3D Biotek
Add Extra Dimension to Your Innovation

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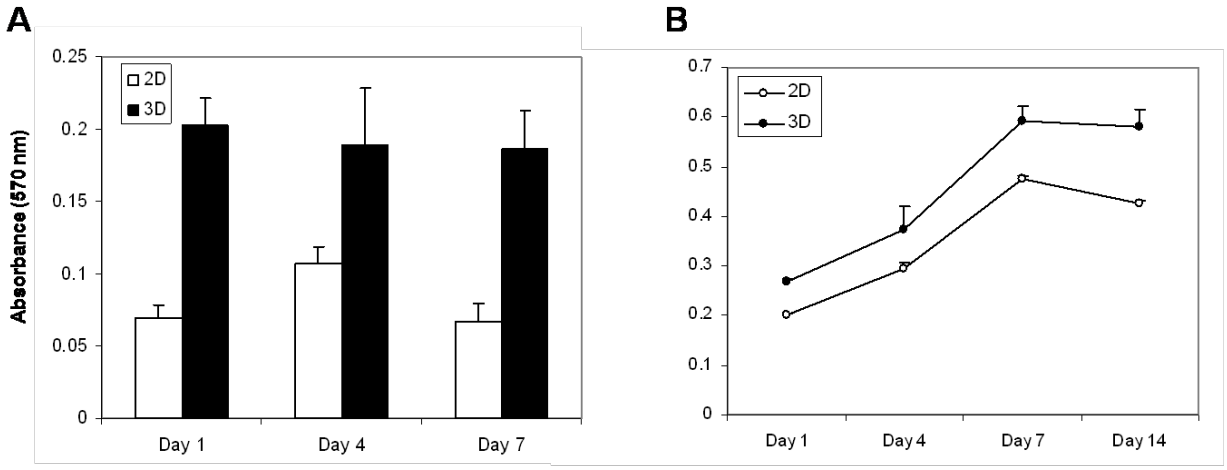
3D Perfusion Bioreactor: The Cumulative Advantages or 3D Scaffold Geometry and Perfusion for Scale-up Processes

1. Metabolism and Proliferation in 3D Static Cell Culture

Cell proliferation is an important measurement of cellular viability. Cell proliferation is sensitive to geometry, as a result of intrinsic cell behavior such as contact inhibition and effective delivery of diffusion-driven nutrient/waste exchange –in the absence of vascularity. These factors are mostly adverse in monolayer cell culture on 2-dimensional plates as a result of lack of 3-dimensional (3D) space availability and media perfusion.

2D vs. 3D: 3D Scaffold Supports Cell Viability

As a result of ample initial surface area on the polymer fiber structure and 3D pore volume, cells have the advantage of grow adherent at early stages and grow as tissues within the 3D porous structure at later times. We have shown a basic comparison highlighting the effect of our scaffold's 3D geometry, i.e. open-pore 3D geometry, and the optimum growth of cells. Here metabolic activity is normally greater than on 2D plates of the same diameter as the 3D scaffolds and 10,000 cells seeded on 2D and 3D.



Sustained cell viability in cells cultured on 3D PS scaffolds. MCF-7 human breast cancer cells were cultured in 2D and on 3D PS scaffolds. Cell vialibity was measured by (A) MTT and (B) Alamar blue assay.

3D vs. 3D: The effect of 3D Geometry on Cell Proliferation

Depending on the type of porous scaffold geometry, cells may have different 3D growth patterns. For example, 3D Biotek's scaffold geometry supports optimum linear growth as a result of its 100% open-pore geometry. *Kumar et al.*, compared the effect of type of porous geometry on

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cellular growth using 3D Biotek's 3D scaffold (PCL_FFF) and other polymer processing methods that rely on random porous geometry such as salt-leeching (PCL_SL), gas foaming (PCL_GF), and gas foaming/phase separation (PCL_GF-PS). First, random porous scaffolds have higher porosity, but the randomness of the porous pattern does not ensure pore interconnectivity. 3D Biotek's scaffolds have 100% pore-to-pore interconnectivity and pore size can be precisely controlled from 100-um to any desired size.

Table I. Porosity and pore size as a function of 3D scaffold fabrication.

