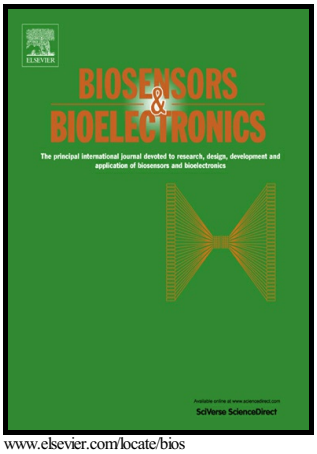

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Sensing Metabolites for the Monitoring of Tissue Engineered Construct Cellularity in Perfusion Bioreactors

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Abstract

As the field of tissue engineering progresses ever-further toward realizing clinical implementation of tissue-engineered constructs for wound regeneration, perhaps the most significant hurdle remains the establishment of non-destructive means for real-time *in vitro* assessment. In order to address this barrier, the study presented herein established the viability of the development of correlations between metabolic rates (specifically oxygen uptake, glucose consumption, and lactate production) and the cellularity of tissue-engineered cultures comprised of rat mesenchymal stem cells dynamically seeded on 85% porous nonwoven spunbonded poly(L-lactic acid) fiber mesh scaffolds. Said scaffolds were cultured for up to 21 days in a flow perfusion bioreactor system wherein α-MEM (supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic) was perfused directly through each scaffold at low flow rates (~0.15 mL/min). Metabolite measurements were obtained intermittently through the use of a fiber-optic probe (for the case of oxygen) and biochemical assays (for glucose and lactate). Such measurements were subsequently correlated with cellularity data obtained utilizing current-standard destructive means. The resulting correlations, all exhibiting high R^2 values, serve as a proof-on-concept for the use of metabolic data for the determination of scaffold cellularity in real-time non-destructively. This study can be easily adapted for use with various cell types, media formulations, and potentially different bioreactor systems. Implementation of more

advanced *in situ* measurement devices could be easily accommodated to allow for true real-time, on-line metabolite monitoring and cellularity estimation.

Keywords: Bioreactor; perfusion; tissue engineering; biosensor; metabolite; monitoring

1. Introduction

Tissue engineering, a field originally developed to overcome the limitations of traditional graft sources for tissue replacement and regeneration, has advanced greatly since its inception, moving ever closer to the realization of clinical application. Perhaps the most predominant hurdle to achieving said goal is the establishment of techniques for the monitoring of tissue engineered constructs in real-time throughout *in vitro* culture without the need for their sacrifice in doing so. In fact, little literature has been published thus far on methods for the non-destructive determination of the quality of constructs cultured within bioreactor systems [1].

The utilization of biosensors for the monitoring of metabolites within cell culture media over time has been studied by many groups [2-5]. In fact, initial investigations have been made into the use of oxygen drop across a construct as a means of determining the number of chondrocytes within [2]. Furthermore, investigations into the cell-specific consumption and production rates of various metabolites in 2D cultures have been performed through the use of assays or flow-through devices, though extension of such data for cellularity determination was not attempted [5-8]. The majority of these studies were performed on cells with high metabolic activities with the investigation of only one or two metabolites [9-14].

This study seeks to demonstrate the feasibility of monitoring metabolites as the sole means for the determination of the cellularity of tissue engineered constructs cultured in flow perfusion bioreactors in real time. Mesenchymal stem cells, widely used for numerous tissue engineering approaches, exhibit fairly high metabolic rates, leading to their selection as the ideal candidate for this study. In particular, the investigation presented herein aims to develop correlations between the rates of oxygen uptake, glucose consumption, and lactate production

and the cellularity of non-differentiating rat mesenchymal stem cell (rMSC) seeded scaffolds cultured in a flow perfusion bioreactor system thereby circumventing the current destructive means cellular quantification of such constructs.

2. Materials and Methods

2.1 Scaffold manufacturing

Nonwoven fiber mesh scaffolds were produced from Poly(L-lactic acid) (PLLA; grade 6251D; 1.4% D enantiomer; 108,500 MW; 1.87 PDI; NatureWorks LLC) via spunbonding [15-19]. Further details on the manufacturing method may be found in our previous publication [20]. Scaffolds were cut from a 5 mm thick nonwoven sheet with an 8 mm diameter circular die. Individual fibers were optically analyzed using a Nikon HFX-II microscope to determine the average fiber diameter, which was found to be 24.5μm. The average porosity of scaffolds was determined to be 88%, with average pore sizes of approximately 250 μm.

2.2 Cell extraction, culture, and passaging

MSCs were extracted from the marrow of the tibias and femurs of adult male Wistar rats (175-199 g in mass; Harlan Laboratories) using established methods [21, 22]. MSCs were isolated from marrow by culturing homogenized marrow suspension in T75 cell culture flasks (Corning) for a period of three days then rinsing the flasks with PBS (Invitrogen) to remove all dead and unattached cells; the remaining cells constituted passage 0 rMSCs. Cells were cultured at 37 °C, 95% relative humidity, and 5% CO_2 in α-MEM (Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 1% antibiotic-antimycotic (Invitrogen). Media was changed within flasks every other day until reaching 70% confluency at which time cells were passaged (through passage 2). Passage 2 cells were lifted and suspended in α-MEM at a density of 1.57×10^7 cells/mL for scaffold seeding.

