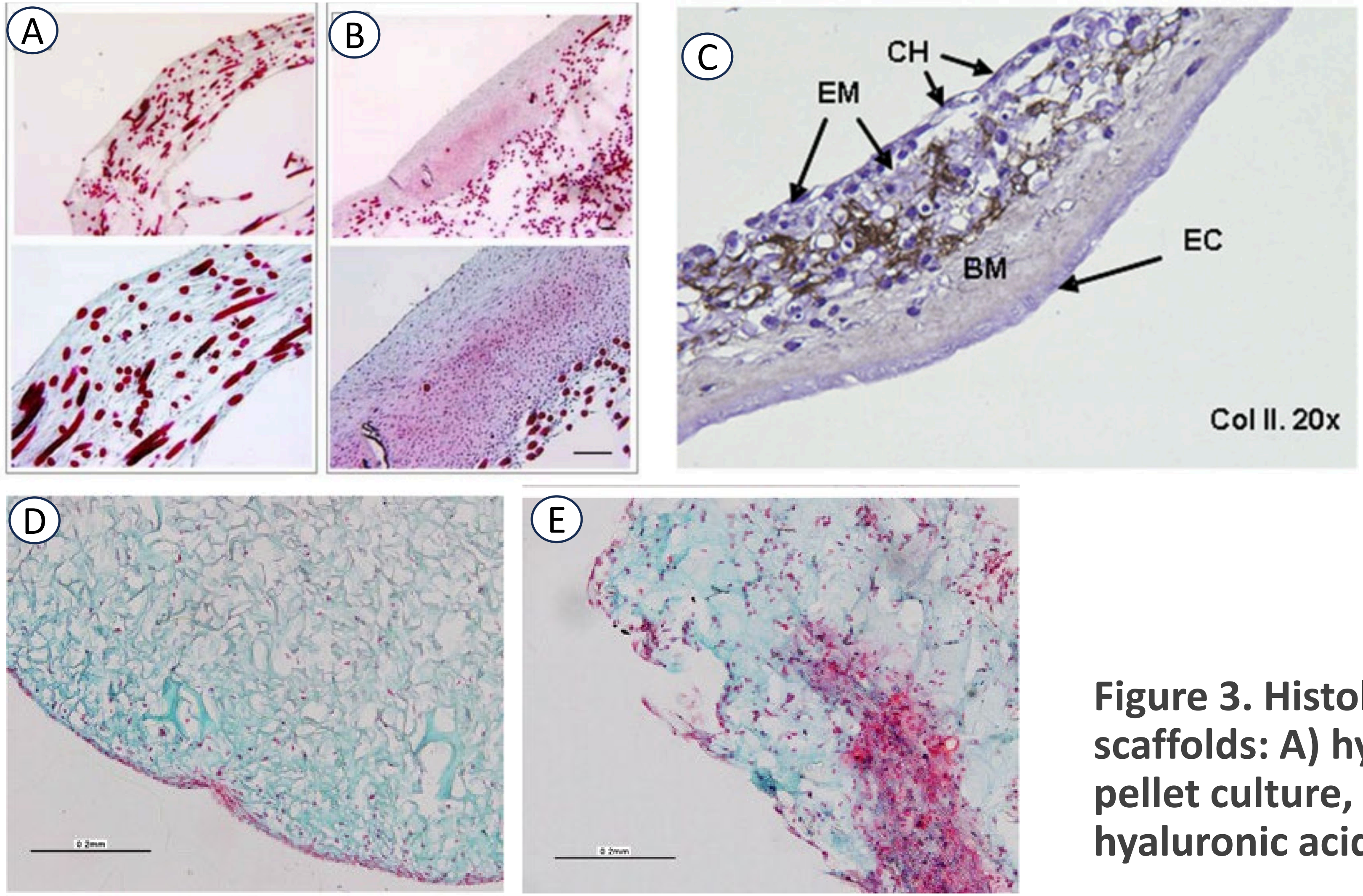
Objectives:

- Analyze articular & osteoarthritic cartilage
- Compare cell populations/properties

Cartilage Functions:

- Low-friction movement
- Shock absorption (protects subchondral bone)
- Load transmission (stabilizes joints)

Motivation: No Engineered Tissues Replicate Tri-Layered Cartilage



Several biopolymer scaffolds

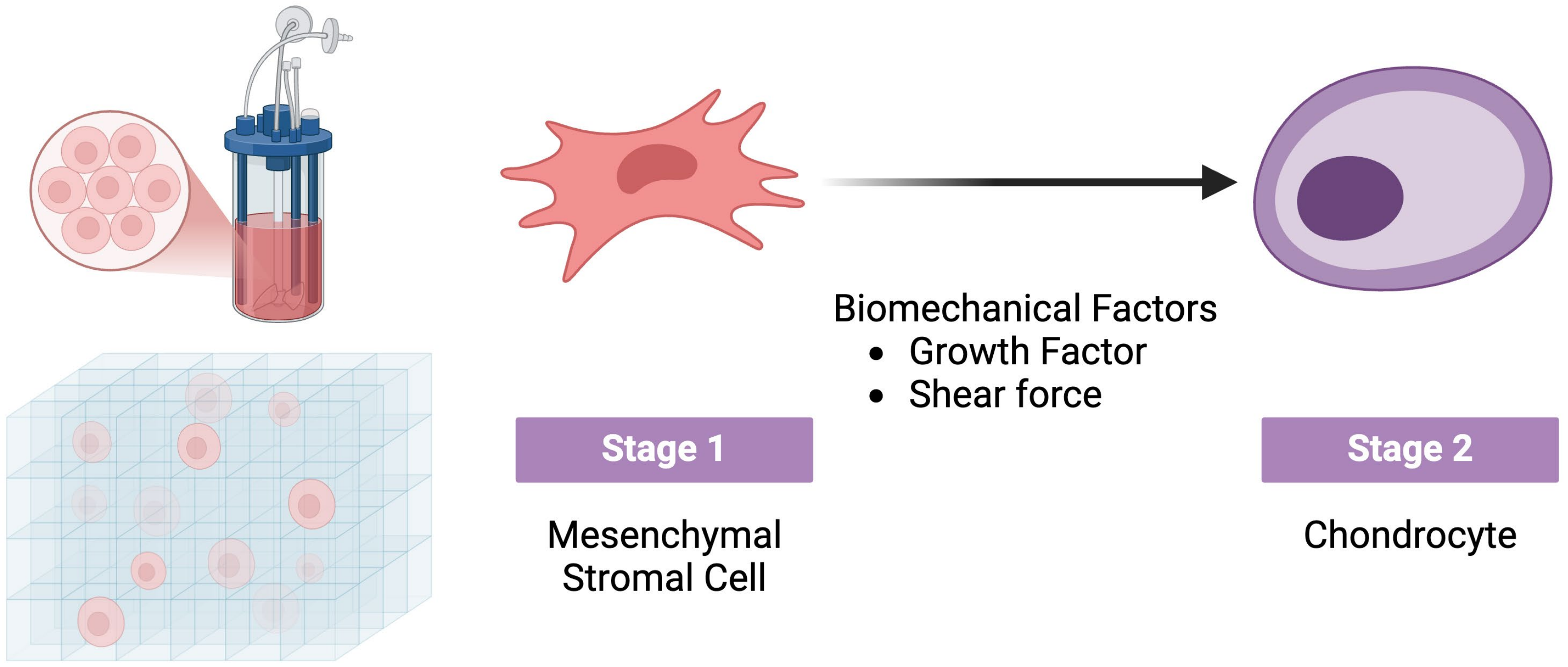
- Wolf et al. (2008): hyaluronan-based polymer
- Silvia et al. (2010): human amniotic membranes (HAMs)
- Amos et al. (2012): collagen-based scaffold

