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Dynamics of Intrinsic Glucose Uptake Kinetics in Human Mesenchymal Stem Cells during Chondrogenesis

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Abstract

Chondrogenesis of human mesenchymal stem cells (hMSCs) is an important biological process in many applications including cartilage tissue engineering. We investigated the glucose uptake characteristics of aggregates of hMSCs undergoing chondrogenesis over a three-week period both experimentally and by using a mathematical model. Initial concentrations of glucose in the medium were varied from 1 to 4.5 g/l to mimic limiting conditions and glucose uptake profiles were obtained. A reaction-diffusion mathematical model was implemented and solved to estimate kinetic parameters. Experimental glucose uptake rates increased with culture time for aggregates treated with higher initial glucose concentrations (3 and 4.5 g/l), whereas they decreased or remained constant for those treated with lower initial glucose concentrations (1 and 2 g/l). Lactate production rate increased by as much as 40% for aggregates treated with higher initial glucose concentrations (2, 3 and 4.5 g/l), whereas it remained constant for those treated with 1 g/l initial glucose concentration. The estimated DNA-normalized maximum glucose uptake rate decreased by a factor of 9 from Day 0–2 (12.5 mmol/sec/g DNA) to Day 6–8 (1.5 mmol/sec/g DNA), after which it started to increase. On Day 18–20, its value (17.5 mmol/sec/g DNA) was about 11 times greater than its lowest value. Further, the extracellular matrix levels of aggregates at Day 14 and Day 21 correlated with their overall glucose uptake and lactate production. The results suggest that during chondrogenesis, for optimal results, cells require increasing amounts of glucose. Our results also suggest that diffusion limitations play an important role in glucose uptake even in the smaller size aggregate model of chondrogenesis. Further, the results indicate that glucose uptake or lactate production can be a tool for predicting the end quality of tissue during the process of chondrogenesis. The estimated kinetic parameters can be used to model glucose requirements in cartilage tissue engineering applications.

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Key terms

Cartilage; Aggregate Cultures; Mass Transport; Glucose Consumption; Diffusional Limitation; Michaelis-Menten Kinetics; Lactate Production; Mathematical Modeling

Introduction

Human mesenchymal stem cells (hMSCs) are an important cell source for tissue engineering (TE) and regenerative medicine applications due to their ability to differentiate *in vitro* into a variety of mesenchymal lineage tissues such as cartilage, bone, muscle and fat ^{4, 5, 9}. Among the TE applications, cartilage has been widely studied since damage to this tissue causes osteoarthritis (OA), a degenerative disease exacerbated in part by the lack of the tissue's regenerative capability ²³.

We and others have shown that transport limitations can significantly affect the function of engineered tissues ^{3, 7, 13, 26, 41, 43, 47}. These limitations are characterized by local availability of nutrients or signaling molecules within the tissue, and can arise due to reduced diffusion or enhanced uptake (*e.g.*, glucose) or both. In hMSC-based cartilage TE, the transport limitations can vary during differentiation as the cells undergo phenotype changes. Understanding such limitations quantitatively is important in developing strategies (*e.g.*, bioreactors) to resolve them.

There have been very few studies that investigated glucose metabolism during hMSC chondrogenesis. Pattappa, *et al.* in a recent study showed that the glucose uptake rate stayed approximately constant during the 21-day period of *in vitro* chondrogenesis of hMSCs ^{27, 28}. No studies, to our knowledge, have investigated intrinsic glucose uptake during chondrogenesis via a kinetic model (*viz.* Michaelis-Menten kinetics). Such kinetic models will be useful in many applications, including as a tool for tissue engineered cartilage design and development, and as a predictive tool for monitoring the quality of the chondrogenesis. Further, unlike aggregates, which are used as a model of hMSC chondrogenesis, engineered cartilage tissue constructs grown from hMSCs can be nutrient-limited due to their larger sizes ⁷. The effect of low nutrient levels on chondrogenesis and nutrient uptake during chondrogenesis are under-investigated.

In this study, we investigated the glucose uptake in hMSCs undergoing chondrogenesis using an aggregate culture system monitoring glucose utilization over time for various initial glucose concentrations. We utilized a reaction mass-transport model and estimated kinetic parameters of uptake from experimental data. Our results show that, overall, glucose uptake rate increased during chondrogenesis and the intrinsic kinetic parameter, $V_{\max}$, normalized to DNA levels, decreased rapidly after aggregation by a factor of 9; it recovered to a level 12 times greater than the lowest level towards the end of the 21-day culture period. Further, our data show that glucose uptake or lactate production can be a predictor of end point-biochemical quality of the chondrogenic construct.

Materials and Methods

Materials

Cell culture medium [Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l - DMEM-HG, 1.0 g/l - DMEM-LG and 0 g/l - DMEM-NG], trypsin, L-glutamine, antibiotic/antimycotic solution (10^5 U/mL penicillin G sodium, 10 mg/mL streptomycin sulfate, and 25 µg/mL amphotericin B in 0.85% saline), nonessential amino acids, dexamethasone, sodium pyruvate, phosphate-buffered saline (PBS) and nuclease-free water were purchased from Invitrogen (Carlsbad, CA, USA). Ascorbate-2-phosphate was purchased from Wako (Richmond, VA). Fetal bovine serum (FBS, Life Technologies, Carlsbad, CA) was lot selected (Lot# 1805387).²⁰ Safranin-O, hydroxyproline, papain, cysteine, 4-(dimethylamino) benzaldehyde, perchloric acid 70% reagent grade, hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), calf thymus DNA standard, cetylpyridinium chloride (CPC), copper(II) sulfate pentahydrate ($CuSO_4$), glycine, hydrazine hydrate solution (24–26% in H_2O), sodium L-lactate, NAD (β -Nicotinamide adenine dinucleotide hydrate), L-lactate dehydrogenase (L-LDH, from rabbit muscle), and Hoechst 33258 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCL), sodium hydroxide (NaOH), sodium phosphate, ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), disodium phosphate (Na_2HPO_4), sodium acetate, isopropanol, bovine serum, 16% formaldehyde and clear 96-well plates were obtained from Fisher Scientific (Pittsburgh, PA, USA) Transforming growth factor- β 1 (TGF- β 1) and human fibroblast growth factor-2 (FGF-2) were obtained from Peprotech (Rocky Hill, NJ, USA). Insulin-transferrin-selenium (ITS)+ Premix was a product of Becton Dickinson (Franklin Lakes, NJ, USA). Chondroitin sulfate C (CS-C, from shark cartilage) was from Seikagaku America (East Falmouth, MA). A Contour Next USB Blood Glucose Monitoring System and glucose test strips were acquired from Bayer AG (Leverkusen, Germany). Nitrocellulose membrane and dot-blot apparatus were bought from BIO-RAD (Hercules, CA, USA). 96-well plates with UV transparent flat bottom and black wells (for DNA assay) were purchased from Corning (Kennebunk, ME, USA). Hemocytometer was from Hausser Scientific (Horsham, PA, USA). 96 well polypropylene plates with a conical bottom were purchased from Evergreen Scientific (Los Angeles, CA, USA). Spectrophotometer was obtained from Molecular Devices (Sunnyvale, CA, USA).