2.3 Scaffold pre-wetting and seeding

Scaffolds were pre-wet to facilitate seeding. This consisted of pulling a vacuum on scaffolds submerged in ethanol then removing them to a beaker of PBS to leach out the ethanol, ensuring the entire scaffold was wetted. Pre-wet scaffolds were then immobilized within cassettes and subsequently placed within a flow perfusion bioreactor and exposed to perfusion of

α-MEM for one hour prior to seeding [21, 22]. Then $2.36.10^6$ MSCs in 150 µL of α-MEM were pipetted on top of each scaffold and perfused directly through the scaffold in alternating directions for a total of two hours with a period of five minutes [23]. After oscillatory seeding, perfusion was suspended as cells were allowed to attach for two hours. Subsequently, α-MEM was continually perfused through scaffolds at a rate of 0.15 mL/min/scaffold for the remainder of the culture period of 7, 14, or 21 days.

2.4 Bioreactor media sampling and replacement

Every other day, the media within the bioreactor was replaced with α-MEM without FBS which was allowed to circulate for three hours before again being replaced with α-MEM with FBS for the next two days.

2.5 Oxygen uptake rate measurements

A fiber optic probe was used for taking oxygen measurements. The device contained a fluorescent source emitting blue light through a fiber-optic probe coated in a ruthenium complex (OceanOptics, Dunedin, FL) overlaid with a hydrophobic sol-gel matrix. Upon contact with molecular oxygen, the ruthenium complex was excited, emitting a red light which was read by a fluorescent detector. A correlation between the fluorescence detected and the partial pressure of dissolved oxygen in the medium was determined by using the Stern-Volmer equation (**Equation 1**).

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + k_q * \tau_0 * [O_2] = 1 + K_{SV} * \mathrm{C}_{\mathrm{O}_2} \qquad \textbf{(Eqn. 1)}$$

Where F_0 = fluorescent intensity in absence of O_2

F = fluorescent intensity in presence of O_2

τ_0 = fluorescent decay time in absence of O_2

τ = fluorescent decay time in presence of O_2

k_q = bimolecular quenching constant

K_{SV} = Stern-Volmer constant for static decay

$\mathrm{C}_{\mathrm{O}_2}$ = concentration of molecular oxygen present at fluorophore

The device was connected to a computer equipped with signal processing software (Tau Theta Software and OOISensors Software), providing a readout of the calculated oxygen concentration.

The device was recalibrated each day with a two-point calibration by immersing the probe in pure CO_2 at 37°C for a 0% oxygen tension standard then submersing it in ambient air at 37°C for a 21% oxygen tension standard. Oxygen measurements were taken every day at the inlet and outlet of each flow chamber of the bioreactor via direct insertion of the probe into the media entering and exiting each chamber through valves in line with the flow. The resulting mass balance on oxygen in the direct perfusion bioreactor, assuming zero-order kinetics for the oxygen uptake rate by cells, can then be used to calculate the cell-specific oxygen uptake rate (see **Equation 2)**.

$$OUR = \frac{N_{cells}}{\nu*(C_{O_2,out}-C_{O_2,in})} \quad \textbf{(Eqn. 2)}$$

Where ν = volumetric flow rate of media through scaffold

C_{O_2} = concentration of O_2 (subscripts "in" and "out" correspond to inlet and outlet of construct, respectively)

N_{cells} = number of cells within scaffold

The cell-specific oxygen uptake rate can thus be calculated if the volumetric flowrate, number of cells, and inlet and outlet oxygen concentrations are known.

2.6 Glucose consumption rate measurements

Glucose assay was performed directly on media samples (obtained daily) using a colorimetric glucose assay kit (Biovision). Media samples were diluted 1:5 in DI water before 25 µl volumes were added to 25 µl of sample buffer in a clear 96-well plate (Corning) alongside standards over the assay range from 1 to 10,000 µM. 50 µl of glucose enzyme reagent was then added to each well and allowed to incubate for 30 minutes at 37°C. After incubation, the plate was read on a Synergy HT Multi-Mode Microplate Reader (Bio-Tek) at an absorbance wavelength of 570 nm. All samples and standards were run in triplicate. Resulting values were then used to calculate the cell-specific glucose consumption rate via **Equation 3**.

$$GCR = \frac{-\Delta C_{glucose}*V_{Media,Total}}{\Delta t*N_{Cells,Total}} \quad \textbf{(Eqn. 3)}$$

Where $\Delta C_{glucose}$ = change in concentration of glucose

$V_{Media,Total}$ = total volume of media within bioreactor system

Δt = time interval between media samples

$N_{Cells,Total}$ = total number of cells within whole bioreactor system

2.7 Lactate production rate measurements

FBS contains lactate dehydrogenase, which degrades lactate rapidly after its production, preventing its ability to be detected; therefore, lactate assays were performed on samples of α-MEM without FBS (obtained every-other day after 3 hours of circulation within the bioreactor) using a colorimetric lactate assay kit (Biovision). 25 µl volumes of each sample were added to 25 µl of sample buffer in a clear 96-well plate (Corning) alongside standards over the assay range from 1 to 10,000 µM. 50 µl of lactate enzyme reagent was then added to each well and allowed to incubate for 30 minutes at 37°C. After incubation, the plate was read on a Synergy HT Multi-Mode Microplate Reader (Bio-Tek) at an absorbance wavelength of 570 nm. All samples and standards were run in triplicate. Resulting values were then used to calculate the cell-specific lactate production via **Equation 4**.

$$LPR = \frac{\Delta C_{lactate} * V_{Media,Total}}{\Delta t * N_{Cells,Total}} \quad \textbf{(Eqn. 4)}$$

