Title: Biocomposite Scaffolds for Cartilage Regeneration
Team Members: Sachi Kurian, Smriti Mumudi, Jahnavi Shah, Emily Sullivan, Ria Suresh
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Professor: Dr. Li Cai

Specific Aims

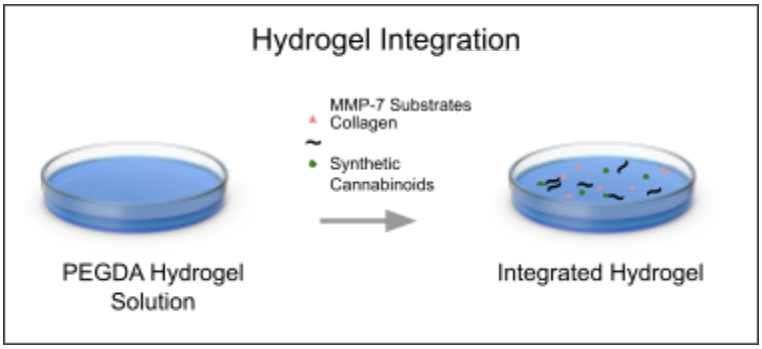
Abstract: Cartilage degeneration poses a significant medical challenge with symptoms like pain, swelling, and limited mobility. Current treatments, such as microfracture and autologous chondrocyte implantation, often fail to restore cartilage's structural and functional integrity, leading to high failure rates and escalating healthcare burdens. To address these gaps, we propose a biocomposite scaffold combining PEGDA, collagen, and synthetic cannabinoids. This innovative design leverages advanced biomaterials to promote chondrogenesis and enable effective cartilage repair and regeneration.

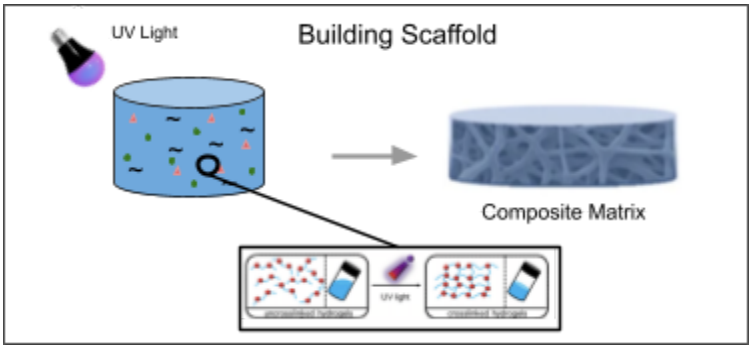
The proposed project is centered on three specific aims designed to overcome existing limitations in cartilage regeneration:

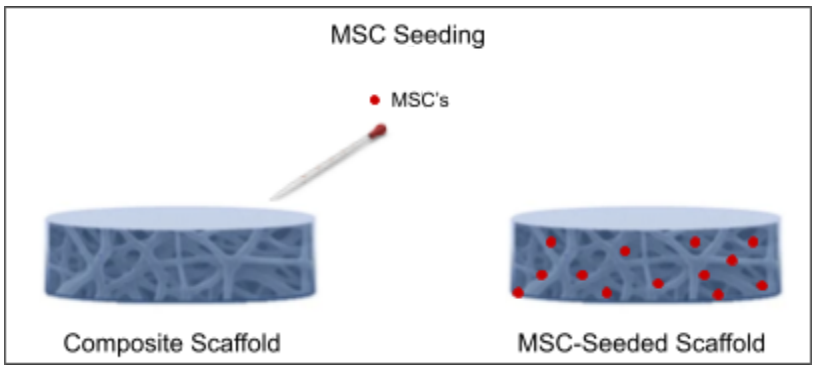
Aim 1 - Reducing Inflammation and Pain: By incorporating synthetic cannabinoids, our scaffold not only mitigates inflammation but also provides a conducive environment for mesenchymal stem cell (MSC) survival and differentiation. This dual action promotes tissue repair while addressing the symptomatic pain of cartilage degeneration, offering a non-invasive alternative to pharmacological treatments.