Methods

Cell Culture—hMSCs were obtained from bone marrow donations by healthy volunteers through collaboration with the Stem Cell Core Facility of the Case Comprehensive Cancer Center and prepared following previously published methods^{11, 36, 37}. The cells were derived from donors after informed consent obtained according to an Institutional Review Board-approved protocol. After primary culture, hMSCs were proliferated in complete medium (DMEM-LG with 10% FBS and 10 ng/mL FGF-2) from an initial density of 15×10^5 cells per T150 flask. The flasks were maintained in an incubator set to 37° C with humidified atmosphere of 95% air and 5% CO_2 . Medium was changed twice every week. The cells were 80–90% confluent after 7–10 days.

Aggregate Culture and Chondrogenic Induction—hMSC aggregates were prepared as initially described^{1, 14, 46}, with modifications as described.^{29, 37} Chondrogenic

differentiation medium with four different glucose concentrations were made as follows: DMEM-HG and DMEM-NG were supplemented with 1% ITS+ Premix, 146 μ M A2P, 10^{-7} M dexamethasone, L-glutamine, antibiotic/antimycotic, nonessential amino acids, and sodium pyruvate at 1% and 10 ng/mL TGF- β 1. The resulting chondrogenic medium high glucose (CM-HG) and glucose-free chondrogenic medium (CM-NG) were mixed to form chondrogenic medium of 1, 2, 3, and 4.5 g/l, glucose concentrations.

Passage 2 cells were used in the experiments. These culture-expanded hMSCs were trypsinized with a 0.05% trypsin EDTA which was subsequently inhibited with Bovine Serum (BS). The cell suspension was centrifuged for 5 min at 500 x g to remove the BS/ trypsin solution and resuspended with complete medium for the cell counting using a hemocytometer. After another centrifugation under same condition, the cells were diluted with four different chondrogenic differentiation medium to a density of 1.25×10^6 cells/mL and were seeded in 96-well, conical bottom, 300- μ L polypropylene microplates at a volume of 200 μ L /well. The plates were gently centrifuged for 5 min at 500 x g to encourage initial aggregation and cell-cell interaction. The plates were then placed in a cell culture incubator overnight. The medium was replaced every other day. Samples of 20 μ L were collected every day and frozen at -20°C for glucose analysis.

Glucose Analysis—The concentration of glucose in the medium aliquots was measured using a Contour Next USB Blood Glucose Monitoring System (Bayer AG). Each aliquot was thawed at room temperature, spun down in a microcentrifuge, and supernatant vortexed. A sample of 3 μ L was then pipetted into a petri dish to be analyzed. This procedure was done one sample at a time to minimize evaporation. A standard curve generated from DMEM with known glucose concentrations was used to calibrate the glucose test results. Experimental glucose uptake rate was calculated from the change in mass/mole of glucose divided by time interval (24h) and normalized by cell numbers.

Lactate Analysis—Lactate levels in the culture medium samples were analyzed using a previously published method.²⁵ Briefly, frozen samples were thawed at room temperature, spun down in a microcentrifuge, and vortexed. 10 μ L samples were added per well to a standard clear flat bottom 96-well plate in duplicates. An assay cocktail of 190 μ L consisting of 320 mM glycine, 320 mM hydrazine, 3 mM NAD⁺, and 20 U/ml freshly-made LDH solution in water was added to each sample well. The plates were sealed by a low-evaporation film and incubated at 37°C for 30 min. The absorbance of the plate was read at 340 nm. A standard curve generated from known lactate standards (0–10 mM) was used to calibrate the absorbance values to obtain lactate levels.

Sample Harvest and Processing—hMSC aggregates under each condition were harvested at day 7, day 14, and day 21 for further analysis. Four aggregates were used to quantify DNA, glycosaminoglycan (GAG) and hydroxyproline (HYP) content while two were used for histology. The aggregates harvested for DNA, GAG and HYP analyses were washed with PBS and frozen in -80°C until analysis was performed.

Histology—Aggregates were fixed in 4% formaldehyde, paraffin-embedded, sectioned, then stained with Safranin-O for a qualitative measurement of glycosaminoglycan (GAG)/

proteoglycan content and distribution³³. A Leica camera was used in conjunction with a Leica fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany) to image the samples under a 10x objective. For aggregates that were too large to be captured in a single picture, a mosaic was compiled using multiple images.

GAG and DNA Assay—GAG and DNA contents of aggregate were determined following previously published methods^{6, 31, 36, 37}. GAG content was normalized to DNA content of the aggregate as a surrogate for cell number. Briefly, for DNA measurement, papain buffer was used to digest aggregates and the digest was combined with Hoechst 33258 to allow fluorescence measurement of each sample at an excitation wavelength of 340 nm and emission wavelength of 465 nm. A standard curve was generated from known concentrations of calf thymus DNA. For GAG analysis, digest of each aggregate was separated and combined with Safranin-O reagent in a dot-blot apparatus. Precipitates on a nitrocellulose membrane were collected after vacuum was applied to the apparatus. Individual dots were cut out, and the dye was eluted using cetylpyridinium chloride. Absorbance of the eluted dye was measured at 536 nm. A standard curve was generated from known concentrations of purified chondroitin sulfate.

