Class Name
Tissue Engineering I

Professor
Dr. Li Cai

Date
Dec 11 2024

Biocomposite Scaffolds

For Cartilage Regeneration

Sachi Kurian, Smriti Mumudi, Jahnavi Shah,
Emily Sullivan, Ria Suresh

Table of Contents

01

Background

Cartilage Degeneration

01

Cartilage is an avascular, aneural tissue.

02

Common causes of cartilage damage include trauma, degenerative disorders, and congenital disorders.

03

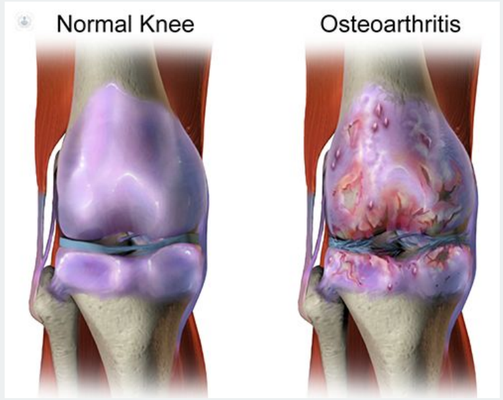
Damage to cartilage results in pain, swelling, and reduced mobility.

04

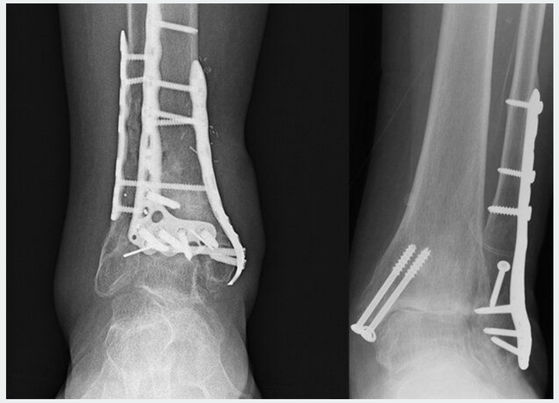
Cartilage has limited regenerative capacity.

Hyaline cartilage lacks blood vessels to facilitate repair.

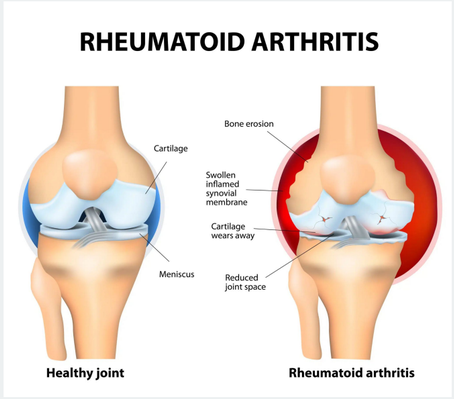
Associated Diseases



Osteoarthritis

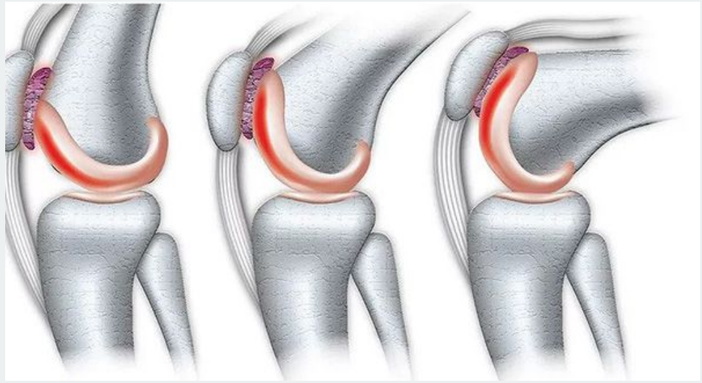


Post-Traumatic Arthritis



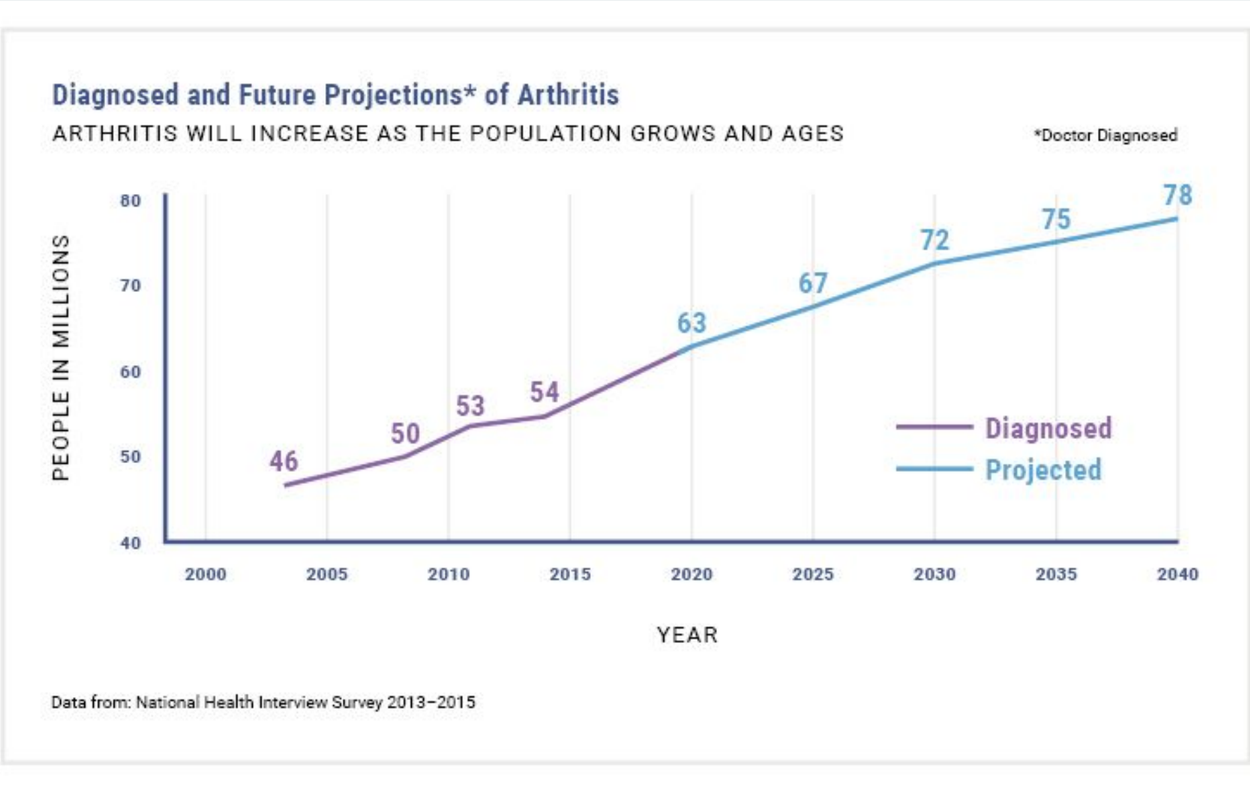
Rheumatoid Arthritis

Joint Dysplasia



Chondromalacia Patellae

Scope



~32.5 million US Adults are affected by osteoarthritis

20% of patients experience chronic pain following total knee arthroplasty

Failure rates for cartilage repair surgeries reported up to 75%

02

Current Treatments

Current Methods
(on Market and Being Studied)

01 Autologous Chondrocyte Implantation

02 Platelet-Rich Plasma Injections

03 Microfracture

04 Osteochondral Grafts

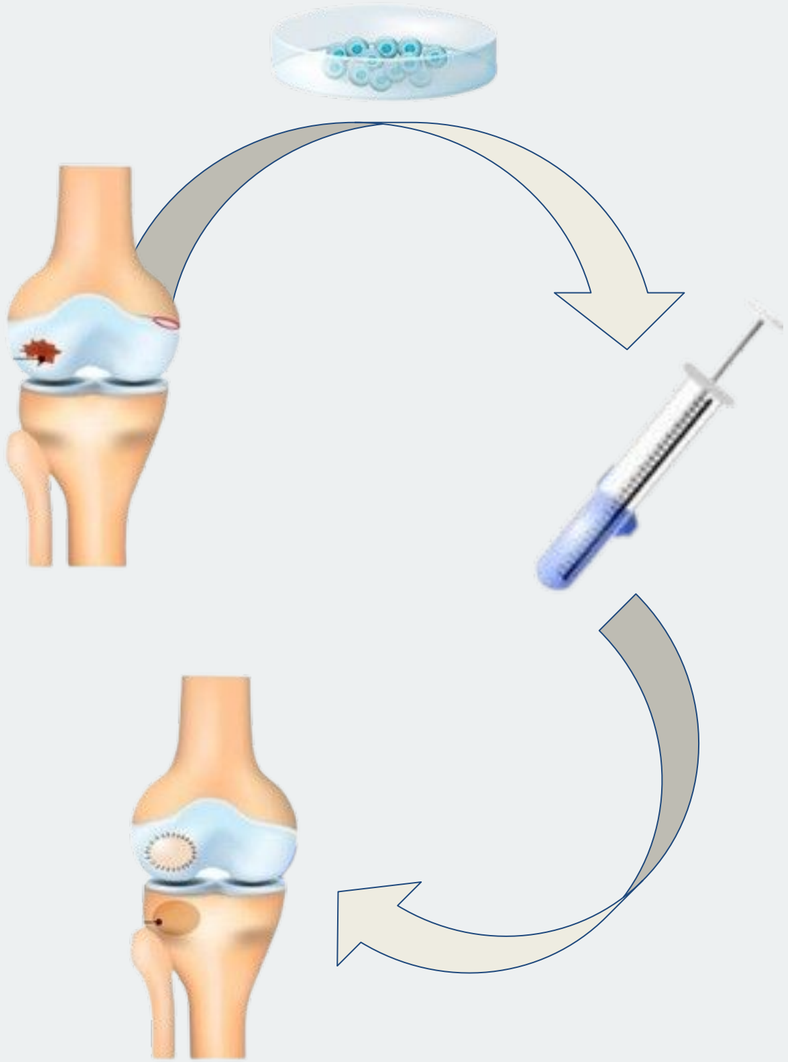
05 CartRevive™ Hydrogel Implant

06 MSC-Based Treatments

Autologous Chondrocyte Implantation

PROS

- Effective for large defects
- Can result in regeneration of hyaline-like cartilage
- Low rejection rates