2.8 Cellularity quantification

The cellularity of constructs sacrificed at different time points was determined via quantification of dsDNA content thereof with the use of a fluorescent PicoGreen® dsDNA assay (Invitrogen). Upon sacrifice, scaffolds were removed from cassettes, rinsed in PBS, and torn apart and submerged in 3 mL of DI water. Samples were then subjected to three freeze/thaw cycles in order to lyse the cells. 43 µl volumes or each sample were pipetted into an opaque 96-well plate (Corning) alongside standards over the assay range from 0.1 to 3 µg/mL. 257 µl of buffered PicoGreen® dye was then added to each well and allowed to incubate for 5 minutes at 25°C. After incubation, the plate was read on a Synergy HT Multi-Mode Microplate Reader (Bio-Tek) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. All samples and standards were run in triplicate. Resulting values were then divided by the previously-determined

dsDNA content per cell of rMSCs which was found to be 7 pg in order to determine the total construct cellularity.

2.9 DAPI and phallacidin staining

Two scaffolds at each time point were subjected to DAPI and phallacidin staining. This was done to confirm the cellularity result from the above dsDNA assay as well as to provide information on cell distribution within the scaffold in addition to matrix deposition. Sections from the top, middle, and bottom of these constructs were separated and stained. Individual layers were fixed in 10% formalin overnight at 4°C, washed twice with PBS, then permeabilized with 0.1 % Triton X-100 before a 20 minute incubation in BODIPY® FL Phallacidin (Invitrogen) at a concentration of 200 U/mL (in the dark). Sections were then rinsed with PBS and incubated for 20 minutes in DAPI at a concentration of 300 nM (in the dark). After incubation, sections were rinsed thoroughly with PBS before imaging on a Nikon Epifluorescence microscope with an excitation wavelength at 558 nm and emission wavelength at 569 nm. Image analysis was performed with MetaMorph 6.2 (Universal Imaging Corporation) and Image J software packages.

2.10 Statistical analysis

All measurements were taken in taken in triplicate. Results are reported as mean ± SD. Linear regression was performed using PRISM 5 (GraphPad).

3. Results and Discussion

Three metabolites were monitored as a means to quantify cell growth within the bioreactor: oxygen, glucose, and lactate. These were quantified in bioreactors run for periods of up to 21 days. At various time points, scaffolds were sacrificed for cellularity determination via dsDNA assay and cell dispersity via DAPI and phallacidin staining.

3.1 Scaffold cellularity

All scaffolds were initially seeded with 2.34 million cells. After dynamic seeding, approximately $6x10^5$ - $7.5x10^5$ cells remained attached to each scaffolds, resulting in a ~29% seeding efficiency.

The resulting seeded scaffolds were then cultured for time period of up to 21 days, with scaffolds being sacrificed approximately every week for cellularity quantification.

After 7 days of culture, a near doubling in cellularity was observed, reaching values of approximately $1.3x10^6$ cells per scaffold. A minor increase in this number was observed by day 14 (to ~$1.4x10^6$ cells per scaffold), followed by a minor decrease by day 21 (to ~$1.2x10^6$ cells per scaffold), though these changes are not statistically significant. It is thought that this number represents the upper cellularity limit of the scaffold, not being able to sustain larger quantities of cells. DAPI and phallacidin staining images (**Figures 1** and **2**, below) reveal more about the cellular dispersity and matrix deposition.