Hydroxyproline Assay—Samples to be analyzed for hydroxyproline content were underwent papain digestion as mentioned above in the GAG/DNA assay section. HYP content was determined by previously published method^{10, 12, 45}. Briefly, samples were hydrolyzed using 6N HCl overnight at 110° C. HCl was then evaporated by heating open samples overnight at 60° C, and the dry samples were redissolved in water. After preparation, 20 µL of each sample were transferred into a 96-well non-tissue culture plate with UV transparent flat bottom in quadruplicate. To each sample, 20 µL of 0.15M CuSO₄ and 2.5N NaOH were added after which they were incubated in an oven for 5 min at 50° C. Each sample then had 20 µL of 6% H₂O₂ added followed by another incubation period of 10 minutes at 50° C. Before a final incubation period of 16 minutes at 70° C, 80 µL of 3N H₂SO₄ and 40 µL of 10% p-dimethyl-amino-benzaldehyde were added to each sample, respectively. The samples were allowed to cool to room temperature between each incubation period. This procedure was performed simultaneously for standards of hydroxyproline between 0–500 µg/ml. As soon as the final incubation-cooling cycle was completed, the absorbance was read at 505 nm.

Mathematical Modeling—To analyze glucose concentrations of cell culture medium and to obtain kinetic parameters of glucose uptake, we developed an axisymmetric (2D) model of glucose transport. The physical model consisted of a chondrogenic aggregate in culture medium contained in a single well (Fig. 1). We modelled the aggregate as a sphere of uniform radius, ‘a’, that is held constant during each 48 hour-simulation (see below). The radius of the aggregate was varied over the culture period. We evaluated the aggregate size at Day 1, 7, 14, and 21. An empirical fit to this experimental data was used to obtain ‘a’ at t=0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 days.

The model assumptions are: between medium changes, the cell culture medium was stationary. Therefore, the dilute species (nominal maximum glucose concentration was 25

mM) transport was governed by diffusion only. Glucose uptake occurs only in the aggregate. The corresponding transport equation in the culture medium is:

$$\frac{\partial C_G^M}{\partial t} = D_G^M \nabla^2 C_G^M \quad (1)$$

Here, C_G^M is the concentration of glucose in the medium, and D_G^M is the diffusion coefficient of glucose in the medium, which is assumed to be a constant.

In the aggregate, glucose transport is governed by diffusion and reaction. The intrinsic glucose kinetics of the aggregate are given by the Michaelis-Menten equation:

$$R_G = -\frac{V_{\max} C_x C_G}{K_M + C_G} \quad (2)$$

Here, R_G represents the rate of reaction of glucose in the aggregate (mole of glucose $\text{m}^{-3} \text{sec}^{-1}$), C_G is the local glucose concentration in the aggregate, C_x is the uniform density of DNA (g of DNA/ m^3), $V_{\max}$ is the intrinsic uptake rate of glucose per DNA (mole of glucose sec^{-1} g $^{-1}$ of DNA), and K_M is the Michaelis constant. DNA levels (g) of the aggregates were experimentally determined on days 1, 7, 14, and 21. Using the aggregate size data (see above), C_x values can be obtained at Days 1, 7, 14, and 21. An empirical fit to this experimental data was used to obtain C_x at $t=0, 2, 4, 6, 8, 10, 12, 14, 16$, and 18 days. The governing transport equation in the aggregate is:

$$\frac{\partial C_G}{\partial t} = D_G \nabla^2 C_G + R_G \quad (3)$$

The boundary conditions included: continuity of diffusional fluxes at the interface between the culture medium and aggregate, and impermeable boundary at all other boundaries (medium-air interphase, medium-well interphase). A jump condition based on solubility was used to relate concentrations at the culture medium aggregate interphase:

$$\text{at } r = a, \quad C_G = \lambda C_G^M \quad (4)$$

Table 1 shows values of parameters used in the model.

Model implementation and solution—We implemented the transport model in COMSOL Multiphysics (COMSOL, Burlington, MA) using backward Euler initialization. The time stepping method was “Backward Differentiation Formula”, using “free steps” taken by the solver with a tolerance of 0.01. Since our model was a 2D axisymmetric model, we implemented a two-dimensional asymmetric unstructured mesh. Maximum element size was 0.2 mm and the minimum was 0.5 μm , while element growth rate was set to 1.2. The

minimum number of mesh nodes in the narrow regions was set to two. The model usually converged between 1000 and 1200 time steps. Further details of solver and mesh optimization is presented in Supplementary Data (Fig. S1–S4). To ensure that the calculated glucose concentration in the model never became negative, which occurred even at the best tolerances, and to thus to subsequently prevent the uptake of glucose to appear to become synthesis of glucose, we replaced any negative value concentration node in the mesh that occurred with an infinitesimally small positive number (10^{-197}) during the simulation.

The model was solved using the time-dependent solver in COMSOL for a period of 48 hours. This represented the time period between medium changes. During this time period, we assumed that the radius of the aggregate ('a') and the DNA density in the aggregate (C_X) were constant. Except for the first medium change model solution – where we assumed the concentration of glucose in the aggregate to be zero – for subsequent solutions, we used the end average concentration in the aggregate of the previous solution as initial conditions for concentration.

Parameter Estimation—The intrinsic kinetic parameters: $V_{\max}$ and K_M were unknowns in the model. We estimated values for these parameters from experimental data of glucose concentration versus time obtained for all initial glucose concentrations using an optimization method. An objective function:

$$Obj = \sum_{i=1}^N (C_{G,i}^{Exp} - \langle C_{G,i}^M \rangle)^2 \quad (5)$$

was evaluated for each simulation run for a given set of parameter values. Here, $C_{G,i}^{Exp}$ represents the experimental values of glucose concentration, and $\langle C_{G,i}^M \rangle$ is the average concentration of glucose in the medium obtained through simulation. A custom-written function in MATLAB (Mathworks, MA) automatically obtained simulation results by running COMSOL with a given set of parameters ($V_{\max}$ and K_M), and used *fminsearch*, an unconstrained optimization function in MATLAB to estimate parameter values that minimized the objective function. For every 48 hours during the culture period, a new set of parameters was estimated. This allowed us to analyze the behavior of glucose kinetics over the entire chondrogenesis period of three weeks.

Statistical Analysis—Statistical analysis was performed using the Origin 2016 software package from Origin Lab (Northampton, MA, USA), Minitab (State College, PA, USA) and Microsoft Office Excel (Microsoft, WA, USA). For select pair-wise comparisons, Student's t-test was used. For multiple comparisons, Tukey's test was employed. For time-dependent data, analysis of variance (ANOVA) was performed to determine significance. Values are represented as means $\pm$ standard deviation. Differences were considered significant when p-value < 0.05.