CONS

- Invasive and complex procedure
- Expensive
- Varied success
- Long-term post-op care required

biopsy → culturing → injection → suturing of graft → cells implanted in graft

Platelet-Rich Plasma Injections

PROS

- Minimal risk for immune reaction
- Reduce pain & increase function
- Less invasive than surgery

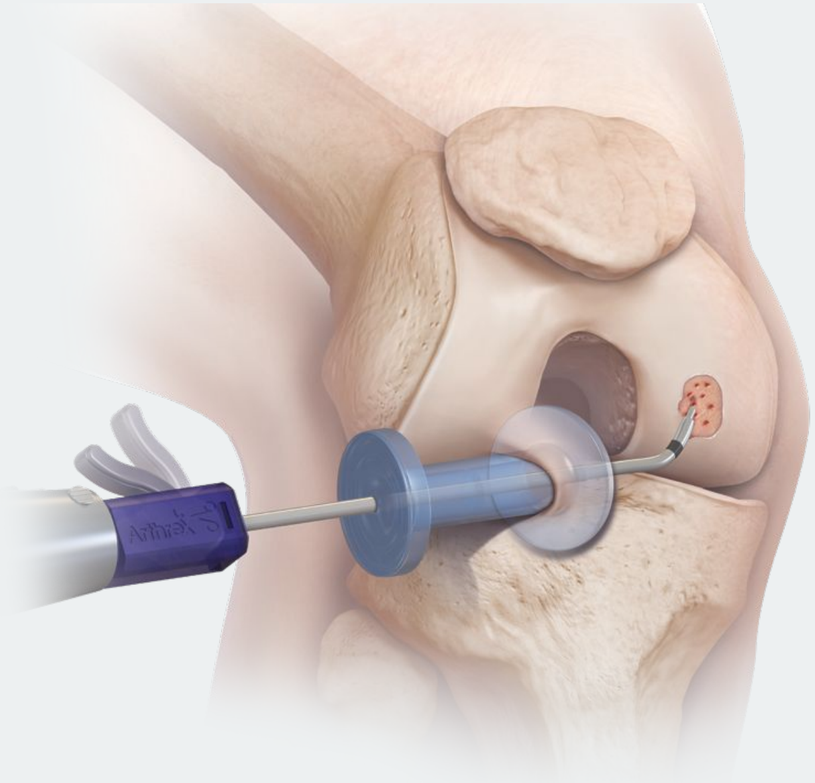
CONS

- Inconsistency in results
- Lack of standardization in protocol
- Symptom-modifying, not structure-modifying

Microfracture

PROS

- Minimally invasive
- Relatively affordable



CONS

- Results in formation of fibrocartilage
- Decline in joint functionality

Osteochondral Grafts

PROS

- **Provides structural support**
- **Minimized risk of immune rejection**

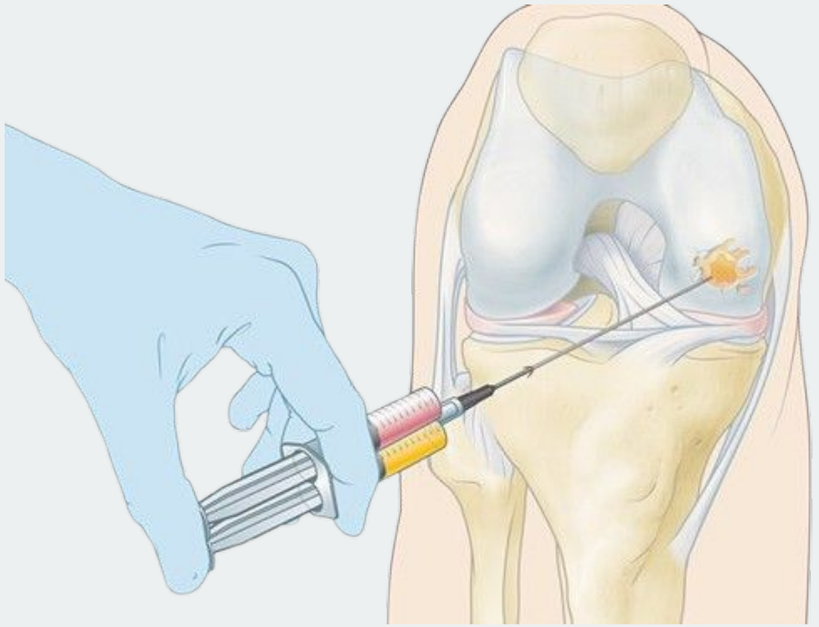
CONS

- **Donor site morbidity**
- **Limited tissue availability**

CartRevive™ Hydrogel Implant

PROS

- Supports autologous regeneration
- Minimally invasive



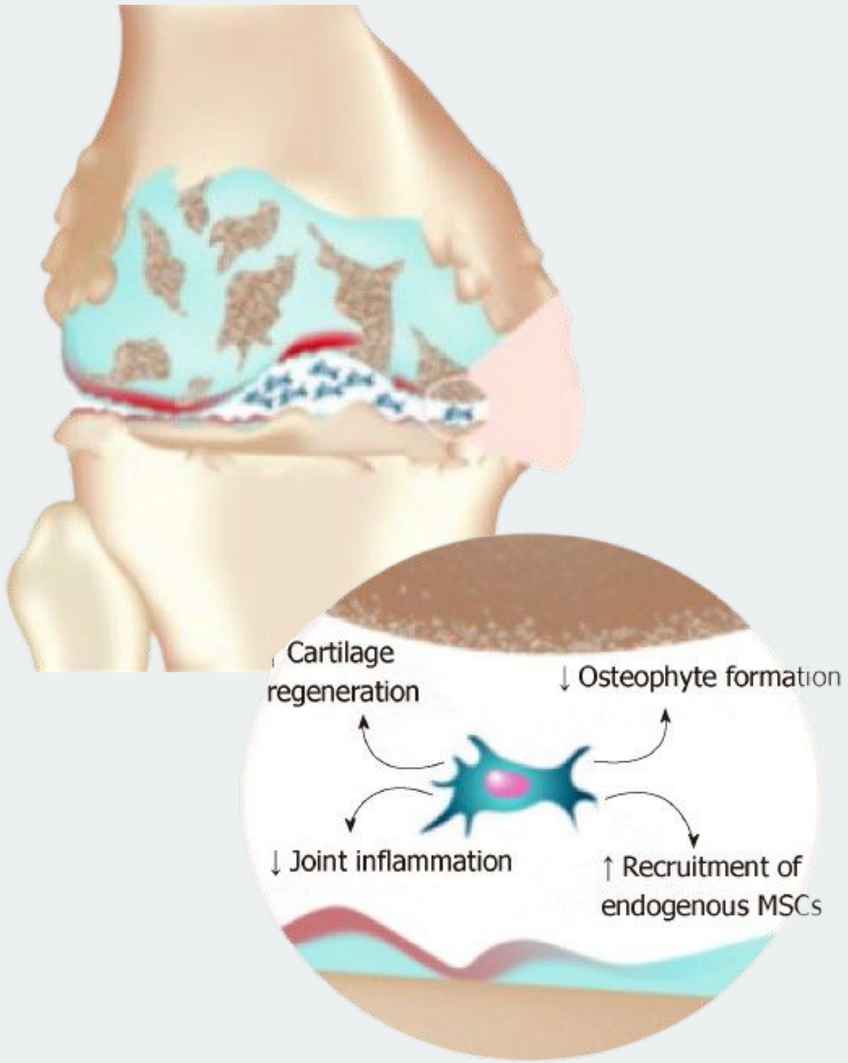
CONS

- Still under clinical investigation

Mesenchymal Stem Cell-Based Treatments

PROS

- MSCs have shown promise in treatment of cartilage damage and repair



CONS

- Long-term effectiveness and safety still under investigation

03

Innovation

Our innovation is a degradable biocomposite scaffold composed of PEGDA and collagen. The scaffold is then cultured in a medium containing synthetic cannabinoids for chondrogenesis of human mesenchymal stem cells in articular cartilage.

Specific Aims

1

Reduce Inflammation and Pain

Improve mobility and quality of life.

2

Patient Specific Temporal Degradation Profiles

Provide customized hydrogel.