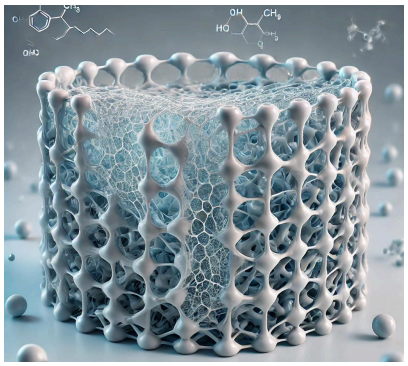
Aim 2 - Developing Patient-Specific Degradation Profiles: Our scaffold utilizes MMP-7-mediated degradation to ensure that the breakdown of the scaffold aligns temporally with cartilage regeneration. This patient-specific tailoring optimizes therapeutic outcomes by synchronizing the scaffold's structural support with tissue growth. The customization process allows clinicians to adjust degradation rates based on individual patient needs, ensuring a personalized and precise therapeutic intervention.

Aim 3 - Preserving Cartilage and Promoting Regeneration: The scaffold's PEGDA-collagen biocomposite structure combines robust structural integrity with superior biocompatibility, enhancing joint stability and mobility while significantly improving patient quality of life. Its mechanical properties are meticulously engineered to mimic those of native cartilage, enabling seamless integration, sustained joint function, and effective cartilage regeneration.









Figures 1–3: *Design Process Schematics;* ***Figure 4:*** *Rendering of Device as Described by Aims and Methods*

Figure 4 shows the scaffold with collagen integrated into the PEGDA matrix, alongside media compounds targeting cannabinoid receptors to reduce inflammation and support MSC growth. Collagen aids healing and cell integration, while PEGDA ensures structural stability during regeneration. Figures 1-3 detail the design process.

Research Strategy

Significance: Cartilage is challenging to repair due to its avascular and aneural nature, which significantly limits its regenerative capacity. Unlike other tissues, cartilage cannot rely on natural wound-healing mechanisms, leaving it susceptible to degeneration and incomplete repair following any injury or disease. Current clinical solutions, including microfracture, autologous chondrocyte implantation, and osteochondral grafts have been inconsistent in their outcomes and at times, failed to restore the tissue's structure and function. The limitations that exist in today's solutions are made worse by the inflammatory joint environment, further impeding healing and contributing to long-term degeneration (Roseti, Redondo).

The proposed solution addresses the stated challenges with an innovative design that integrates PEGDA and collagen to mimic the mechanical and biological properties of cartilage. PEGDA provides structural stability while collagen promotes cellular attachment and tissue remodeling, creating a supporting environment for regeneration. Furthermore, synthetic cannabinoids add to this approach by leveraging their anti-inflammatory properties through the reduction of harmful cytokines. A favorable environment is then created for MSC survival and differentiation (Atalay).

The scaffold features a controlled degradation mechanism through MMP-7 mediated cleavage, which allows for its breakdown to align with the progression of tissue regeneration. This approach overcomes the limitations of traditional materials that degrade unpredictably, resulting in harmful by-products and inconsistent healing (Hu). By synchronizing support along with the repair process, this scaffold enhances the likelihood of forming durable, functional cartilage.

By addressing both the biological and structural deficits of damaged cartilage, this scaffold targets critical unmet needs in orthopedic care, offering a comprehensive solution and promising alternative to current therapies.

Innovation: The proposed biocomposite scaffold introduces several elements that differentiates it from existing approaches to cartilage regeneration. It integrates several biomaterials and leverages several biological pathways to address any limitations that may exist in current treatments. The scaffold incorporates synthetic

cannabinoids, uses MMP-7 mediated enzymatic degradation, and uses a PEGDA-collagen matrix to create an environment that is both biologically functional as well as mechanically stable.

The scaffold contains synthetic cannabinoids which target CB1 and CB2 receptors, reducing inflammation by suppressing pro-inflammatory cytokines while promoting the secretion of anti-inflammatory cytokines (Atalay). Cannabinoids create a favorable environment for MSCs survival and differentiation by reducing oxidative stress and preventing apoptosis while enhancing chondrogenic differentiation. Unlike traditional scaffolds which degrade through ester hydrolysis, the use of MMP-7 allows for a more controlled, patient specific degradation that aligns with tissue regeneration, ensuring proper structural support throughout the healing process (Hu, Moreno). The PEGDA-collagen matrix combines mechanical stability with biocompatibility, ensuring adaptability to patient-specific needs by mimicking human cartilage properties and facilitating cell attachment. These innovations collectively aim to offer an adaptable approach to cartilage repair through the amalgamation of tissue engineering and regenerative medicine research.