Results

Glucose consumption characteristics

The glucose concentrations in the culture medium of the aggregates decreased with time over the 48 hours after all medium changes (Fig. 2). For all initial concentrations of glucose tested, the reduction in glucose concentrations over 48-hour window after every medium change increased with subsequent medium changes. For experiments with initial concentrations of 1 g/l, this decrease was such that after Day 2, the concentration of glucose in the well before medium change was essentially zero. For experiments with 2 g/l initial concentrations, this occurred from Day 12. For experiments with 3 and 4.5 g/l initial concentrations, the glucose concentrations in the well never reached low values obtained with 1 and 2 g/l initial concentrations; the lowest values in the well for experiments with 3 and 4.5 g/l initial concentrations were 0.34 and 1.69 g/l respectively, reached on Day 21.

Experimental rates of glucose consumption calculated from mass balance show that the first day consumption rate after medium change increased with time for all experiments (Fig. 3A). The increase was small for 1 g/l and ranged from 150 to 200% for other concentrations. However, when consumption rates were calculated for the second day after medium change, only experiments with 3 and 4.5 g/l initial concentrations led to increased consumption rates (Fig. 3B). Experiments with an initial concentration of 2 g/l had decreased rates of glucose consumption after Day 18. In the 1 g/l experiments, the rates were the lowest and essentially unchanged over time.

In general, glucose consumption over 24 hours increased with time (Fig. 4) for all conditions. Up to Day 7, the cumulative glucose consumption profiles were approximately similar for all initial concentrations. After around Day 7, aggregates treated with 1 g/l initial glucose concentration consumed significantly less compared to aggregates treated with 2, 3, and 4.5 g/l. Between medium changes, when glucose consumption in the first 24 hours was compared to the next 24 hours, interesting trends emerged (Fig. 3). The absolute glucose consumption increased from the first 24 hours to the next 24 hours after a medium change for 0 out of 10 media changes for 1 g/l, 2 out of 10 media changes for 2 g/l, 4 out of 10 media changes for 3 g/l and ~5 out of 10 media changes for 4.5 g/l. The last four media changes for all initial glucose levels led to decreasing consumption from the first 24 hours to the next 24 hours. Overall, the 21-day net glucose consumption was lowest for aggregates with initial concentration 1 g/l whereas for aggregates with all other initial concentrations, the 21-day consumption values were similar (data not shown). Percent glucose utilization, the ratio of cumulative glucose consumption (moles) to the total amount of glucose (moles) supplied in the medium (Fig. 5), also increased for all initial concentrations. For 4.5 g/l, especially towards the last week of culture, the percent consumption did not change from the first 24 hours to the next 24 hours after a medium change. For 1 g/l, the utilization was nearly 100% 48 hours after a medium change, whereas for 2 g/l it increased from about 65% to 100% from Day 2 to Day 20, for 3 g/l aggregates it increased from 40% to 75%, and for 4.5 g/l aggregates, it increased from 25% to 40% from Day 2 to Day 12 and remained constant at about 40% from Day 14 to Day 20.

Lactate production characteristics

Lactate production rate significantly increased by about 50% from Day 2 to Day 20 for aggregates treated with 2, 3, and 4.5 g/l initial glucose concentrations, whereas it remained constant for those treated with 1 g/l (Fig. 6). Further, lactate production for the latter condition was significantly lower by about 40% to about 60% when compared to lactate production by aggregates treated with 3 and 4.5 g/l initial glucose concentrations. Further, at every time, on average the lactate production rate is about twice that of glucose production rate.

Aggregate biochemical properties

Fig. 7 shows GAG and HYP contents of the aggregates at Day 7, Day 14 and Day 21 normalized to the DNA. While 2, 3, and 4.5 g/l treatments led to increasing levels of GAG/DNA and HYP/DNA from Day 7 to Day 21, 1 g/l treatment showed such a trend for HYP/DNA only; it, however, had a significantly increased GAG/DNA content at Day 7. The Day 21 results show that both GAG/DNA and HYP/DNA contents of aggregates with initial glucose concentrations 2, 3, and 4.5 g/l were significantly about 10 times greater for GAG and about 60% greater for HYP than those with initial glucose concentration 1 g/l. There was no significant difference between GAG/DNA and HYP/DNA contents of aggregates treated with 2, 3, and 4.5 g/l. The DNA content of aggregates remained unchanged as a function of time for 1 and 2 g/l (Supplementary Material, Table S1), whereas for 3 and 4.5 g/l, there was an increase for Day 21 levels compared to other time points. All the aggregates were small on Day 7, but all increased in size with time (Supplementary Material, Table S2). On Day 14 and Day 21, aggregates treated with 1 g/l initial glucose concentration were significantly smaller than aggregates treated with 2, 3, and 4.5 g/l.

Histology

Aggregates treated with 2, 3, and 4.5 g/l glucose concentrations exhibited very good Safranin-O staining on Day 14 and Day 21, indicative of GAG, whereas aggregates treated with 1 g/l glucose concentrations exhibited inferior staining for GAG (Fig. 8). Aggregates were also significantly smaller in the latter when compared to the former experiments. The difference in the size is unlikely due to differences in DNA content, which are very small (Table S1). The results show reduced staining for all Day 7 aggregates. The enhancement in the quality of the aggregate from Day 14 to Day 21 was more when compared to Day 7 to Day 14.

Numerical model and parameter estimation

The numerical model was verified for accuracy by solving for time-dependent glucose concentration profiles within the aggregate in an unsteady-diffusion transport model for which an analytical solution can be obtained (see Supplementary Material, Fig. S5). The analytical solution agreed with the simulation results with less than 1% error with an average error of 0.36%. The numerical model was then used to estimate the parameters $V_{\max}$ and K_M . $V_{\max}$ decreased significantly (by a factor of 9 from 12.5 to 1.5 mmol/sec/g DNA) with time from Day 0–2 to Day 6–8, after which it started to increase. On Day 18–20, the value of $V_{\max}$ was 17.5 mmol/sec/g DNA, 12 times greater than its lowest value on Day

6–8 (Fig. 9). K_M value did not change with time and was 10^{-3} mol/m³. Overall, the estimated parameters led to good prediction of concentration in the medium for all the experiments (Fig. 2). The average percentage errors ranged from as small as 11% to as large as 695% (Table S3); the latter arose when experimental values were essentially zero (typically, for initial glucose concentrations of 1 g/l and 2 g/l). The model over-predicted concentration for experiments with 1 g/l and 2 g/l initial glucose concentrations. Elimination of 1 g/l data in parameter estimation had very little effect on the parameter estimates (average error < 1%, data not shown). V_{max} values estimated from data at 24-hour intervals (open circles in Fig. 9) assuming constant K_M were similar to those estimated from data over 48-hour intervals (filled circles in Fig. 9).

