Seventh International Conference on CFD in the Minerals and Process Industries
CSIRO, Melbourne, Australia
9-11 December 2009

BIOREACTOR DEVELOPMENT FOR CARTILAGE TISSUE ENGINEERING: COMPUTATIONAL MODELLING AND EXPERIMENTAL RESULTS

Timothy M. WICK[1*] and Tanya FAROOQUE[2]

[1] Department of Biomedical Engineering, The University of Alabama at Birmingham, Birmingham, Alabama, USA
[2] School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA
*Corresponding author, E-mail address: tmwick@uab.edu

ABSTRACT

Tissue engineering is an emerging technology to replace tissues and organs damaged due to trauma of disease with fully functional living tissue equivalents. Tissue development is facilitated by bioreactors, devices engineered to deliver appropriate spatial and temporal nutrient transport and mechanical loading in a well-defined and controlled environment. Bioreactors that provide well-defined and controllable environments are useful for fundamental studies to optimize tissue growth and as production units for large-scale tissue production bioprocesses.

We have developed a concentric cylinder bioreactor and a perfusion concentric cylinder bioreactor suitable for growth of relatively thin (~1-3 mm thick) tissues under well-defined and controllable bioprocessing conditions. Using cartilage as a model tissue, we identify the role of mechanical loading, tissue perfusion and growth factors on tissue development. Since hydrodynamic profiles and nutrient gradients are complex during tissue growth, we demonstrate the utility of computational fluid dynamics (CFD) modelling of bioreactor growth conditions on bioreactor design, bioreactor scale-up and tissue growth.

NOMENCLATURE

CC concentric cylinder bioreactor

PCC perfusion concentric cylinder bioreactor

INTRODUCTION

Tissue engineering has the potential to address diseases and disorders that significantly impact existing health care costs (Vacanti and Langer 1999). Engineered tissues are revolutionizing disease therapy in many areas of medicine, but for most engineered tissues, including cartilage; current production practices adhere closely to the original bench-top processes developed during the early discovery research phase of tissue engineering technology. Across the industry, limitations in scale-up and cumbersome or ineffective preservation methods for engineered tissue production lead to high costs of goods and batch-to-batch variability that may impair clinical outcomes (Pancrazio, Wang et al. 2007).

Tissue engineering consists of culturing appropriate cells on a biodegradable scaffold under conditions that promote development of tissue architecture, function and mechanical properties suitable for integration and function in vivo. Differentiated cells such as chondrocytes (for cartilage) or multipotent stem cells are used for engineering tissues (Hwang and Elisseeff 2009). Biocompatible biodegrade polymer scaffolds define the tissue geometry and provide the initial support for cell seeding and growth (Chan and Leong 2008). Scaffolds are highly porous (80-95%) with 'pore' sizes on the order of 10-100 microns to allow cells and nutrients to penetrate the scaffold thickness during seeding. As cells grow, differentiate, secrete and organize extracellular matrix the scaffold degrades, so that at the end of culture cells have developed mature tissue architecture (Chung and Burdick 2008). Tissue growth is complex and regulated by nutrients, growth factors and mechanical loading that vary temporally and spatially across the tissue (Wendt, Jakob et al. 2005; Vinatier, Mrugala et al. 2009).

Our approach involves the use of bioreactor technology. Bioreactors provide the appropriate culture environment to grow three-dimensional tissue using biologically relevant scaffolds. The bioreactor environment can be programmed to modulate and preserve cartilage growth, especially when applying mechanical stimuli such as shear, perfusion, and compression. The premise that the mechanical and chemical stimuli that modulate chondrocyte behavior *in vivo* will also influence their behavior *in vitro* (Saini and Wick 2003; Sharma and Elisseeff 2004; Schulz and Bader 2007) has led to a number of studies showing that cell-seeded scaffolds are influenced by their surrounding environment, stimulating chondrogenesis and matrix synthesis (Pazzano, Mercier et al. 2000; Darling and Athanasiou 2003; Saini and Wick 2003). Bioreactors allow changes in the culture environment to affect the kinetics and properties of tissue growth, in a well-defined fluid regime to elucidate mechanotransduction pathways and the relationship between mechanical forces and tissue growth kinetics. To understand and identify the relationship between culture conditions and tissue properties that achieve suitable cartilage growth in a bioreactor requires knowledge of the interaction between cell behavior and their mechanical and chemical stimuli (Waldman, Spiteri et al. 2003; Bilodeau and Mantovani 2006).

Shear stress (Gemmiti and Guldberg 2006; Raimondi, Moretti et al. 2006), compression (Jung, Kim et al. 2008; Kock, Schulz et al. 2009), tissue perfusion (Pazzano, Mercier et al. 2000; Schulz, Wustneck et al. 2008) or hydrostatic pressure (Carver and Heath 1999; Hu and Athanasiou 2006; Elder and Athanasiou 2009) increase

chondrogenesis and improve the quality of tissue engineered cartilage in culture. Dynamic or intermittent loading increases chondrogenesis compared to steady loading for any of the mechanical loading regiments studied (Saini and Wick 2003; Hu and Athanasiou 2006; Villanueva, Weigel et al. 2009).