Motivation

- Reproduce tri-layer articular cartilage-like from MSCs hydrogel cultured by bioreactor

Figure 3. Histological Section of biopolymer scaffolds: A) hyaluronan monolayer, B) hyaluronan pellet culture, C) HAMs, D) collagen-based, E) hyaluronic acid scaffold

Motivation and Problem Statement



- Reproduce tri-layered articular cartilage culture in bioreactor
- Evaluate tri-layered MSC hydrogel
  - RNA-seq provides gene expression profiles
  - validate tri-layer articular cartilage culture

Figure 4. MSCs hydrogel culture in bioreactor for articular cartilage regeneration [2]

Background

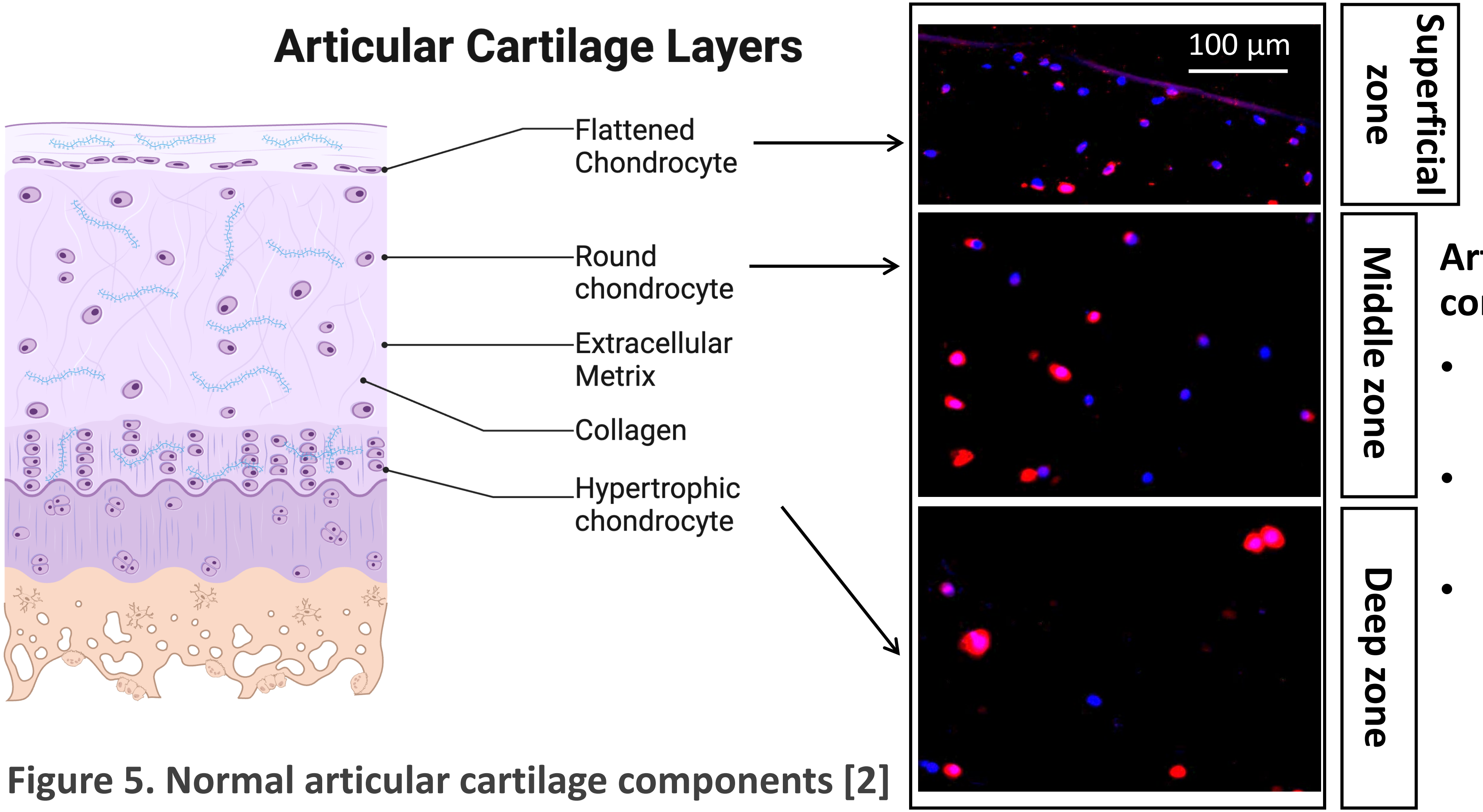


Figure 5. Normal articular cartilage components [2]

Articular cartilage components:

- Chondrocytes: maintain cartilage matrix
- ECM: supports structure/function
- Collagen: provides tensile strength