Biochemical properties, and glucose consumption and lactate production

GAG and HYP contents of aggregates evaluated at Day 14 and Day 21 increased with the overall glucose consumption regardless of initial glucose concentrations (Fig. 10A). These increases were linear. For every μ g of GAG synthesized, an increase in glucose consumption of about 170 μ g was observed. Similarly, for every μ g of HYP synthesized, an increase in glucose consumption of about 460 μ g was observed. Fig. 10B shows GAG and HYP contents of aggregates as a function of lactate production. Similar to glucose consumption, lactation production increased with the biochemical content. For every μ g of GAG synthesized, an increase in lactate production of about 160 μ g was observed. Similarly, for every μ g of HYP synthesized, an increase in lactate production of about 440 μ g was observed.

Discussion

The effect of initial glucose concentrations (or available glucose concentrations) on hMSC chondrogenesis and glucose uptake were evaluated. The results show that glucose consumption increased with time for all initial glucose concentrations; except for the lowest initial concentration, all initial concentrations led to GAG and HYP synthesis. The estimated intrinsic kinetic parameter, V_{max} , decreased by 80% initially after aggregation; however, V_{max} increased 12-fold from its lowest value. Further, glucose consumption related directly with the GAG and HYP contents of the resultant aggregates independent of the initial glucose concentrations.

Our results (Figs. 2–5) showing an increase in glucose consumption suggest that the glycolytic metabolism is accelerated during chondrogenesis. Such enhancement in glucose uptake rate (~200% increase, Fig. 3A) occurred with only a small enhancement (~25%) in DNA (Table S1) suggesting that the uptake is unrelated to cell proliferation. Greater consumption rates occurred towards the end of the 21-day culture period; this coincides with ECM synthesis which has been shown to occur in the last 7–10 days of culture⁴⁴. Our results are different from those of Pattappa, *et al.*²⁷, where the glucose uptake rate was approximately constant. The difference could be due to higher ECM content of the resultant aggregates in the experiments (Fig. 7) reported here. The reduction in ECM in Pattappa, *et al.*²⁷ could be due to higher passage numbers (P3–P5) used in their study. GAG synthesis depends on passage number; at higher passage numbers, MSCs chondrogenic potential

(GAG synthesis) is significantly reduced.³⁵ In addition, the stem cells used in this study (P2) were cultured in serum, selected for its ability to enhance adhesion, proliferation and chondrogenic/osteogenic differentiation.¹⁹

An important feature of our experimental design is the effect of initial glucose concentrations. Our result showing that 1 g/l initial glucose concentrations led to almost 100% glucose utilization and subsequently inferior quality of the aggregates (Fig. 5), suggests that the glucose availability to the aggregate was limited and can be improved by either enhanced medium volume or more frequent medium changes independent of glucose level. In addition, the results showing that the simulations had improved agreement with results from aggregates exposed to 2, 3, and 4.5 g/l when compared to that from aggregates exposed to 1 g/l suggests that a combination of exposure to low glucose concentration and decreased glucose availability can change the glucose consumption behavior of the cells. Even though the simulation results show that the predicted glucose concentrations within the aggregate can be significantly lower than 1 g/l for initial glucose concentrations of 2, 3 and 4.5 g/l (data for 4.5 g/l shown in Fig. S6), due to higher glucose availability and transport rate to the cells, the aggregates from 2, 3, and 4.5 g/l experiments exhibited exuberant ECM and were considered a success when compared to those from 1 g/l experiments. We can, therefore, conclude that it is not the local glucose concentrations but rather the transport rate of glucose that determine the quality of the aggregate. Small changes in glucose uptake can lead to metabolic disorders that have been suggested to be a trigger for cartilage degeneration. For example, OA is found to occur coincident with metabolism diseases such as type 2 diabetes.^{8, 23, 24, 34}

Our lactate production results show that the rate of lactate production increased during chondrogenesis for aggregates exposed to 2, 3, and 4.5 g/l initial glucose concentrations whereas for aggregates exposed to 1 g/l had a constant production rate. This trend was consistent with the glucose consumption rate. This suggests that lactate production is a result of enhanced glucose consumption and is likely due to the differentiation of MSCs into chondrocytes; the latter are known to utilize oxygen-independent glycolysis mechanism thus producing lactate as a waste product.^{15–18} Also, since all the experiments were conducted under normoxia conditions, these results suggest that even under normoxia glycolysis is the preferred mechanism under conditions of increasing glucose availability during chondrogenesis.

Intrinsic kinetics of nutrients are important not only for understanding the general mechanisms of glucose uptake but also for developing accurate mathematical models of transport for bioreactor design. Michaelis-Menten kinetics is a widely-used model to describe glucose uptake by cells^{2, 21, 30}. A mathematical model of mass transport involving reaction and diffusion to estimate kinetic parameters of the M-M model from the experimental data was used. The DNA-specific maximum uptake rate ($V_{\max}$, mol/s/g DNA) started high but precipitously decreased from Day 0–2 to Day 2–4 (Fig. 9). The high initial rate is likely due to the energy-intensive aggregation process. Further, there are significant differences in gene expression profiles of proliferating hMSCs and hMSCs undergoing chondrogenesis³⁸. The reduction can be due in part to glucose transporters hidden by the aggregation of cells. After Day 2–4, $V_{\max}$ decreased slightly during early chondrogenesis

suggesting that after aggregation, the glucose requirements of the cells were decreasing. Towards the late chondrogenesis period, $V_{\max}$ increased steadily to a maximum value at Day 18–20; this is likely due to ECM synthesis, which has been known to occur in weeks 2 and 3 of chondrogenesis⁴⁴. Further, since GAG is a significant part of cartilage ECM, we argue that the increase in $V_{\max}$ is due in large part to enhanced glucose uptake for GAG synthesis. When only 24-hour data were used to estimate parameters (Fig. 9), the parameter values did not change significantly from the value estimated using the entire 48-hour data set. This suggests that intrinsic glucose consumption characteristics of aggregate do not change appreciably over a period of 48 hours.