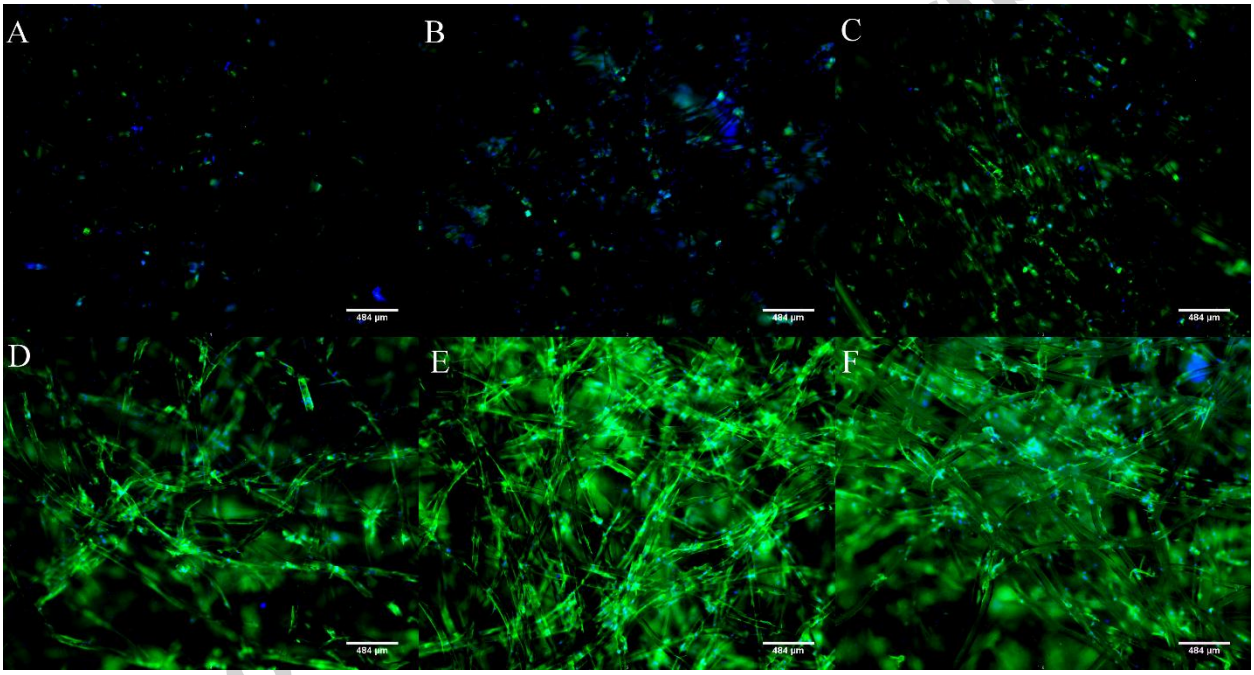

Figure 1: DAPI and phallacidin staining of day 0 (A-C) and day 7 (D-F) scaffolds. F-actin cytoskeletal structures fluoresce in green. Cell nuclei fluoresce in blue. From left to right, images are of the bottom, middle, and top sections of each scaffold, respectively. Images taken with a Nikon Epifluorescence microscope. Image analysis was carried out with MetaMorph 6.2 (Universal Imaging Corporation). Scale bars in all images are 480 µm.

Figure 1, above, reveals the uniform distribution of cells throughout the scaffold for both day 0 and day 7, proving the efficacy of the oscillatory seeding protocol utilized, as confirmed by the literature [24]. A doubling in cellularity by day 7, as determined by the dsDNA assay, is

confirmed by the staining. Furthermore, cell stretching and minor matrix deposition is evident by day 7.

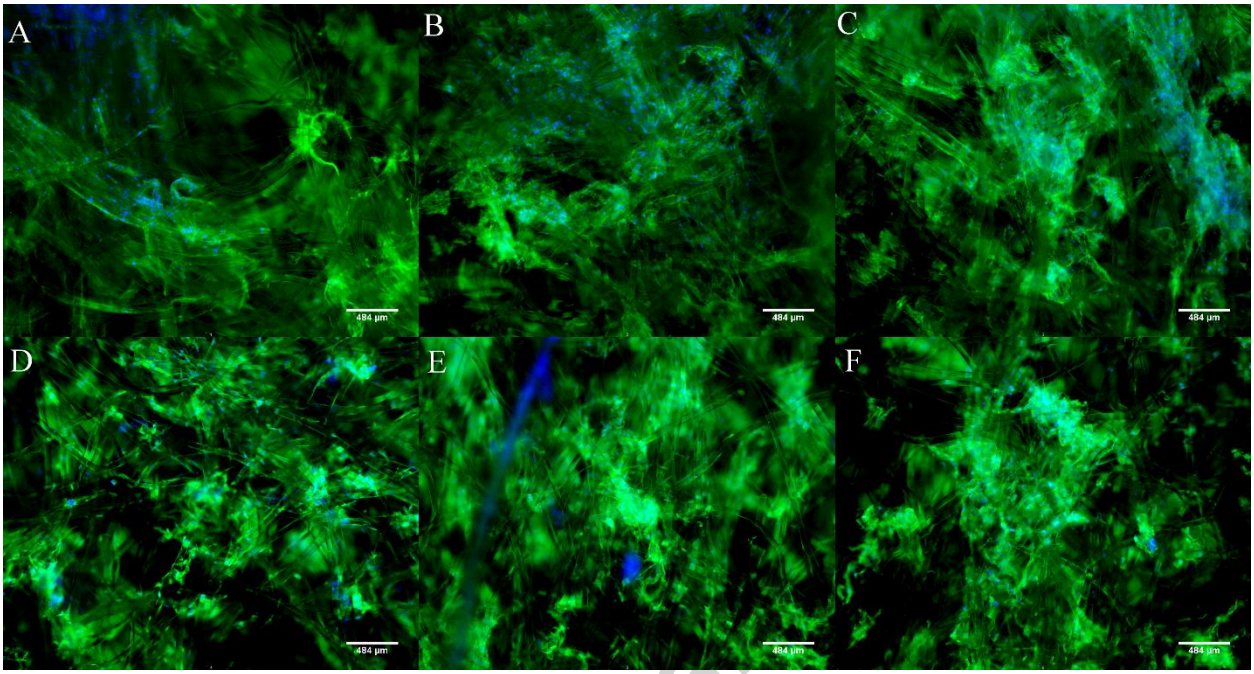

Figure 2: DAPI and phallacidin staining of day 14 (A-C) and day 21 (D-F) scaffolds. F-actin cytoskeletal structures fluoresce in green. Cell nuclei fluoresce in blue. From left to right, images are of the bottom, middle, and top sections of each scaffold, respectively. Images taken with a Nikon Epifluorescence microscope. Image analysis was carried out with MetaMorph 6.2 (Universal Imaging Corporation). Scale bars in all images are 480 µm.

Figure 2 reveals increased cell stretching and much greater matrix deposition as compared with earlier time points shown in **Figure 1**. As confirmed by the dsDNA assay, cellularity is not seen to have changed much between day 7 and day 21, though much more tissue is present, resulting from increased matrix deposition by the attached MSCs.