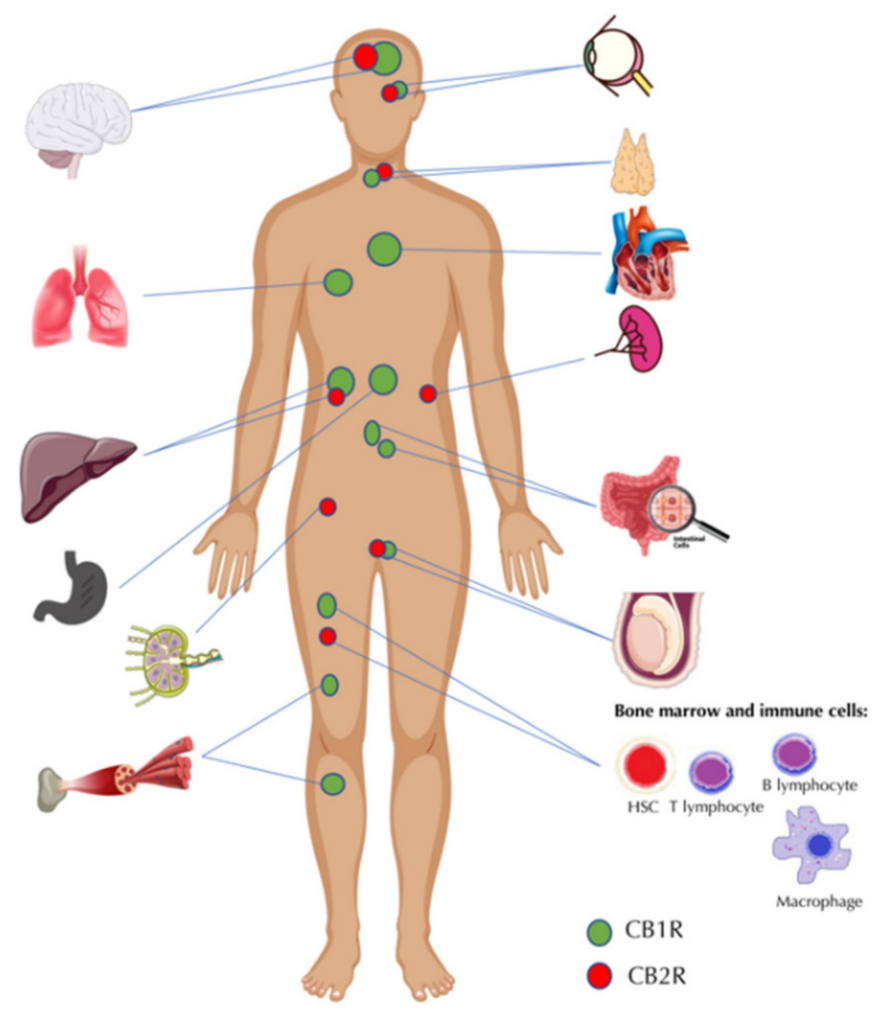
3

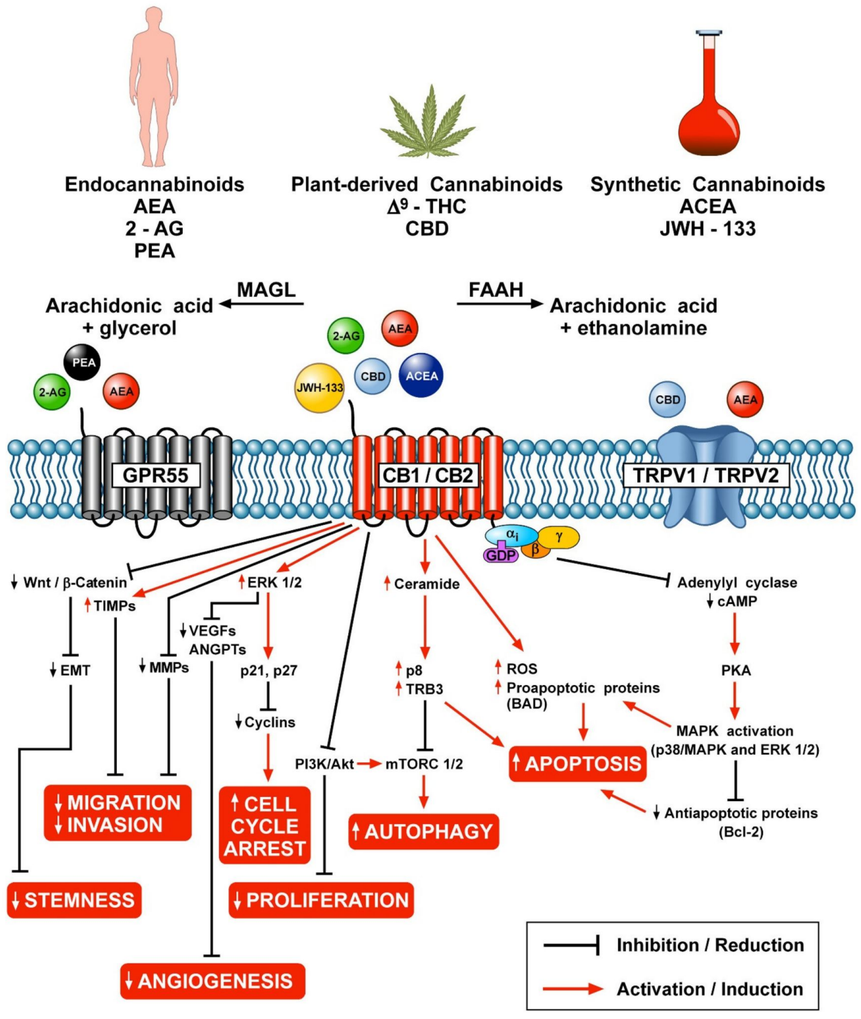
Preventing Further Degradation and Facilitating Cartilage Regeneration

Improve joint stability.

Aim 1: Endocannabinoid System

Mechanisms of Success:





Aim 1: Endocannabinoid System

Mechanisms of Success:

MSCs express cannabinoid receptors CB1 and CB2.

Activation of CB2 through cannabinoid exposure reduces secretion of pro-inflammatory cytokines (such as TNF-α and IL-1β) while also promoting release of anti-inflammatory cytokines (such as IL-10).

Anti-inflammatory environment promotes MSC survival and function.

Aim 1: Endocannabinoid System

Mechanisms of Success:

Wnt/β-catenin pathway and P38 MAPK pathway are critical for stem cell differentiation.

Exposure to cannabinoids activates these pathways.

Cannabinoids promote differentiation into chondrocytes, which then aid cartilage repair and regeneration.

Aim 1: Endocannabinoid System

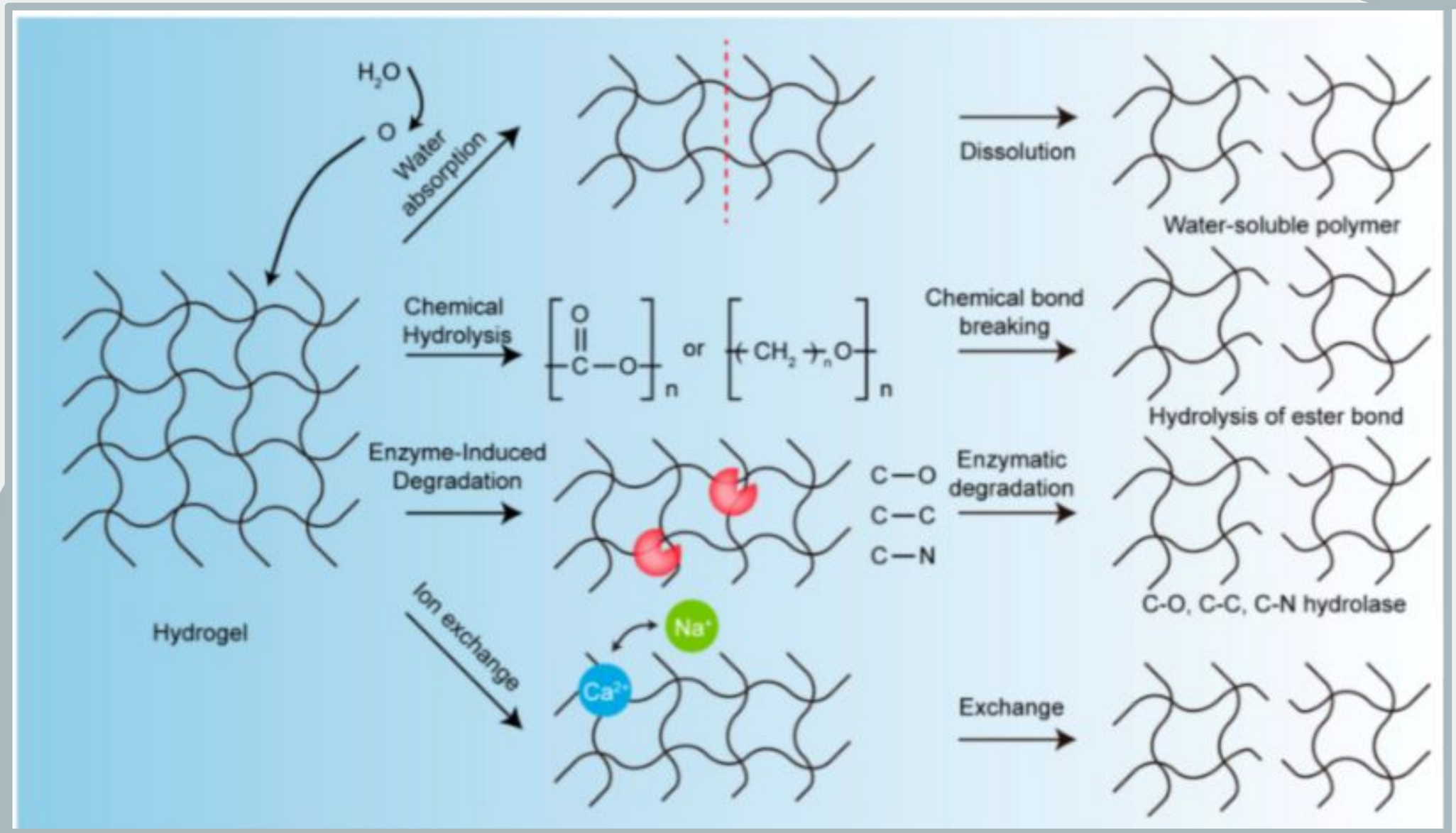
Mechanisms of Success:

Cannabinoids exert antioxidant effects once receptors are activated.

Reducing oxidative stress helps protect MSCs from apoptosis.

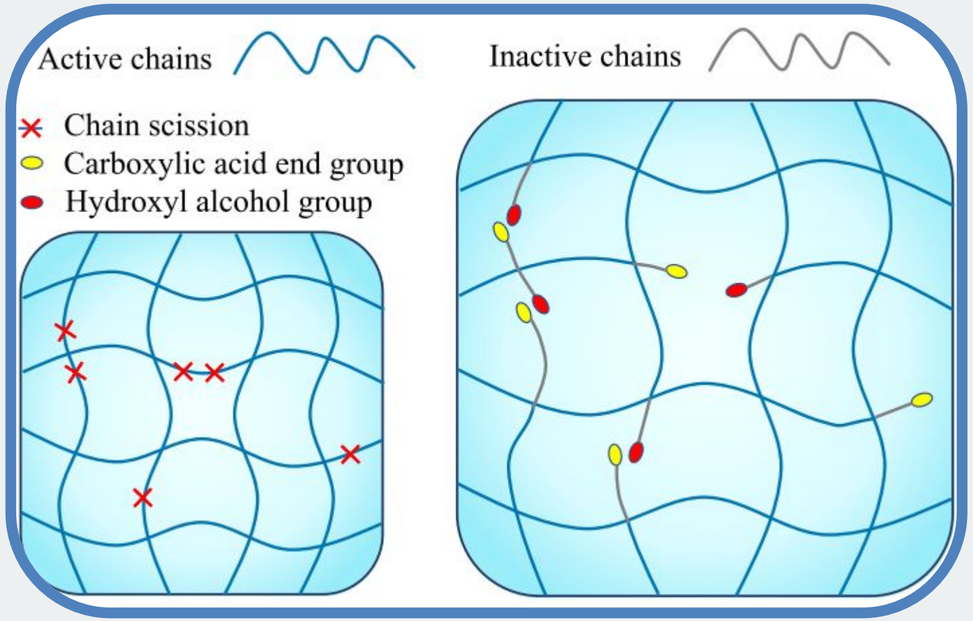
MSC survival is increased and regenerative potential is maintained, despite the hostile environment of the inflamed joint.