$V_{\max}$ values from these hMSCs chondrogenesis experiments are approximately 35–467 larger than the $V_{\max}$ values reported for hMSCs in proliferation.^{32, 42} This suggests that glucose uptake is greatly enhanced during chondrogenesis. This is supported by significant lactate production (Fig. 6), which indicates glycolysis as the preferred pathway of ATP production by the cells.

In our simulations for parameter estimations, we used data from all initial concentrations to evaluate $V_{\max}$. The premise behind the approach was that the cells consume glucose at a local concentration-dependent manner as described by the Michaelis-Menten kinetics. The concentration-dependent kinetic model is thus presumed to be applicable to all concentration ranges. However, the results show that low uptake rate of glucose by aggregates exposed to 1 g/l initial concentration changes the glucose kinetic behavior of the cells. The simulation models show low levels of glucose within the aggregate even when exposed to 4.5 g/l initial concentrations. Such rate-dependent behavior suggests other mechanisms besides Michaelis-Menten kinetics play a role at very low concentration levels. This is a limitation of the kinetic model employed in this work and needs to be explored further in a future work. Specifically, transporter-based kinetic models of glucose uptake can help to address this limitation.⁴⁰

Both experimental data and simulation results (Figs. 2 and S6) indicate that glucose transport is limited by diffusion within the aggregates even when exposed to 4.5 g/l initial concentration of glucose. Indeed, the Damköhler number⁴⁰ ($\frac{V_{\max} C_X a^2}{C_{G,0}^M D_G}$, where $C_{G,0}^M$ is the

initial glucose level used to make the concentration dimensionless), typically used to determine the relative contributions of diffusion and reaction to transport, ranged from 260 to 1775. This suggests the glucose transport to the cells is limited by diffusion within the aggregate. This is an important finding as aggregates are a well-established small-sized model to study chondrogenesis. Future investigation of intra-aggregate glucose levels via imaging techniques (*e.g.*, PET imaging using fluorodeoxyglucose (¹⁸F)) will further strengthen this finding.

The results showing both GAG synthesis and HYP synthesis are related to glucose uptake and lactate production (Fig. 10) suggest that both glucose uptake and lactate production can be used as a marker of chondrogenic quality. The kinetics of chondrogenesis can significantly impact the quality of engineered cartilage developed using hMSCs. A non-

invasive chondrogenesis monitoring tool based on glucose uptake can be used to optimize strategies for improving the quality of the engineered tissue.

Conclusions

The intrinsic parameters of glucose uptake kinetics during human mesenchymal stem cell *in vitro* chondrogenesis were studied. Experimental results show that during chondrogenesis, increased glucose uptake and lactate production led to improved chondrogenesis end points. The DNA normalized maximum glucose uptake rate ($V_{\max}$) decreased rapidly followed by a steady increase to a plateau. This period of increase coincides with the primary ECM synthesis period during chondrogenesis. For tissue engineering researchers, the increasing glucose uptake rate during hMSC chondrogenesis means that culture conditions need to be optimized to provide increasing amounts of glucose. Further, the biochemical content of the resultant tissue correlates with the glucose uptake and lactate production, suggesting that either glucose uptake or lactate production can be a useful parameter to monitor and to predict tissue quality during chondrogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ballock RT, Reddi AH. Thyroxine is the serum factor that regulates morphogenesis of columnar cartilage from isolated chondrocytes in chemically defined medium. *J Cell Biol.* 1994; 126:1311–1318. [PubMed: 8063865]
2. Bizzotto R, Natali A, Gastaldelli A, Muscelli E, Krssak M, Brehm A, Roden M, Ferrannini E, Mari A. Glucose uptake saturation explains glucose kinetics profiles measured by different tests. *Am J Physiol Endocrinol Metab.* 2016; 311:E346–357. [PubMed: 27245333]
3. Brown DA, MacLellan WR, Laks H, Dunn JC, Wu BM, Beygui RE. Analysis of oxygen transport in a diffusion-limited model of engineered heart tissue. *Biotechnol Bioeng.* 2007; 97:962–975. [PubMed: 17195988]
4. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol.* 2007; 213:341–347. [PubMed: 17620285]
5. Caplan AI, Bruder SP. Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. *Trends Mol Med.* 2001; 7:259–264. [PubMed: 11378515]
6. Carrino DA, Arias JL, Caplan AI. A spectrophotometric modification of a sensitive densitometric safranin-o assay for glycosaminoglycans. *Biochemistry International.* 1991; 24:485–495. [PubMed: 1772427]
7. Cigan AD, Durney KM, Nims RJ, Vunjak-Novakovic G, Hung CT, Ateshian GA. Nutrient channels aid the growth of articular surface-sized engineered cartilage constructs. *Tissue Eng Part A.* 2016; 22:1063–1074. [PubMed: 27481330]
8. Denko CW, Malemud CJ. Metabolic disturbances and synovial joint responses in osteoarthritis. *Front Biosci.* 1999; 4:D686–693. [PubMed: 10525474]