As cartilage and other tissue engineered products are accepted for use in humans, large scale production methods must be developed to meet patient demand. For example, approximately 3.8 million procedures to repair cartilage lesions in the knee are performed annually world-wide. With an aging population, this number is estimated to grow to 5.3 million next decade (InteLab Corporation 2009). Similar demand exists for replacement of cardiovascular, musculoskeletal and other tissues. While an individual patient typically requires one or a small number of tissues for a procedure, efficient manufacturing requires large-scale production and preservation with the ability to deliver viable produce "off-the-shelf'" to clinical facilities that treat the patient(s). Thus, well-defined, controlled tissue growth conditions are required to determine operating conditions (nutrient transport, mechanical loading, etc.) for production of functional tissue as well as for large scale production of tissue with properties suitable for implantation for patients.

Bioreactors capable of providing a well controlled, uniform and quantifiable growth environment are important for fundamental studies of tissue development and ultimately for large-scale production of engineered tissue to meet patient demand. The overall goal of our lab is to develop bioreactors that provide tight spatiotemporal regulation of nutrient transport and mechanical loading to produce cartilage with architecture and functional properties suitable for implantation. In this paper, we describe the concentric cylinder bioreactor which supports steady or intermittent hydrodynamic loading (shear stress) on growing cartilage constructs. The concentric cylinder bioreactor provides spatially uniform loading of all constructs in the bioreactor. Nutrient transport and hydrodynamic loading can be changed as needed throughout scaffold seeding, construct growth and tissue maturation. The concentric cylinder bioreactor can operate for at least 4-6 weeks of continuous operation in fed batch mode without handling of constructs.

RESULTS – BIOREACTOR DEVELOPMENT AND MODELLING

Concentric Cylinder Bioreactor-Design

Important bioreactor design considerations are 1) the ability to seed constructs with cells under dynamic conditions in the same vessel that will support construct growth, 2) well-defined, uniform hydrodynamic loading of constructs, 3) no construct handling or bioreactor manipulations except media exchange. To achieve these goals, constructs must be fixed in space. A simple bioreactor geometry will ensure uniform nutrient transport and construct loading. The concentric cylinder bioreactor consists of a stationary inner cylinder and an outer rotating cup (Figure 1). The gap between the inner and outer cylinders is 3 mm. Porous scaffolds are mounted on the inner cylinder and protrude into the space between the

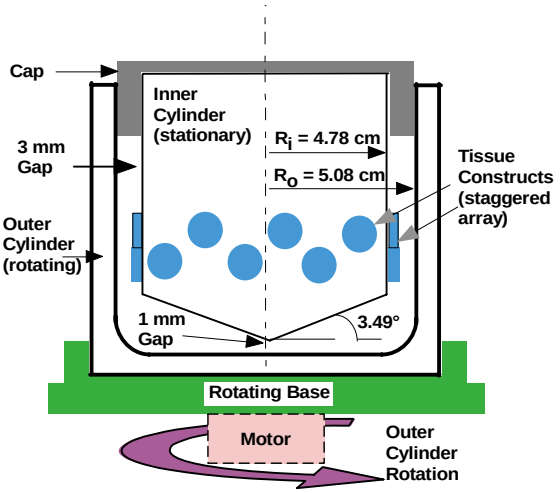

Figure 1: Concentric Cylinder Bioreactor. 16 constructs (arrows) are staggered in 2 rows of 8 on the inner cylinder (Saini and Wick 2003).

inner and outer cylinder to facilitate cell seeding and nutrient transport.

The concentric cylinder bioreactor is operated in a fed-batch mode. Poly-L-lactic acid scaffolds 1.0 cm in diameter and 1.87 mm thick are affixed to the inner cylinder. Constructs are spaced uniformly around the inner cylinder in two rows of eight staggered as shown in figure 1. Culture media containing 100 million freshly-isolated juvenile bovine chondrocytes are added to the bioreactor. The bioreactor is closed, assembled in the motor mount and placed in a 95% air/5% CO_2 incubator. The outer cup rotates at 38 RPM, providing mixing and motion to seed scaffolds with cells.

Cell seeding of scaffolds occurs under dynamic conditions by adding the chondrocyte suspension to the bioreactor containing the 16 porous constructs. Typically the bioreactor is seeded with 60-100 million bovine chondrocytes in 62 ml tissue culture medium (corresponding to 3.75-6.25 million chondrocytes per construct). After chondrocyte addition and bioreactor assembly, the outer cylinder rotates at 38 RPM to provide mixing and hydrodynamic loading. The rotation direction is changed every 12 hours during the first 96 hours to ensure uniform seeding of constructs with chondrocytes. Following seeding for 96 hours, the media is completely replaced. Thereafter, 80% of the media is replaced every 48 hours and rotation direction is reversed every 24 hours to ensure that all cells within the construct experience, on average, the same shear stress over the 28 day experiment. Scaffolds (4) are harvested weekly and analysed for cell proliferation and matrix deposition (Saini and Wick 2003).

