EFFECTS OF MEDIUM AND TEMPERATURE ON CELL MECHANICS IN OSMOTICALLY CHALLENGED ARTICULAR CARTILAGE

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SUMMARY
The aim of this study was to compare the influence of two different media (DMEM and PBS) and temperatures (21°C and 37°C) on the cell volume changes in hypo-osmotically challenged intact articular cartilage. Calcein-AM and propidium iodide stained chondrocytes were imaged with confocal laser scanning microscope through cartilage surface at 21°C or 37°C before and after hypo-osmotic challenge. Half of the samples were measured in Phophate Buffered Saline (PBS) and half in Dulbecco's Modified Eagles Medium (DMEM). In all groups, cell volumes were increased significantly ($p < 0.05$) three minutes after the hypo-osmotic challenge. After this time point, cell volumes decreased significantly ($p < 0.05$) close to the volumes before the osmotic challenge. Normalized cell volumes were not significantly different ($p > 0.05$) for the samples measured in PBS or DMEM. Further, normalized cell volumes were not significantly different ($p > 0.05$) for the samples measured at 21°C or 37°C. Our results suggest that cell volume changes following hypo-osmotic challenge of cartilage are not dependent whether the experiments are conducted in PBS or DMEM, and whether the room temperature or 37°C is used. This kind of quality control study is very important for the interpretation of and comparison between earlier studies and for the planning of future research.

INTRODUCTION
One of the earliest macroscopic events of osteoarthritis (OA) is swelling of articular cartilage [1]. OA leads to decreased extracellular osmolarity and increased chondrocyte volume [1,2]. Thus, cell responses have been typically studied within osmotically challenged cartilage. Both Dulbecco´s Modified Eagles Medium (DMEM) and Phosphate Buffered Saline (PBS) have been used for the measurements of the cell volume changes following osmotic challenges [3,4]. These kind of measurements have been conducted mainly at room temperature (~21°C) [2,3,4]. However, it is generally thought that in physiological temperature (37°C) changes in chondrocyte volumes following osmotic loading are more rapid than at ~21°C [5]. Also it is generally thought that active cell volume regulation processes are inhibited by the use of PBS. However, it has not been directly shown whether different media and temperatures affect cell volume changes in a relatively short osmotic loading experiment.

METHODS
For both temperature groups (21°C and 37°C), seven bovine knee joints were obtained from the local slaughterhouse. Articular cartilage samples with subchondral bone were released from the lateral patellar groove (LPG) of the femur and two smaller cylindrical plugs were further prepared; the other block for the tests in DMEM (low glucose serum-free with 2 mM L-glutamine) and the other for the tests in PBS (Figure 1). In each group, chondrocytes were studied at the center of the cylindrical plugs through the intact cartilage surface.

Chondrocytes were stained with both Calcein-AM and propidium iodide for live and dead cells, respectively. Confocal laser scanning microscope (CLSM) (Nikon Eclipse TE-300, Nikon co., Japan) with ultraVIEW confocal laser scanner (Perkin-Elmer, UK) was used for cell imaging. Stacks of images were obtained at a 0.3 µm vertical z-axis spacing using an objective with 60x magnification. The pixel size in x-y plane was 0.2 µm x 0.2 µm. Chondrocytes were first imaged in isotonic medium (~290 mOsm). After this, the medium concentration was changed to hypotonic (~170 mOsm). Image stacks were further captured at two different time points, i.e., at short-term (3 min) and steady-state time point (120 min).

Five to ten cells from each image stack were chosen for the analysis. The Visualization Toolkit 5.2.0 (Kitware Inc.) was used to reconstruct 3D-images of the cells, and a code programmed with Python was used to calculate cell volumes [6]. Threshold of 40% of the maximum fluorescence intensity, the same as used earlier [2,3,4,7] was applied in the calculations.

Wilcoxon signed rank test (adjacent samples from the same joint, comparison between media) and Mann-Whitney U test (samples from different joints, comparison between

temperatures) were used for statistical analysis using SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA).

