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Heparin-Conjugated Bioactive Glue for Regeneration of Lubricin-infiltrated Meniscus Tears by Recruitment of Stem/Progenitor Cells

A. Rationale

Knee meniscus plays indispensable roles in joint congruence, shock absorption, and stress transmission. Meniscus injuries are extremely common with approximately one million patients undergoing surgical treatment annually in the U.S. alone. Upon injury, the outer zone of the meniscus can be repaired and expected to functionally heal but tears in the inner avascular region are unlikely to heal. In a previous study, we showed that controlled applications of connective tissue growth factor (CTGF) and transforming growth factor beta-3 (TGFβ3) can induce seamless healing of avascular meniscus tears by inducing recruitment and stepwise differentiation of synovial mesenchymal stem/progenitor cells (syMSCs) [1]. However, there are limitations to this method when introduced into a biological system. The presence of lubricin around the knee joint provides necessary functions for load-bearing and load transferring between bones. Additionally, lubricin possesses extremely strong surface adhesion given the extreme force that it must withstand [2]. This result is an issue for the regeneration of meniscus tears that attempts to join together two parts of the meniscus. Lubricin works against the regeneration of the tears by lubricating the meniscus tear and preventing the growth factors from binding both sides. Therefore, a bioglue that contains joining properties to lubricin need to be incorporated in order to introduce a successful regeneration technique of avascular meniscus tears.

B. Research Questions, Hypotheses, Expected Outcomes

a. Research Question

i. What are the mechanical properties of a heparin conjugated fibrinogen and genipin cross-linked hydrogel when applied to incised bovine knee samples in the presence and absence of lubricin?

ii. How will tensile strength, compression, GAG and collagen content and formation of fibrocartilaginous tissue change after being treated with heparin conjugated fibrinogen hydrogel containing CTGF/TGFβ3uS after 4-6 weeks of being cultured with mesenchymal stem cells (MSCs)?

b. Hypotheses/Goals/Expected Outcomes

i. Goal: To successfully manufacture a bioglue coupled with growth factors that will be able to sequester the anti-healing properties of lubricin and optimize meniscal tear healing.

ii. Hypotheses/Expected Outcome: The incorporation of heparin into a fibrin-based bio-glue will sequester lubricin through interacting with its heparin-binding domains and thus, promote fibrocartilaginous tissue healing induced by MSCs with CTGF/TGFβ3uS. The addition of genipin into the bioglue will act as a stabilizer and prolong the release rate and degradation rate of the bioglue. CTFG/TGFβ3uS will display cohesive regeneration of the meniscal tissue and work together with the MSCs to promote the growth of a complex tissue by step-wise differentiation of different sections of the meniscus.

C. Research Procedures

a. Procedure

i. Synthesis of Heparin Bioglue

1. Bioglues will be prepared by combining a pre-made fibrinogen mixture and a pre-made thrombin genipin mixture at various concentrations. Plasminogen-free fibrinogen, N-hydroxysuccinimide solution (NHS solution) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride solution (EDCA solution) will be used to synthesize the heparin-conjugated fibrinogen. Thrombin mixtures will be prepared by dissolving varying concentrations of genipin and thrombin. Bioglues will be applied through FibriJet® dual-injector with a blending applicator.

ii. TGFβ3 encapsulation in PLGA μS

1. PLGA μS encapsulating recombinant human TGFβ3 will be prepared by a modified double-emulsion technique. Briefly, 500 mg PLGA, 5 mL chloroform, and 250 μL of diluted TGFβ3 will be combined. This solution will then be emulsified (primary emulsion) by ultrasonicating for 5 minutes to reduce the size of μS. The primary emulsion (w/o) will be added to 10 mL 4% (w/v) PVA (poly vinyl alcohol) solution to form the second emulsion (w/o/w). This double emulsion solution will then be added to 250 mL of 0.3% PVA

solution and continuously stirred for 2 hours to evaporate the solvent. Finally, the microspheres (μS) will be filtered, washed with DI water, resuspended in DI water and then lyophilized.

iii. Explant Model for Avascular Meniscus Healing

1. Menisci will be isolated from skeletally mature bovine knee joints from a local butcher shop. The isolated meniscus will be sterilized with antibiotics and rinsed with PBS. Then full-thickness longitudinal incisions will be made in the middle of the inner third zone, and various gel combinations will applied co-injected between the incised tissue surfaces using FibriJet® dual-injector with a blending applicator to glue the incised tissues. Then the meniscus explants will be placed on the monolayer cultured P5 - P6 human syMSCs and cultured for four weeks, two weeks with fibrogenic induction supplement followed by two week of chondrogenic induction supplement. The samples will be observed weekly with bright-field microscopy.

iv. Tensile Tests

1. Mechanical properties of the gels and of the healed meniscus will be conducted using lap shear tests with a BioDynamics test system. Various meniscus samples will be mounted onto the machine and the test will be conducted with a 0.02-N tare load and will be elongated at 10%/min until failure.