Construct seeding is homogeneous among the constructs in the bioreactor and chondrocyte seeding efficiency is 85-95% over 48 hours of continuous rotation. Chondrocytes are uniformly distributed throughout the construct (Saini and Wick 2003). For bioreactors seeded with $100x10^6$ chondrocytes (~$6.x10^6$ chondrocytes/construct), cell growth and matrix deposition was robust over 28 days in culture. After 28 days bioreactor culture, constructs contain $13.9\pm0.6x10^6$ chondrocytes, 3.8±0.2 mg proteoglycan and 1.8±0.2 distributed uniformly throughout the construct (Figure 2). Shear stress (Saini and Wick 2003, low oxygen tension {Saini, 2004 #468),

low oxygen tension (Saini and Wick 2004), dynamic mechanical loading (Rangamani 2005) and growth factors (Rangamani 2005) regulate chondrogenesis in the bioreactor. Additional construct analyses validate the modeling assumption of dense tissue formation with no flow through the construct during bioreactor operation (see below).

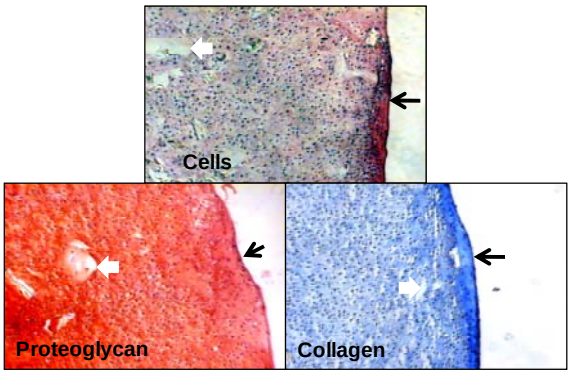

Figure 2: Construct Histology After 28 days Bioreactor Culture. Histological stains showing distribution of cells, proteoglycan and collagen (10x magnification). White arrows indicate undegraded polymer. Black arrows point to the construct surface exposed to fluid flow (Saini and Wick 2003).

Concentric Cylinder Bioreactor – CFD Modelling

The exact geometry of the bioreactor was used as the basis for the computational mesh. Because of radial symmetry, only one-eighth (45°) of the bioreactor is modelled. Constructs are modelled as nonporous discs (1.0 cm dia x 1.75 mm thick) affixed to the surface of the inner cylinder. This represents the experimental condition of a solid tissue representative of late culture conditions (Saini and Wick 2003). In the computational model, the construct is assumed to have the same radius of curvature as the inner cylinder. The media volume is defined as the 3mm gap between the inner and outer cylinder from the bottom of the outer cup to the media/gas interface 4.0 cm from the bottom of the inner cylinder. The fluid is discretized using an unstructured hexahedral grid with approximately 270,000 cells and 315,000 nodes (Figure 3). Streamlines and species gradients (nutrients or oxygen) are expected to vary greatest near the construct edges and surfaces; these areas were assigned a more finely structured mesh. Grid size is increased away from the constructs and corners without loss of computational accuracy (Williams, Saini et al. 2002).

Three-dimensional fluid velocity and oxygen concentration were calculated from the incompressible Navier-Stokes equations (continuity, momentum and oxygen-species) using the commercial CFD code Fluent® (Version 5.4, Lebanon, New Hampshire). Model assumptions were the no-slip fluid condition at all surfaces, zero shear stress at the gas-liquid interface, and

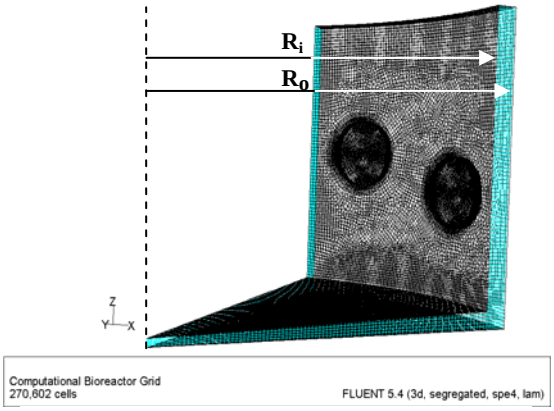

Figure 3: Bioreactor Computational Grid. (Williams, Saini et al. 2002)

no fluid flow through the constructs. Concentration profiles were calculated from the oxygen species equation using the dilute solution approximation, incompressible fluid and constant diffusivity. To account for oxygen transport, the liquid phase oxygen concentration was assigned the value of the equilibrium concentration for O_2 in the media calculated using Henry's law constant for oxygen in water (Incropera and DeWitt 1996). The binary diffusivity for oxygen in water was estimated to be 3.23 x 10^{-9} m^2/s (Macpherson, O'Hare et al. 1997). Under experimental culture conditions, the media Schmidt number is large (>200), indicating that momentum diffusivity dominates in this flow field. The experimental oxygen consumption was assumed constant at 0.5 μmol $O_2/10^6$ cells/hr (Lee, Trindade et al. 2002). This value was used with the observed cell density/construct to assign a rate of oxygen consumption to the surface of each construct in the computational mesh.