Fabrication Methods	Porosity	Pore Size (um)
PCL_SL	94.3 +/- 0.7	*337
PCL_GF	92.6 +/- 0.8	*337
PCL_GF/PS	90.3 +/- 0.7	*337
PCL_FFF	65.6 +/- 0.9	569

**Average crystal size range (250 – 425 um) of sieved NaCl and NH_4HCO_3.*

It was also noted that geometric differences between various 3D porous scaffolds had an effect on the cellular growth, as seen in the following DNA vs time graphs (Figure 2 and 3).

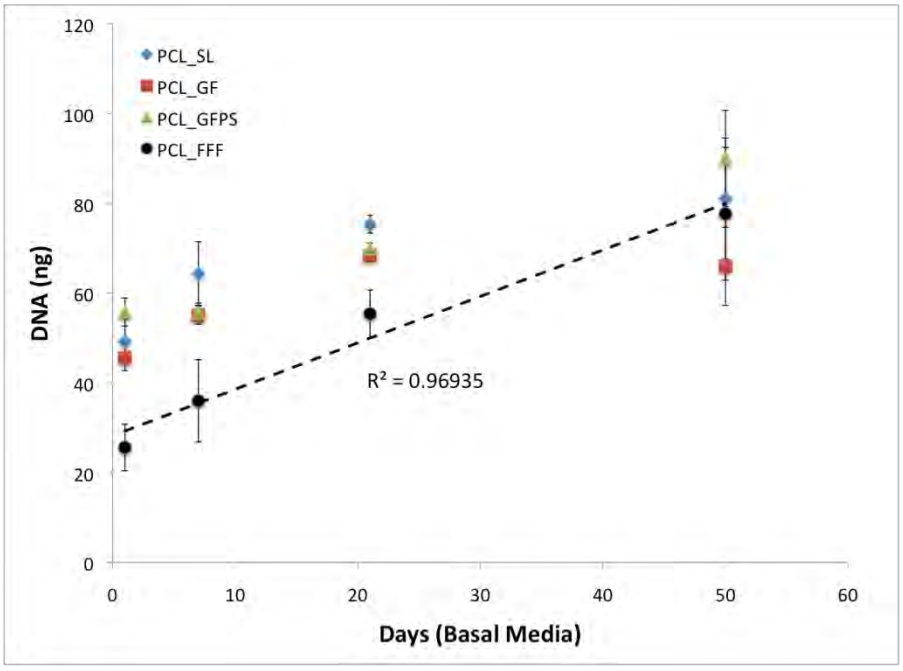

Figure 2. Human Bone Marrow Cells (hBMCs) growth in 3D scaffolds. Cell growth was linearly consistent for hBMSCs growing in 3D Biotek's Inserts (PCL_FFF, solid black dots) than cells growing on other 3D scaffolds.

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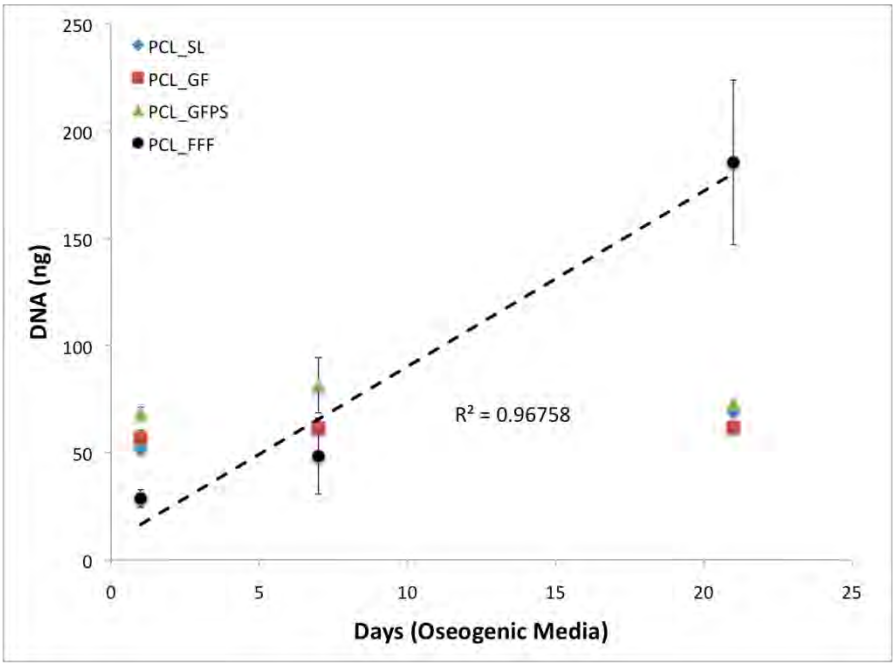

