THU0058

ENHANCEMENT OF CARTILAGE REGENERATION EFFICIENCY WITH HUMAN ADIPOSE STEM CELL THREE-DIMENSIONAL SPHEROID

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Background: 3D (three-dimensional) cell culture technology has been researched steadily because of its high potential of biocompatibility compared to single cells since 1990s, and is being developed to 3D spheroids recently. Spheroids are considered to reflect the natural organization of cells better than 2D cell cultures, and stem cells spheroids have been studied extensively in therapeutic transplantation. Stem cells were considered as a method of replacing autologous chondrocyte in regenerative treatment of articular cartilage. Compared to conventional single cells, 3D cell culture is artificially created an environment similar to a living body in vitro so that all cells collectively, a cell culture model that allows growth or interaction with the environment. Therefore, the findings of this study indicate that enhancement of treatment efficiency of stem cells caused by potential of survival and proliferation of hASC spheroid in Osteoarthritis. In conclusion, spheroid positive subpopulation of hASCs has high cell proliferation and survival but not apoptosis and cell death potential, which may contribute to successful cartilage regeneration and the development of stem cell therapies in the future.

Objectives: Studied for 3D spheroids to investigate the mechanism of enhancement of survival and proliferation of hASC (human adipose stem cells) spheroid, which may contribute to successful improvement of therapeutic efficacy of stem cells.

Methods: Cell isolation and culture / 3D cell culture dish preparation / hASCs culture on 3D cell culture dish / Real-time PCR analysis / Western blotting / Alcian blue staining / ACLT + MM (Anterior cruciate ligament transection with Medial meniscectomy) model / In vivo fluorescence for cell tracking / In vivo effects of spheroids in OA joint / Histological analysis / Enzyme-linked immunosorbent assay (ELISA) results for inflammatory cytokines in rat synovial fluid / Statistical Analysis

Results: In order to see how the spheroid showed more residual than single, and how effective it was in actual cartilage regeneration, the result of paraffin tissues were confirmed by safranin O staining for each condition. The tendency of cartilage regeneration efficiency was good for spheroid. Although the differences between the single and spheroid groups were small, they reaffirmed that they could somewhat protect cartilage and help regeneration treatment. However, immunohistochemistry of HN(Human nucleic antigen) staining showed that cells of single and spheroid were not observed in the wound but disappeared by the paracrine effect.

Conclusion: Spheroids do not exhibit differentiation characteristics, but they could be seen as a result of expression of related genes such as Bax, Bcl-XL and Alcian blue staining. Spheroids tend to have low potential of cell death rather than proliferation and reduction in the proliferation. So, we conclude the fact that instead of hASCs going directly to the surgical site to regenerate cartilage, they can help cartilage regeneration.

Figure 1. General scheme of *in vitro* and *in vivo* experiments using spheroid. (A) *in vitro* experimental procedures to identify single and spheroid. (B) *in vivo* experimental procedures in surgically-induced OA model to identify the cartilage regeneration efficiency of spheroid.

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THU0059

INCREASING SURFACE LUBRICATION WITH POLY(2-METHYL-2-OXAZOLINE) HALTS DEGENERATIVE CHANGES IN THE CARTILAGE IN A COLLAGENASE INDUCED OSTEOARTHRITIS (CIOA) RAT MODEL

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Background: Degenerative lesions of articular cartilage (AC) surface are related to disruption of the well-organized collagen network and allow proteoglycans to escape from the tissue. Ultimately, this leads to the development of osteoarthritis (OA). Targeted therapy for early AC lesions could provide an effective way to halt the OA development process.

Objectives: This study aims to evaluate the effectiveness of an engineered surface lubricant; poly(2-methyl-2-oxazoline) (PMOXA)¹ to prevent the destruction of the AC surface. Our recently developed contrast-enhanced μ CT (CE μ CT) method was used to quantify AC surface erosion².