Bioreactor Computational Modelling

CFD modelling parameters were based on the cartilage growth conditions (Williams, Saini et al. 2002). Outer cup rotation at 38RPM provides steady-state flow across the construct surfaces. Velocity vectors (Figure 4A) indicate uniform fluid flow around constructs and good fluid mixing throughout the bioreactor. The flow patterns around the construct are characterized by a stagnation region on the upstream side and a recirculation region on the down-stream edge of the constructs. Particle trajectory plots (Figure 4B) approximate cell trajectories during bioreactor seeding. The particle trajectories demonstrate significant recirculation around the constructs. Fluid and cell circulation among constructs promotes delivery of chondrocytes to the constructs and enhances transport of cells into the constructs. This continuous circulation of chondrocytes among constructs accounts for the high seeding efficiencies and uniform seeding of constructs with chondrocytes observed experimentally.

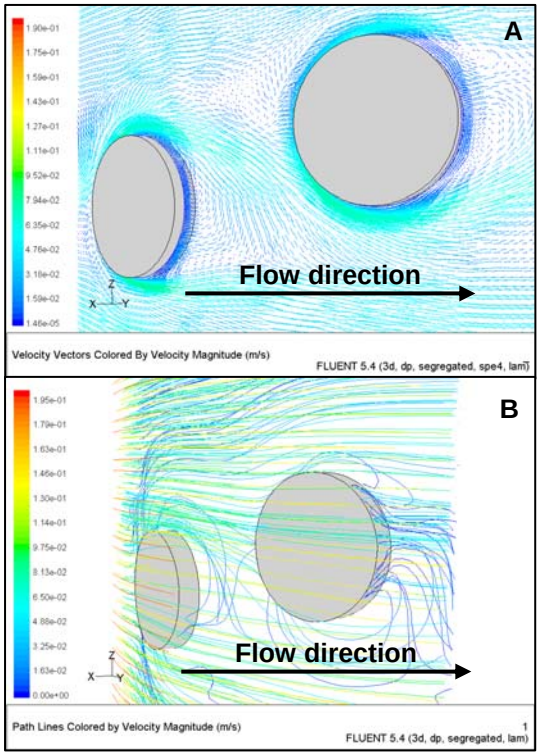

Figure 4: Steady-State Bioreactor (A) Velocity Vectors (B) and Particle Trajectories (from Williams, Saini, et. al. 2002).

Since chondrocytes are responsive to mechanical loading, and specifically shear stress, it is important to calculate the shear stresses on the construct surface under typical experimental conditions. In addition, if all constructs in the bioreactor experience the same shear stress under steady-state operating conditions, the assumption of uniform hydrodynamic loading is valid and the concentric cylinder bioreactor is suitable for tissue production bioprocess in which mechanical loading is a parameter to regulate tissue growth and maturation.

With the exception of a small region of high shear stress at the leading edge, constructs are exposed to relatively uniform shear stress (Figure 5). The maximum shear stress is 11.7 dyn/cm^2 for the constructs in the lower row and 10.9 dyn/cm^2 for the constructs in the upper row. The mean shear stress on the construct face is 2.47±1.04 dyn/cm^2 for constructs in the bottom row and 2.27±0.91 dyne/cm^2 for constructs in the upper row. Eighty percent of the total construct surface areas exposed to flow experiences shear stresses in the range of 1.5-4.0 dyne/cm^2 (Williams, Saini et al. 2002).

Oxygen is sparingly soluble in aqueous media and one concern of high density tissue culture is depletion of oxygen and formation of metabolically inactive or necrotic regions within the tissue construct. Although neither histology nor mechanical testing of cartilage constructs harvested from the bioreactor revealed any tissue heterogeneity, it was important to calculate oxygen profiles in the bioreactor to determine conditions leading to oxygen depletion. Steady-state oxygen profiles were calculated based on 15x10^6 chondrocytes per construct consuming oxygen at a constant rate of 1.39x10^{-16} mol

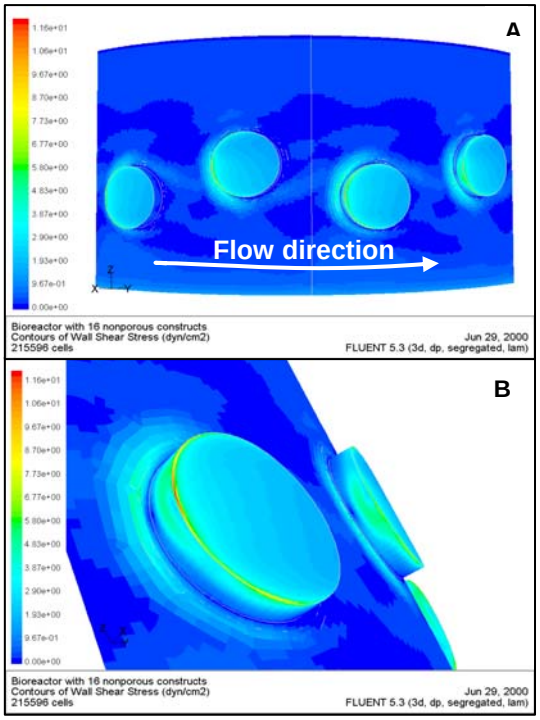

Figure 5: Bioreactor Steady-State Shear Stress Profiles for 16-Construct Bioreactor. Panel (B) is enlargement of (A) showing location of maximum shear stress on the leading edge of the upper and lower constructs (from (Williams, Saini et al. 2002).