Aim 2: Hydrogel Degradation

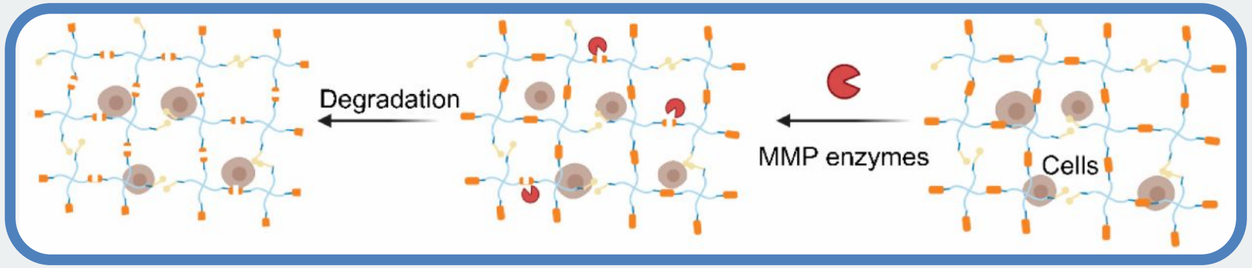


Ester hydrolysis degradation

- Lack spatial specificity
- Biodegradation forms COOH and OH which causes
  - acidic environment which can hinder chondrogenesis.
  - accelerated degradation



VS.

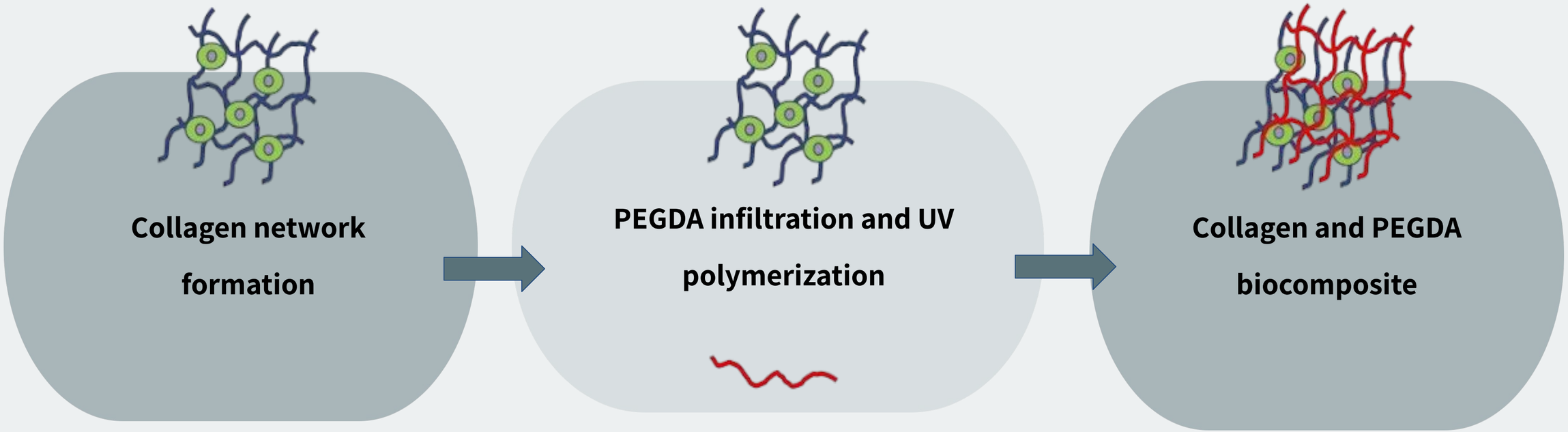


MMP-7 mediated degradation

- Cell-differentiation mediated
- Spatiotemporal control
- Mechanical stability for load bearing joints.

Aim 3: Collagen and PEGDA Biocomposite

Mechanisms of Success:



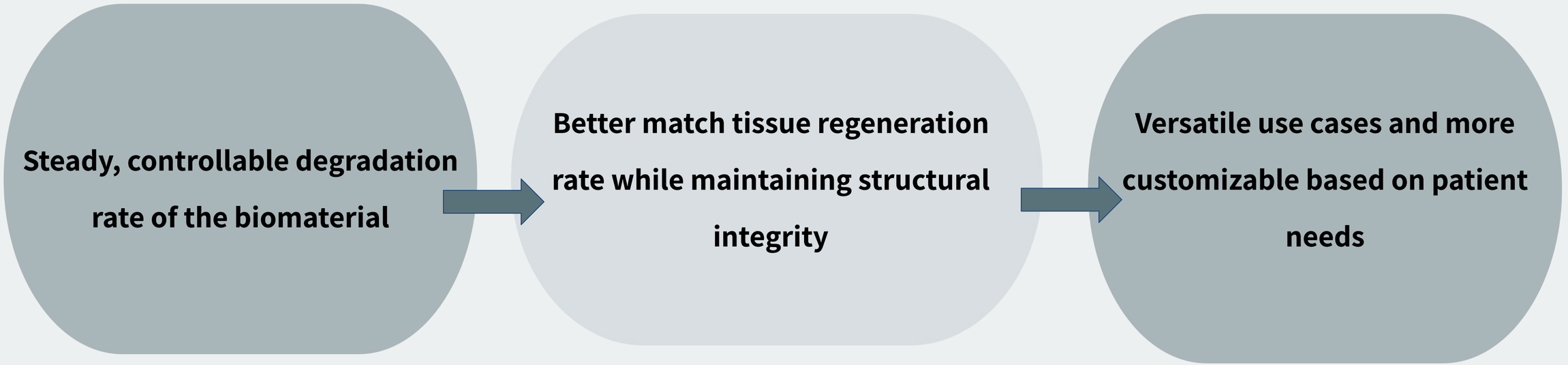
Aim 3: Collagen and PEGDA Biocomposite

Mechanisms of Success:

High swelling ratio → **Better mimics natural collagen, increases surface area and porosity of the matrix** → **Improves biocompatibility, creates more space for cells to form new tissue and enhances diffusion of materials through the scaffold**

Aim 3: Collagen and PEGDA Biocomposite

Mechanisms of Success:



Methodology of Development

Phase I:

Hydrogel Preparation

Phase II:

Bioprinting and Crosslinking

Phase III:

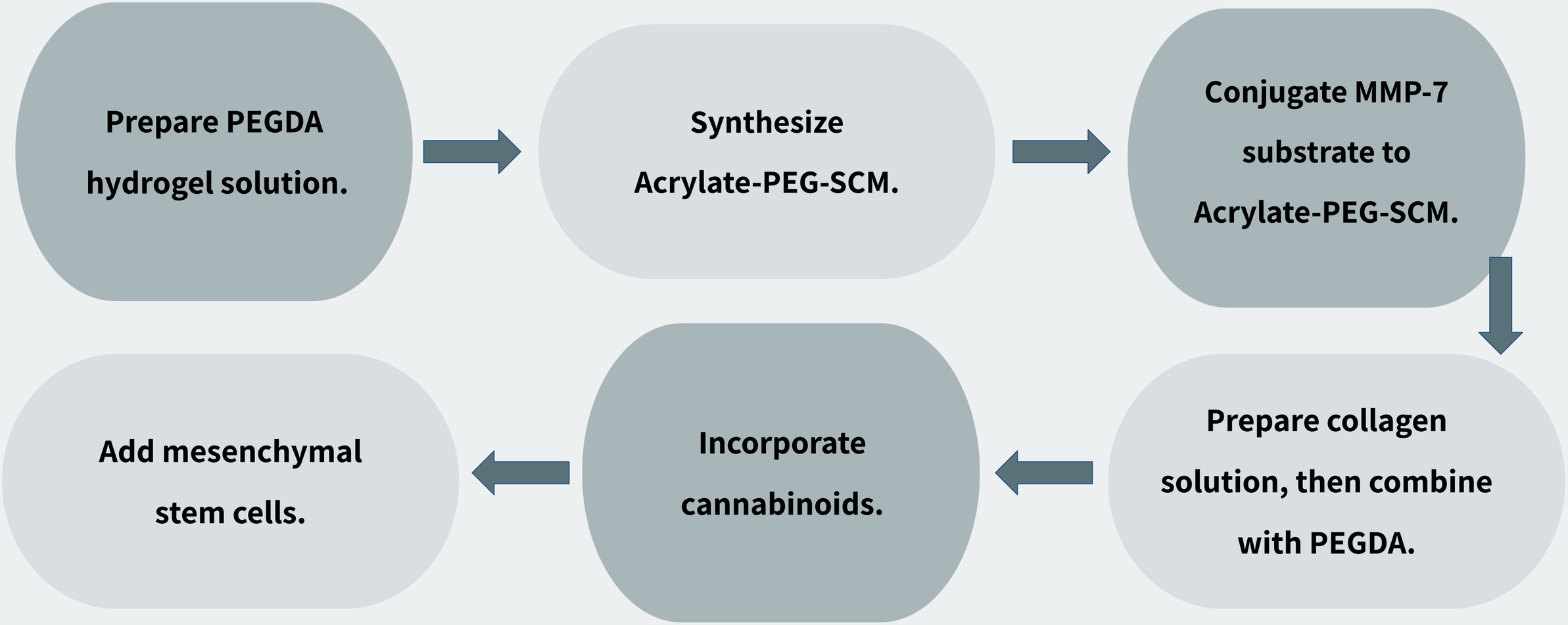
Culture and Differentiation

Phase IV:

Assessment of Outcomes

Methodology

Hydrogel Preparation



Methodology

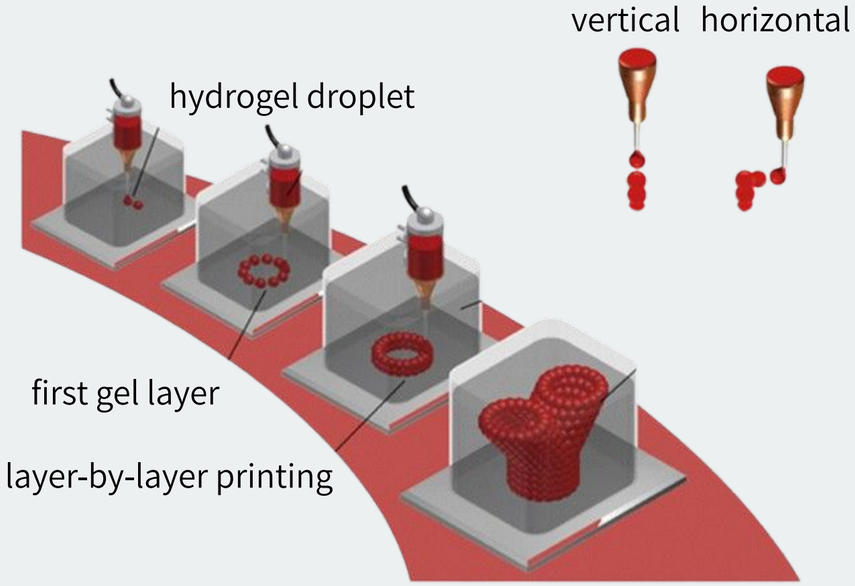
Bioprinting and Crosslinking

Load hydrogel-cell mixture into 3D bioprinter cartridge.

