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COMPLEX OSTEOCHONDRAL TISSUE ENGINEERING

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Purpose: Proper zonal organization of cartilage is important for maintaining proper functions of joint tissues. Complex tissue engineering approaches recapitulating the cartilage zonal organization have great potential in treating cartilage injuries and diseases particularly those with compromised osteochondral interface. Here, we aim to develop a stem cell-based biomaterial-assisted tissue engineered osteochondral tissue with proper zonal organization and evaluate its efficacy by comparing with bone marrow stimulation and autograft in a rabbit osteochondral defect model.
Methods: Rabbit bone marrow mesenchymal stem cell were isolated and expanded before microencapsulating them in collagen biomaterials to form cell-matrix microspheres. The mesenchymal stem cells were induced to differentiate towards both the chondrogenic and the osteogenic lineages using chemically defined culture medium. The chondrogenic and the osteogenic micro-tissues so obtained were assembled to form an osteochondral tissue with proper zonal organization. A surgically induced osteochondral defect was created in the load-bearing femoral condyle of adult New Zealand rabbits before the autologous MSC-derived engineered osteochondral tissue was implanted. Osteochondral defect alone (bone marrow stimulation) and autograft were used as the control groups. The articular joint tissues were harvested at 1, 3 and 6 months post-operation for histological, biochemical and biomechanical analyses.
Results: The engineered osteochondral tissue group and the autograft group showed full volume filling immediately after operation. At 1 month post-operation, the engineered osteochondral group showed excellent regeneration outcomes with type II collagen and glycosaminoglycan-rich hyaline cartilage and with partial regeneration of the tide-mark while the defect in the bone marrow stimulation (natural healing) group was still a big hole slightly filled with fibrous tissues without cartilage matrix. The autograft group showed some remodeling but was also positive for cartilage matrix. The histological characteristics of the engineered osteochondral tissues were similar to that of the autograft, with full thickness hyaline cartilage and proper osteochondral zonal organization throughout the 6 months follow up period while the natural healing group showed some regeneration particularly at 3 months post-operation but the nature of the repaired cartilage was still fibrocartilage with mixed type I and II collagen matrix. Moreover, at 6 months post-operation, the natural healing group showed obvious cartilage thinning while the engineered osteochondral tissue group and the autograft group showed nice hyaline cartilage. The O'Driscoll histological score of knees showed that the engineered osteochondral tissue group was the highest among all groups and was statistically significantly different from the bone marrow stimulation group. Biochemical compositional analysis of the cartilage tissues in various groups showed that the glycosaminoglycan to hydroxyproline (marker for collagen) ratio of the engineered osteochondral tissue group was increasing over time and was statistically similar to that of the autograft group throughout the 6 months follow up period but was statistically different than the natural healing group. Biomechanically, the aggregate modulus and the permeability of the engineered osteochondral tissues were statistically similar to that in autograft throughout the 6 months follow up period and the values were even within the 95% confidence interval of the mechanical properties of uninjured (healthy) rabbit cartilage. The natural healing group showed significantly different (lower) biomechanical properties as compared with the other groups.
Conclusions: The engineered osteochondral tissue showed excellent regeneration outcomes histologically, biochemically and biomechanically and these outcomes were statistically comparable with the clinical gold standard the autograft group, throughout the 6 months follow up period. This work demonstrates the promising potential of using complex engineered osteochondral tissues for cartilage regeneration.