O_2/cell/s (Cartwright 1994). Oxygen concentration is essentially uniform throughout the bioreactor under tissue growth conditions (Figure 6), with the exception of a small region near the inner cylinder wall on the downstream side of the construct, where oxygen saturation falls to 23% (Williams, Saini et al. 2002) which is well above the 1-8% oxygen saturation values reported for cartilage *in vivo* (Brighton and Heppenstall 1971; Shapiro, Mansfield et al. 1997).

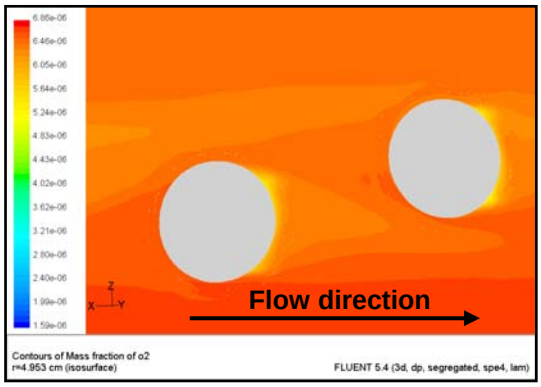

Figure 6: Bioreactor Oxygen Concentration Profile. (Williams, Saini et al. 2002).

Bioreactor Scale-Up: Computational Modelling

Cartilage *in vivo* is avascular and under low oxygen. Oxygen tension in cartilage is reported to be between 1% and 8% of saturation (Brighton and Heppenstall 1971; Shapiro, Mansfield et al. 1997). Cartilage growth studies (Saini and Wick 2003) and computational modelling

(Figure 5) (Williams, Saini et al. 2002) demonstrate that under the operating conditions described above with the bioreactor operating in a 5% CO_2/95% air incubator with 20% oxygen saturation, the developing cartilage tissue is not oxygen depleted. This suggests that the bioreactor can accommodate additional constructs without oxygen depletion. Since oxygen is likely to be the limiting nutrient in bioreactor culture (since the concentration of other nutrients and growth factors or more frequent media replenishment can be increased to meet the greater metabolic demand of addition constructs in the bioreactor).

Additional CFD simulations were performed to determine the effect of adding 1 or 2 additional rows of 8 constructs in the bioreactor. For these simulations, construct chondrocyte density was assumed constant (15 million/construct). Additional rows of constructs (8 constructs per row) are centered one-half construct diameter above the previous row. Constructs in the third row (24-construct bioreactor) were directly above the constructs in the first row and constructs in the fourth row (32-construct bioreactor) were directly above the second row. The steady-state shear stress profile for the 24-construct bioreactor is similar to the 16-construct bioreactor (Figure 7). Maximum shear stress calculated at the leading edge of the construct is 11.8, 7.2 and 9.1 dyn/cm^2 from the bottom to top row of constructs. Steady-state shear stress profiles for the 32-construct bioreactor were similar to Figure 5. Maximum shear stresses in the 32-construct bioreactor ranged from 9.1-11.8 dyn/cm^2 for the four rows of constructs (Williams, Saini et al. 2002)

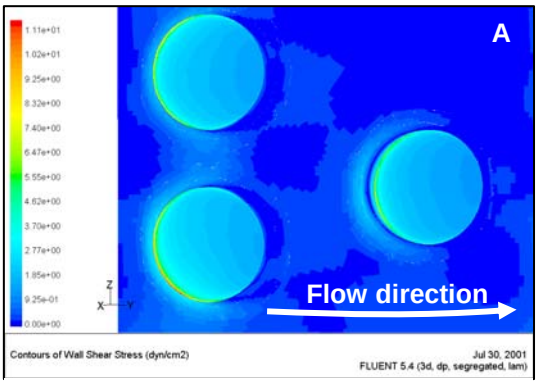

Figure 7: Bioreactor Steady-State Shear Stress Profiles for 24-Construct Bioreactor (from (Williams, Saini et al. 2002).

Steady-state oxygen profiles calculated for 24-construct bioreactor are similar to the 16-cosntruct bioreactor (Figure 8a), however oxygen concentrations are lower throughout the bioreactor as expected. Constructs are not oxygen limited, with the exception of a small region of oxygen depletion near the bioreactor inner cylinder at the down-stream stagnation points (Figure 8b).

Concentric Cylinder Perfusion Bioreactor

The concentric cylinder bioreactor was developed to provide a uniform hydrodynamic loading and nutrient transport environment to identify and validate bioreactor conditions that promote robust construct development