Print the construct layer by layer, according to scaffold architecture.

Expose printed scaffold to UV light for crosslinking.

Immerse in cell culture media.



Methodology

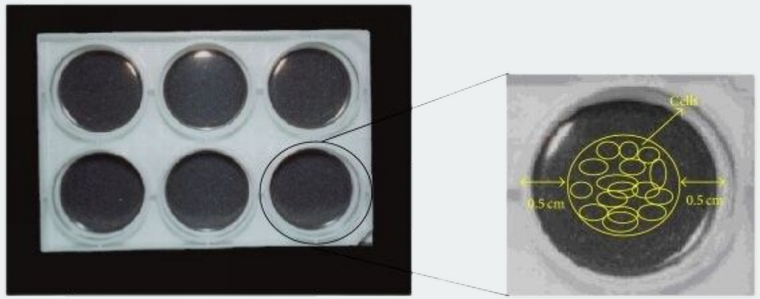
Culture and Differentiation

Place printed constructs into culture plates.

Add chondrogenic differentiation media.

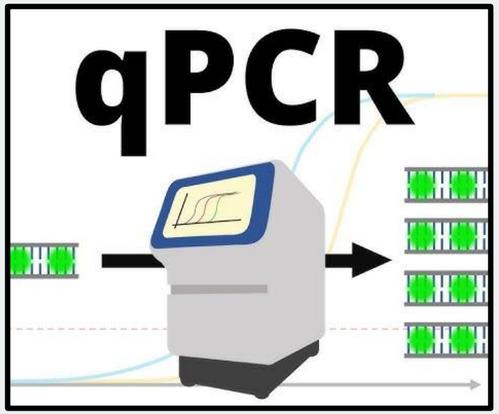
Add cannabinoids at desired concentration to the media.

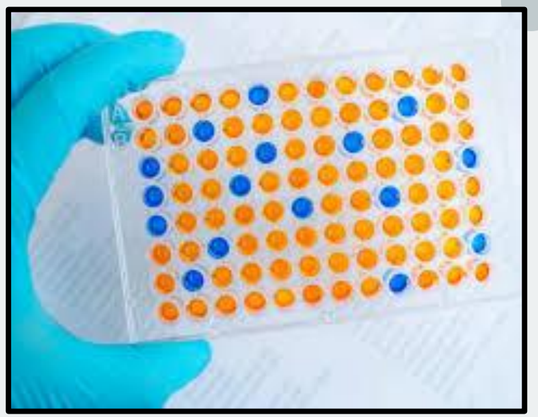
Change media every 2-3 days.

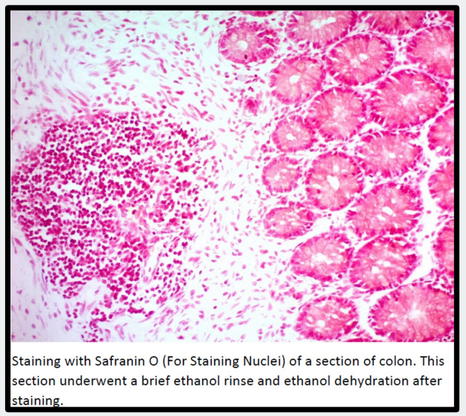


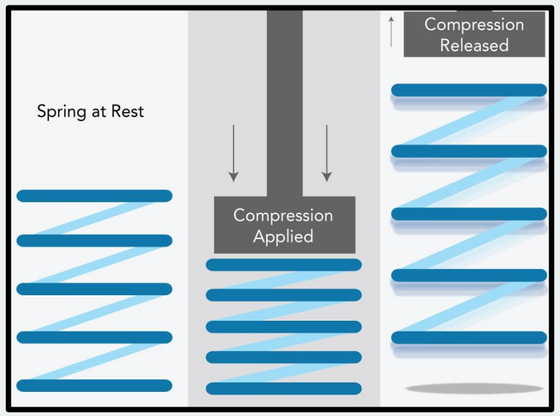
Assessment of Outcomes

- **Quantify chondrogenic markers:**
  - *Gene expression*: qPCR for COL2A1, ACAN, and SOX9.
  - *Protein expression*: ELISA or immunohistochemistry
- **Histological evaluation:**
  - *Proteoglycan content*: Safranin O and Alcian Blue staining
  - *Collagen deposition and alignment*: Picrosirius Red staining
- **Mechanical Properties:**
  - *Young's Modulus:* Compression tests
- **Cannabinoid Effect Validation:**
  - *Cell proliferation:* DNA quantification, BrdU assay
  - *ECM deposition:* GAG, collagen quantification
  - *Anti-inflammatory markers:* IL-10 and PGE2



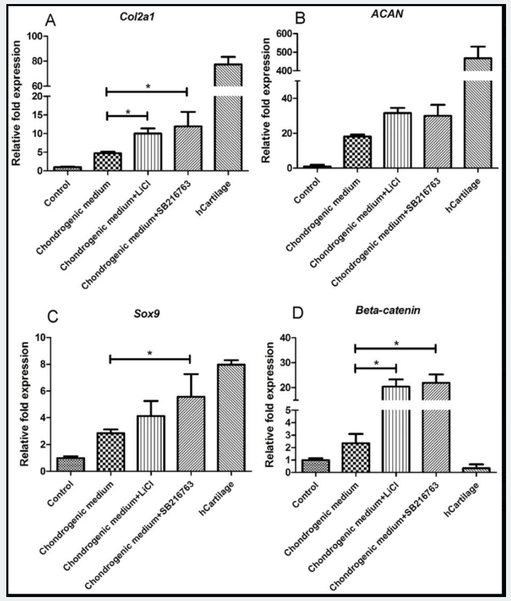


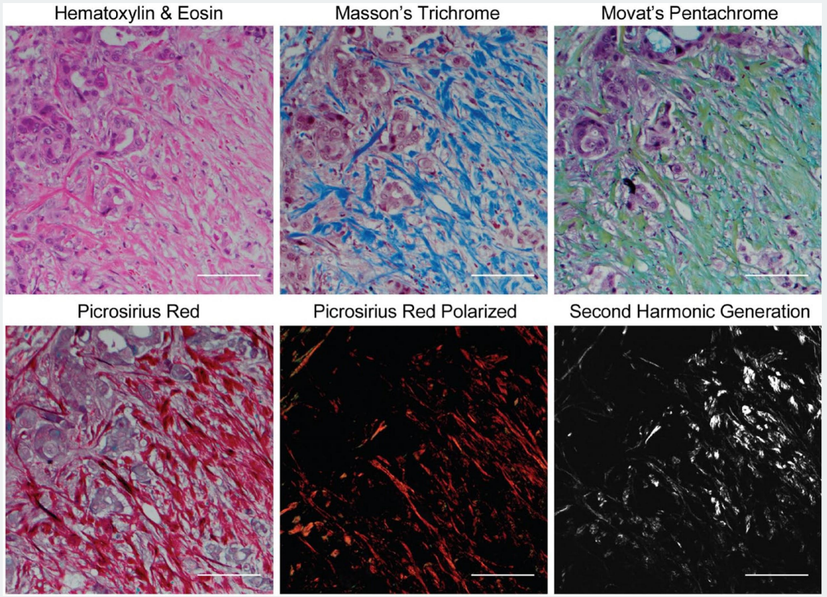

Staining with Safranin O (For Staining Nuclei) of a section of colon. This section underwent a brief ethanol rinse and ethanol dehydration after staining.

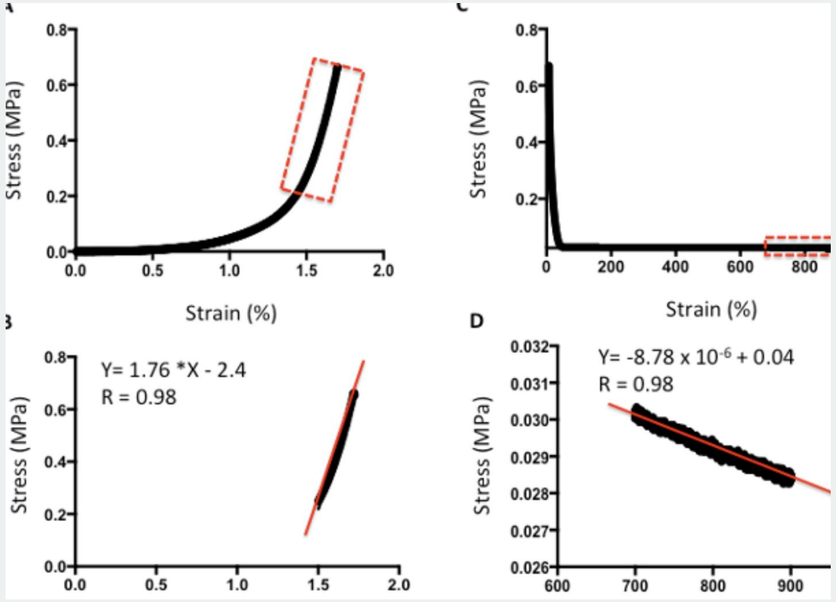


Expected Results

- **Chondrogenic markers**
  - Upregulation of chondrogenic markers in MSCs
  - High levels of protein levels and collagen II
    - COL2A1: >10-fold increase compared to baseline.
    - ACAN: >5-fold increase compared to baseline.
    - SOX9: >5-fold increase during early differentiation.
- **Histologic Evaluation**
  - Intense staining = high proteoglycan synthesis
  - Well-aligned and dense collagen fibrils
- **Mechanical Properties**
  - Similar young's modulus to native: 0.1–2 MPa
- **Cannabinoid Therapeutic Effects**