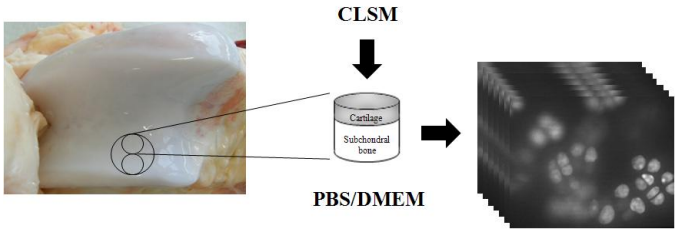

Figure 1: Two adjacent samples with subchondral bone were freed from the lateral patellar groove. The other sample was measured in PBS and the other in DMEM. This same procedure was carried out at 21°C or 37°C.A representative image stack of CLSM is indicated on the right.

RESULTS AND DISCUSSION

In all groups, the cell volumes were increased significantly ($p < 0.05$) three minutes after the medium concentration was changed from isotonic to hypotonic (Figure 2). After this time point, the cell volumes decreased significantly ($p < 0.05$) close to the reference volumes, i.e., the volumes before the osmotic challenge. The cell volume increase and recovery back to the original volume were consistent with earlier findings [4].

In both observation time points after the hypo-osmotic challenge, there were no statistically significant differences ($p > 0.05$) in normalized cell volumes between the samples immersed in PBS or DMEM (Figure 2). This result was the same at 21°C and 37°C. This finding is in disagreement with a general assumption that in hypo-osmotic loading experiments, cell volume regulation is inhibited by the used of PBS, especially due to the lack of glucose [8,9]. It may be that two hours is still such a short measurement period that cells receive enough nutrients even without the use of DMEM.

Even though it was hypothesized that cell volumes would recover back to the original volume faster at 37°C than 21°C [5,10], our results showed that normalized cell volumes were not significantly different ($p > 0.05$) for the samples measured at 21°C or 37°C. This behavior may be due to the fact that we measured cells in the intact tissue, while earlier suggestions were based on measurements conducted for isolated cells [10]. Mechanisms in the intact tissue may be slower, for example, due to tightly packed collagen fibrils in the superficial zone. On the other hand, at the short-term time point, cells may have not yet been fully activated for volume regulation, and cells at least in the lower temperature group may have still been in the growing phase compared to the cells in the higher temperature group.

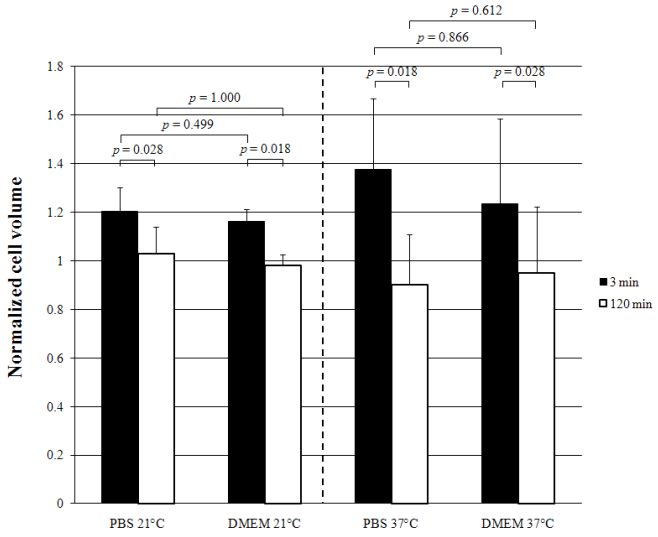

Figure 2: The cell volume change in the samples immersed in two different media (PBS and DMEM) and at temperatures of 21°C and 37°C after changing the medium concentration from isotonic to hypotonic. The cells were imaged and cell volumes analyzed before the osmotic challenge and at two different time points (3 min and 120 min) after the osmotic shock. The cell volumes at 3 and 120 min time points were normalized with those recorded before the osmotic challenge.

CONCLUSIONS

Our results suggest that the cell volume changes in hypo-osmotically challenged intact articular cartilage are not dependent whether the experiments are conducted in PBS or DMEM, and whether the temperature of 21°C or 37°C is used. This is an important quality control finding for interpretation of the results in the previous studies and for future research plans.

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