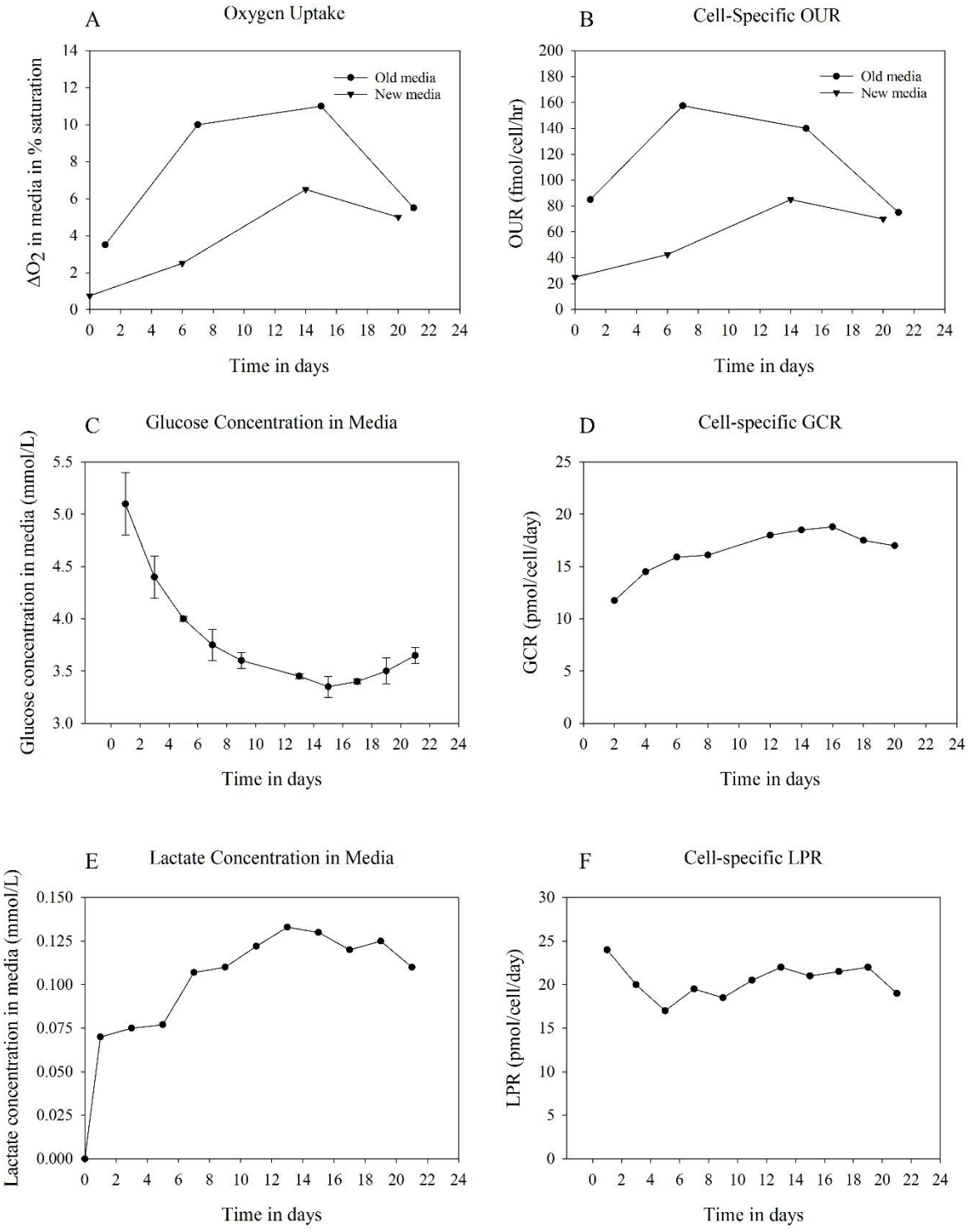

Figure 3: Metabolite monitoring trends over the 21 day culture period. The left column shows per-scaffold (or per-bioreactor in the case of glucose and lactate) change in metabolite concentration (where A is the oxygen uptake rate, C is the change in glucose concentration over time, and E is the lactate

concentration over time). The right column shows the cell-specific rates (where B is the oxygen uptake rate, D is the glucose consumption rate, and F is the lactate production rate). Error bars are present for all graphs, though not visible for many. It is important to note that the media entering each scaffold was fully re-oxygenated with media whereas glucose levels continually decreased and lactate levels continually increased between media changes (with initial values immediately after media replacement of 5.3 and 0 mM, respectively). "New media" corresponds to measurements taken immediately after the period of non-FBS supplemented media circulation; "old media" corresponds to those taken at least 24 hours after the reintroduction of FBS-supplemented media.

2.2 Oxygen study

Oxygen measurements were taken at the entrance and exit of each scaffold daily. The average change in oxygen concentration across the scaffolds is reported in **Figure 3a**. It is important to note that measurements at the inlet remained quite constant at 17.45 ± 1.76 % O_2 confirming that the re-circulating media was fully re-oxygenated to saturation levels in incubator conditions (i.e., 18 % O_2) prior to re-entering the scaffolds. "New media" corresponds to measurements taken immediately after the period of non-FBS supplemented media circulation (required for lactate quantification); "old media" corresponds to those taken at least 24 hours after the reintroduction of FBS-supplemented media. The first immediate observation is the difference between these two curves. The oxygen consumption is much lower for the "new media" samples, a result attributed to the stress induced by the three-hour culture period in the absence of FBS (which itself contains numerous proteins and growth factors required by the cells). These per-scaffold values were divided by the known scaffold cellularity at the sacrificial time points of days 0, 7, 14, and 21 in order to determine the cell-specific OUR at said time points. These values are provided in **Figure 3b**.