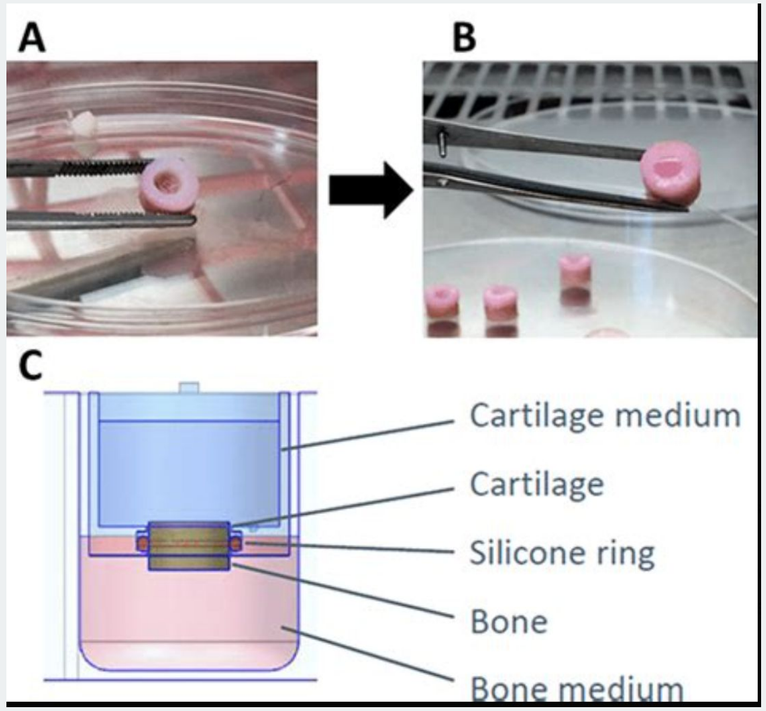


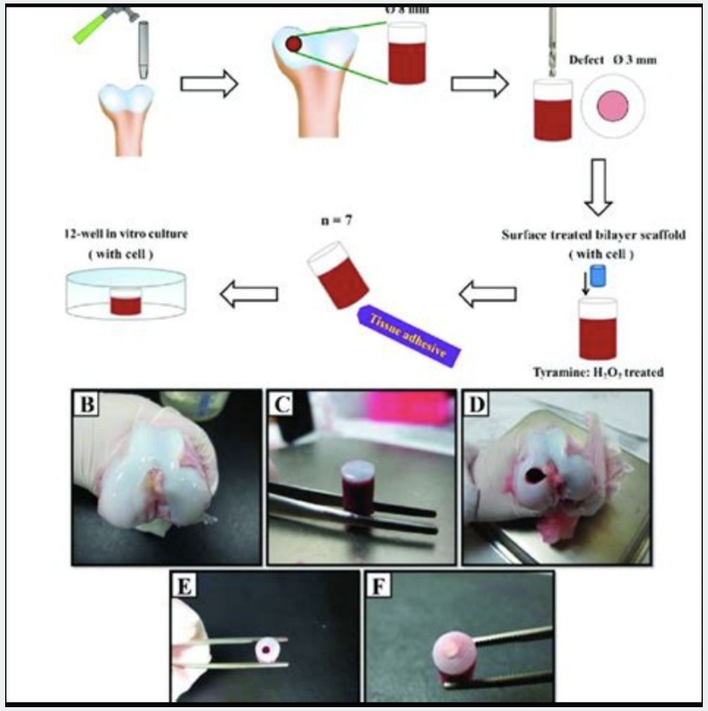




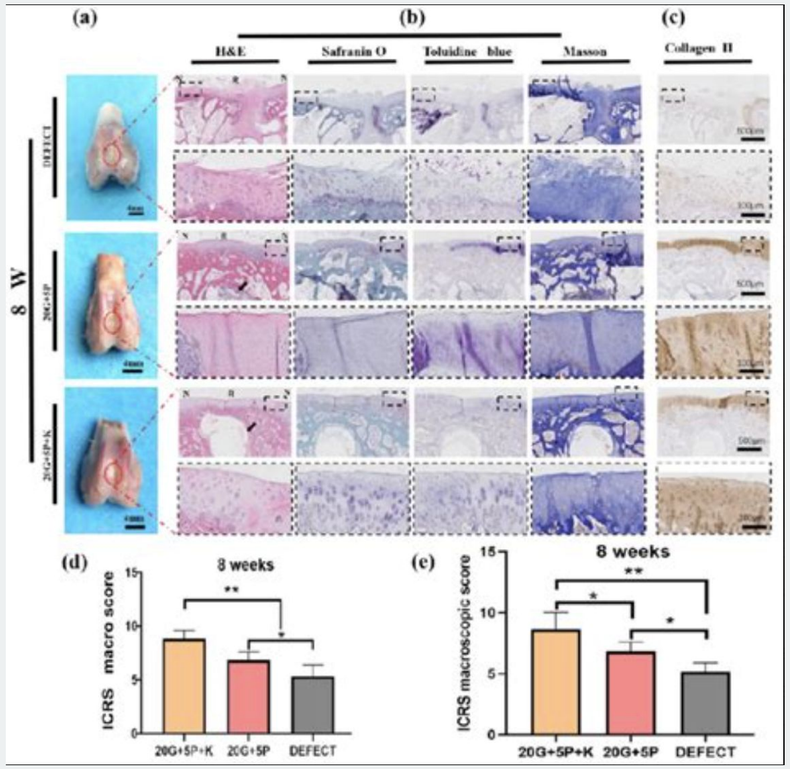
Ex Vivo Testing

- **Ex Vivo - Osteochondral Explants from Porcine Knees:**
  - Assesses integration with native cartilage and ECM deposition.





Results



In Vivo Testing

Small Animal Model (Mouse)

- Biocompatibility of the scaffold and its immune response subcutaneously
- Choose multiple osteoarthritis models

Results: Histology

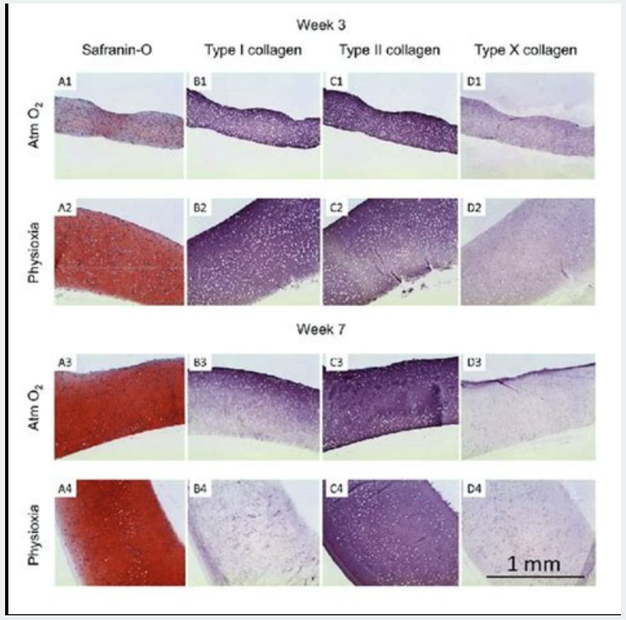


Table 1. Mouse models of osteoarthritis.

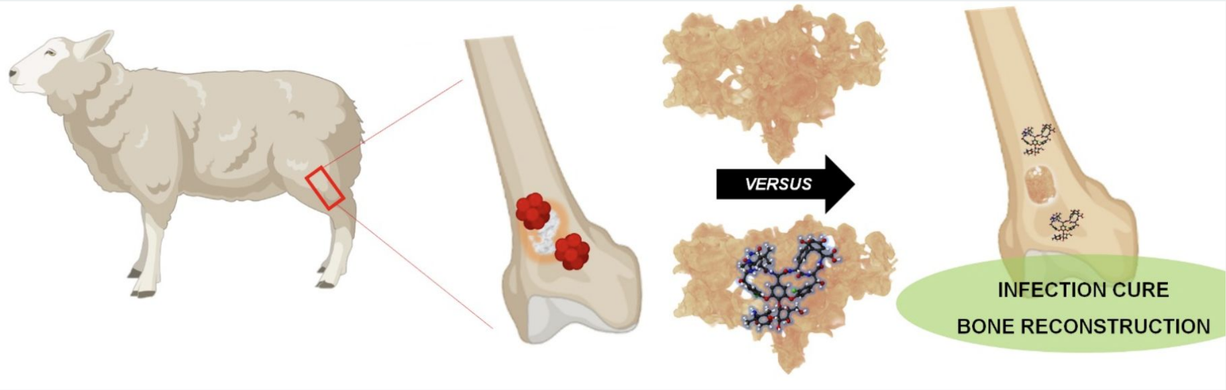
Models		Pros	Cons
Spontaneous	Wild type mice[7,9,59,67,68,70,72,74,80,85,87,115,118,119,120]	- Model of aging phenotype - The less invasive model - Physiological relevance: mimics human pathogenesis - No need for technical expertise - No need for specific equipment	- Variability in incidence - Large number of animals at baseline - Long-term study: Time consuming (time of onset: 4 -15 mo) - Expensive (husbandry)
	Genetically modified mice[2,7,25,40,50,52,67,72,79,80,89,120]	- High incidence - Earlier time of onset: 18 wk - No need for specific equipment - Combination with other models	- Time consuming for the strain development - Expensive
Chemical-induced	Mono-iodoacetate injection[7,11,46,47,60,66,90,91,101,128]	- Model of pain-like phenotype - To study mechanism of pain and antalgic drugs - Short-term study: Rapid progression (2-7 wk) - Reproducible - Low cost	- Need for technical expertise - Need for specific equipment - Systemic injection is lethal - Destructive effect: does not allow to study the early phase of pathogenesis
	Papain injection[66,67,120]	- Short-term study: rapid progression - Low cost	- Need for technical expertise - Need for specific equipment - Does not mimic natural pathogenesis
	Collagenase injection[7,65,67,98]	- Short-term study: rapid progression (3 wk) - Low cost	- Need for technical expertise - Need for specific equipment - Does not mimic natural pathogenesis
Non-invasive	High-fat diet (Alimentary induced obesity model)[5,8,43,45,57,96,124]	Model of metabolic phenotype No need for technical expertise No need for specific equipment Reproducible	Long-term study: Time consuming (8 wk–9 mo delay) Expensive
	Physical activity and exercise model[45,73]	Model of post traumatic phenotype No need for technical expertise	Long-term study: time consuming (18 mo delay) Expensive Disparity of results
	Mechanical loading models Repetitive mild loading models Single-impact injury model[7,16,23,24,32,35,104,105,106]	Model of post traumatic phenotype Allow to study OA development Time of onset: 8-10 wk post injury Noninvasive	Need for technical expertise Need for specific equipment Heterogeneity in protocol practices Repetitive anesthesia required or ethical issues
Surgical	Ovariectomy[114]		Contested.
	Meniscectomy model[7,32,63,67,87]	Model of post traumatic phenotype High incidence Short-term study: early time of onset (4 wk from surgery) To study therapies	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human
	Anterior cruciate ligament transection (ACLT)[7,39,40,61,48,67,70,87,126]	Model of posttraumatic phenotype High incidence Short-term study: early time of onset (3-10 wk from surgery) Reproducible To study therapies	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human
	Destabilization of medial meniscus (DMM)[7,32,39,40]	Model of post traumatic phenotype High incidence Short-term study: early time of onset (4 wk from surgery) To study therapies The most frequently used method	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human

Since all animal models have strengths and weaknesses, it is often best to plan using a number of models and techniques together to combine the results.

