positive control and results are expressed in Newtons case shoulder less Newtons control shoulder.

Results are expressed in median [percentil25 – percentil75], non-parametric Mann-Whitney U test was used for statistical analysis. P values were obtained for comparison between non-treated animals (chronic rotator cuff tears model) and treated groups.

Results: Treatment was well tolerated in all cases, no adverse effects were observed in treated animals. Non-treated animals (rotator cuff tear model) showed a resistance of – 3.9N [−8.4–0.71]. A significant improvement of resistance was observed in treatment groups.

In the case of MSCs with collagen type I membranes the resistance was 4.8N [1.3–7.1] and 3.3N [−0.17–7.67] in their respective controls (type I collagen membrane without cells). In the case of MSCs with type I collagen gel the resistance was 11.3N [9.7–19.0] and 7.8N [−2.1–14.3] in their controls (type I collagen gel without cells).

Conclusions: We have observed a significant recovery after repairing SE chronic lesions via the use of MSCs in the context of type I collagen vehicles. This “injury-repair” animal model using allogeneic MSCs could be extrapolated as an alternative surgery in rotator cuff tears in human subjects.

Table 1

<table>
<thead>
<tr>
<th>Change</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Non-treated animals</td>
<td>−3.97 N [−8.4, 0.71]</td>
</tr>
<tr>
<td>MSCs + membrane</td>
<td>4.89 N [1.36, 7.08]</td>
</tr>
<tr>
<td>Membrane</td>
<td>3.30N [−0.17, 7.67]</td>
</tr>
<tr>
<td>MSCs + gel</td>
<td>11.35 N [9.77, 19.00]</td>
</tr>
<tr>
<td>Gel</td>
<td>7.81 N [2.10, 14.33]</td>
</tr>
</tbody>
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ENGINEERING AN OSTEOCHONDRAL COMPOSITE FOR THE TOTAL JOINT RESURFACING

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Purpose: This laboratory is developing methods to engineer clinical-scale, scaffold-free, tissue engineered cartilage for joint repair, as an alternative to metal-based joint prostheses. The purpose of this study is to combine engineered cartilage sheets, human bone marrow derived mesenchymal stem cells (hMSCs) and trabecular tantalum metal (TTM) into a single construct and, thus, enable its integration into native bone.

Methods: Rabbit auricular chondrocytes were isolated and culture-expanded in 10% FBS in DMEM (low glucose), and used at primary to 4th passage. To fabricate scaffold-free cartilage sheets combined with hMSCs, rabbit auricular chondrocytes were applied to a “Double-Diffusion Bioreactor” at 1.8 × 10^6 cells/cm² and, 1 or 3 days later, hMSCs were added at 3.1 × 10^6 cells/cm². The reverse order of cell seeding was also performed with hMSCs added first, and chondrocytes applied 1 or 3 days later. The cell composites were cultured for 4 weeks in serum-free DMEM (high glucose), containing of 1% ITS-premix, 10 nM Dexamethasone, 50μM ascorbate-2-phosphate, 1% sodium pyruvate, 1% non-essential amino acid, 1% glutamax, and 1% Penicillin/Streptomycin. The medium was changed daily on the inside of the bioreactor and every other day on the outside reservoir of the bioreactor. To test conditions for integrating TTM with engineered cartilage, 1.0 cm disks of sterile, porous TTM were applied on top of the engineered cartilage at days 2, 4, 6, with or without hMSCs applied at day 2, and cultured for an additional 4 weeks under the same conditions as above. The tissues were fixed with 10% formalin and embedded in paraffin alone, for in situ hybridization, or in plastic for imaging both TTM metal and cellular components. Paraffin sections were stained with safranin-O and Fast Green and plastic sections were stained with toluidine-blue. In order to detect the human-derived cells, Alu in situ hybridization to Alu sequences was performed using an automated system (Vantana, Inc.).

Results: Histologic sections showed that hMSCs and chondrocytes integrated well under all conditions studied (Figure 1). In situ hybridization images (Figure 2) showed a thin region, 50–80 microns thick, that contained Alu-positive cells, which corresponds to the Fast Green positive/safranin-O-negative regions in Figure 1. The cell application order of chondrocyte-first and hMSC-second gives a smoother surface than of hMSC-first and chondrocyte-second, which more closely resembles the native articular surface. The toluidine blue-stained plastic sections showed robust expression of a cartilage-like matrix in both the cartilage-only samples and in the combined cartilage-MSC samples (Figure 3). The MSC-containing constructs tended to stain more lightly and were thicker than the cartilage-only samples, suggesting there is cross-talk between the MSCs and chondrocytes. Integration of TTM with engineered cartilage was seen in the all combinations except that of TTM applied to chondrocytes at day 6 without MSCs (Figure 4).
Conclusions: hMSCs were well integrated with the engineered cartilage, and chondrocytes maintained their phenotype. hMSCs improved integration into TTM. This results show significant progress towards mimicking the native structure of the calcified zone to the subchondral bone in the articular cartilage.