The cell-specific OUR curve shows an increase in the cell-specific oxygen uptake rate during the first 7 days before reaching a plateau (maximum cell-specific consumption) followed by a decrease after day 14. The shape of this curve is validated by the literature [25, 26]. This can be explained by the cells acclimating to their new environment over the first few days in culture and proliferating to fill the scaffold. Once the scaffold becomes highly populated and extracellular matrix is deposited, there is less space for the cells to grow and media to circulate, resulting in decreased oxygen availability and therefore decreased oxygen uptake by the cells. The calculated

cell-specific OUR for "old media" samples is 120 ± 40 fmol/cell/hr which is consistent with literature values, in the range of 50-500 fmol/cell/hr [27].

2.3 Glucose study

Glucose measurements were performed on samples taken every other day immediately prior to changing the media. The resulting glucose concentrations in circulating media are provided in **Figure 3c**. It can be seen that increasingly more glucose is consumed during the first week in culture (as evinced by a marked decrease in the glucose content of the media over this period), followed by a plateau in glucose consumption for the remainder of the culture period. Utilizing the equation outlined in the materials and methods section, the cell-specific glucose consumption rate was calculated, the resulting values are provided in **Figure 3d**. This curve demonstrates a gradual increase in the cell-specific GCR over the first week in culture, followed by a plateau, which can again be attributed to cellular acclimation. The averaged calculated cell-specific over the entire culture period GCR was found to be 15 ± 6 pmol/cell/day. This value is higher than that found in the literature of 6 ± 3 pmol/cell/day [25]. It is important to note, however, that this literature value was obtained for cells cultured on microcarriers in flasks rather than in a perfusion system. Due to the stagnant nature of such a culture system, gradients in glucose concentration within the system could have resulted in decreased glucose availability and therefore decrease glucose consumption. Such gradients are mitigated within a direct perfusion system, thereby accounting for a possible reason for the much higher values determined herein.

2.4 Lactate study

Lactate measurements were performed on media samples taken every other day from non-FBS supplemented α-MEM allowed to circulate for three hours prior to replacement with FBS-supplemented media. Samples were taken after the three hour culture period prior to changing the media. Results are shown on the **Figure 3e**. It is important to note that the short time period allowed for lactate accumulation, coupled with the stress induced on the cells by the removal of FBS during this period may well have resulted in larger errors than presented in **Figure 3**. Utilizing the equation outlined in the materials and methods section, the cell-specific lactate production rate was calculated, the resulting values are provided in **Figure 3f.**

The cell-specific lactate production rate was seen to remain fairly steady throughout the entire culture period, with perhaps slightly higher rates experienced during the first week of culture. These higher rates can potentially be attributed, again, to the acclimation of the cells to their new environment.

The averaged cell-specific lactate production rate over the entire culture period was calculated to be 19 ± 6 pmol/cell/day. This value is fairly close to that found in the literature of 11 ± 5 pmol/cell/day [28]. Furthermore, another important value to look at is the lactate yield from glucose ($Y_{lac/gluc} = q_{lactate}/q_{glucose}$), which was found to be 1.2 ± 1.0 mol lactate/mol glucose. This value agrees with the literature value of 1.9 ± 0.2 mol lactate/mol glucose [27]. This ratio serves as an indirect measurement of the glycolytic metabolism of a cell, with a value of 2 representing a fully glycolytic culture, as is reported for MSCs [25].

2.5 Correlation between oxygen consumption and cellularity

In order to determine scaffold cellularity without the need to perform destructive analysis, metabolite rates were plotted against cellularity in order to check for goodness of fit. Due to the low number of data points available for such fit, the resulting correlations merely serve as a proof-of concept, but do not have enough significance to be validated. The first correlation attempted was that for cellularity as a function of the per-scaffold oxygen consumption rate, as provided in **Figure 4**.

