

molecular mechanism of OA development and have reported the effects of mechanical stress to human synovial cells. Recently, there are many evidences that show the expression of inflammatory mediators and pain-sensitizing molecules are increased in tissues including cartilage, meniscus, and synovium from joints of OA patients. The purpose of this study is to examine the expression of inflammatory mediators (Interleukin-1 β ; IL-1 β , Interleukin-6; IL-6, Interleukin-8; IL-8, prostaglandin E2; PGE2) and pain-sensitizing molecules (nerve growth factor; NGF, tachykinin receptor 1; Tac1, bradykinin receptors B1 and B2; Bradykinin B1, B2) by cyclic compression loading on 3D cultured constructs of human knee articular chondrocytes to clarify the effects of mechanical stress on OA development.

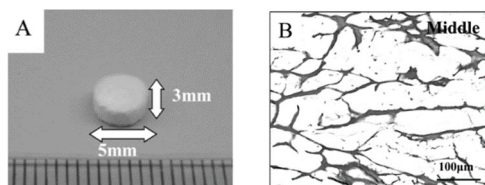
Methods: Human articular chondrocytes were isolated from the knee joint cartilage of patients (n=3, 63, 66, 78 years old women) who underwent total knee Arthroplasty. Cells were cultured in growth media of DMEM containing 10% FBS and 1% penicillin-streptomycin at 37°C and in 5% CO₂ atmosphere. Cells from the fourth passage were used to produce 3D constructs. The cultured cells (5x10⁵ /scaffold) were suspended in a growth medium and then mixed with an equal volume of 1% Atelocollagen gel (Koken, Tokyo, Japan) on ice to produce cell suspension in 0.5% collagen solution. The cell suspension was incorporated into collagen scaffolds (AteloCell® Atelocollagen sponge, MIGHTY; KOKEN, Tokyo, Japan) (5mm diameter, 3mm thick) (Fig.1) by centrifugation. 3D constructs were maintained in growth media for three days in free-swelling conditions. On day three, cyclic compressive loading was applied to the 3D constructs for one hour using cyclic load stimulator (CLS-5J-Z, Technoview, Osaka, Japan) (Fig.2) (40kPa, 0.5Hz). After six hours, culture supernatant was collected, and the concentrations of PGE2, IL-6 and IL-8 were measured with the homogeneous time-resolved fluorescence (HTRF). In addition, the mRNA expression of NGF, TAC1, Bradykinin1, Bradykinin2 genes were quantitatively measured using a real-time polymerase chain reaction (PCR). RNA expression levels were normalized to that of GAPDH. 3D constructs without loading were considered to be the control. Statistical analysis was performed with t-test. Statistical significance was set at p<0.05.

Results: The concentrations of PGE2 and IL-6 in culture supernatant of loading samples were significantly higher compared with that of un-load groups (PGE2, 1 vs 9.23 (SD 10.04); IL-6, 1 vs 2.25 (SD 0.41), p<0.01) (Fig.3). However, the concentration of IL-1 β and IL-8 were unchanged between loaded and unloaded. The mRNA levels of NGF and TAC1 genes of loaded samples were significantly higher compared with that of unloaded samples. (NGF, 1 vs 23.1 (SD 5.65); TAC1, 1 vs 4.0 (SD 1.85), p<0.01) (Fig.4). BradykininB2 genes of loaded samples were significantly lower compared with that of unloaded samples. (1 vs 0.7 (SD 0.12, p<0.01). BradykininB1 genes were unchanged between loaded and unloaded.

Conclusions: In this study, we directly demonstrated that cyclic compressive loading on a 3D-cultured construct of human chondrocytes upregulated inflammatory mediators (IL-6, PGE2) and pain-sensitizing molecules (NGF, Tac 1). In contrary, pro-inflammatory cytokine IL-1 β protein that is regarded as one of key factors for OA development, was not promoted by cyclic compression as reported in our previous study performed using human synovial cells. These results suggest that excessive mechanical stress alone is possible to initiate OA development without stimulating pro-inflammatory cytokines via an unknown mechanism.

□ (Fig.1) Three-dimensional constructs made using collagen scaffolds

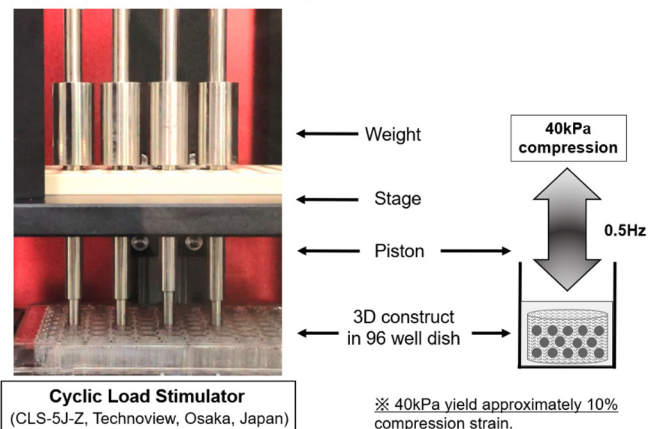
(AteloCell® Atelocollagen sponge, MIGHTY; KOKEN)



