cartilage extracellular matrix (ECM). Pro-inflammatory signaling contributes to the degradation of the cartilage matrix. Under OA conditions, synoviocytes produce a range of inflammatory mediators, including IL-1β. Upon binding to chondrocytes, these mediators activate specific signal transduction cascades, such as the nuclear factor-kappa-B (NF-kB) pathway, which acts as one central regulator of the catabolic processes that suppresses the synthesis of ECM components, and induces the expression of matrix metalloproteinases and enzymes of the ADAMTS family, leading to perpetuated cartilage breakdown. This study aimed to evaluate the effects of brazilin in osteoarthritic chondrocytes and synoviocytes with particular focus on the NF-kB pathway.

**Methods:** Brazilin was isolated from Caesalpinia sappan extract (CSE) and identified using HPLC and NMR methods. Chondrocytes and synoviocytes were isolated from OA patients undergoing total knee arthroplasty. The isolated human chondrocytes and synoviocytes were resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin/streptomycin. Cells were cultured for 3 days at 37°C in a humidified atmosphere containing 5% CO2.

**Results:** Nine NF-kB pathway-related genes (BIRC3, CCL2, CSF1, CSF3, IL1B, IL8, NFkB1, NFkB2 and TNFAIP3) were found to be significantly up-regulated by IL-1β-stimulation and significantly down-regulated after pre-incubation with brazilin. Pathway analysis revealed NFkB1 as one major gene regulating the anti-inflammatory activities of brazilin. RT-qPCR assays confirmed that the up-regulation of NFkB1 mRNA in IL-1β-stimulated primary chondrocytes and synoviocytes was significantly reduced by pre-treatment with brazilin. Western blotting showed that IL-1β treatment increased p105 protein in chondrocytes with a peak after 1 h followed by a steady decline, while increasing constantly the amount of p50 over 24 h. Brazilin suppressed the IL-1β-mediated induction of NFkB1/p50 in chondrocytes and synoviocytes.

**Conclusions:** The present study suggests that brazilin effectively blocks the induction of NFkB1/p50 in cytokine-stimulated primary human chondrocytes and synoviocytes, pointing towards a chondroprotective potential of brazilin, which may be beneficial for reducing cartilage breakdown in OA.

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**EFFECT OF BONE MARROW SURGICAL APPROACH AND RAPIDLY DEGRADING PRESOLIDIFIED SUBCHONDRAL CHITOSAN/BLOOD IMPLANT ON RESURFACING OF CHONDRAL DEFECTS IN A SHEEP MODEL**

**A. Bell** | **M. Hurtig** | **G.-E. Rivard** | **C.D. Hoemann** | **Univ. of Guelph, Guelph, ON, Canada; 1 Hosp. Sainte-Justine, Montreal, QC, Canada; 5 Ecole Polytechnique, Montreal, QC, Canada**

**Purpose:** Bone marrow stimulation for cartilage repair is partly limited by inadequate stem cell recruitment from the trabecular bone marrow to the cartilage lesion. We tested the hypothesis that the cartilage repair elicited by marrow stimulation can be improved by subchondral delivery of rapidly degrading pre-solidified chitosan/blood implants to bone defects with free communication with the trabecular bone marrow. Jamshidi needles and a drill burr were used to create 2mm diameter bone defects with cleanly removed bone to maximize cell recruitment.

**Methods:** Full-thickness 10x10mm cartilage defects were created in the medial femoral condyle in both knees of 11 mature sheep. Based on a pre-planned template, three 2-mm diameter bone holes were generated systematically at the center of each defect with a Jamshidi biopsy needle and a drill burr, and the area between the holes was perforated with six smaller microfracture holes (Fig. 1A-B). 10 kDa chitosan/alginate-chitosan whole blood implant was pre-solidified ex vivo with coagulation factor (recombinant human Factor VIIa, rhFVIIa, or Tissue Factor, TF) and inserted into each of the three 2-mm diameter holes. Contralateral control defects were treated with whole blood presolidified with rhFVIIa or TF. Day 1 (N = 1 1) and 6.5 months repair (N = 10) was evaluated by macroscopic scoring, and micro-CT, histomorphometry for cartilage matrix and bone, and histological scoring (ICRS-2). Four intact retrieved condyles were micro-CT scanned for baseline bone parameters. 6.5 month cartilage repair tissues were also analyzed by biochemistry for glycosaminoglycan (GAG) and collagen (% weight per wet weight). The General Linear Model (Statistica, Statsoft, V6.2, NB, USA) was used to analyze differences due to surgical approach (drill hole), marrow stimulation method and defect size.