Collecting Tissue

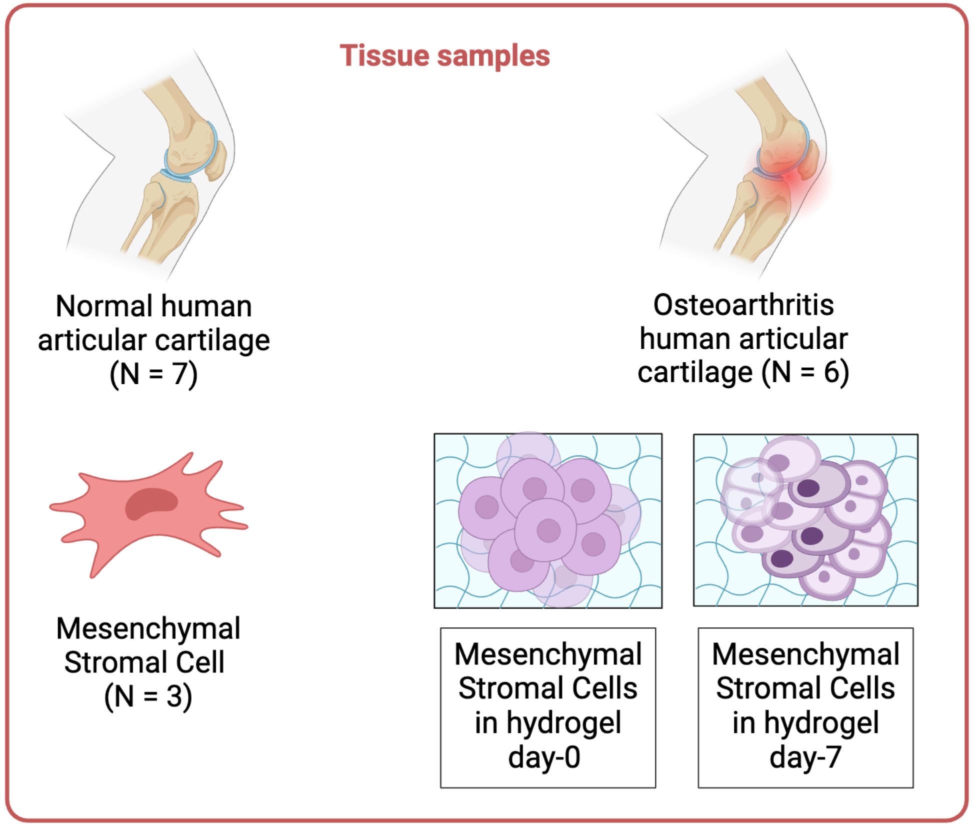
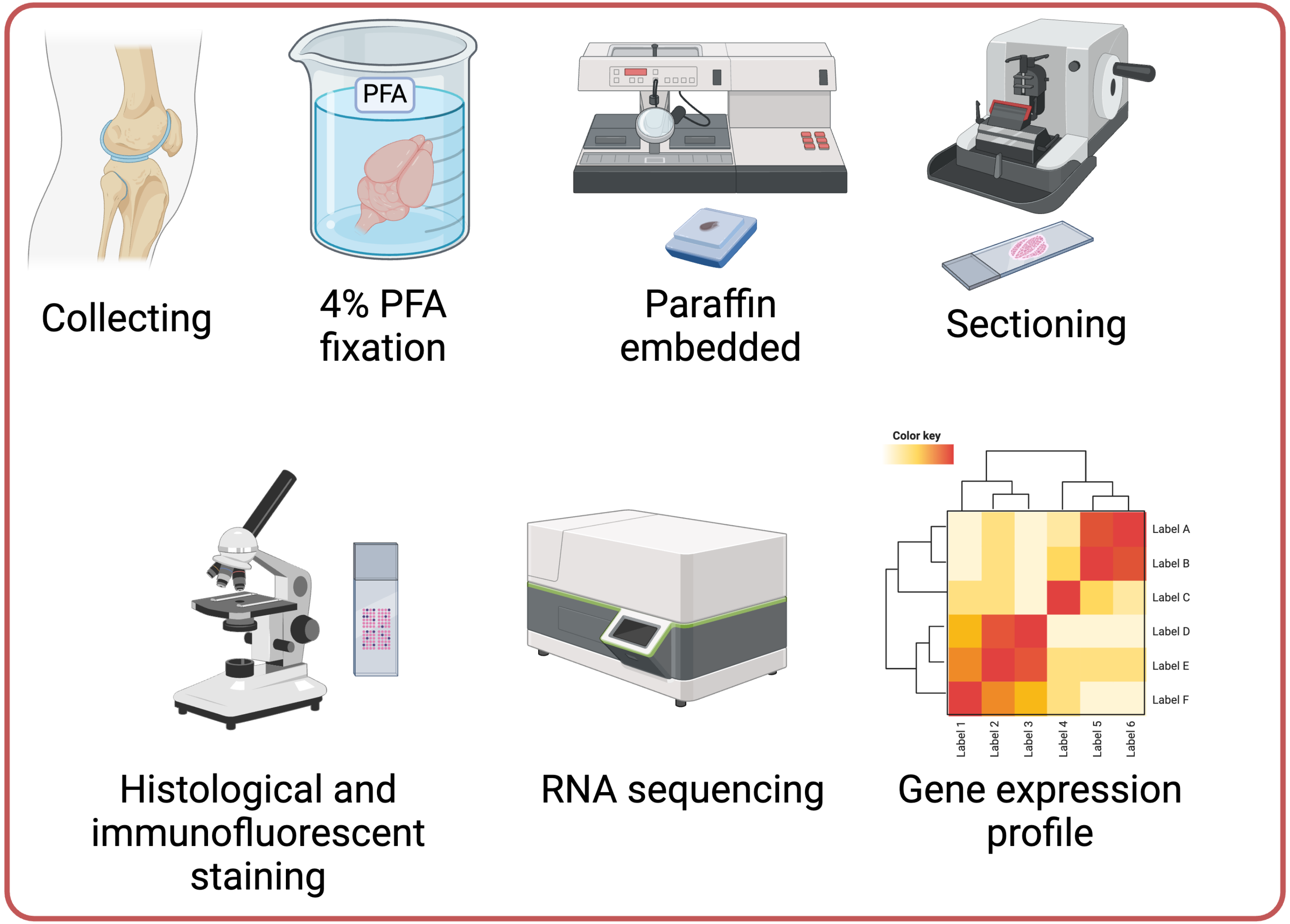


Figure 6. Collecting [2]

Table of articular cartilage sample collection

	Category 1	Category 2	Category 3	Category 4	Category 5
Age	10-20	20-40	40-50	50-60	60-70
Zone	Superficial		Middle	Deep	
Type	Mesenchymal Stromal Cells	MSC-derived day-0	MSC-derived day-7	Normal articular cartilage	Ostearthritis articular cartilage

Processing of Tissue

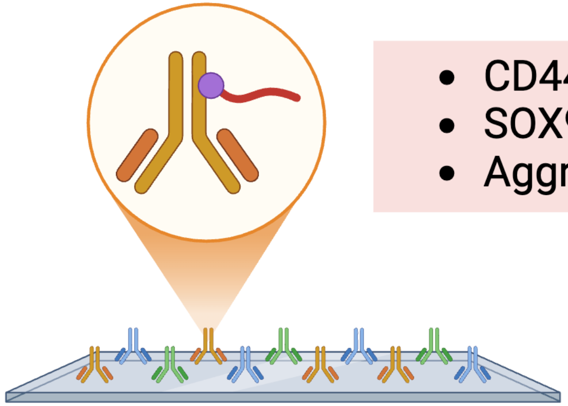


Procedures

1. Collect nHAC tissue samples
2. Fix tissues: 4% PFA fixative, 24 h
3. De-calcify: 20% EDTA (2 D)
4. Preserve: 70% ethanol
5. Paraffin embed

Figure 7 Handling & processing [2]

Nano String



- CD44
- SOX9
- Aggrecan

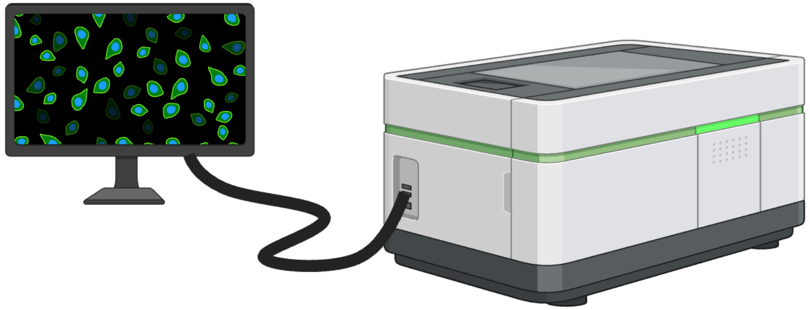


Figure 8 Immunofluorescence staining [2]

Table 2 of protein/cell markers

	Cell/protein markers
Mesenchymal Stromal Cells	• CD44
Normal human articular cartilage	• CD44 • SOX9 • Aggrecan
Osteoarthritis articular cartilage	• CD44 • RUNX2 • Aggrecan

Immunofluorescence staining

- Protein/cell markers (CD44/Sox9/Aggrecan)
- Visualize MSC and nHAC-specific proteins under nano string