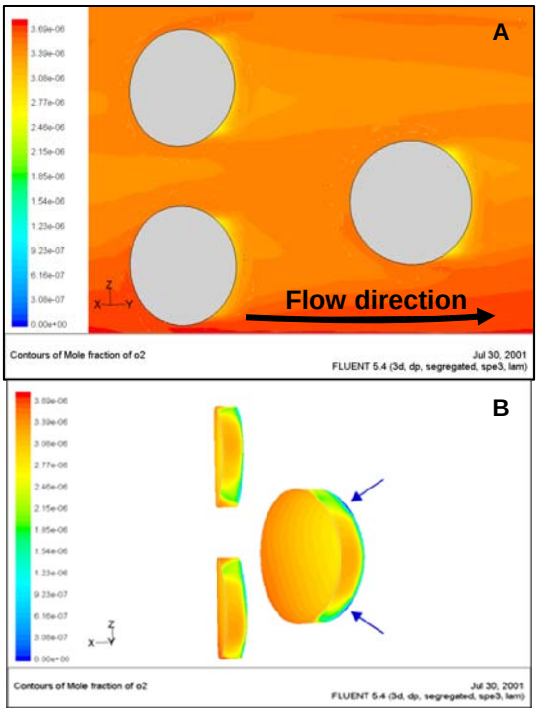

Figure 8: Bioreactor Oxygen Concentration Profile. Panel (B) shows small region of oxygen depletion (arrows)(Williams, Saini et al. 2002).

under well-defined and controllable conditions. Using this bioreactor, dynamic hydrodynamic loading, low oxygen tension and growth factors have been shown to contribute to chondrogenesis over four weeks of tissue growth (Saini and Wick 2003; Saini and Wick 2004; Rangamani 2005).

Articular cartilage is highly organized, with three distinct architectural and functional zones from top to bottom called the superficial, middle and deep zones (Brama, Tekoppele et al. 2000; Rogers, Murphy et al. 2006; Klein, Malda et al. 2009). The superficial zone comprises the upper 10-20% of articular cartilage and encompasses the articulating surface. In the superficial zone, chondrocytes and collagen are aligned parallel to the articulating surface. Chondrocytes are at higher density in the superficial zone. In the middle zone, collagen fiber orientation is more random. Chondrocytes are at a lower density, more rounded and randomly oriented. In the deep zone, chondrocytes are oriented perpendicular to the articulating surface. Nutrient transport, mechanical properties and chondrocytes phenotype changes with tissue depth (Schinagl, Gurskis et al. 1997; Poole, Kojima et al. 2001; Hunziker, Quinn et al. 2002).

To control zonal organization into tissue engineered cartilage, the concentric cylinder bioreactor was modified to deliver tissue perfusion and surface shear stress simultaneously to the growing construct. The *perfusion concentric cylinder bioreactor* (**PCC**) is shown schematically in Figure 9. All major geometric parameters were maintained between the concentric cylinder (**CC**) and perfusion concentric cylinder bioreactor. Notably, the outer rotating cylinder, motor and housing are identical. The PCC bioreactor has the same capacity (16 constructs) as the CC bioreactor. Notable differences between the CC and the PCC are: (1)

the two rows of constructs are aligned above each other and not staggered as in the PCC, (2) media volume increased from 62 ml (CC) to 400 ml (PCC) to fill the space around the construct arms, and (3) while the gap between the construct arm and the outer cylinder is the same in the PCC and the CC bioreactor (3 mm), in the PCC, constructs are recessed 2 mm into the construct arm and thus do not protrude into the flow field like in the PCC. The PCC bioreactor was designed to stimulate chondrogenesis by simultaneously applying low shear stress (~0.4 dyne/cm^2) across the surface of cell-seeded scaffolds (by rotation of the outer cup) and media perfusion (0.6 ml/construct/min) through the construct thickness by recirculating media from the outer cup through the (hollow) inner tube.

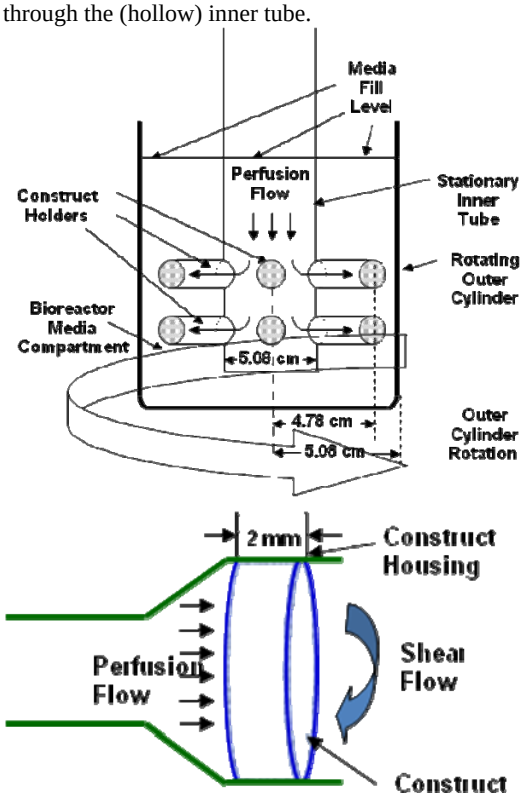

Figure 9: Perfusion Concentric Cylinder Bioreactor. Bioreactor schematic (upper panel) and close-up of an individual construct & housing showing construct placement (lower panel).