467 A PHYSIOLOGIC ROBOT REACTOR SYSTEM TO SIMULATE IN VIVO CONDITIONS
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Purpose: Success in cartilage tissue engineering and cartilage repair strategies depends on the formation of a hyaline cartilage tissue. In any circumstances, the interaction of at least the four components cells, scaffold matrix, biochemical, and biomechanical factors, is of utmost importance to induce or maintain the cells in a chondrocytic phenotype, which is a prerequisite to form hyaline cartilage tissue. Any significant in vitro evaluation, such as testing different cell-scaffold constructs, has to be performed under the harsh conditions encountered in vivo within synovial joints. Therefore, many different bioreactor systems have been developed with the aim to simulate these conditions. However, two main shortcomings have been identified in these systems: (i) the mechanical stimulation units do not operate within a physiological stress range and are limited in the applicable motion pattern, and (ii) most systems lack an ambient control and therefore no hypoxic environment is generated as encountered in synovial joints. We have addressed these shortcomings by designing a fully autonomic modular Physiologic Robot Reactor System (PRRS).

Methods: We have engineered a reactor system that comprises a mechanical stimulation unit (MSU), an automatic sample changer (ASC), and an environmental control box (ECB) (Figure 1). The MSU is designed with three linear (orthogonal axes) and one rotational degree of freedom (around z-axis; a rotational component around y-axis is pending). The load generated by the MSU is transferred via an exchangeable plunger on a sample tissue placed in a sample holder (Figure 2). Highly accurate force-feedback and motion systems are controlled by ultra-fast Field Programmable Gate Array (FPGA) and real-time components which continuously monitor all system parameters. The ASC is designed as a carousel providing space for 24 sample holders, which allows for individual piloting of the samples with their own stimulation pattern. The ASC and the MSU are integrated in the ECB in which humidity, temperature, gas composition (O2, CO2), and pressure are actively controlled. In addition, an automated media exchange is also implemented in the system, which enables a prolonged uninterrupted cultivation of sample tissues.

Results: The complex physiological motion and load pattern of a knee joint were closely simulated by combining sinusoidal and linear motions. Loading forces of up to 500 N in z-axis were achieved, closely matching the physiological forces encountered in the knee. Within the ECB, the climate is accurately controlled and maintained (deviations of less than 0.1% and 0.1°C from given gas concentrations and temperature, respectively) within the range of the detectors.

Fig. 1.

Conclusions: The PRRS has the potential to be a convenient and flexible tool for screening and evaluation of cartilage repair strategies in vitro performed under the harsh conditions encountered in vivo within synovial joints. The PRRS is designed modularly, thus it is a very flexible system that may also be used for the stimulation of other sample tissues than cartilage, with much different mechanical and environmental parameters.

468 MICROSCALE GUIDANCE FOR OPTIMAL HUMAN MESENCHYAL STEM CELL-BASED CHONDROGENESIS

Purpose: Tissue engineering has tremendous potential for long-term repair of cartilage lesions, but current tissue engineered cartilage constructs, while similar in biochemical features, have inferior mechanical properties compared to native cartilage. This problem may be due to a lack of an oriented structure in the constructs at the microscale that is present in the native tissue. The goal of this study is to test the hypothesis that microscale features on scaffolds will cause the differentiating mesenchymal stem cells (MSCs) to preferentially arrange themselves and to create a microscale-oriented extracellular matrix similar to the native tissue structure. Another goal of the study is to investigate a method to convert the findings to form larger 3D cartilage constructs.

Methods: Channels of varying microscale dimensions (25–1000 micrometers) were formed in collagen-based scaffolds via microfabrication. The channel quality was confirmed through image analysis. Human MSCs were seeded in these channels, and Live/Dead staining was used to confirm the viability of cells within the channels. Selective attachment and spreading of MSCs within the channels was ensured by modifying the plateau regions with triblock copolymer F108. The chondrogenic potential of MSCs seeded in these channels was investigated by culturing them in chondrogenic medium for three weeks, then evaluating them by type II collagen immunohistochemistry, and for mechanical behavior.

Results: We show selective adhesion of MSCs in microchannels. MSCs aligned along the length of the channel at optimal channel dimensions of 25–100 micrometers. We further show mature oriented type II collagen formation in smaller channels. In addition, we show the mechanical properties (modulus of elasticity and ultimate stress) were significantly improved in scaffolds with MSCs in smaller channels. More importantly, a new method was developed to obtain large 3D constructs that contain these microscale guidance features.

Conclusions: Microscale channels of collagen can guide MSCs and lead to improved mechanical biochemical function of the construct. In addition, we demonstrate that large cartilage constructs with such microscale guidance features can be fabricated for in vivo testing. This work paves the way for developing cartilage constructs with ultrastructure similar to native tissue.