Nano String

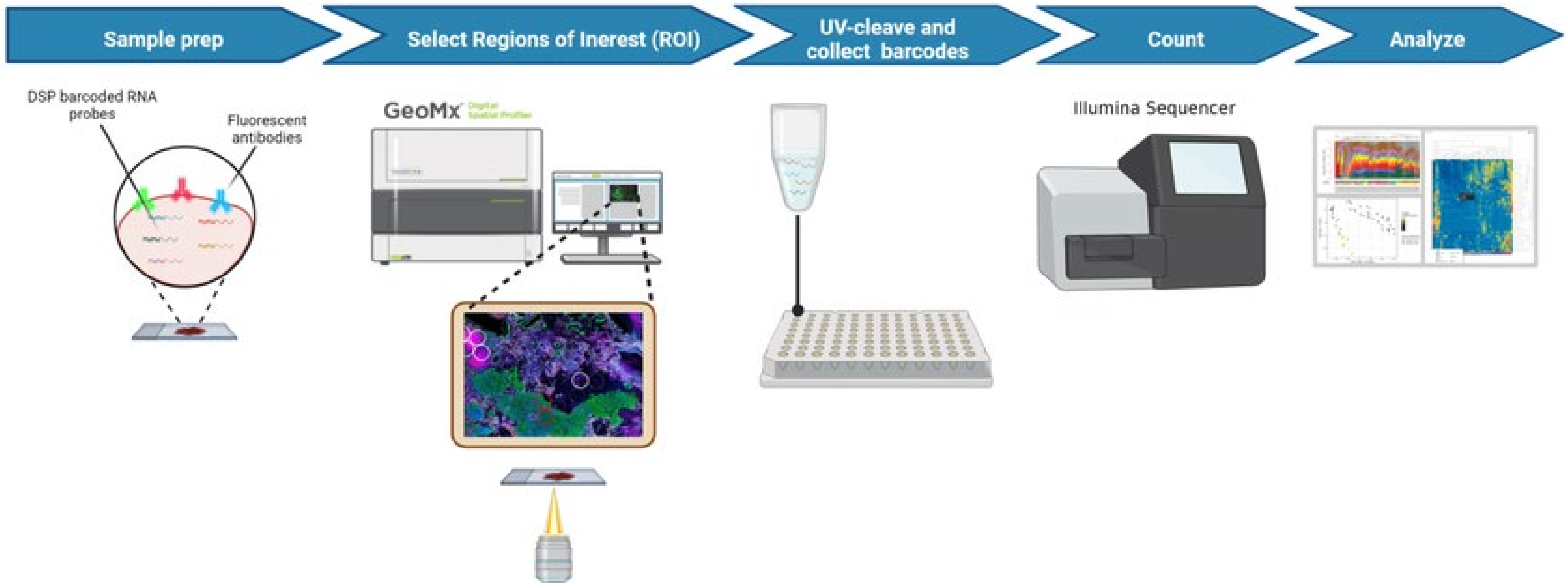
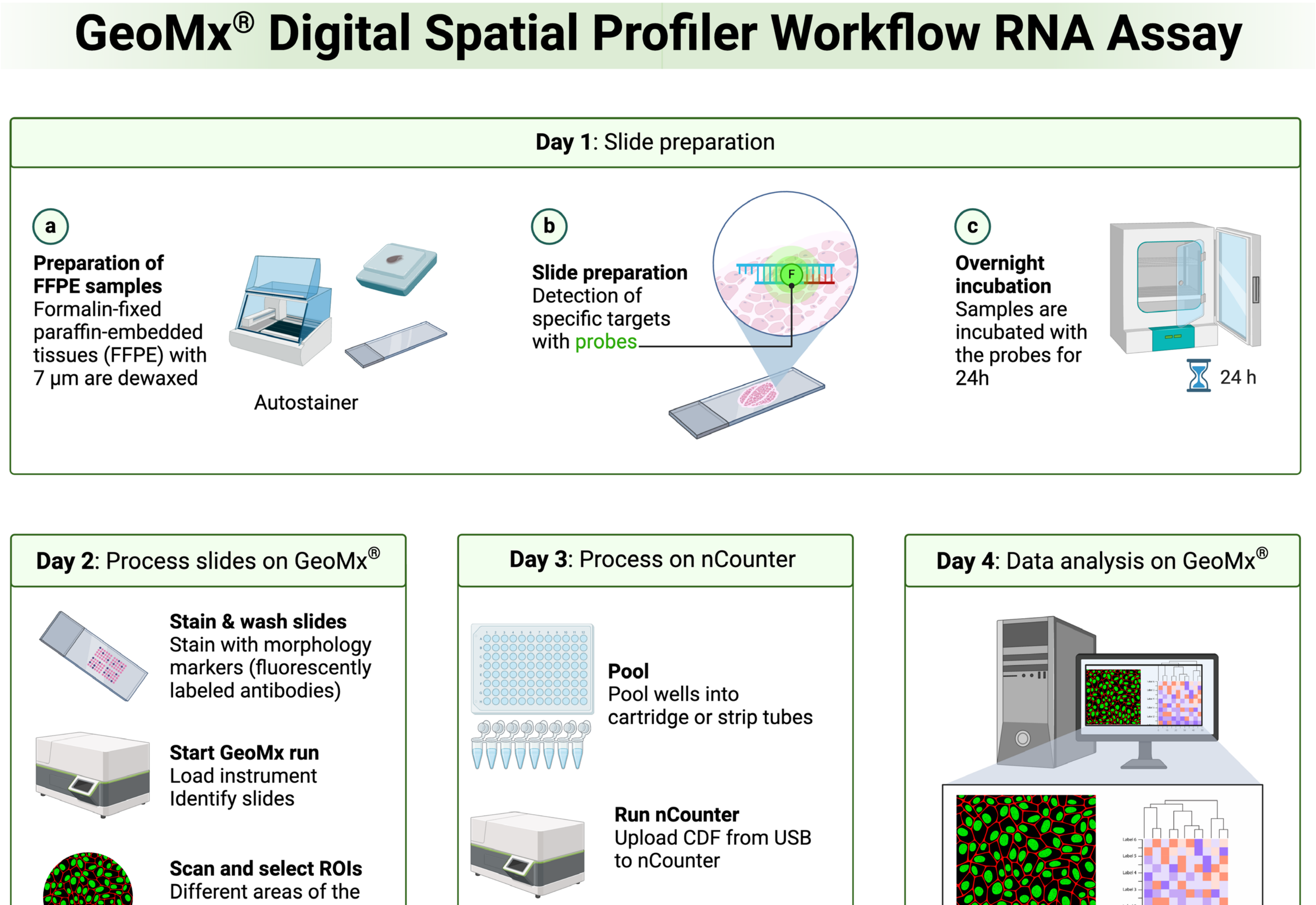


Figure 9. Nano string GeoMx Digital Spatial Profiling (DSP) workflow overview [7]