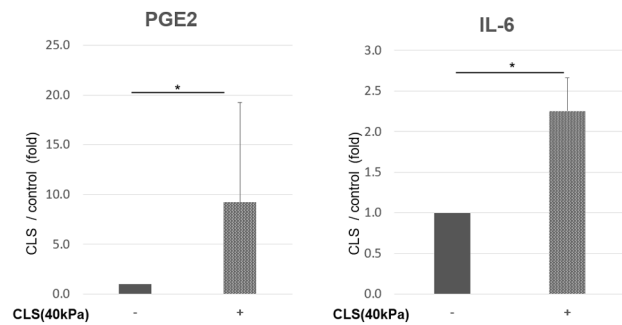
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(A) The pore size was designed to be 30-200 μ m, and the pores were inter-connected. (B) Haematoxylin and eosin stained sections after cell seeding: middle layer of construct. The cells in the 3D construct were evenly embedded in the collagen scaffold.

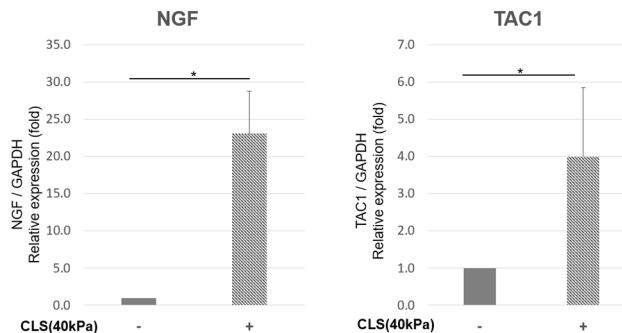
□ (Fig.2) Cyclic compressive loading on 3D constructs



□ (Fig.3) The expressions of PGE2 and IL-6 proteins by cyclic compressive loading.



□ (Fig.4) The expressions of NGF and TAC1 genes by cyclic compressive loading.



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INTRA-ARTICULAR INJECTION OF TRIAMCINOLONE ACETONIDE IN A SUSTAINED RELEASE POLYESTERAMIDE FORMULATION SHOWS PROLONGED ANTI-INFLAMMATORY EFFICACY IN A RAT MODEL

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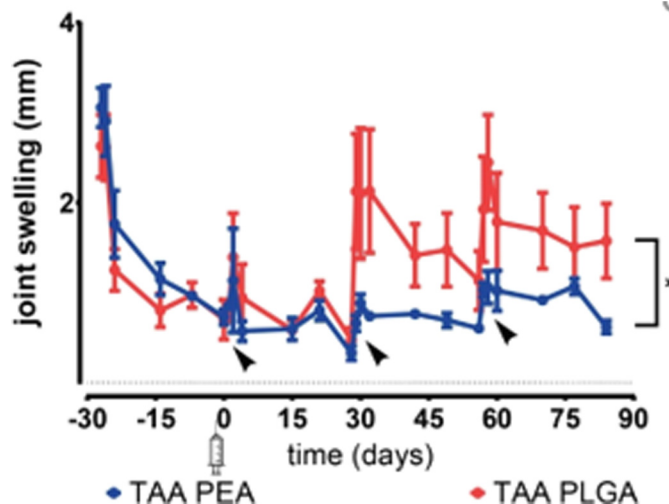
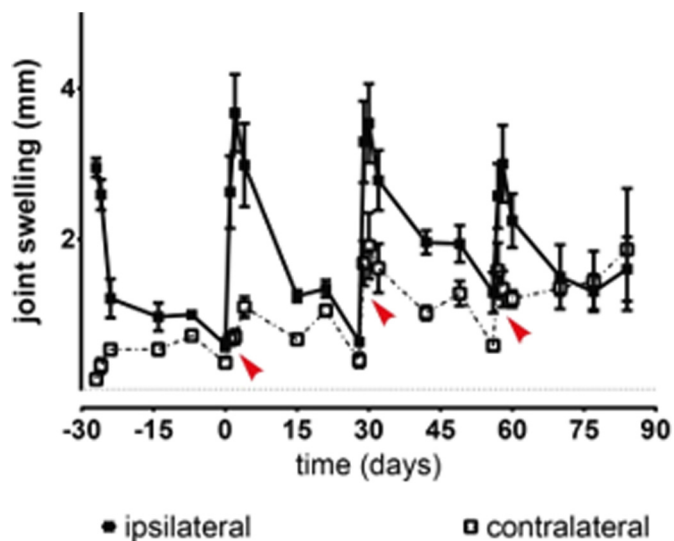
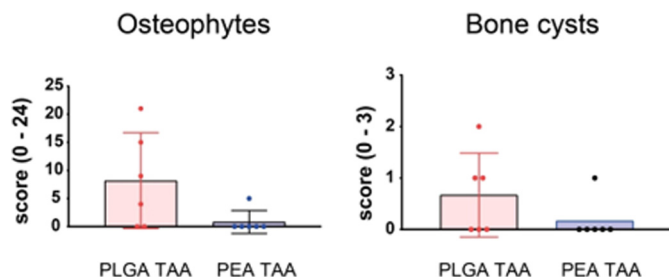
Purpose: Inflammation in an osteoarthritic joint predominantly causes pain and stimulates cartilage matrix breakdown. Tri-