Figure 3. hBMCs proliferation during osteogenic differentiation. Consistently with the linear growth observed under basal media growth conditions, hBMSCs in 3D Biotek's Inserts (PCL_FFF, solid black dots) grew optimally when compared to other scaffolds.

Biologically, pore size and pore-to-pore interconnectivity will affect cellular infiltration into the scaffold's 3D porous structure, especially the central porous regions. In addition, these geometric properties will affect the exchange of waste/nutrients within the porous scaffold.
The result from the study by kumar et al., demonstrates that porosity is not the only factor that needs to be considered when choosing suitable geometries for 3D cell culture. Pore-to-pore interconnectivity played a critical role in facilitating cell growth in the 3D PCL_FFF scaffold (3D Biotek's Insert).

Citation: Kumar et al., The Determination of Stem Cell Fate by 3D Scaffold Structures Through the Control of Cell Shape. Biomaterials, (2011) 32(35):9188.

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2. The Effect of 3D Perfusion on Cellular Growth

Cell Proliferation and Viability in the Bioreactor Chamber

One of the advantages of the 3D Biotek's scaffolds is the open pore structure, which facilitates the exchange of waste and nutrients even in static conditions. In the same manner, the open pore geometry can facilitate the perfusion of media through the 3D scaffolds. Perfusion would facilitate waste removal and replenish nutrients at a faster rate than cells growing under static conditions. Thus, active perfusion would benefit cellular proliferation and cell viability.