Nano String

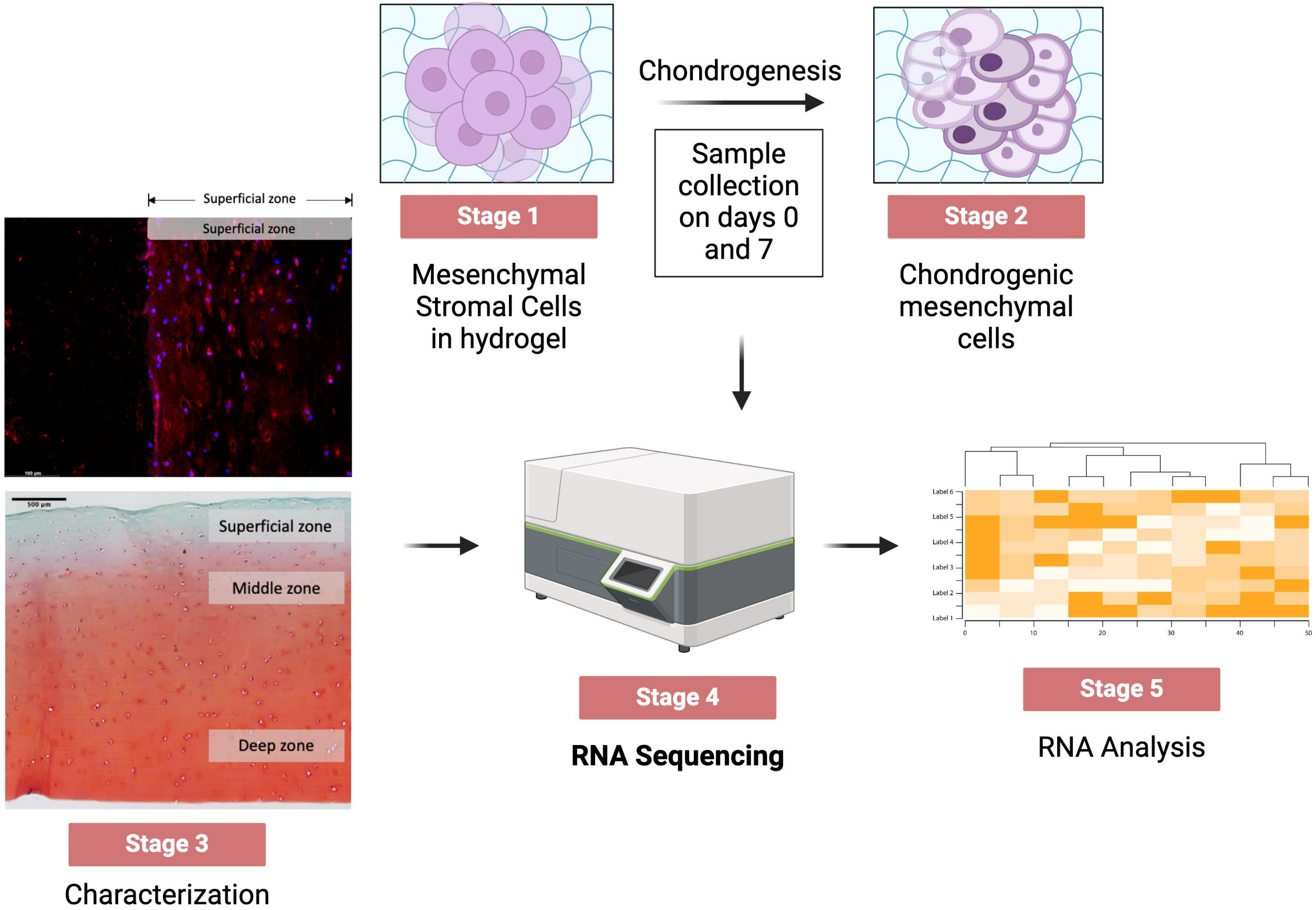


Procedures

1. Section tissue into 7 µm
2. Fluorescence stain tissue
3. Scan and Select tri-layer tissue
4. Collect RNA into 96 well plates
5. Sequence RNA
6. Analyze gene expression

Figure 10. Processing of Nano string spatial profiler [2]

Overview of RNA Sequencing

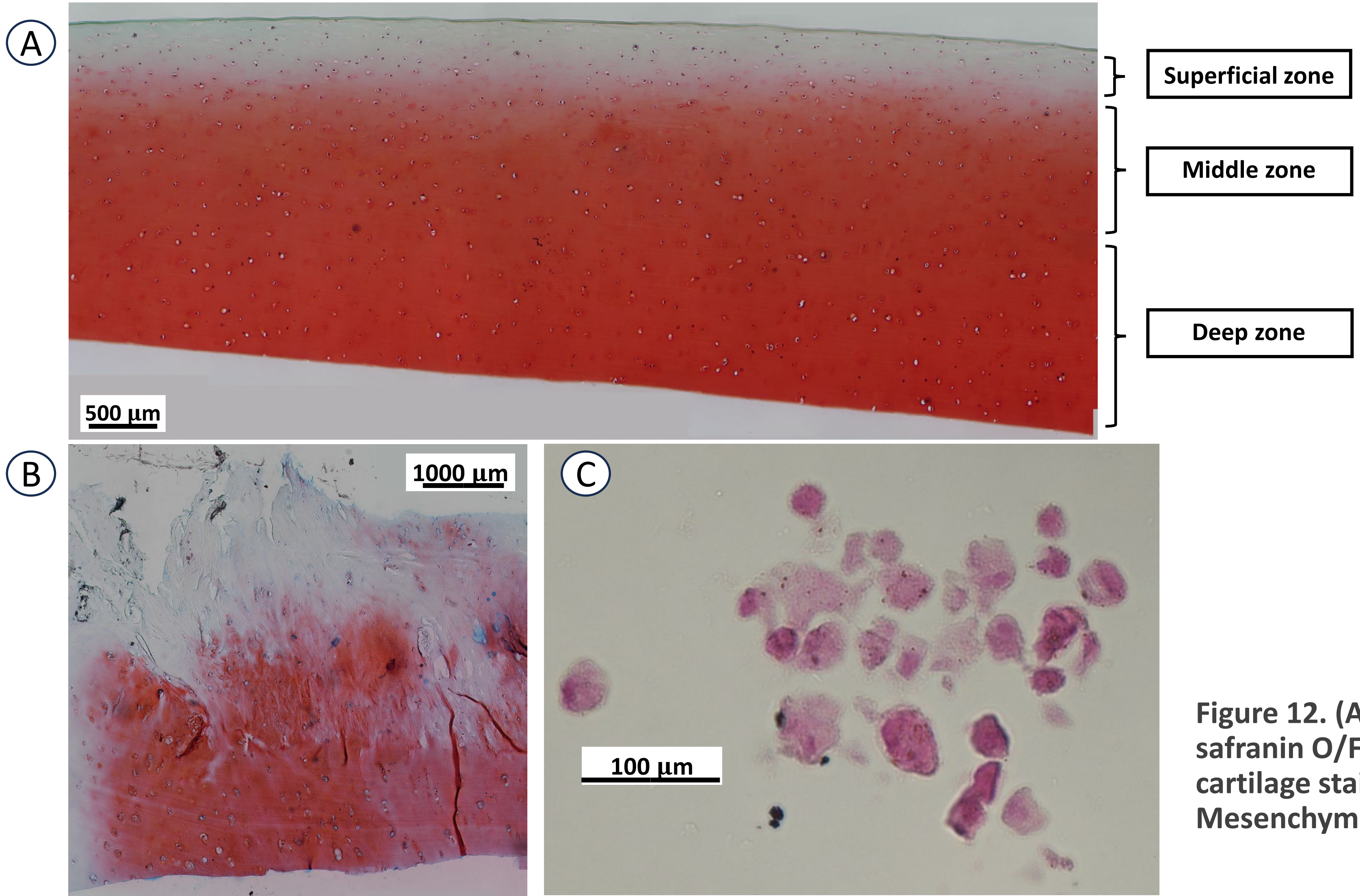


Procedures

1. Tissue structure: histology & immunofluorescence
2. Nanostring: collect RNA by cell/protein markers
3. Gene expression: tri-layer comparison

igure 11. Overview tissue processing [2]