Approach: To address the structural and biological challenges associated with cartilage regeneration, the biocomposite scaffold would integrate synthetic and biological components to achieve optimal mechanical and biochemical properties while maintaining biocompatibility.

1. Hydrogel Development

Aim 1: Reducing Inflammation and Pain

Synthetic Cannabinoid Functionalization: Synthetic Cannabinoids such as WIN55,212-2 will be conjugated to PEGDA through covalent linkages, this ensures its uniform distribution throughout the hydrogel matrix and avoids burst release for sustained therapeutic effect. These cannabinoids would target CB1 and CB2 receptors on MSCs, enhancing its chondrogenic differentiation, through Wnt/β-catenin and p38 MAPK. For its incorporation into the hydrogel, the desired cannabinoid compound (1-10 μM) is added to the hydrogel mixture and is thoroughly mixed for uniform dispersion of cannabinoid.

Aim 2: Developing Patient-Specific Degradation Profiles

MMP-7 substrate: To achieve enzymatic degradation, MMP-7-cleavable peptide sequences are synthesized with reactive functional groups at the N- or C-terminus. The peptide is then reacted with SCM-functionalized PEG (e.g., acrylate-PEG-SCM) in an organic solvent (e.g., DMSO) or aqueous buffer at room temperature. Then the MMP-7-Acrylate-PEG-SCM is added to PEGDA solution and mixed until homogeneous.

Aim 3: Preserving Cartilage and Promoting Regeneration

PEGDA Synthesis and Optimization: PEGDA is a photopolymerizable synthetic polymer capable of forming crosslinked networks. In order to mimic Young's modulus of 0.1-2 MPa of native scaffold, optimum molecular weight should be investigated. Higher MW ensures flexibility and porosity. Hence a concentration of 10-20% w/v with MW of 3.4kDa or higher would provide optimal balance between mechanical strength and nutrient diffusion. PEGDA is first dissolved in phosphate-buffered saline (PBS) to create a precursor solution (typically 10-20% w/v). Then the photoinitiator (e.g., 0.05% w/v Irgacure 2959) is added and mixed thoroughly. Non crosslinkable PEG is reacted with Succinimidyl Carboxymethyl Precursors: NHS (N-hydroxysuccinimide) and a coupling agent like EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) to activate the carboxylic acid precursor of SCM. This step converts PEG into SCM-functionalized PEG.

Collagen integration: Collagen type I is a major ECM protein and incorporating that into the hydrogel would ensure that the scaffold mimics a natural one through cell attachment enhancement, proliferation and ECM

deposition. Preparing collagen by dissolving it in acidic solution (0.01N HCl) to a desired concentration (e.g., 3-5 mg/mL). Then neutralize the solution to physiological pH using NaOH while keeping it on ice to prevent premature gelation. Slowly mix the neutralized collagen solution into the PEGDA precursor solution. Ensure uniform dispersion by gentle mixing to avoid air bubbles.

2. Bioprinting and Crosslinking

Aims 2 and 3: Patient-Specific Degradation Profiles, Preserving Cartilage, and Promoting Regeneration

3D Bioprinting: The hydrogel-MSC mixture is first loaded into a temperature-controlled bioprinter cartridge. The nozzle diameter is set to 100 µm for high resolution, print speed is set to 5-20 mm/s to avoid shear stress on cells and temperature is maintained at 4-10°C to preserve hydrogel viscosity.

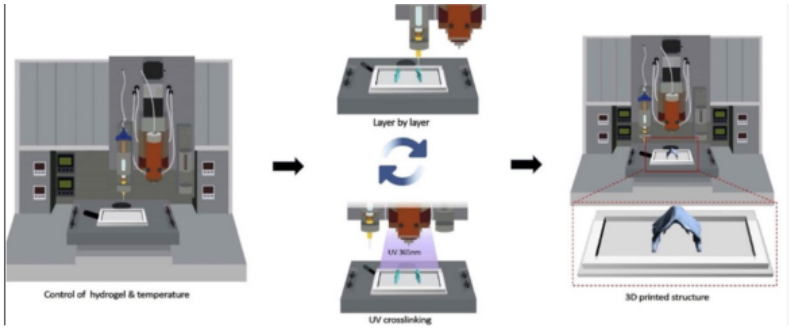


Figure 5*: Schematic of 3D bioprinting technique from Zhou et al., 2017*

Layer-by-layer construction:The superficial zone is printed with lower stiffness to account for shear forces, while the deeper zones are reinforced for compressive loads. (Fig 5) Gradients in pore size (100-300 µm) are incorporated for nutrient diffusion and ECM deposition.