9. Dos Santos F, Andrade PZ, Boura JS, Abecasis MM, da Silva CL, Cabral JM. Ex vivo expansion of human mesenchymal stem cells: A more effective cell proliferation kinetics and metabolism under hypoxia. *J Cell Physiol.* 2010; 223:27–35. [PubMed: 20020504]
10. Ham KD, Oegema TR, Loeser RF, Carlson CS. Effects of long-term estrogen replacement therapy on articular cartilage igfbp-2, igfbp-3, collagen and proteoglycan levels in ovariectomized cynomolgus monkeys. *Osteoarthritis Cartilage.* 2004; 12:160–168. [PubMed: 14723875]
11. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. *Bone.* 1992; 13:81–88. [PubMed: 1581112]
12. Ignat'eva NY, Danilov NA, Averkiev SV, Obrezkova MV, Lunin VV, Sobol' EN. Determination of hydroxyproline in tissues and the evaluation of the collagen content of the tissues. *Journal of Analytical Chemistry.* 2007; 62:51–57.
13. Janakiraman V, Mathur K, Baskaran H. Optimal planar flow network designs for tissue engineered constructs with built-in vasculature. *Ann Biomed Eng.* 2007; 35:337–347. [PubMed: 17203399]
14. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res.* 1998; 238:265–272. [PubMed: 9457080]
15. Lane JM, Brighton CT, Menkowitz BJ. Anaerobic and aerobic metabolism in articular cartilage. *J Rheumatol.* 1977; 4:334–342. [PubMed: 604473]
16. Lee RB, Urban JP. Evidence for a negative pasteur effect in articular cartilage. *Biochem J.* 1997; 321(Pt 1):95–102. [PubMed: 9003406]
17. Lee RB, Urban JP. Functional replacement of oxygen by other oxidants in articular cartilage. *Arthritis Rheum.* 2002; 46:3190–3200. [PubMed: 12483723]
18. Lee RB, Wilkins RJ, Razaq S, Urban JP. The effect of mechanical stress on cartilage energy metabolism. *Biorheology.* 2002; 39:133–143. [PubMed: 12082276]
19. Lennon DP, Caplan AI. Isolation of human marrow-derived mesenchymal stem cells. *Exp Hematol.* 2006; 34:1604–1605. [PubMed: 17046583]
20. Lennon DP, Haynesworth SE, Bruder SP, Jaiswal N, Caplan AI. Human and animal mesenchymal progenitor cells from bone marrow: Identification of serum for optimal selection and proliferation. *In Vitro Cellular & Developmental Biology - Animal.* 1996; 32:602–611.
21. Lewko B, Bryl E, Witkowski JM, Latawiec E, Golos M, Endlich N, Hahnel B, Koksich C, Angielski S, Kriz W, Stepinski J. Characterization of glucose uptake by cultured rat podocytes. *Kidney Blood Press Res.* 2005; 28:1–7. [PubMed: 15383720]
22. Maroudas A. Distribution and diffusion of solutes in articular cartilage. *Biophys J.* 1970; 10:365–379. [PubMed: 4245322]
23. Mobasheri A, Vannucci SJ, Bondy CA, Carter SD, Innes JF, Arteaga MF, Trujillo E, Ferraz I, Shakibaei M, Martin-Vasallo P. Glucose transport and metabolism in chondrocytes: A key to understanding chondrogenesis, skeletal development and cartilage degradation in osteoarthritis. *Histol Histopathol.* 2002; 17:1239–1267. [PubMed: 12371151]
24. Okma-Keulen P, Hopman-Rock M. The onset of generalized osteoarthritis in older women: A qualitative approach. *Arthritis Rheum.* 2001; 45:183–190. [PubMed: 11324783]
25. Passonneau JV, Lowry OH. Enzymatic analysis a practical guide. Humana Press; 1993.
26. Pathi P, Ma T, Locke BR. Role of nutrient supply on cell growth in bioreactor design for tissue engineering of hematopoietic cells. *Biotechnol Bioeng.* 2005; 89:743–758. [PubMed: 15696509]
27. Pattappa G, Heywood HK, de Bruijn JD, Lee DA. The metabolism of human mesenchymal stem cells during proliferation and differentiation. *J Cell Physiol.* 2011; 226:2562–2570. [PubMed: 21792913]
28. Pattappa G, Thorpe SD, Jegard NC, Heywood HK, de Bruijn JD, Lee DA. Continuous and uninterrupted oxygen tension influences the colony formation and oxidative metabolism of human mesenchymal stem cells. *Tissue Eng Part C Methods.* 2013; 19:68–79. [PubMed: 22731854]
29. Penick KJ, Solchaga LA, Welter JF. High-throughput aggregate culture system to assess the chondrogenic potential of mesenchymal stem cells. *Biotechniques.* 2005; 39:687–691. [PubMed: 16312217]

30. Perriott LM, Kono T, Whitesell RR, Knobel SM, Piston DW, Granner DK, Powers AC, May JM. Glucose uptake and metabolism by cultured human skeletal muscle cells: Rate-limiting steps. *Am J Physiol Endocrinol Metab.* 2001; 281:E72–80. [PubMed: 11404224]
31. Ponticello MS, Schinagl RM, Kadiyala S, Barry FP. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. *J Biomed Mater Res.* 2000; 52:246–255. [PubMed: 10951362]
32. Raghunand N, Dale BE. Alteration of glucose consumption kinetics with progression of baculovirus infection in *spodoptera frugiperda* cells. *Appl Biochem Biotechnol.* 1999; 80:231–242. [PubMed: 10488553]
33. Rosenberg L. Chemical basis for the histological use of safranin o in the study of articular cartilage. *J Bone Joint Surg Am.* 1971; 53:69–82. [PubMed: 4250366]
34. Rosenbloom AL, Silverstein JH. Connective tissue and joint disease in diabetes mellitus. *Endocrinol Metab Clin North Am.* 1996; 25:473–483. [PubMed: 8799711]
35. Solchaga LA, Penick K, Goldberg VM, Caplan AI, Welter JF. Fibroblast growth factor-2 enhances proliferation and delays loss of chondrogenic potential in human adult bone-marrow-derived mesenchymal stem cells. *Tissue Eng Part A.* 2010; 16:1009–1019. [PubMed: 19842915]
36. Solchaga LA, Penick K, Porter JD, Goldberg VM, Caplan AI, Welter JF. Fgf-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. *J Cell Physiol.* 2005; 203:398–409. [PubMed: 15521064]
37. Solchaga LA, Penick KJ, Welter JF. Chondrogenic differentiation of bone marrow-derived mesenchymal stem cells: Tips and tricks. *Methods Mol Biol.* 2011; 698:253–278. [PubMed: 21431525]
38. Somoza RA, Correa D, Labat I, Sternberg H, Forrest ME, Khalil AM, West MD, Tesar PJ, Caplan AI. Transcriptome-wide analyses of human neonatal articular cartilage and human mesenchymal stem cells (hmscs)-derived cartilage provide a new molecular target for evaluating engineered cartilage. *Tissue Eng Part A.* 2017
39. Suhaimi H, Wang S, Das DB. Glucose diffusivity in cell culture medium. *Chemical Engineering Journal.* 2015; 269:323–327.
40. Ultman JS, Baskaran H, Saidel GM. Biomedical mass transport and chemical reaction: Physicochemical principles and mathematical modeling. New York: Wiley; 2016.
41. Webber MJ, Khan OF, Sydlik SA, Tang BC, Langer R. A perspective on the clinical translation of scaffolds for tissue engineering. *Ann Biomed Eng.* 2015; 43:641–656. [PubMed: 25201605]
42. Weber C, Freimark D, Portner R, Pino-Grace P, Pohl S, Wallrapp C, Geigle P, Czermak P. Expansion of human mesenchymal stem cells in a fixed-bed bioreactor system based on non-porous glass carrier--part a: Inoculation, cultivation, and cell harvest procedures. *Int J Artif Organs.* 2010; 33:512–525. [PubMed: 20872346]
43. Weber C, Freimark D, Portner R, Pino-Grace P, Pohl S, Wallrapp C, Geigle P, Czermak P. Expansion of human mesenchymal stem cells in a fixed-bed bioreactor system based on non-porous glass carrier - part b: Modeling and scale-up of the system. *Int J Artif Organs.* 2010; 33:782–795. [PubMed: 21140354]
44. Welter JF, Solchaga LA, Penick KJ. Simplification of aggregate culture of human mesenchymal stem cells as a chondrogenic screening assay. *Biotechniques.* 2007; 42:732–737. 734–737. [PubMed: 17612296]
45. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys.* 1961; 93:440–447. [PubMed: 13786180]
46. Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, Johnstone B. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg Am.* 1998; 80:1745–1757. [PubMed: 9875932]
47. Zhao F, Pathi P, Grayson W, Xing Q, Locke BR, Ma T. Effects of oxygen transport on 3-d human mesenchymal stem cell metabolic activity in perfusion and static cultures: Experiments and mathematical model. *Biotechnol Prog.* 2005; 21:1269–1280. [PubMed: 16080711]