amcinolone acetonide (TAA) is injected intra-articularly to alleviate pain and temporarily reduce inflammation. Since repeat bolus steroid injections entail risks, local sustained TAA release can overcome such disadvantages. PLGA formulations of TAA has been shown to effectively prolong pain inhibition in OA but is limited to 12 weeks. A novel polyesteramide (PEA) microsphere platform based on natural α -amino acids previously showed release in the joint for over 3 months and release in vitro for more than 12 months. To evaluate its capacity for sustained inhibition of pain and inflammation, PEA microspheres loaded with TAA (PEA/TAA) were locally delivered in an adapted rat model of acute inflammatory arthritis and compared to PLGA (PLGA/TAA)

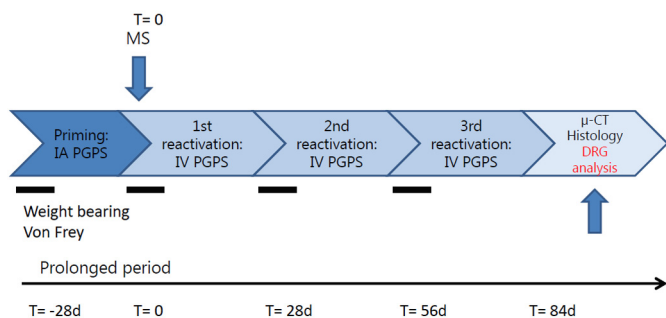
Methods: Localized synovitis was induced in the left knee of 18 adult Sprague-Dawley rats by intra-articular injection of streptococcal-cell-wall-peptidoglycan-polysaccharide (PGPS with 5 mg rhamnose/mL) (priming, day -28). Rats were divided in 3 groups with n=6. Control group, injection of PEA/TAA and injection of PLGA/TAA delivery system. Synovitis flare-ups were reactivated on day 0, 28 and 56 by administration of intravenous PGPS. 2.5 hours prior first reactivation (day 0), 25 μ l unloaded and loaded microspheres with 2.5 mg/ml TAA, using PEA and PLGA polymer as carrier (2.5 mg TAA/ml) were intra-articularly delivered in the affected knee. Joint thickness and signs of pain-like behavior such as lameness and referred mechanical hypersensitivity were measured every 0, 1, 2, 4, 15 and 21st day. Dynamic weight bearing was measured on day 0, 2 and 15 after PGPS administration and served as non-evoked pain-like behavior. Rats were terminated after 12 weeks, scanned with μ CT and knees histologically analyzed. Macroscopic and microscopic analysis of Spleen and liver served as identification method of potential systemic side effects. Observer of read outs was blinded at day of injection.

Results: The control group showed that the modified animal model was able to function as a suitable pain model related to arthritis. An increase of joint swelling in the affected knee and minor swelling in the contralateral knee was observed. Reactivation of inflammation was performed via the tail vein of the animal and therefore systemic effects established. Regardless this phenomena, a difference in joint swelling and synovitis of treated and untreated collateral knee was observed but without statistical significance. For PLGA and PEA releasing particles, the effect after the first reactivation at day 0 days is similar as the inflammation was effectively suppressed. PEA platform continued with this behavior for the upcoming two activations at day 28 and 56 whereas PLGA platform could not prevent the flare up of the inflammation. The priming with PGPS showed that the swelling is retrogressive back to the baseline within the 28 days before the reactivation, even without treatment with TAA. PEA/TAA suppresses the swelling and related inflammation for all three reactivations which might be an indication for the retention of TAA. This result is strengthened by the fact that PLGA/TAA doesn't show this behavior at the second reactivation or third reactivation but can be rather compared to the control group which was not treated. After a period of 12 weeks the osteophyte and bone cyst formation were identified in the knees treated with PLGA/TAA but not in PEA/TAA treated joints.

Conclusions: Sustained suppression of synovitis by controlled TAA release from the PEA platform provides prolonged reduction of joint inflammation, functional improvement and pain relief over an extended period after single injection. In addition, an improvement of PEA platform over PLGA MPs was observed with respect to joint thickness, osteophyte and bone cyst formation.



Experimental set-up



596 IN VITRO EFFECTS OF SOLUBLE EPOXIDE HYDROLASE INHIBITION ON CHONDROCYTE APOPTOSIS AND SENSITIZATION OF PERIPHERAL SENSORY NERVES

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Purpose: Chondrocyte apoptosis and neuronal sensitization are prominent features of osteoarthritis (OA) that are difficult to control with currently available therapies.