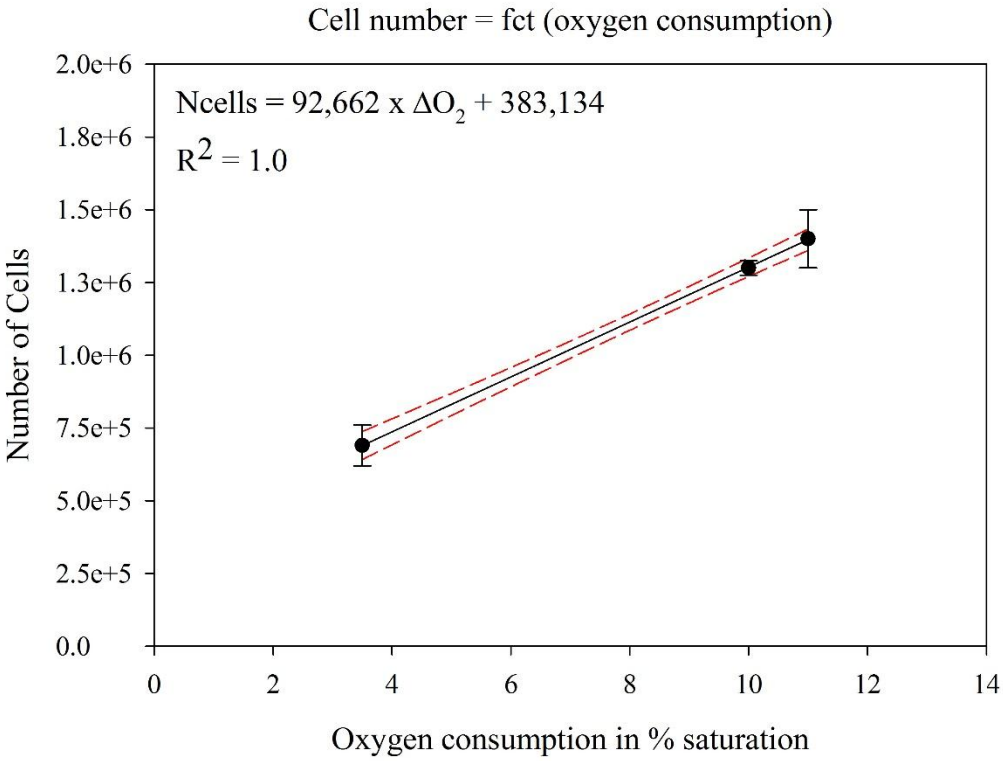

Figure 4: Scaffold cellularity as a function of oxygen consumption rate. A linear regression was calculated; the resulting equation was found to be N_{cells} = 92,662 x ΔO_2 + 383,134, with an R^2= 1.0. Dashed lines represent 95% confidence bands. One point was deemed an outlier and subsequently removed prior to the regression shown above.

Figure 4 resulted in a very good curve fit, exhibiting an R^2 of 1.0 after the removal of one point deemed an outlier. Although due to the low number of data points, this correlation lacks the significance required for true confidence in the goodness of fit, it demonstrates the feasibility of such a method for the determination of the cellularity of a scaffold mid-culture. These results show that oxygen monitoring is a potentially viable method to evaluate the live cellularity of a tissue-engineered construct without its sacrifice.

2.6 Correlation between glucose consumption and cellularity

Similarly to the above, a correlation between the glucose consumption and cell number was attempted. Only data obtained for the four time points of known cellularity (days 0, 7, 14 and 21) was used. The resulting correlation is presented in **Figure 5**.

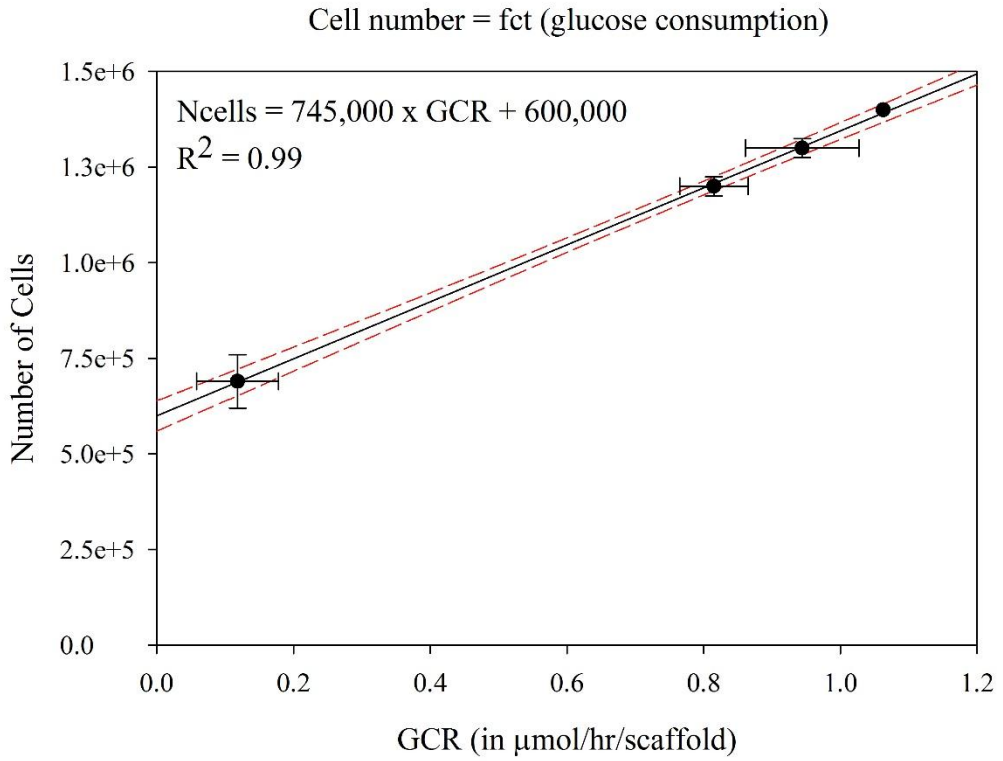

Figure 5: Scaffold cellularity as a function of glucose consumption rate. A linear regression was calculated; the resulting equation was found to be N_{cells} = 745,000 x GCR+ 600,000, with an R^2= 0.99. Dashed lines represent 95% confidence bands.

The linear regression resulted in an R^2 of 0.99. Again, although a low number of data points does not allow for conclusive determination, **Figure 5** demonstrates the feasibility of such a method for the determination of the cellularity of a scaffold mid-culture. These results show that glucose monitoring is a potentially viable method for the evaluation of the live cellularity of a tissue-engineered construct without its sacrifice.

2.7 Correlation between lactate production and cellularity

Finally, a correlation between the lactate production and cellularity was attempted. Again, only data obtained for the four time points of known cellularity (days 0, 7, 14 and 21) was used. The resulting correlation is presented in **Figure 6**.