Figure 1.

Model schematic used for simulation and parameter estimation. The model represents an aggregate cultured in a single well of a 96-well plate. Due to axisymmetry, only half of the cross section of the plate from top to bottom is shown. Aggregate (Region II) and medium (Region I) are shown. Region I has no glucose uptake, whereas Region II has. Partitioning of glucose occurs between Region I and Region II.

Figure 2.

Dynamics of glucose concentrations in the medium during chondrogenesis. Markers show glucose concentrations in medium. Initial glucose concentrations were varied: 1 g/l (black squares), 2 g/l (red circles), 3 g/l (green triangles) and 4.5 g/l (inverted blue triangles). Data for ten medium exchanges are represented as ten sets of three markers for each initial glucose level. Error bars represent means $\pm$ standard deviation (SD) (N varied from 6 to 23 per condition). Predicted glucose concentrations from simulations are shown as solid lines. Results from 40 separate simulations representing four different initial glucose concentrations and ten consecutive medium changes using the parameter estimated by fitting the experimental data to the model are shown.

Figure 3.

Glucose uptake profiles from aggregates cultured under different glucose concentrations: 1 g/l (squares), 2 g/l (circles), 3 g/l (triangles) and 4.5 g/l (inverted triangles). Uptake rate ($\text{fmol hr}^{-1} \text{cell}^{-1}$) profiles are shown separately for (A) the first day and (B) second day after each medium change. Lines connecting the markers are used for clarity to distinguish results from individual initial glucose concentrations. Error bars represent means $\pm$ SD (N varied from 6 to 23 per condition).

Figure 4.

Glucose consumption by aggregates over 24 hours for 1 (Top Left), 2 (Top Right), 3 (Bottom Left), and 4.5 g/l (Bottom Right) initial glucose concentrations. Lines connecting markers for 24- and 48-hour consumption are used to show whether the consumption increased (upward trend) or decreased (downward trend). Error bars represent means $\pm$ SD (N varied from 6 to 23 per condition). Consumption data are separated (lines) for each medium change.

Figure 5.

Fractional glucose consumption (percent of amount of glucose consumed divided by the amount of glucose supplied) by aggregates over 24 hours for 1 (Top Left), 2 (Top Right), 3 (Bottom Left), and 4.5 g/l (Bottom Right) initial glucose concentrations. Lines connecting markers for 24- and 48-hour consumption are used to show whether the consumption increased (upward trend) or decreased (downward trend). Error bars represent means $\pm$ SD (N varied from 6 to 23 per condition). Consumption data are separated (lines) for each medium change.

Figure 6. Lactate production by aggregates. The rates were calculated over 48 hours. Initial glucose concentrations were varied: 1 g/l (squares), 2 g/l (circles), 3 g/l (triangles) and 4.5 g/l (inverted triangles). Error bars represent means $\pm$ SD (N=5–6 per condition).

Figure 7. Biochemical properties of chondrogenic aggregates harvested at Day 7, Day 14 and Day 21 cultured under different glucose concentrations: 1 g/l, 2 g/l, 3 g/l and 4.5 g/l. (A) GAG and (B) HYP contents of chondrogenic aggregates normalized by DNA content. Error bars represent means $\pm$ SD (N=3–4/condition). The * indicates significant difference ($p<0.05$).

Figure 8.

Histology results. Safranin-O staining for chondrogenic aggregates harvested at Day 7 (A–D), Day 14 (E–H), and Day 21 (I–L). Scale bar = 200 μ m. Aggregates cultured under various initial glucose concentrations: 1 g/l (A, E, I), 2 g/l (B, F, J), 3 g/l (C, G, K), and 4.5 g/l (D, H, L).

Figure 9.

Dynamics of DNA-normalized maximum glucose uptake rate, $V_{\max}$, during chondrogenesis. $V_{\max}$ values were estimated for each medium change over 48 hours (Day 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, 14–16, 16–18, 18–20) using experimental values of glucose concentrations for all initial glucose concentrations (see Methods for details) and shown as filled circle. Open circles represent values estimated from consumption data over 24 hours.

Figure 10.

A: GAG (circles, Pearson's $r = 0.919$) and HYP (squares, Pearson's $r = 0.978$) contents of Day 14 and Day 21 aggregates as a function of cumulative glucose consumption. Markers represent experimental data and lines (dashed: GAG, solid: HYP) represent linear fits. Error bars represent standard deviation. **B:** GAG (circles, Pearson's $r = 0.908$) and HYP (squares, Pearson's $r = 0.995$) contents of Day 14 and Day 21 aggregates as a function of cumulative lactate production. Markers represent experimental data and lines (dashed: GAG, solid: HYP) represent linear fits. Error bars represent means $\pm$ SD ($N=3-4/\text{condition}$)

Table 1

Parameters used in the simulation

Parameter	Value	Reference
D_G^M	$6.73 \times 10^{-10} \text{ m}^2/\text{sec}$	³⁹
D_G	$2.217 \times 10^{-10} \text{ m}^2/\text{sec}$	²²
λ	0.643	²²