Under seeding and culture conditions similar to those described for the CC bioreactor above, chondrocyte seeding and matrix deposition in two-week pilot studies using the PCC were similar to that observed in the concentric cylinder bioreactor ((Saini and Wick 2003; Saini and Wick 2004; Rangamani 2005). The PCC bioreactor stimulates cartilage growth over the course of four weeks, supported by the appearance of glycosaminoglycan (GAG) and collagen type II; markers for articular cartilage. The shear plus perfusion condition in the PCC bioreactor results in a 12-fold increase in cell number per construct compared to 7-fold increase in the CC bioreactor under otherwise identical conditions. However, constructs cultured in the PCC bioreactor had a significantly less glycosaminoglycan and collagen content (0.7 mg and 0.8 mg per construct, respectively) compared to constructs grown in the CC bioreactor for 28 days (1.3 mg of GAG and 1.8 mg of collagen per construct, respectively) (Farooque 2008). CFD simulation of flow fields in the PCC bioreactor revealed that 80% of the scaffold is exposed to fluid velocities in the range of 0.02 and 0.04 cm/s and approximately 20% at the edge experienced recirculation with an average flow rate of 0.6 cm/s (Farooque 2008). The shear stress on the construct surface in the PCC bioreactor ranges from 0.02 dyn/cm^2 to 3 dyn/cm^2. Because the outer cup rotation direction is reversed every 12 hours, the center of the scaffold surface experiences a constant shear stress of 0.4 dyn/cm^2, while the construct periphery is exposed to oscillations between high and low shear stresses (Farooque 2008).

Comparison of the two fluid flow regimes (shear stress only vs. shear stress plus perfusion) demonstrates that hydrodynamic loading is a variable that regulates cell proliferation and matrix deposition. By varying the magnitude and duration of the applied shear stresses in tissue composition architecture and function is regulated. Thus, along with growth factors, nutrient transport, scaffold properties and cell sourcing, hydrodynamic loading (shear stress) is an important parameter available to optimize tissue growth and maturation in tissue engineering bioprocesses.

CONCLUSIONS

- Hydrodynamic loading of tissues in bioreactor culture is an important process variable to regulate tissue growth and maturation.
- Bioreactors with well-defined and mechanical loading and nutrient transport are suitable for tissue growth for bench scale experiments and large-scale tissue production.
- While bioreactor operating conditions must be validated experimentally, accurate CFD modelling of mechanical loading and nutrient transport are important for bioreactor and bioprocess design
- Bioreactor transport coupled with tissue growth models are necessary to accurately model tissue development 3-D architecture.

REFERENCES

BILODEAU, K. and D. MANTOVANI (2006). "Bioreactors for tissue engineering: focus on mechanical constraints. A comparative review." Tissue Eng **12**(8): 2367-83.

BRAMA, P. A., J. M. TEKOPPELE, et al. (2000). "Topographical mapping of biochemical properties of articular cartilage in the equine fetlock joint." Equine Vet J **32**(1): 19-26.

BRIGHTON, C. T. and R. B. HEPPENSTALL (1971). "Oxygen tension in zones of the epiphyseal plate, the metaphysis and diaphysis. An in vitro and in vivo study in rats and rabbits." J Bone Joint Surg Am **53**(4): 719-28.

CARTWRIGHT, T. (1994). Animal cells as bioreactors. New York, NY, USA, Cambridge University Press.

CARVER, S. E. and C. A. HEATH (1999). "Semi-continuous perfusion system for delivering intermittent physiological pressure to regenerating cartilage." Tissue Eng **5**(1): 1-11.

CHAN, B. P. and K. W. LEONG (2008). "Scaffolding in tissue engineering: general approaches and tissue-specific considerations." Eur Spine J **17 Suppl 4**: 467-79.

CHUNG, C. and J. A. BURDICK (2008). "Engineering cartilage tissue." Adv Drug Deliv Rev **60**(2): 243-62.

DARLING, E. M. and K. A. ATHANASIOU (2003). "Articular cartilage bioreactors and bioprocesses." Tissue Eng **9**(1): 9-26.

ELDER, B. D. AND K. A. ATHANASIOU (2009). "Effects of temporal hydrostatic pressure on tissue-engineered bovine articular cartilage constructs." Tissue Eng Part A **15**(5): 1151-8.

FAROOQUE, T. (2008). Biochemical and Mechanical Stimuli for Improved Material Properties and Preservation of Tissue-Engineered Cartilage. School of Chemical and Biomolecular Engineering. Atlanta, GA, Georgia Institute of Technology. **PhD**.

GEMMITI, C. V. AND R. E. GULDBERG (2006). "Fluid flow increases type II collagen deposition and tensile mechanical properties in bioreactor-grown tissue-engineered cartilage." Tissue Eng **12**(3): 469-79.

HU, J. C. AND K. A. ATHANASIOU (2006). "The effects of intermittent hydrostatic pressure on self-assembled articular cartilage constructs." Tissue Eng **12**(5): 1337-44.

HUNZIKER, E. B., T. M. QUINN, ET AL. (2002). "Quantitative structural organization of normal adult human articular cartilage." Osteoarthritis Cartilage **10**(7): 564-72.

HWANG, N. S. AND J. ELISSEEFF (2009). "Application of stem cells for articular cartilage regeneration." J Knee Surg **22**(1): 60-71.

INCROPERA, F. P. AND D. P. DEWITT (1996). Fundamentals of heat and mass transfer. New York, Wiley, p. 850.

