Controlled Studies of Alginate Hydrogels under Biomimetic Bioreactor Conditions for Cartilage Tissue Engineering
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Introduction
Tissue engineering bioreactors mimicking in vivo environment of the specific tissue, may provide relevant models for biomaterial assessment. In this work, we have utilized a novel bioreactor with dynamic compression and interstitial medium flow at physiological regimes for evaluation of alginate hydrogels as cell supports in cartilage tissue engineering.

Materials and Methods
Alginate discs (13 mm x 3 mm thick, 1.5 % w/v) were made by immersing a holder filled with aqueous solution of low viscosity Na-alginate (Protanal LF 20/40, FMC Biopolymer, USA), in 1.5 % w/w CaCl$_2$ solution for 48 h. Alginate microbeads (~800 µm, 1.5% w/v) with and without immobilized bovine calf chondrocytes were produced by electrostatic droplet generation. Degradation of control microbeads was studied in Na-citrate solutions (0.05-0.5 mM) in DMEM. The novel bioreactor (Fig. 1a,b) was used to test the discs and packed beds of microbeads at 10% strain and (i) at a loading rate of 337.5 µm/s and (ii) at sequential increments of 50 µm displacement every 30 min. Microbeads with cells (33x10$^6$ cells/ml) were cultivated for up to 4 weeks (dynamic compression: 1 hr on/1 hr off, 0.42 Hz, 10% strain; continuous perfusion: 0.28 ml/min). Imposed loads were measured over time, while the resulting beads were evaluated regarding size, biochemical composition and histology.

Results
Bioreactor compression tests yielded stress-strain curves, used to determine compression (CM) (Fig. 1e, Table 1) as well as equilibrium unconfined compression (Young's, YM) moduli. Packed beds of control microbeads exhibited significantly higher CM as well as YM than the discs (31.6 ± 8.3 vs. 20.9 ± 1.6 kPa, respectively) indicating the effects of interstitial water and specimen geometry. Microbeads with cells exhibited initially lower CM than the control beads but over the cultivation, cells proliferated and produced extracellular matrix while the packed bed compacted deforming the beads (Fig. 1d) and inducing mechanical strengthening (up to 40%). Degradation studies have shown that alginate gels swell proportionally to the concentration of Na-ions and rapidly lose the mechanical strength until spontaneous burst.

![Image](image324x526to541x578)

Fig. 1. a,b) Bioreactor cartridge and holder; c) control microbead; d) microbead with cells, 2 weeks of bioreactor cultivation (c,d: bar=200 µm); e) stress-strain curves at a loading rate of 337.5 µm/s for discs (1), packed beds of control microbeads (2), of microbeads with cells, day 1 (3), and of control microbeads after 3 days in 0.2 mM Na-citrate solution (4) (e: n=3).

<table>
<thead>
<tr>
<th>Alginate specimen (1.5 % w/v)</th>
<th>CM [kPa]</th>
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<tbody>
<tr>
<td>disc</td>
<td>77.3 ± 1.1</td>
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<tr>
<td>control microbeads</td>
<td>136.6 ± 2.8</td>
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<tr>
<td>microbeads with cells, day 1</td>
<td>114.3 ± 1.0</td>
</tr>
<tr>
<td>control microbeads after 3 days in 0.2 mM Na-citrate</td>
<td>36.3 ± 1.4</td>
</tr>
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Table. 1. Compression moduli (CM)

Discussion and Conclusions
Results of this study show potentials of the novel bioreactor in conjunction with alginate microbeads to monitor, control, and adjust cultivation conditions for cartilage tissue engineering.

Acknowledgments
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Disclosures
Authors have nothing to disclose.