Methods: OA was induced in 12-18 week-old male Wistar rats (N=17) with an injection of 250 U Collagenase within 25 μ L solution into the left hind limb. Both hind legs were treated with a second injection three days after the collagenase injection (CI). Three groups were formed by using either PMOXA (N=5), hyaluronic acid (HA; N=6), or saline (N=6) during the second injection. The animals were sacrificed after 45 days, and harvested knees were fixed in phosphate-buffered formalin for a week. Knees were stored in 70% ethanol, and tibia and femur were carefully dissected free of other tissue, stained with 1% phosphotungstic acid³, and scanned with a desktop μ CT with 2.8 μ m pixel size. The medial and lateral AC surfaces were manually segmented from 3D projections using an in-house developed program (Matlab software). These surfaces were analyzed by iteratively fitting a reference surface (RS) to a median-filtered smoothed surface representing a perfectly smooth surface, capturing the realistic shape AC. An offset of 5 pixels (14 μ m) was added between the RS and the original surface (OS). Two quantitative parameters were calculated from the data: Average of Maximum Void Depth (MVD) (depth of lesion) and Degeneration-% (area exceeding 20 μ m MVD / whole area) *100. Estimates of mean differences from all groups against the CI+Saline -group were determined using a linear mixed model.

Results: Boxplots from tested groups are shown in Fig. 1A and MVD results are visualized in Fig. 1B. Collagenase caused structural defects only on the medial and lateral tibial AC surfaces, which was seen as increased MVD and Degeneration-%. CI changes were not seen in PMOXA or HA treated groups. Furthermore, MVD and Degeneration% were lower in CI knees that were treated with PMOXA.

Conclusion: Our CE μ CT analysis method was able to detect subtle changes of the AC surface in the medial and lateral tibial cartilage, caused by the CI. In contrast, the CI did not cause detectable changes in the AC of the femur, which indicates that in the CIOA model, the tibia is more susceptible to structural degradation. Our results show that early intervention with HA or PMOXA can halt the degenerative AC changes caused by CI. However, HA did not suppress the effects of CI in the medial tibia, which indicates that PMOXA could be more effective to prevent the development of OA.

Figure 1. A) Boxplots of Maximum Void Depth (MVD) and Degeneration-%. Lateral and medial side are analyzed separately for both tibias and femurs. Stars indicate if a group was statistically different from control group (CI+Saline). CI= red, no CI= blue. B) Representative visualizations for maximum void depth overlaid on top of the 3D AC surface.

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THU0060 KNEE JOINT DISTRACTION INDUCED SHIFT FROM CATABOLIC TO ANABOLIC STATE OCCURS AFTER DISTRACTION PERIOD

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Background: Knee joint distraction (KJD) is a validated joint-preserving treatment strategy for severe osteoarthritis (OA) that provides long-term clinical and structural improvement. Human trials and animal models indicate clear cartilage regeneration from 6 months and onwards post-KJD [1]. Recent work showed that during distraction, the balance between catabolic and anabolic indicators is directed towards catabolism, as indicated by collagen type 2 markers, proteoglycan (PG) turnover and a catabolic transcription profile.

Objectives: To investigate the cartilage changes directly and 10 weeks after joint distraction in order to elucidate the shift from a catabolic to an anabolic cartilage state.

Methods: Knee OA was induced bilaterally in 8 dogs according to the groove model. After 10 weeks of OA induction, all 8 animals were treated with knee joint distraction, employing the left knee as an OA control. After 8 weeks of distraction, 4 dogs were euthanized (KJD_{direct}) and after 10 weeks of follow-up the 4 remaining dogs (KJD₊₁₀). Macroscopic and microscopic cartilage degeneration was assessed using the OARSI canine scoring system. RT-qPCR was used to determine relative expression of aggrecan (ACAN), collagen type II (COL2a1), cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-3 (MMP3) in the cartilage. PG content was determined by the Alcian Blue assay and the synthesis of PGs was determined using ³⁵SO₄²⁻ as a tracer, as published before.

Results: Macroscopic cartilage damage of the tibial plateau in the KJD_{direct} group was higher as compared to the OA control (OARSI score: 1.7±0.2 vs 0.6±0.3; *p* < 0.001). For KJD₊₁₀ this difference persisted (OARSI score: 1.4±0.6 vs 0.6±0.3; *p* = 0.05). Microscopically, an increase in the total OARSI score was seen after 10 weeks post-KJD. This was mainly due to an increase of chondrocyte clusters at 10 weeks of follow-up, resulting in an increased sub score chondrocyte pathology. Remarkably the sub score intensity of proteoglycan staining decreased directly after KJD (indicating a loss of PGs) but increased after 10 weeks of follow-up suggesting a mixed response depending on the item scored.