b. Risk and Safety

i. This study will involve the use of potentially hazardous chemicals such as sodium hydroxide (NaOH), hydrochloric acid (HCl), 100 % glacial acetic acid, formaldehyde, glutaraldehyde, ethanol, methanol, acetone, and chloroform. These chemicals can potentially contaminate environmental systems whenever discarded inappropriately and may cause disturbance and have dangerous biological impacts. There may potentially also be physical harm such as irritation. Thus, the utilization of PPE equipment, such as goggles, nitrile gloves, respirator, and a lab coat, can help limit injury due to chemical exposure. Working under a fume hood for volatile substances will help reduce inhalation-related issues. Working under direct lab supervision will also help increase safety. (See Item #4 for additional information)

c. Data Analysis

i. Tensile Tests

1. Data from the tensile tests will be analyzed in Excel and all quantitative mechanical properties will be measured

ii. Samples from the explant culture will be stained with H&E and Saf-O/Fast Green and further tensile tests as described before. Images with bright-field microscopy of stained samples will be used to observe the regenerative properties of each gel.

D. References

1. Athanasiou KA, Sanchez-Adams J. Engineering the Knee Meniscus. Athanasiou KA, Kent Leach JK, editors: Morgan and Claypool Publishers; 2009 March 30, 2009.

2. Lee CH, Rodeo SA, Fortier LA, Lu C, Erisken C, Mao JJ. Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep. Science translational medicine. 2014;6(266):266ra171.

3. Cheung HS. Distribution of type I, II, III and V in the pepsin solubilized collagens in bovine menisci. Connective tissue research. 1987;16(4):343-56.

4. Makris EA, Hadidi P, Athanasiou KA. The knee meniscus: structure-function, pathophysiology, current repair techniques, and prospects for regeneration. Biomaterials. 2011;32(30):7411-31.

5. Tarafder S, Gulko J, Kim D, Sim KH, Gutman S, Yang J, et al. Effect of dose and release rate of CTGF and TGFbeta3 on avascular meniscus healing. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2019;37(7):1555-62.

6. Tarafder S, Gulko J, Sim KH, Yang J, Cook JL, Lee CH. Engineered Healing of Avascular Meniscus Tears by Stem Cell Recruitment. Sci Rep. 2018;8(1):8150.

7. Zhang D, Cheriyan T, Martin SD, Gomoll AH, Schmid TM, Spector M. Lubricin distribution in the torn human anterior cruciate ligament and meniscus. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2011;29(12):1916-22.

Item #1. Human Participants Research:

a. No human participants were used

Item #2. Vertebrate Animals Research:

a. No vertebrate animals were used

Item #3. Potentially Hazardous Biological Agents Research:

a. The bovine meniscus tissue was obtained from a local butcher shop, Harlem Shambles. The mesenchymal stem cells from human synovial fluid were purchased from articular engineering and were named Human Synoviocytes, Cryopreserved Item #: CDD-H-2910. The BSL assessment process determined the level of biological containment to be BSL-2. Both the bovine meniscus tissue and mesenchymal stem cells from human synovial fluid pose moderate risk to the personnel and environment. If exposure occurs in a laboratory situation, the risk of spread is limited and it rarely would cause infection that would lead to serious disease. Effective treatment and preventive measures are available in the event that an infection occurs.

b. The site of research conduct will be equipped with all necessary protections. Personal protectives (safety goggles, nitrile gloves and lab coats), chemical hoods, cell and tissue culture hoods will be used to minimize risk of procedures. All necessary training will be given on how to use the tissue culture hood and equipment. Biological safety cabinets will be available. An autoclave will also be readily available for decontaminating waste materials. Potentially hazardous biological agents will be disposed following policy and regulation enforced by Columbia University.

Item #4. Hazardous Chemicals, Activities & Devices:

As a minor visitor, Columbia University strictly regulates all the potential safety issues. As a part, you are never allowed to do any activities without direct supervision by a Columbia employee. Potentially hazardous agents will include sodium hydroxide (NaOH), hydrochloric acid (HCl), 100 % glacial acetic acid, formaldehyde, glutaraldehyde, ethanol, methanol, acetone, and chloroform. Proper training will be undertaken in order to learn techniques for safe operation and handling of necessary chemicals and machinery. Proper equipment will be worn to minimize chemical exposure.

Ethyl Alcohol - 200 Proof

- Product E7023, Sigma-Aldrich
    - Ethanol is highly combustible and a major fire hazard in the lab. It is also a skin and eye irritant, and will require safety measures and precautions. The chemical will be stored in a tightly-closed container in a cool, well-ventilated area, away from all possible sources of ignition and oxidizing agents. All containers will be grounded for electrostatic charge to avoid fire. The chemical will not be stored 23°C. PPE will include eye/face protection (appropriate safety goggles at appropriate government standards for eye protection) and skin protection (nitrile gloves and lab coat). Inhalation of vapor or mist will be avoided by working under fume hood for adequate ventilation and using a respirator if necessary. The

chemical will be disposed to an approved waste disposal plant in proper containers.

NO ADDENDUMS EXIST