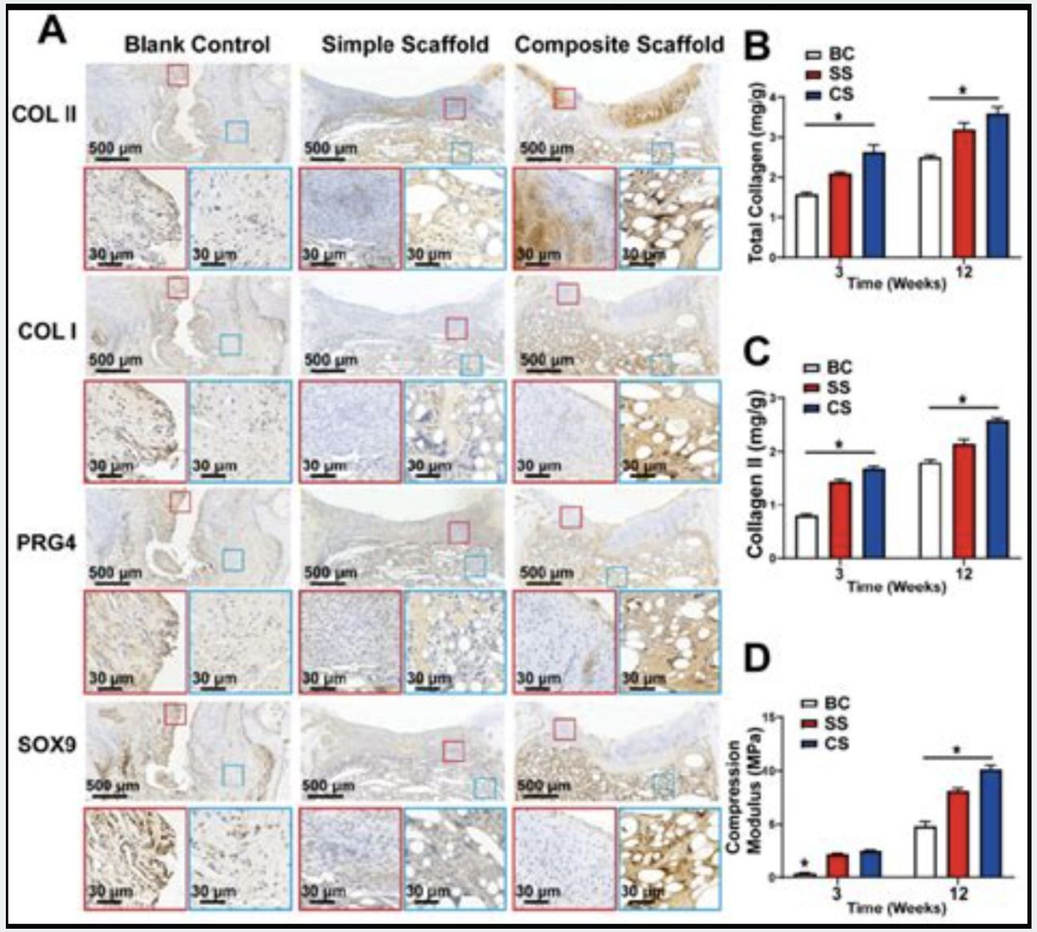
In Vivo Testing

Large Animal Model (Sheep)

- Create osteochondral defects (5–10 mm diameter) in the femoral condyles of sheep knees
- Surgically implant scaffold
- Restrict joint movement for 1–2 weeks, followed by gradual reintroduction of load-bearing activities.
- If possible obtain samples at different time points or have multiple animals euthanized at different time points.



Results

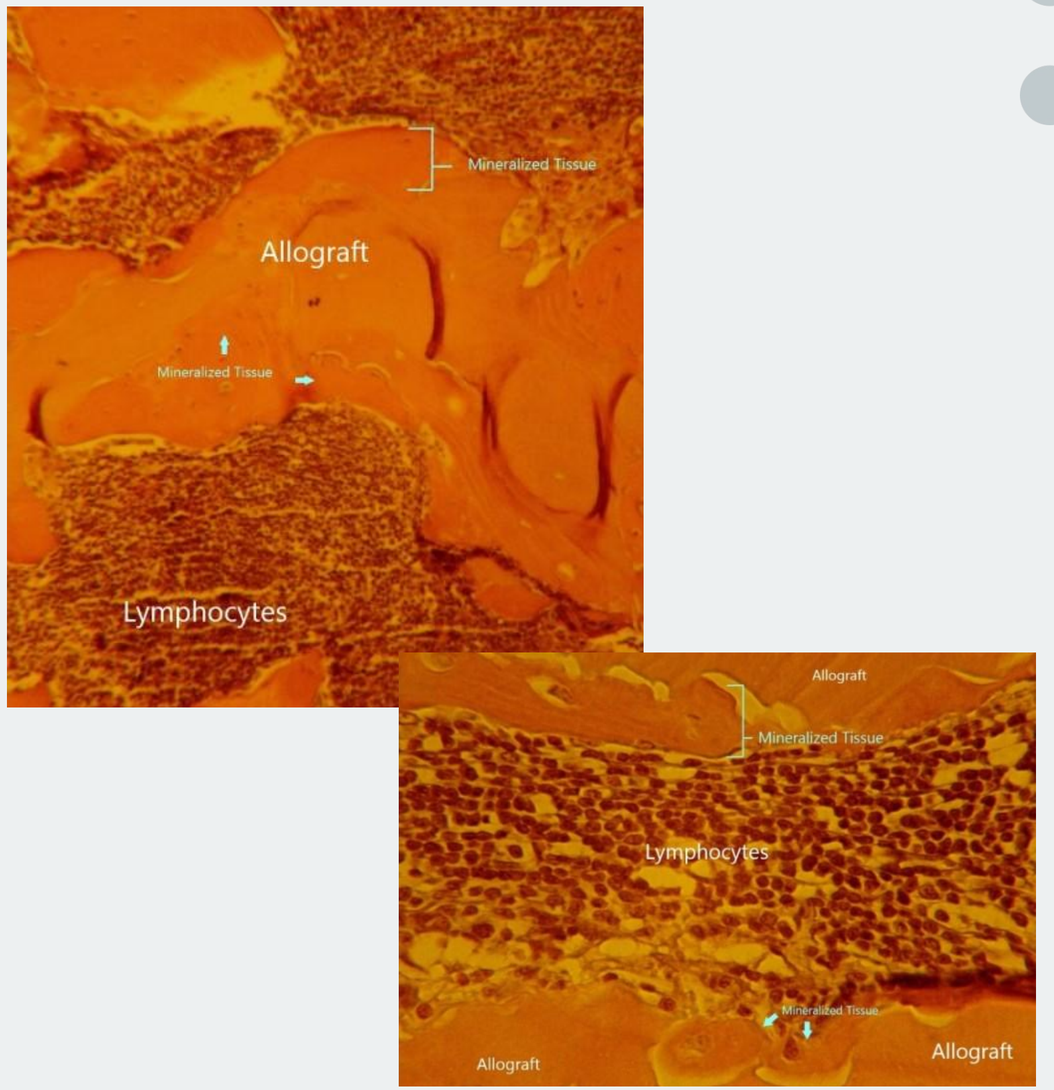


Limitations and Challenges

1. **Immune Rejection, Inflammation, Side Effects**
   a. Carcinogenicity, cytotoxicity, excessive inflammatory response

2. **Scalability and Cost**
   b. Small scale we will be using 3D bioprinters to print scaffolds for crosslinking, how will we increase the scale?
   c. How will we ensure sterility?

3. **Long-Term Integration and Mechanical Strength**
   d. Is the regenerated cartilage mechanically stable?
   e. Is the integration mechanism replicable?