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LORECIVIVINT (SM04690), A POTENTIAL DISEASE-MODIFYING TREATMENT FOR KNEE OSTEOARTHRITIS, DEMONSTRATED CARTILAGE-PROTECTIVE EFFECTS ON HUMAN OSTEOARTHRITIC EXPLANTS

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Purpose: Wnt pathway upregulation contributes to knee osteoarthritis (OA) through osteocyte differentiation, cartilage thinning, and inflammation. Lorecivivint (LOR; SM04690), a novel, small-molecule CLK/DRYK1A inhibitor, demonstrated disease-modifying potential for knee OA via Wnt pathway modulation in preclinical studies. Further studies were performed to evaluate cartilage-protective effects of LOR on human OA explants from total knee replacement (TKR) donors.
Methods: Knee joint tissue from 22 TKR donors was obtained. IRB approval was obtained from Scripps Health. Cartilage was scored using the Outerbridge classification system based on gross appearance (grade 1=least-damaged tissue, grade 4=most-damaged tissue). Cartilage explants (4 mm in diameter) with Outerbridge grades 2-3 were harvested and cultured for 48 hours to reach metabolic stability. These were treated with LOR (10 nM, 30 nM) or DMSO and stimulated with either IL-1β (10 ng/ml) or TNF-α (20 ng/ml) and oncostatin M (OM) (10 ng/ml) or left unstimulated. After 72 hours, supernatants and explants were collected. Gene expression of matrix metalloproteinases (MMP) 1, 3, and 13 were measured by qPCR and levels of MMP1, MMP3, MMP13, ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs), and ADAMTS5 protein were measured in supernatants by ELISA. Glycosaminoglycan (GAG) and nitric oxide (NO) levels were measured in supernatants using the Dimethylmethylene Blue Assay (DMMB) assay and Griess assay, respectively. One-way ANOVA was used for multiple group comparisons.
Results: Treatment with IL-1β or TNF-α and OM led to statistically significant increases in gene expression of MMP1, MMP3, and MMP13 and increased secretion of GAG, MMP1, MMP3, MMP13, ADAMTS4, ADAMTS5, and NO in supernatants compared to unstimulated control. Treatment with LOR decreased both IL-1β-stimulated or TNF-α and OM-stimulated gene expression of MMP1, MMP3, and MMP13 and secretion of GAG, MMP1, MMP3, MMP13,

A

	IL1-β stimulation			TNF-α + OM stimulation		
Protein levels (Supernatants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP1	4.49 (0.2)	**1.24 (0.03)**	**1.1 (0.02)**	8.23 (0.44)	**1.01 (0.01)**	**1.0 (0.01)**
MMP3	5.97 (0.37)	**1.02 (0.03)**	**1.35 (0.07)**	5.17 (0.32)	**1.1 (0.02)**	**1.08 (0.04)**
MMP13	14.8 (0.49)	**4.57 (0.7)**	**1.93 (0.2)**	70.78 (3.31)	**2.56 (0.17)**	**1.47 (0.08)**
ADAMTS4	1.28 (0.02)	1.04 (0.01)	1.09 (0.02)	1.45 (0.03)	1.03 (0.01)	0.91 (0.02)
ADAMTS5	1.91 (0.1)	**1.03 (0.03)**	**1.15 (0.03)**	2.28 (0.11)	**1.24 (0.02)**	**0.87 (0.03)**

B

	IL1-β stimulation			TNF-α + OM stimulation		
Gene expression (Explants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP1	1.04 (0.01)	0.81 (0.04)	0.71 (0.05)	1.04 (0.01)	**0.67 (0.03)**	**0.50 (0.03)**
MMP3	1.02 (0.01)	0.82 (0.02)	**0.56 (0.02)**	1.03 (0.01)	**0.73 (0.03)**	**0.71 (0.03)**
MMP13	1.05 (0.01)	**0.60 (0.02)**	**0.60 (0.05)**	1.05 (0.01)	**0.60 (0.03)**	**0.63 (0.03)**

C

	IL1-β stimulation			TNF-α + OM stimulation		
Supernatants	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
GAG	1.35 (0.04)	1.05 (0.03)	**0.87 (0.02)**	2.4 (0.07)	2.02 (0.05)	**1.54 (0.04)**
NO	490.46 (18.69)	**0.89 (0.05)**	**0.44 (0.03)**	144.2 (9.6)	**19.79 (2.71)**	**20.09 (2.65)**