Results 1: Histological Staining



Safranin O—Fast green

- Black dot — Nuclei
- Green — Cytoplasm
- Red — Proteoglycan

Alcian blue/safranin O

- Alcian blue — Glycosaminoglycans
- Safranin O — Proteoglycan, aggrecan

Figure 12. (A) Normal articular cartilage stained with safranin O/Fast green, (B) Osteoarthritis articular cartilage stained with Alcian blue/safranin O, (C) Mesenchymal Stromal Cells stained with H&E

Results 2: Immunofluorescence Staining

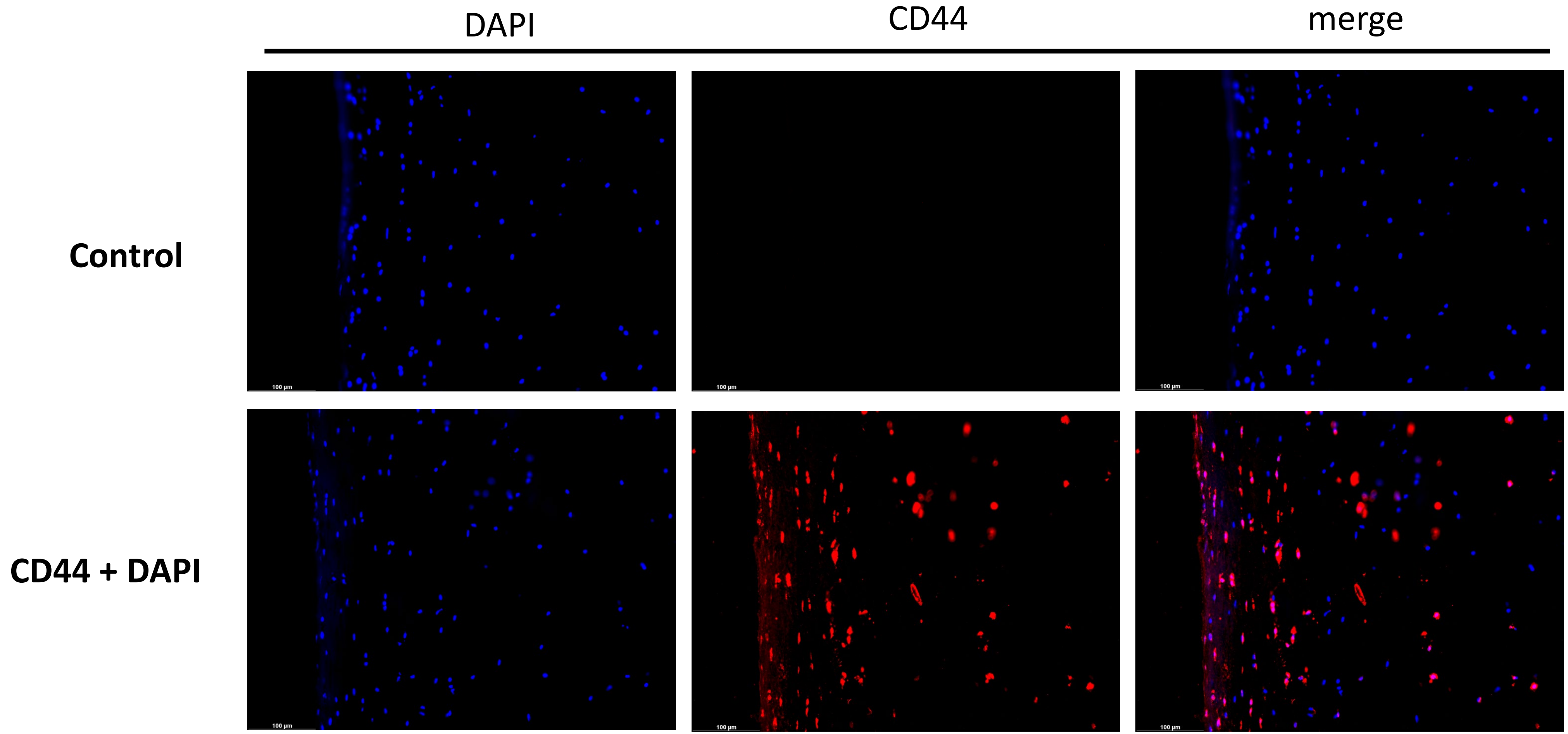


Figure 13. Representative images of immunofluorescence staining (IF) of CD44 and nuclei for normal human articular cartilage (hAC41) under fluorescence microscopy (magnification: 20x).