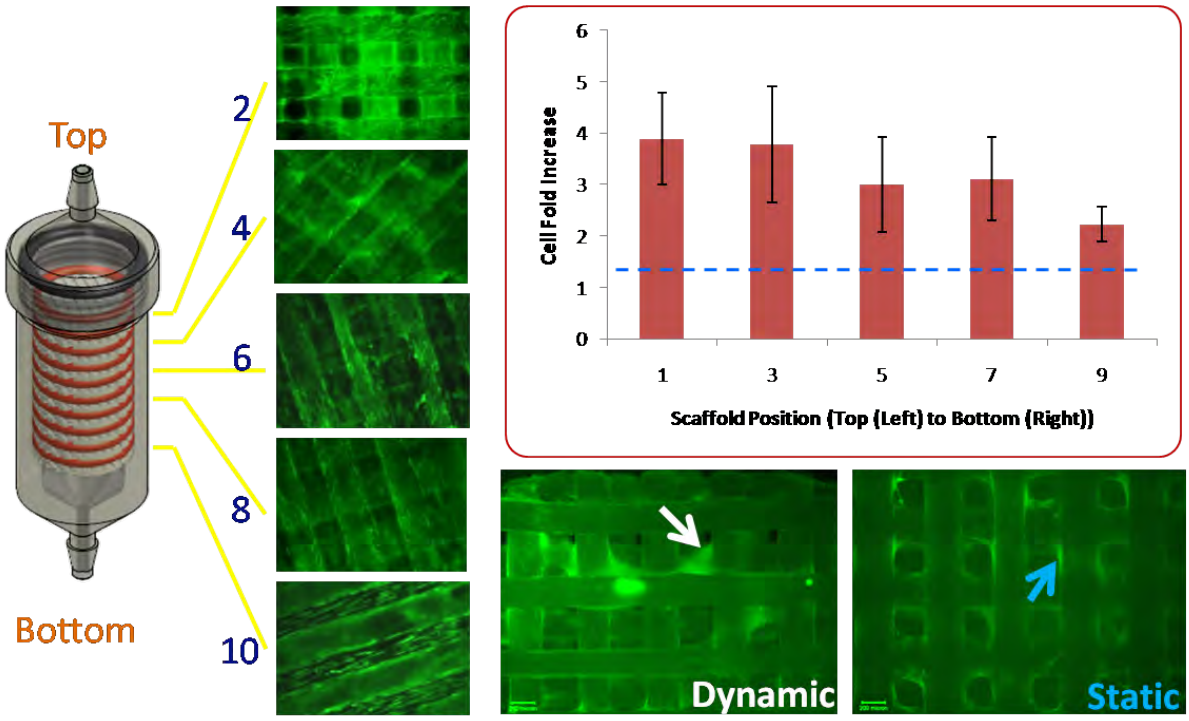

Figure 4.Assessment of cell viability and growth as a function of 3D scaffold position and presence of perfusion at day 6. Cells were viable along the length of the bioreactor chamber (even numbered scaffolds, left image). There was a significant fold increase on odd numbered scaffolds, in comparison to static growth (blue dotted line, top right. Dynamic culture also increases the formation of 3D cell sheets faster than in 3D static (white and blue arrows, bottom right).

Figure 4 shows proliferation fold cell increase and cell viability from foreskin fibroblast grown in 3D static and 3D perfusion for 6 days. For example, live fluorescent viability staining on even-numbered scaffolds in the bioreactor chamber showed healthy fibroblast growing independent of scaffold position (Fig. 4, Left). In addition, total cell number after cell dissociation was consistent under 3D perfusion and higher than cells growing in 3D scaffolds under static conditions (Fig 4. Right: blue dotted line). Further assessment of cell growth showed that cells developed larger cell sheets than static conditions for cell culture period. As will be described in

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the following section, cells on scaffolds would naturally grow into its 3D porous structure. The effect of perfusion is that it will make this process faster because it provides mechanical stimulation and active transport of waste/nutrients.

3. Scaling Parameters: Comparison between T300 Flask and Bioreactor Chambers

The following comparison is based on absolute values of surface area from T300 2D cell culture flasks and total surface area from 4 bioreactor chambers using 6-well inserts PS(1520) with a 33-mm Insert diameter, 0.150-mm fiber diameter, and 0.200-mm fiber-to-fiber spacing. From Table II, 4 fully loaded bioreactor chambers are equal to 9 T300 2D flasks. However, the amount of available space in 3D is exponentially greater than in 2D plates. For example, a common feature in the 3D scaffolds is continuous growth into the porous structure of the scaffold. This is defined as true 3D geometry. During this transition cells change shape and secrete extracellular matrix (ECM) as the ground substance to growth in the absence of the polymer surface. Figure 4 shows a visual description of 3D growth behavior using cell lines such as HepG2, NIH-3T3, and stem cells (we have used other cell lines as well).

Table II. Direct Scaling Comparison between 2D Cell Culture Flask and 3D Bioreactor.

Cell Culture	3D Perfusion	2D Flask
Format	6-Well PS(1520)	T-300 Flask
Unit Surface Area	54.02- cm^2	300- cm^2
Total Scaffolds in 4 Chambers	52 scaffolds	N/A
Total Surface Area 4 Chambers	2,809.04- cm^2	N/A
Total T-300 Flaks equal to Total Surface Area in 4 Chambers	N/A	9 Flasks

Initial growth on polymer fibers is similar to growth on 2D surfaces. However, cells on 3D scaffolds naturally grow from the fibers into the 3D porous space as sheets or cell agglomerates. As seen in figure 5, NIH-3T3 at day 21, hMSCs at day 7, and Hep-G2 at day 5 all display 3D growth after growing on the polymer fibers (Fig. 5, white arrows)