Cartilage gene expression analysis showed downregulation of COL2a1 (-1.3 ± 0.3), ACAN (-4.4 ± 1.0, *p* < 0.01) and COMP (-1.7 ± 0.5) in the _{direct} group compared to OA control suggesting enhanced catabolic activity during KJD. In contrast, after 10 weeks of follow-up the expression of COL2a1 and COMP were increased as compared to the OA control (2.6 ± 1.1 and 2.5 ± 1.2 respectively) as well as compared to the KJD_{direct} situation (3.3 ± 1.4 and 4.2 ± 2.0). Expression of MMP3 was upregulated directly after KJD (4.4 ± 0.8) and downregulated after 10 weeks of follow up (-3.3 ± 0.8).

Biochemical analysis of the tibia cartilage of the KJD_{direct} group revealed a lower PG content compared to the OA joint (20.1±10.3mg/g vs 23.7±11.7mg/g). At 10 weeks post-KJD this difference in PG content was gone (24.8±6.8mg/g vs 25.4±7.8mg/g). The PG synthesis rate directly after KJD appeared significantly lower vs. OA (1.4±0.6 nmol/h.g vs 5.9±4.4 nmol/h.g; *p* < 0.001). 10 weeks post-KJD this difference was not detected (3.7±1.2 nmol/h.g vs 2.9±0.8 nmol/h.g), and the synthesis rate in the distracted knee was increased compared to directly after distraction (*p* < 0.01) indicating a shift upon follow-up.

Conclusion: Further in-depth investigation of the material is ongoing and also includes the other joint tissues such as the bone and the synovial tissue. Irrespective, these first results on cartilage changes suggest that the shift from a catabolic to an anabolic state occurs within the weeks after joint distraction. As such, the post-distraction period seems to be essential in identifying key-players that support intrinsic cartilage repair.

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THU0061 EVALUATION OF SAFETY AND EVOLUTION OF OSTEOARTHRITIC JOINTS AFTER THE ADMINISTRATION OF HETEROLOGOUS MESENCHYMAL STROMAL CELLS IN AN ANIMAL MODEL

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Background: One of the main osteoarthritis (OA) consequences is the cartilage loss. Nowadays there is not cure for the OA, so there is an intense research focused on finding a therapy to solve this problem. In this paradigm heterologous mesenchymal stromal cells (MSCH) rise as a solution. Different studies used them as a cellular therapy in order to regenerate damaged cartilage. Furthermore, MSCH have shown anti-inflammatory effects.

Objectives:

- 1) Study the safety of a single intraarticular injection of MSCH derived from healthy canine fat tissue (adMSCH) in OA joints of dogs
- 2) Observe the evolution of functionality and range of articular mobility of these treated joints 6 months after the injection.

Methods: adMSCH obtained (n=10) were phenotypically characterized by flow cytometry, including the Major Histocompatibility Complex type II (MHC-II). Those with the best morphology and growth (n=5) were used for the injection.

The infiltrated dogs (n=7) met our inclusion/exclusion criteria. The adMSCH were injected in 11 joints by 1 million of cells/kilogram of weight.

Before the infiltration we evaluated the joints (basal visit, BV), the vital signs and the animal pain by the owner (Visual Analogical Scale, VAS). The same data were collected one week after the infiltration (V1) and also studied the injected zone. Six months after treatment (V2), joint functionality and range of articular mobility were evaluated, also data of behavioural changes and pain observed by the owners.

Table 1. Functionality and range of articular mobility comparison between BV and V2. Visits column show the number of joints in that state

	Variable	Answers	Visits		t-student (p-value)	n
			BV	V2		
FUNCTIONALITY	Load changes	Normal	3	7	0.095	11
		Change load	8	4		
		Support fingers	0	0		
		No support	0	0		
	Load changes when getting up	Correctly	1	6	0.024*	11
		Position modification	10	5		
		Difficulty to get up	0	0		
		No get up	0	0		
	Lameness in cold	No lameness	1	2	0.062	11
		Mild lameness	6	9		
		Intense lameness	4	0		
		No support	0	0		
RANGE OF ARTICULAR MOBILITY	Lameness when warmed	No lameness	1	3	0.106	11
		Mild lameness	8	8		
		Intense lameness	2	0		
		No support	0	0		
	Articular passive manual mobility	Without pain	0	0	0.400	11
		Mild pain	3	5		
		Raised pain	8	6		
		Disabling pain	0	0		
	Flexion limitation	Total flexion	9	8	0.750	11
		Mild limitation	1	2		
		Severe limitation	1	1		
		No limitation	0	0		
	Extension limitation	Total extension	0	3	0.749	11
		Mild limitation	9	4		
		Severe limitation	2	4		
		No limitation	0	0		

*. Statistical significance established at *P* ≤ 0.05