Results 3: Immunofluorescence Staining

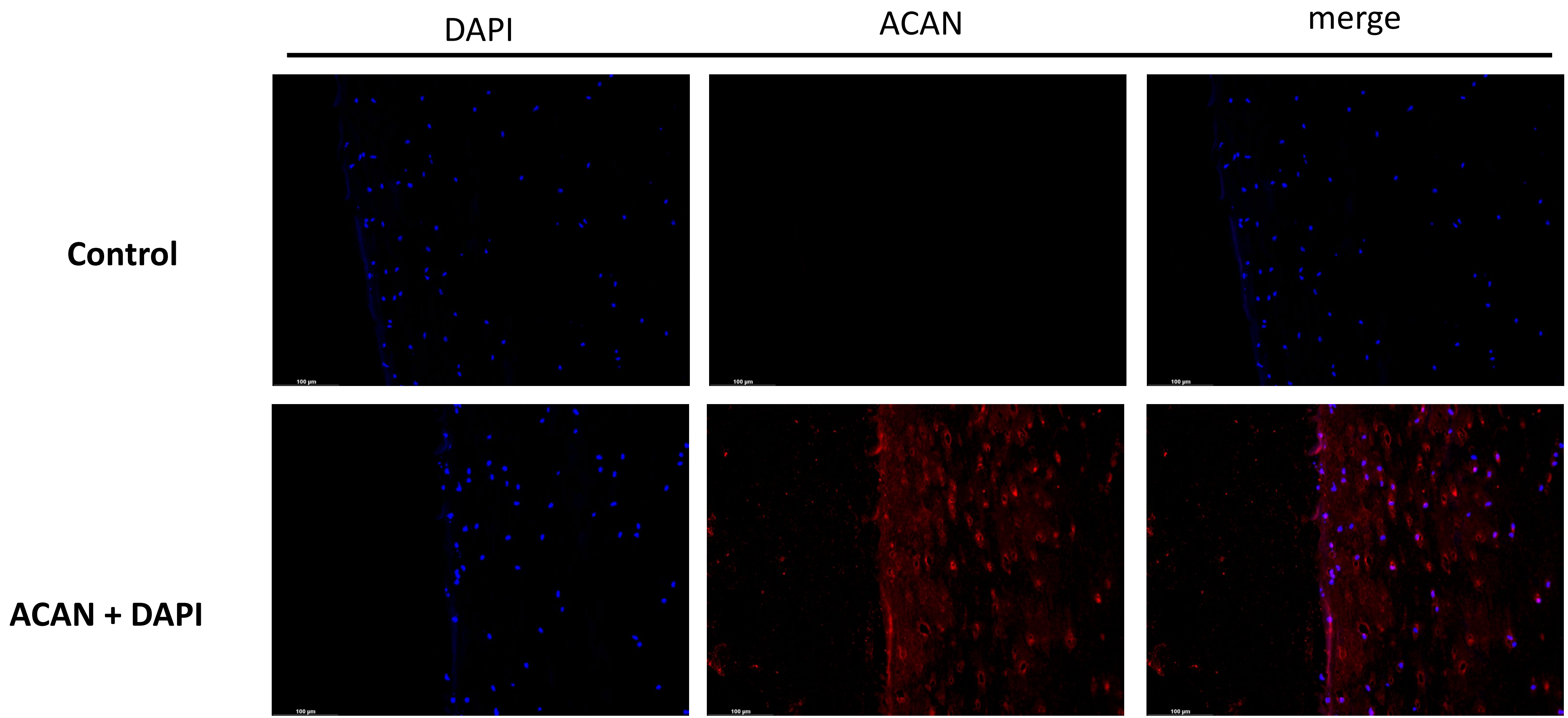


Figure 14. Representative images of immunofluorescence staining (IF) of Aggrecan and nuclei for normal human articular cartilage under fluorescence microscopy (magnification: 20x).

Results 4: Immunofluorescence Staining

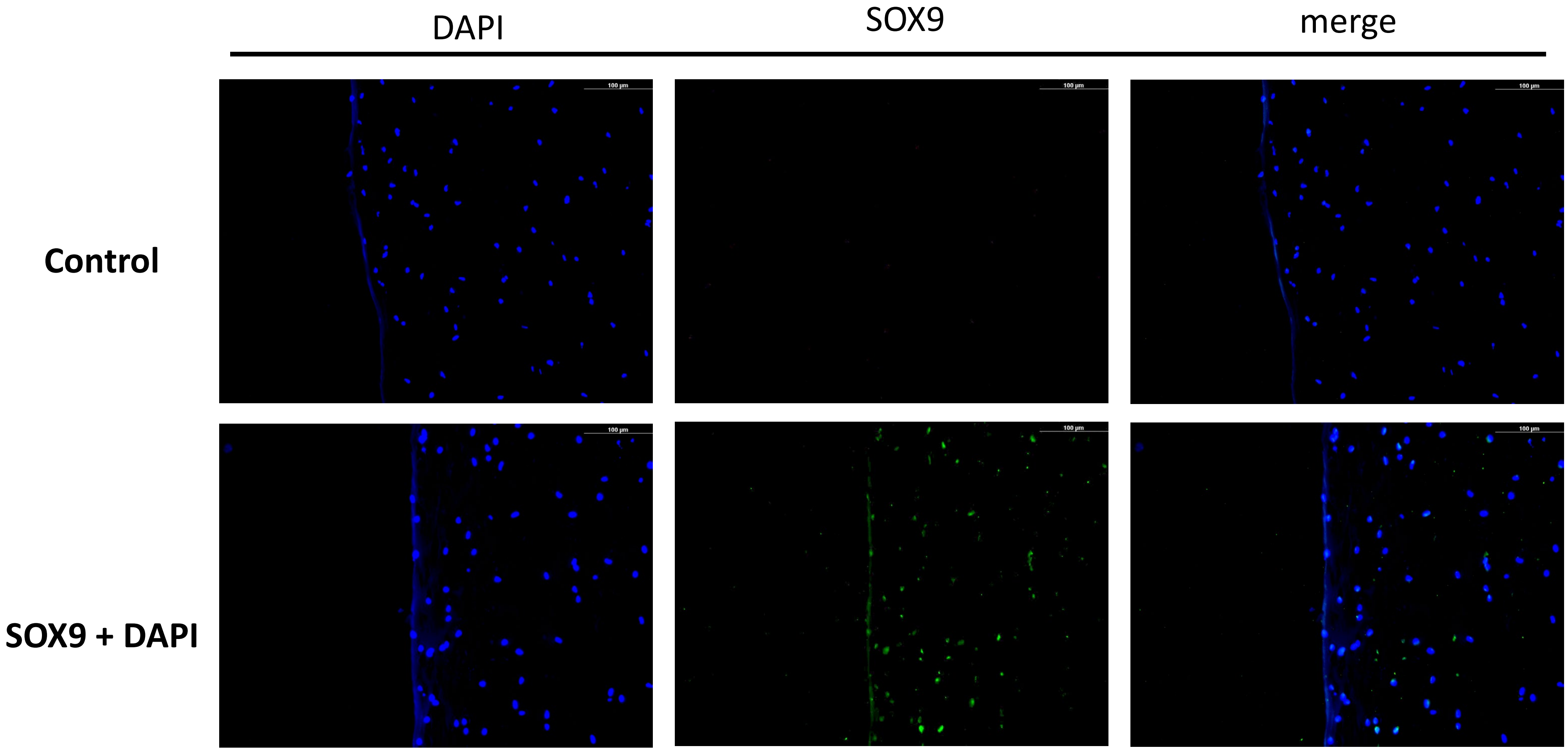


Figure 15. Representative images of immunofluorescence staining (IF) of SOX9 and nuclei for normal human articular cartilage in 5% BSA under fluorescence microscopy (magnification: 20x).