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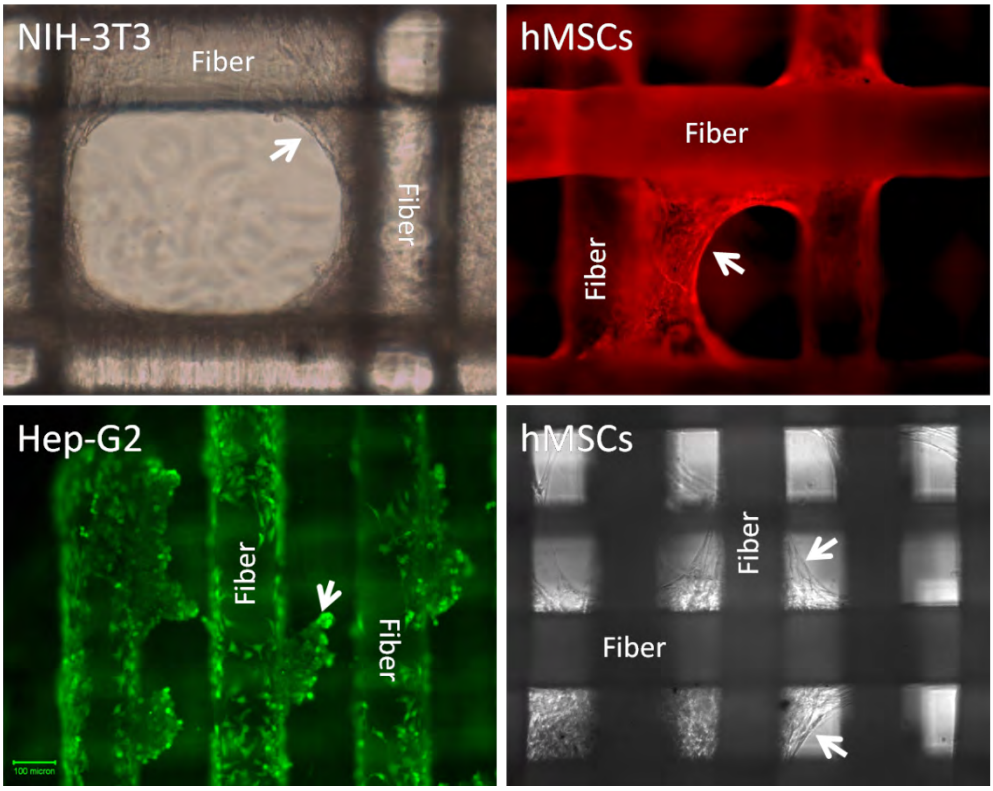

Figure 5. Images of cells naturally growing into the 3D porous structure of 3D Biotek's scaffolds. Initially, cells grow on the polymer fibers. Following, cells grow towards the 3D porous space mimicking in vivo-like growth conditions.

Transition towards cell growth in the 100% interconnected 3D porous structure defines several advantages:

- Cells will grow closer to in vivo conditions.
- Cells in 3D tend to become less spread than on 2D plates.

Thus, cells in 3D grow more efficiently because of the use of space when making cell contact with other cells. As opposed to spread morphologies on plates that become contact inhibited over time (Fig. 5 HepG2: spread on fibers and become round as they grow into the 3D pore). For example, renal tubular cells on flat surfaces will have an approximate volume of ~6 x 10^{-6} ul in contact with other cells (Korchev YE., et al.,). Assuming the same cell volume and cell growth towards the empty 3D pore, the following is an approximate number of cells that would need to populate several percent volume fractions in a single 3D PS(1520) 6-well scaffold with a total internal wetting volume of 250-ul:

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Table III. Approximated Growth Surplus of Cells into 3D Porous Geometry

3D Scaffold's Percent Volume Fraction	Approximate Cell Number
50%	2.1×10^7
25%	1.0×10^7
10%	4×10^6
5%	0.8×10^6

As described in table III, the approximated growth is defined by the natural tendency of cells to grow into the 3D open pore structure. A value as low as 5% 3D growth will translate into 0.8×10^6 cells per scaffold, for an approximate 43×10^6 cells in all 4 bioreactor chambers. This approximate cell number would be in addition to normal growth on the available total surface area in the 3D scaffold and would not represent the same extra cell growth on regular 2D cell culture plates.

Citation: Korchev YE et al., Cell Volume Measurement Using Scanning ion Conductance Microscopy. Biophysical Journal, (2000) (78):451-457.

Conclusions

- 3D Biotek's scaffolds have 100% open pore geometry facilitating passive and active transport of nutrients and transition towards 3D growth within the 3D porous structure
- Growth curves on the available surface area should be comparable to 2D as cell morphology on the fibers is approximate to cell morphology on regular 2D plates. However, the real benefit comes from growth into the 3D open pore structure.
- Cell growth into the 3D porous structure as little as 5% of the 3D porous structure can translate into exponential cell growth, as opposed to the linear cell growth from increasing surface area.
- Perfusion will speed up cell growth, which will benefit transition into 3D cell growth.