INTELAB CORPORATION (2009). Worldwide Markets and Emerging Technologies for Tissue Engineering and Regenerative Medicine. Huntington Beach, CA, Life Science Intelligence, Inc.

JUNG, Y., S. H. KIM, ET AL. (2008). "Cartilaginous tissue formation using a mechano-active scaffold and dynamic compressive stimulation." J Biomater Sci Polym Ed **19**(1): 61-74.

KLEIN, T. J., J. MALDA, ET AL. (2009). "Tissue Engineering of Articular Cartilage with Biomimetic Zones." Tissue Eng Part B Rev.

KOCK, L. M., R. M. SCHULZ, ET AL. (2009). "RGD-dependent integrins are mechanotransducers in dynamically compressed tissue-engineered cartilage constructs." J Biomech **42**(13): 2177-2182.

LEE, M. S., M. C. TRINDADE, ET AL. (2002). "Effects of shear stress on nitric oxide and matrix protein gene expression in human osteoarthritic chondrocytes in vitro." J Orthop Res **20**(3): 556-61.

MACPHERSON, J. V., D. O'HARE, ET AL. (1997). "Quantitative spatially resolved measurements of mass transfer through laryngeal cartilage." Biophys J **73**(5): 2771-81.

PANCRAZIO, J. J., F. WANG, ET AL. (2007). "Enabling tools for tissue engineering." Biosens Bioelectron **22**(12): 2803-11.

PAZZANO, D., K. A. MERCIER, ET AL. (2000). "Comparison of chondrogensis in static and perfused bioreactor culture." Biotechnol Prog **16**(5): 893-6.

Poole, A. R., T. Kojima, et al. (2001). "Composition and structure of articular cartilage: a template for tissue repair." Clin Orthop Relat Res(391 Suppl): S26-33.

RAIMONDI, M. T., M. MORETTI, et al. (2006). "The effect of hydrodynamic shear on 3D engineered chondrocyte systems subject to direct perfusion." Biorheology **43**(3-4): 215-22.

RANGAMANI, P. (2005). Bioprocessing Conditions for Improving Material Properties of Tissue Engineered Cartilage. School of Chemical and Biomolecular Engineering. Atlanta, GA, Georgia Institute of Technology. **MS**.

ROGERS, B. A., C. L. MURPHY, ET AL. (2006). "Topographical variation in glycosaminoglycan content in human articular cartilage." J Bone Joint Surg Br **88**(12): 1670-4.

SAINI, S. AND T. M. WICK (2003). "Concentric cylinder bioreactor for production of tissue engineered cartilage: effect of seeding density and hydrodynamic loading on construct development." Biotechnol Prog **19**(2): 510-21.

SAINI, S. AND T. M. WICK (2004). "Effect of low oxygen tension on tissue-engineered cartilage construct development in the concentric cylinder bioreactor." Tissue Eng **10**(5-6): 825-32.

SCHINAGL, R. M., D. GURSKIS, ET AL. (1997). "Depth-dependent confined compression modulus of full-thickness bovine articular cartilage." J Orthop Res **15**(4): 499-506.

SCHULZ, R. M. AND A. BADER (2007). "Cartilage tissue engineering and bioreactor systems for the cultivation and stimulation of chondrocytes." Eur Biophys J **36**(4-5): 539-68.

SCHULZ, R. M., N. WUSTNECK, ET AL. (2008). "Development and validation of a novel bioreactor system for load- and perfusion-controlled tissue engineering of chondrocyte-constructs." Biotechnol Bioeng **101**(4): 714-28.

SHAPIRO, I. M., K. D. MANSFIELD, ET AL. (1997). "Chondrocytes in the endochondral growth cartilage are not hypoxic." Am J Physiol **272**(4 Pt 1): C1134-43.

SHARMA, B. AND J. H. ELISSEEFF (2004). "Engineering structurally organized cartilage and bone tissues." Ann Biomed Eng **32**(1): 148-59.

VACANTI, J. P. AND R. LANGER (1999). "Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation." Lancet **354 Suppl 1**: SI32-4.

VILLANUEVA, I., C. A. WEIGEL, ET Al. (2009). "Cell-matrix interactions and dynamic mechanical loading influence chondrocyte gene expression and bioactivity in PEG-RGD hydrogels." Acta Biomater.

VINATIER, C., D. MRUGALA, ET AL. (2009). "Cartilage engineering: a crucial combination of cells, biomaterials and biofactors." Trends Biotechnol **27**(5): 307-14.

WALDMAN, S. D., C. G. SPITERI, ET AL. (2003). "Long-term intermittent shear deformation improves the quality of cartilaginous tissue formed in vitro." J Orthop Res **21**(4): 590-6.

WENDT, D., M. JAKOB, ET AL. (2005). "Bioreactor-based engineering of osteochondral grafts: from model systems to tissue manufacturing." J Biosci Bioeng **100**(5): 489-94.

WILLIAMS, K. A., S. SAINI, ET AL. (2002). "Computational fluid dynamics modeling of steady-state momentum and mass transport in a bioreactor for cartilage tissue engineering." Biotechnol Prog **18**(5): 951-63.