Expected results

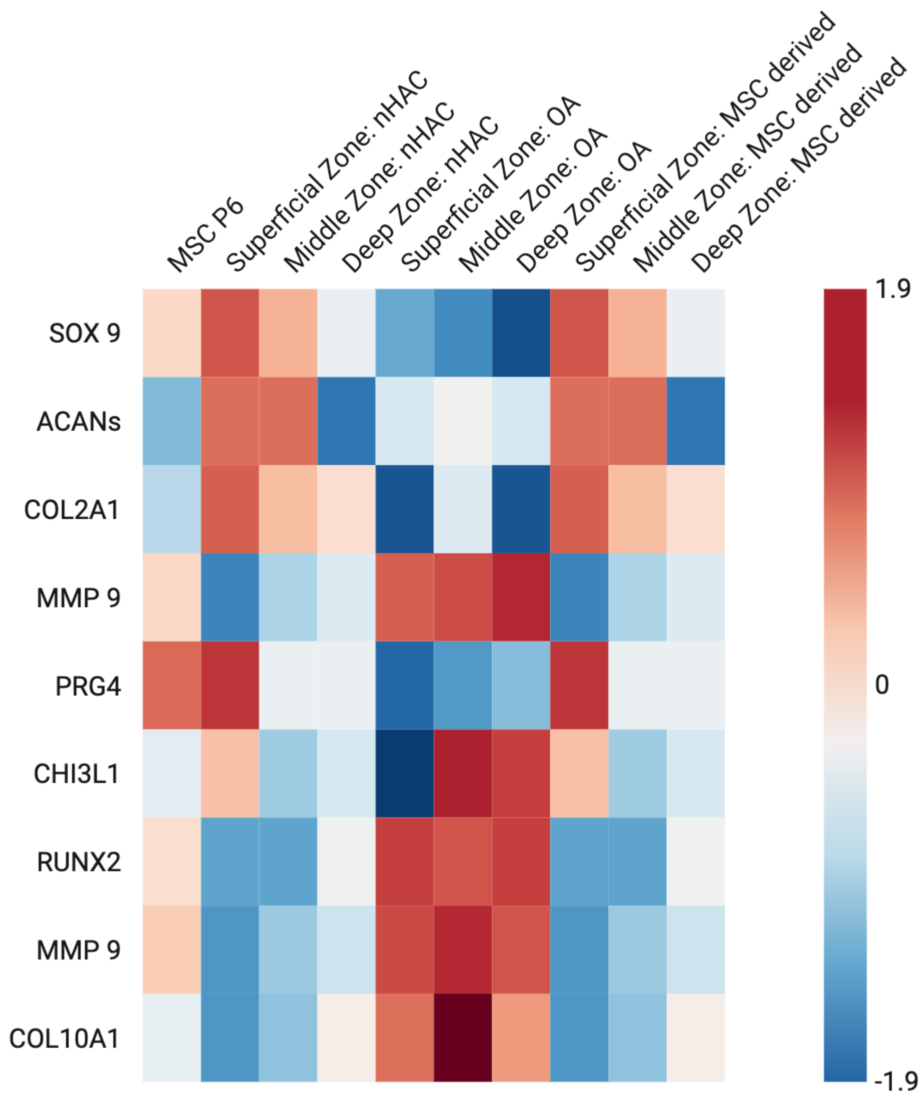


Figure 16 Heat map gene expression [2]

Expected results:

- **SOX9, COL2A1, ACAN**: high in nHAC& MSC-derived bioreactor
- **RUNX2**: elevated in OA.
- Similar gene profiles between nHAC& MSC-derived bioreactor.

Project Summary

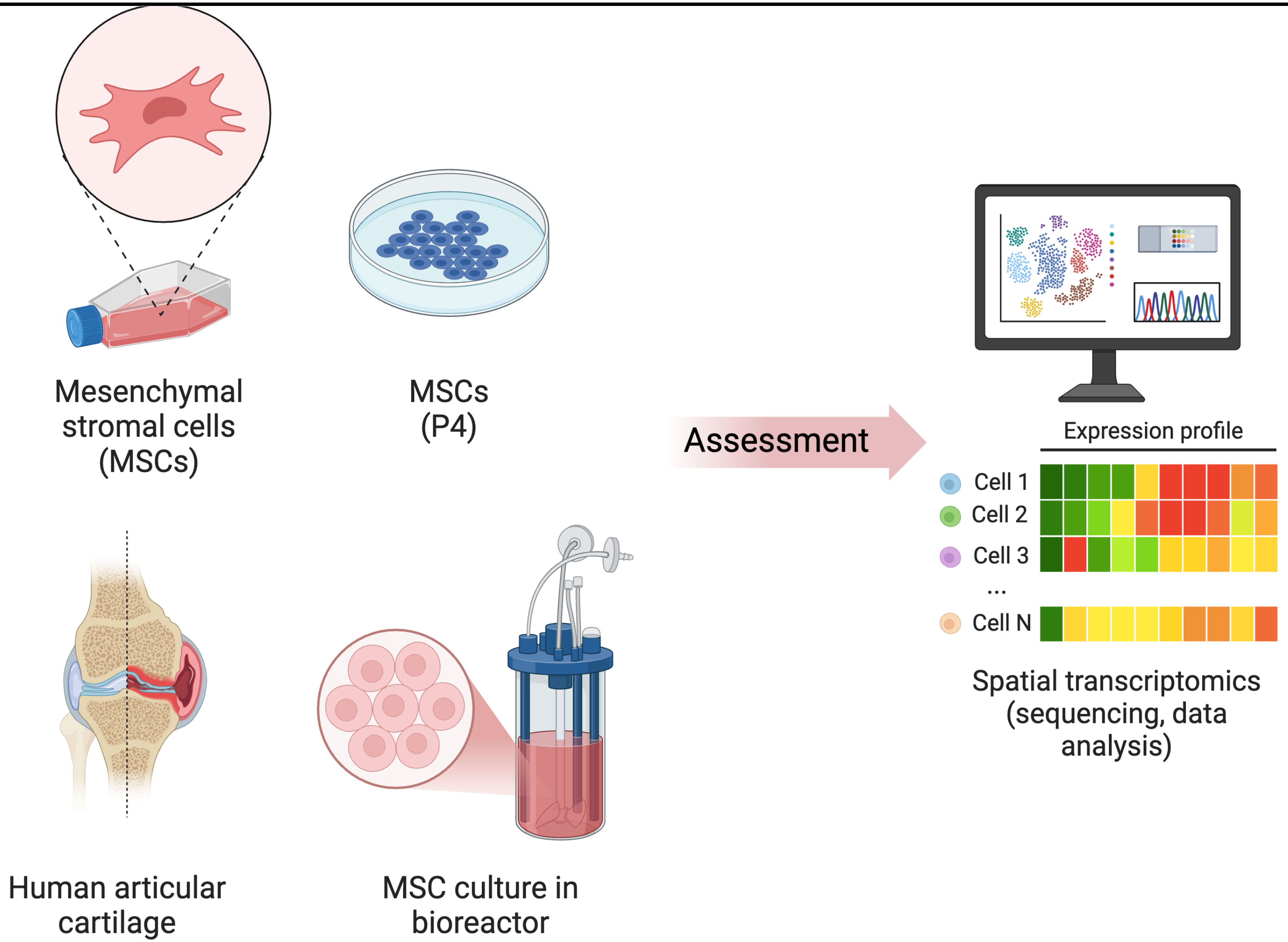
Objective:
- Analyze properties & cell populations
- Assess MSCs & tri-layer constructs in bioreactors
- Validate MSCs with mechanical forces & TGF-B3

Tissue samples:
- nHAC: 9 patients (N = 9)
- OA: 6 patients (N = 6)
- MSCs in hydrogel (cultured in bioreactor 2 weeks)

Methodology:
- Immunofluorescence
- NanoString for RNA sequencing
- Python & R Studio: Data Analysis

Expected results:
- Gene Expression Profiles
- Assess replication of NHAC properties.

Future plans for RNA sequencing



Sequence RNA

- Compare zones: superficial, middle, deep
- Analyze normal, osteoarthritic, and MSC-derived types

17. MSC, normal, OA, and chondrogenesis in bioreactor [2]

Collaborators

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Acknowledgments

Research support:

- WSU NIH Protein Biotechnology Training Grant #T32 GM008336-31
- NSF RECODE #2225528 (WSU) and # 2225559 (Cornell)
- WSU Biotechnology Life Sciences Staff: Imagining, Bioanalysis Lab
- WSU Franceschi Microscopy & Imaging Center (FMIC) Staff: staining preparation & cryotome

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THANK YOU!