UV Crosslinking: The scaffold is then exposed to UV light (365 nm) to polymerize PEGDA. Exposure duration is set to 30-120 seconds to optimize the mechanical strength.

3. Cell Culture and Differentiation

Aim 1: Reducing Inflammation and Pain

Synthetic Cannabinoid Stimulation: Synthetic Cannabinoids are added to cultured medium at concentration of 10 µM. Their effects on MSCs are observed by measuring expression of chondrogenic markers (SOX9, COL2A1, ACAN) and cytokines (IL-10, TNF-α).

Aim 3: Preserving Cartilage and Promoting Regeneration

MSC preparation and seeding: MSCs are harvested from adipose tissue or bone marrow and expanded under standard culture conditions. The cells are then suspended in a chondrogenic differentiation medium containing transforming growth factor-beta 3 (TGF-β3), dexamethasone, and ascorbic acid. Seeding density is standardized at 10^6 cells/mL.

Dynamic culture conditions: The scaffold is then placed in a bioreactor system (equipped with perfusion system) with cyclic compressive forces (1-5 MPa at 1Hz) to simulate the mechanical environment of cartilage and enhance ECM deposition and promote collagen fiber alignment.

Outcome Assessment and Testing

To validate the efficacy of our biocomposite scaffold for cartilage regeneration, we propose a comprehensive testing strategy encompassing in vitro, ex vivo, and in vivo methods. These approaches allow us to systematically assess the scaffold's biological, structural, and mechanical performance in simulated and physiological environments. By employing diverse models and evaluation methods, we aim to ensure robust preclinical evidence supporting the scaffold's therapeutic potential.

In-Vitro Testing:

In vitro studies employ 3D bioprinted constructs with MSCs encapsulated in the PEGDA-collagen matrix, cultured with TGF-β3 to induce chondrogenesis. Key outcomes include increased COL2A1, ACAN, and SOX9 expression (qPCR/ELISA) and proteoglycan deposition (biochemical assays). Cytotoxicity testing, per ISO 10993-5 standards, ensures biocompatibility by assessing cell viability and proliferation through assays like Alamar Blue and MTT. Histological stains such as Picrosirius Red and Safranin O evaluate collagen alignment and ECM formation, while confocal microscopy tracks cell viability and distribution. Data are analyzed using ANOVA and regression models to identify significant trends.

Ex-Vivo Testing:

Using osteochondral explants from porcine knees, the hydrogel scaffold will be injected into defects and cultured under simulated physiological conditions. Cytotoxicity is evaluated through direct contact tests with explant tissue. Histological stains (H&E, Masson's Trichrome) and immunohistochemistry for type II collagen and proteoglycans evaluate tissue integration and ECM deposition. Mechanical testing assesses scaffold stability, and paired t-tests alongside multivariate analysis verify significant outcomes.

In-Vivo Testing:

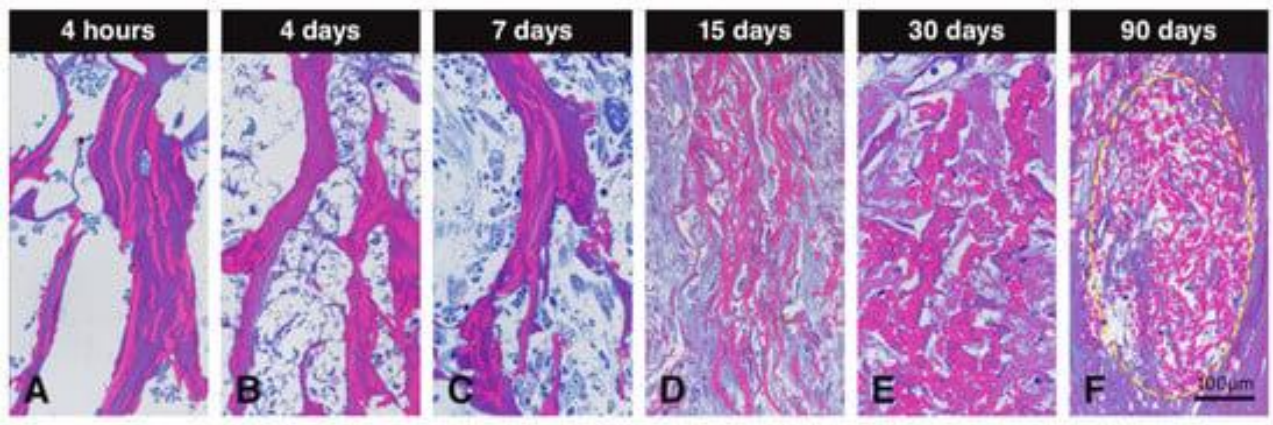
We chose mice as our small animal model to test biocompatibility through subcutaneous implantation, analyzing cytokine levels (IL-6, TNF-α via ELISA/qPCR) and cartilage formation (Alcian Blue). Mice were selected for their well-established osteoarthritis models, including non-invasive exercise and mechanical loading models. Additionally, surgical models like anterior cruciate ligament transection (ACLT) can provide parallels to our large animal sheep model. For the sheep model, osteochondral defects (5–10 mm diameter) are created in the femoral condyles to validate functional properties through gait analysis, range-of-motion tests, and mechanical strength assays. Histological evaluation confirms ECM deposition, with data analyzed using two-way ANOVA and Tukey tests. The use of ISO 10993-6 ensures proper evaluation of immune responses and inflammation.

Biochemical Analysis: ELISA and qPCR are utilized to quantify chondrogenic markers such as COL2A1 and SOX9, providing insights into cellular functionality within the scaffold.

Mechanical Testing: Compression tests assess the scaffold's mechanical properties, ensuring they align with native cartilage which has a young's modulus of 0.1 - 2 MPa. This will include dynamic compression testing to assess the scaffold's performance under stimulated joint movement, tensile strength testing to evaluate the ability to withstand stretching forces, and swelling ratio/water retention to measure the scaffold's ability to absorb and retain fluid. Stress-strain analysis will be conducted to evaluate the durability and elasticity of the scaffold under physiological loads.

Histological Evaluation: Picrosirius Red staining evaluates collagen alignment, providing insights into the structural integration of the scaffold. Advanced imaging techniques such as confocal microscopy will be used to visualize cellular distribution and matrix deposition. ECM Deposition will evaluate extracellular matrix components. Additional parameters that will be assessed include cell morphology and viability to assess the

spreading of MSCs within the scaffold. Polarized light microscopy will be used to analyze the orientation of collagen fibers. The spatial distribution of the cells will be evaluated via confocal microscopy to ensure uniform cell seeding and penetration throughout the scaffold.



Images of expected results sourced from existing papers can be seen below:

Figure 5: Figure of expected scaffold integration over time from Cabelle-Serrano et al., 2020

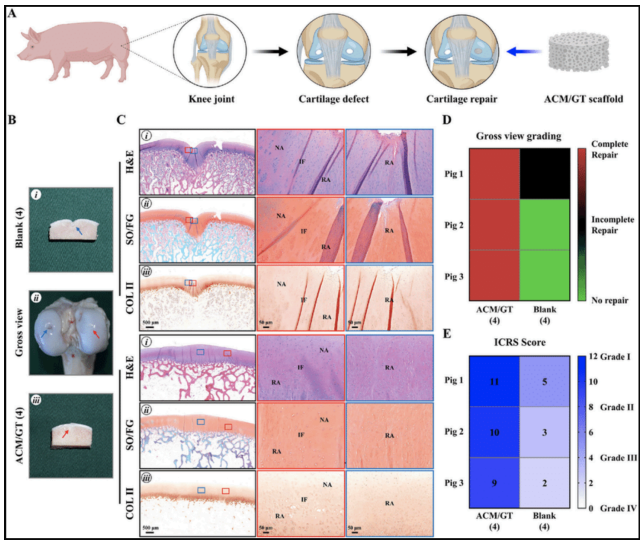


Figure 6: Shows cartilage graft histology in a porcine model from Silva et al., 2022

This rigorous approach ensures that the scaffold meets both biological and mechanical requirements for successful cartilage repair, with iterative refinements informed by experimental data.

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