**Results:** At day 1 post-operative, the marrow stimulation approach led to a 65% decrease in the subchondral bone volume fraction (BFV, %) compared to intact condyles, along with extensive subchondral bleeding (Fig. 1A-D). Rapidly degrading chitosan implant was retained near the surface of the bone defects at day 1, with no significant effects on bone or cartilage repair features at 6.5 months. At 6.5 months post-operative, most medial femoral condyles (but not lateral femoral condyles) developed large osteophytes. Only modest subchondral bone repair was observed in the defect area (13% increase in BFV compared to acute defects), and in 3 out of 20 defects, bone resorption around the Jamshidi biopsy hole was observed (Fig. 1C). The larger 2mm bone holes were consistently resurfaced with collagen type II repair with higher collagen content (18% vs 15% w/w) and lower GAG (2.4% vs 4.4% w/w) than tissue outside the defect, while shallow microfracture holes were poorly resurfaced (Fig. 1F). Compared to Jamshidi biopsy holes, drill burr elicited a higher overall cartilage repair histological quality (33 ± 28 vs 49 ± 25, p = 0.041). N = 20 marrow stimulation holes, Fig. 1H-A.

**Conclusions:** Blood vessels sheared by Jamshidi surgical marrow stimulation can devitalize the subchondral bone plate and provoke sporadic bone necrosis. In large animal condyles, bone cleanly removed by Jamshidi biopsy or drilling is slow to repair with mineralized tissue. 2-mm diameter drill holes are resurfaced with soft repair tissue whereas shallow microfracture holes have inadequate marrow communication and are poorly resurfaced. Marrow stimulation surgical approaches need to find a balance between bone damage/blood vessel rupture and subchondral implants that create optimal inflammatory responses that elicit cell influx and osteochondral repair.

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**A TISSUE ENGINEERED OSTEOCHONDRAL COMPOSITE FOR CARTILAGE REPAIR: AN IN VIVO STUDY**

**C. Sosi** | **A. Di Giancamillo** | **D. Deporti** | **M. Agnoletto** | **F. Gervaso** | **M. Domenicucci** | **A. Addis** | **M. Campagnol** | **C. Domenech** | **A. Sammio** | **G. Peretti** | **ICRCS Istituto Ortopedico Galeazzi, Milan, Italy; 1 Dept. of Engineering for Innovation, Univ. of Salento, Lecce, Italy; 2 Istituto Sperimentale Italiano Lazzaro Spallanzani, Rivolta d’Adda (CR), Italy; 3 Dept. of Hlth., Animal Sci. and Food Safety, Univ. of Milan, Milan, Italy; 4 Dept. of BioMed. Sci. for Hlth., Univ. of Milan, Milan, Italy**

**Purpose:** The aim of this work is to validate the efficacy of a tissue engineered osteochondral composite for the treatment of cartilage lesion produced in adult pigs. The osteochondral composite was manufactured by combining an osteo-compatible cylinder and a neo-cartilaginous tissue obtained by seeding swine articular chondrocytes in a collagen scaffold.

**Methods:** Articular cartilage was harvested from the trochlea of six adult pigs and chondrocytes were isolated; after the in vitro expansion, chondrocytes were seeded onto a collagen scaffold that was pre-integrated in vitro to an osteo-compatible cylinder. The seeded osteochondral scaffolds were cultured in chondrogenic medium for 3 weeks, then they were surgically implanted in osteochondral lesions performed in the trochlea of the same pigs from which the cartilage was

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**Footnotes:**

Panel A shows survival surgical approach (A, acute defect); day 1 subchondral hematoma (C) and bone damage (D), 4.5 months post-operative macroscopic appearance of best-in-word repair (E-J), and collagen type II histology of the defect shown in panel G over the drill hole (arrow) in AA chondrocyte-implanted cartilage.