Addressing Limitations

1. **Immune Rejection, Inflammation, Side Effects**
   a. Biocompatible coatings/hydrogels
   b. Dose-controlled release of cannabinoids
   c. Extensive preclinical safety testing

2. **Scalability and Cost**
   a. Automated bioprinting systems
   b. Closed-loop sterile manufacturing processes
   c. Partner with manufacturers

3. **Long-Term Integration and Mechanical Strength**
   a. Gradient mechanical properties to match tissue
   b. Enhance cross linking techniques for durability
   c. Perform in vivo validation to ensure long-term functionality

04

Additional Considerations

Additional Considerations:

Synthetic vs. Natural Cannabinoids

WIN55, 212-2

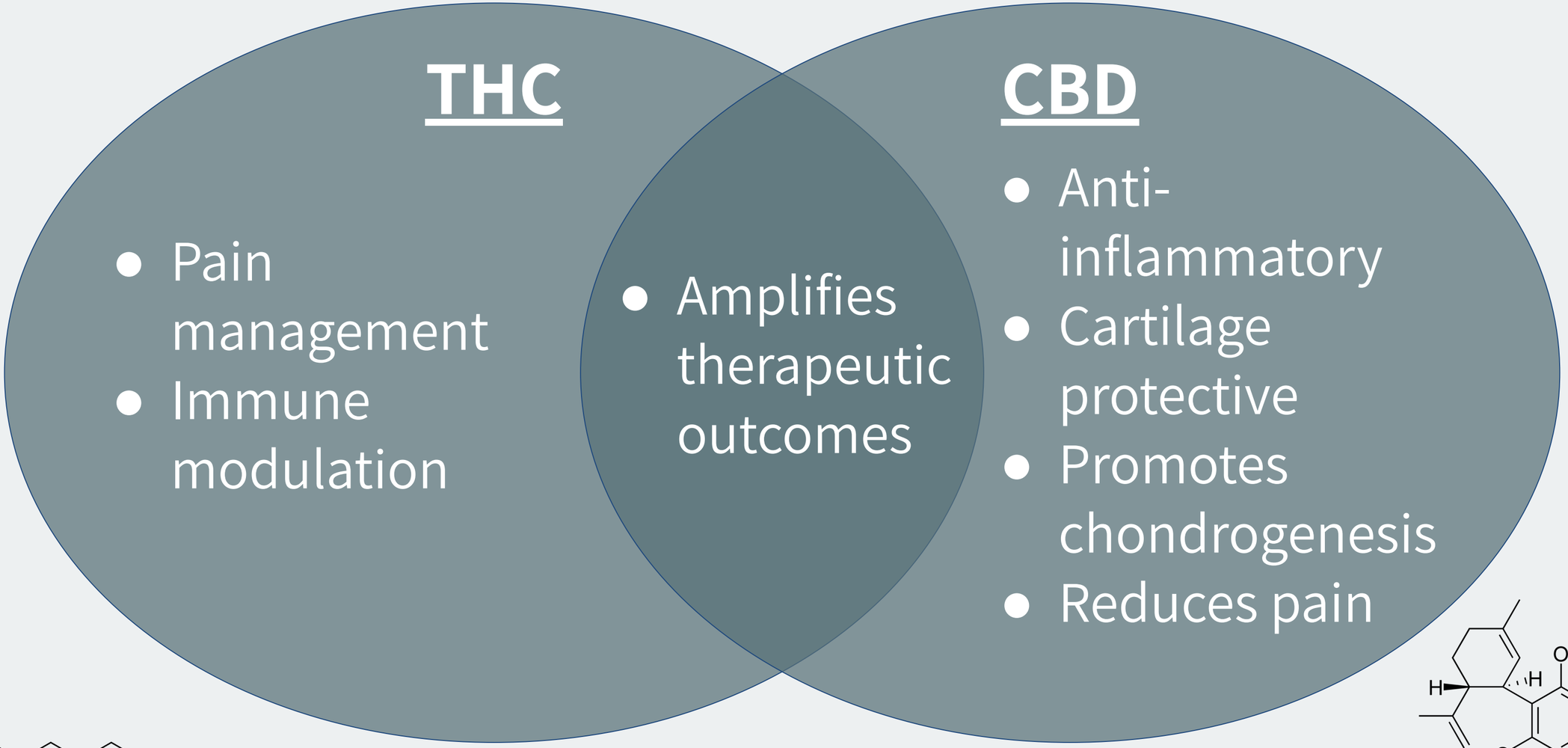
- No psychoactive effects
- Suitable for in vitro/vivo studies
- Limited long term safety data challenging clinical adoption

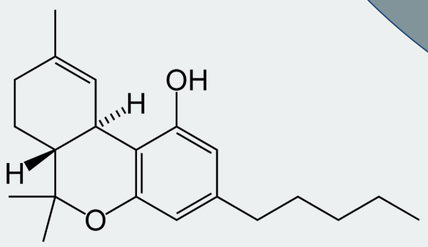
Delta9-THC

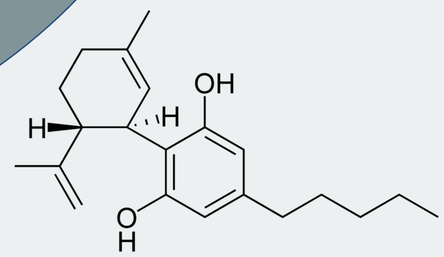
- Widely studied, naturally occurring
- Established medical frameworks for use
- Psychoactive and risk of tolerance and dependency

Additional Considerations:

Varying THC and CBD Combinations





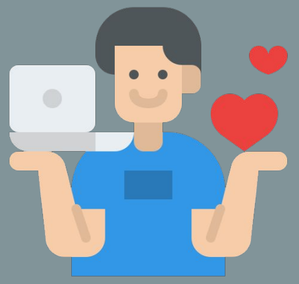


05

Conclusion

Conclusion

- **Our Solution:**
  - Designed a biocomposite scaffold combining PEGDA and collagen cultured in a medium containing synthetic cannabinoids for patient-specific cartilage regeneration
- **Our Intended Outcomes:**
  - Enhanced tissue integration, structural stability, and quality of life for patients
- **Possible Next Steps:**
  - Address scalability, long-term safety, and clinical testing for real-world applications
- **Our Key Takeaway:**
  - This innovative scaffold merges advanced materials and biological insights to address critical challenges in cartilage repair



Thank You

Any Questions?

References

Arthritis Foundation. (n.d.). Osteoarthritis. Arthritis Foundation. Retrieved December 13, 2024, from https://www.arthritis.org/diseases/osteoarthritis

Arthritis Foundation. (n.d.). The future of joint repair. Arthritis Foundation. Retrieved December 13, 2024, from https://www.arthritis.org/health-wellness/treatment/joint-surgery/preplanning/the-future-of-joint-repair

Atalay, S., Jarocka-Karpowicz, I., & Skrzydlewska, E. (2020). Antioxidative and Anti-Inflammatory Properties of Cannabidiol. *Antioxidants*, 9(1), 21. https://doi.org/10.3390/antiox9010021

Bahney, C. S., Hsu, C. W., Yoo, J. U., West, J. L., & Johnstone, B. (2011). A bioresponsive hydrogel tuned to chondrogenesis of human mesenchymal stem cells. FASEB Journal, 25(5), 1486–1496. https://doi.org/10.1096/fj.10-165514

Chen-Chan Hsieh, B. Linju Yen, Chia-Chi Chang, Pei-Ju Hsu, Yu-Wei Lee, Men-Luh Yen, Shaw-Fang Yet, Linyi Chen, Wnt antagonism without TGFβ induces rapid MSC chondrogenesis via increasing AJ interactions and restricting lineage commitment, iScience, Volume 26, Issue 1, 2023, 105713, ISSN 2589-0042, https://doi.org/10.1016/j.isci.2022.105713.(https://www.sciencedirect.com/science/article/pii/S2589004222019861).

Cong, B., Sun, T., Zhao, Y., & Chen, M. (2023). Current and Novel Therapeutics for Articular Cartilage Repair and Regeneration. Therapeutics and Clinical Risk Management, 19, 485–502. https://doi.org/10.2147/TCRM.S410277

Gowran A, McKayed K, Kanichai M, White C, Hammadi N, Campbell V. Tissue Engineering of Cartilage; Can Cannabinoids Help? Pharmaceuticals (Basel). 2010 Sep 6;3(9):2970-2985. doi: 10.3390/ph3092970. PMID: 27713386; PMCID: PMC4034107.

Hy2Care. (n.d.). Home. Hy2Care. Retrieved December 13, 2024, from https://www.hy2care.com/en

Hu B, Gao J, Lu Y, Wang Y. Applications of Degradable Hydrogels in Novel Approaches to Disease Treatment and New Modes of Drug Delivery. *Pharmaceutics*. 2023; 15(10):2370. https://doi.org/10.3390/pharmaceutics15102370

Kalapa Clinic. (n.d.). Cannabinoids for cartilage tissue repair. Kalapa Clinic. Retrieved December 13, 2024, from https://www.kalapa-clinic.com/en/cannabinoids-cartilage-tissue-repairing/

Lee, M., & Kwon, Y. (2014). The effect of a synthetic cannabinoid on cartilage repair. European Journal of Pharmacology, 730, 51-58. https://doi.org/10.1016/j.ejphar.2014.03.014

Liao, M., & Zhang, X. (2016). Chondrogenic gene expression profile by in vitro matured constructs: RT-qPCR analysis. ResearchGate. https://doi.org/10.13140/RG.2.2.15609.12640

Mazzantini C, El Bourji Z, Parisio C, Davolio PL, Cocchi A, Pellegrini-Giampietro DE, Landucci E. Anti-Inflammatory Properties of Cannabidiol and Beta-Caryophyllene Alone or Combined in an In Vitro Inflammation Model. *Pharmaceuticals*. 2024; 17(4):467. https://doi.org/10.3390/ph17040467

Moreno-Castellanos, N., Cuartas-Gómez, E., & Vargas-Ceballos, O. (2023). Functionalized Collagen/Poly(ethylene glycol) Diacrylate Interpenetrating Network Hydrogel Enhances Beta Pancreatic Cell Sustenance. *Gels*, 9(6), 496. https://doi.org/10.3390/gels9060496

Redondo, M. L., Beer, A. J., & Yanke, A. B. (2018). Cartilage restoration: Microfracture and osteochondral autograft transplantation. The Journal of Knee Surgery, 31(03), 231–238. https://doi.org/10.1055/s-0037-1618592

Roseti, L., Grigolo, B. Current concepts and perspectives for articular cartilage regeneration. J EXP ORTOP 9, 61 (2022). https://doi.org/10.1186/s40634-022-00498-4

Ruhl T, Karthaus N, Kim BS, Beier JP. The endocannabinoid receptors CB1 and CB2 affect the regenerative potential of adipose tissue MSCs. Exp Cell Res. 2020 Apr 1;389(1):111881. doi: 10.1016/j.yexcr.2020.111881. Epub 2020 Jan 29. PMID: 32006556.

Schlüter, J. M., & Luedeke, M. (2017). Chondrogenic gene expression profile by in vitro matured constructs. Thieme Connect. https://doi.org/10.1055/s-0037-1618592

Sharma, S., & Gupta, A. (2017). Inflammation in the context of endocarditis of a Contegra® graft (EvG staining). ResearchGate. https://doi.org/10.13140/RG.2.2.14289.62560

SteinerBio. (n.d.). How the immune system determines bone graft success or failure. SteinerBio. Retrieved December 13, 2024, from https://www.steinerbio.com/how-the-immune-system-determines-bone-graft-success-or-failure/