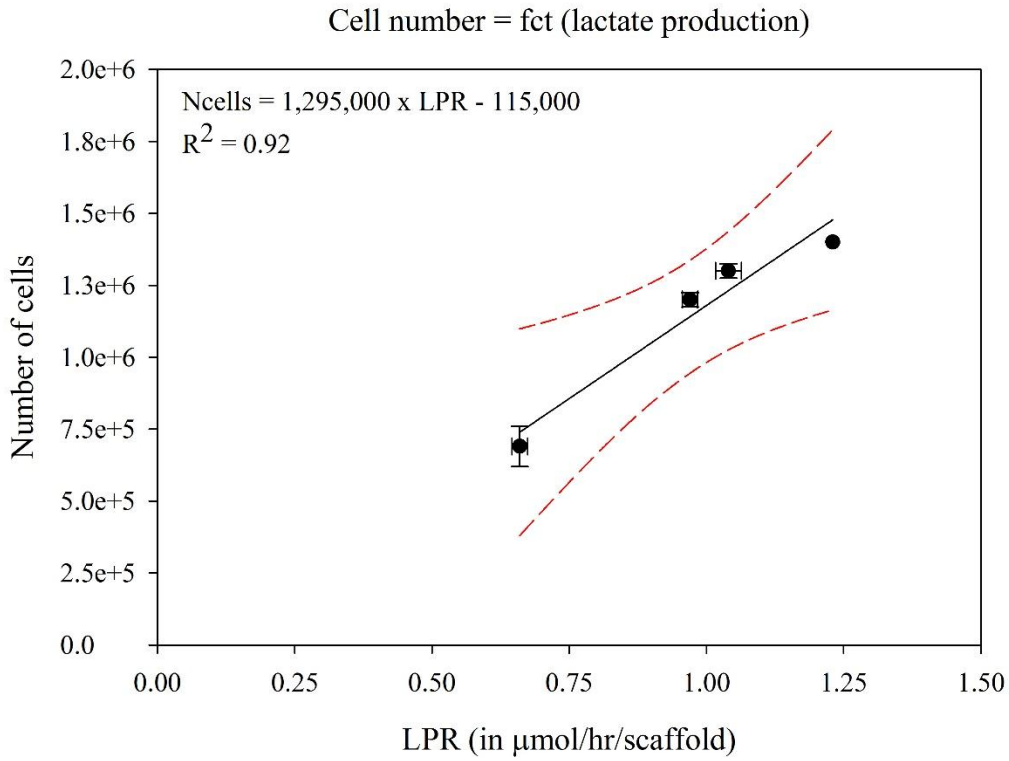

Figure 6: Scaffold cellularity as a function of lactate production rate. A linear regression was calculated; the resulting equation was found to be N_{cells} = 1,295,000 x LPR - 115,000, with an R^2= 0.92. Dashed lines represent 95% confidence bands.

The linear regression resulted in an R^2 of 0.92. Again, although a low number of data points does not allow for conclusive determination, **Figure 6** demonstrates the feasibility of such a method for the determination of the cellularity of a scaffold mid-culture. These results show that lactate monitoring is a potentially viable method for the evaluation of the live cellularity of a tissue-engineered construct without its sacrifice.

2.8 Comparison of cellularity correlations

The three above correlations for the determination of the cellularity of a tissue-engineered construct mid-culture show that such a method could potentially be used. The data presented herein, however, are very limited, serving as a proof-of concept for such correlative monitoring. As it was found that the cells were potentially stressed during circulation of non-FBS supplemented media, and such stress drastically altered their metabolic profile (at least with

respect to oxygen uptake), the above correlations may only be valid under the specific conditions of their determination herein, although the idea of utilizing metabolites as a key indicator of scaffold cellularity still holds. Based on the fairly steady glucose consumption and lactate production rates for the duration of the culture period (with exception for the first few days wherein the cells were acclimating to their new environment), these seem to be the most promising metabolites for the determination of scaffold cellularity. Of these, glucose seems to stand out as the most feasible candidate as it does not require special considerations (as did lactate) for its quantification in cell culture media.

4. Conclusion

The study detailed herein demonstrates the possibility of utilizing metabolic rates easily obtainable mid-culture for the determination of the cellularity of a tissue engineered construct without the need for its destruction (as is the current standard). Three key metabolites – oxygen, glucose, and lactate – were studied as prime candidates for this purpose due to their applicability to nearly all cell types and the current existence of reliable methods for their quantification in cell culture media. It was found that all three of these molecules show strong potential, with glucose coming to the forefront as the most promising. Although both glucose and lactate were quantified offline at a later time via an assay, the analysis could easily be adapted for use with *in situ* measurement devices allowing for their continual, real-time quantification.

It must be noted that the specific results presented herein are limited to the culture of non-differentiating rat MSCs cultured within a flow perfusion bioreactor under normoxia. This said, similar studies could be performed with numerous different cell types – either differentiating or not – under several different oxygen tension conditions and these could potentially be adapted for different bioreactor systems as well.

Finally, other metabolites or molecules (proteins, hormones, etc.) could be studied as potential candidates for use as non-destructive markers for construct cellularity and perhaps other quality factors as well. Such chemicals would ideally be consumed or produced at fairly constant rates over the entire course of culture if used for the determination of cellularity or exhibit drastically altered rates over the culture period if used for determination of other factors of construct quality (such as degree of differentiation, etc.).

Acknowledgements

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Highlights

- A real-time means of determining cellularity of 3D tissue constructs is proposed.
- It overcomes the current standards which require construct destruction.
- This method is based on the monitoring of key metabolites throughout culture.
- It can be adapted to accommodate more sensors for better quantification.
- Correlations have been developed between cellularity and metabolic flux.