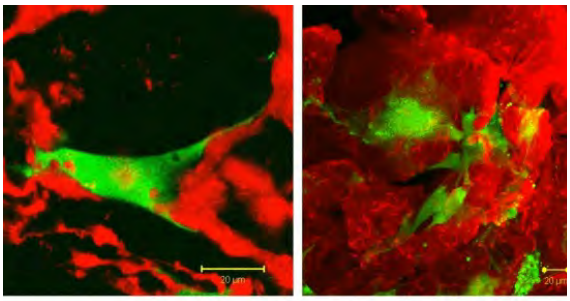

Confocal images showing osteoblast cells (green): (a) bridging and, (b) flatly attached, to struts in a highly porous collagen-GAG scaffold (red). Our results demonstrate that the mechanism of attachment has important consequences for the response of the cells to biophysical stimulus.
(Jungreuthmayer et al. Tissue Eng Part A. 2009 15(5):1141-1149)

(7.O1) MICROBIOREACTORS FOR CARDIAC TISSUE ENGINEERING

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In contractile tissues such as myocardium, functional properties are directly related to cellular orientation and elongation. Thus, tissue engineering of functional cardiac patches critically depends on our understanding of the interaction between multiple guidance cues such as topographical, adhesive and electrical cues.

One of our goals was to determine the interactive effects of contact guidance and electrical field stimulation on elongation and orientation of cardiomyocytes and fibroblasts, major cell populations of the myocardium. We developed a precise microfabricated system, incorporating topographical and electrical cues on a single chip. The cell culture chips were created by hot embossing of polystyrene, with microgrooves and microridges of precisely defined depth, width and periodicity. The two gold electrodes were electrodeposited 1cm apart such that the microgrooves in between were oriented either parallel or perpendicular to the electrodes. Importantly, simultaneous application of biphasic electrical pulses and topographical cues resulted in gap junctions confined mainly at the cell-cell ends rather than the punctuate distribution normally found in neonatal cells. Overall, we observed that i) cardiomyocyte and fibroblast elongation on smooth surfaces was significantly enhanced by electrical field stimulation and ii) topographical cues were a significantly stronger determinant of cardiomyocyte orientation than the electrical field stimulation. The orientation and elongation response of cardiomyocytes was completely abolished by inhibition of actin polymerization and only partially by inhibition of phosphatidyl-inositol 3 kinase pathway.

Our current efforts focus on development of a microarray of cardiac organoids for drug and cell testing, where tissues are created by self-organization of embryonic stem cell derived cardiomyocytes around two microposts. Additionally, to create biological wires of 1-10cm scale capable of propagating electrical impulses, we employ self organization of cardiomyocytes around sutures placed in microbioreactor wells. Thus, the three microbioreactor configurations we developed provide control of cellular microenvironment to enable engineering of functional cardiac organoids.

Keywords. Bioreactor, microenvironment, cardiomyocyte, electrical stimulation

(7.O2) NEW GENERATION BIOREACTOR FOR IN VITRO ENGINEERING OF TUBULAR STRUCTURES

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Introduction. The clinical need to replace tubular organs with functional substitutes, where conventional reconstruction techniques are inadequate, is growing exponentially. Recently, there has been a growing optimism that cell-based tissue engineering methods may provide effective solutions and early promising results have been reported. We have already shown in a clinical setting that our previously developed double-chamber rotating bioreactor allowed multiple cell types to be grown onto a decellularized trachea [1,2]. Here we introduce a second-generation bioreactor for tubular construct engineering with improved functionalities.

Methods. Major aims of the bioreactor design were to: allow proper seeding and culturing of different cell types on both sides of a tubular matrix, promote efficient mass transport within a construct of clinically relevant dimensions and stimulate cells with hydrodynamic stimuli. Modularity, optimization of assembly procedures, control and automation over the entire process were further key requirements.

Results. Our bioreactor combines scaffold pre-tensioning, rotation and luminal perfusion, exposing cells alternatively to liquid and gas phases if half immersed in culture medium. A novel apparatus for the automatic medium exchange was also realised and coupled to the bioreactor, significantly contributing to minimize contamination risks and to protect homeostasis of the culture milieu. The manufactured system was bench-tested under different operating conditions, and preliminary cell culture trials were performed with positive outcome: higher cell survival and much better colonisation throughout the scaffolds thickness with respect to static controls were obtained.

Conclusions. The improved bioreactor is an effective and versatile system that could be used with different scaffolds (Ø, L) to in vitro engineer tubular structures, e.g., trachea and blood vessels. Based on the collected promising results, we have been in further experimental sessions to better investigate its role in driving cell response.

Acknowledgements: Supported by Regione Lombardia and by Harvard Bioscience, Inc. through a sponsored research agreement.

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Keywords. bioreactor, tubular structures, enabling technologies

(7.O3) AUTOMATED, ONLINE, REAL-TIME MONITORING OF CULTURE PARAMETERS IN MULTIPLE INDEPENDENT CHAMBERS OF A PERFUSION BIOREACTOR

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Introduction. Perfusion bioreactors represent a promising possibility for the development of automated, standardized, cost-effective, and safe manufacturing processes of engineered tissue substitutes.

Based on a previously developed perfusion bioreactor for seeding and culture of cell-scaffold constructs (NASA techbrief), in this study we developed and tested an automated device for online, real-time monitoring of critical culture parameters.

Materials and Methods. The bioreactor has been equipped with a motor driven automated sensing system (Fig.1a) and with a customized software (Fig.1b) able to monitor up to 18 independent culture chambers.

Validation tests were performed to monitor pH value within buffers and non pre-equilibrated culture medium without cells, with reference to induced environmental changes monitoring capability.

Moreover, expanded primary human articular chondrocytes were seeded and cultured on collagen (UltraFoam) scaffolds for 7 days in DMEM+10%FBS, with different cell densities. pH and pO2 were optically monitored and ΔpH (difference between pH upstream and downstream the scaffold) was calculated for each chamber. Inoculation of bacteria was performed, so as to simulate possible contamination and detect related changes in pH or pO2.

Results and Discussion. The sensing system was able to detect induced environmental changes due to incubator door opening, medium change and accidental blackout, in non pre-equilibrated DMEM+10%FBS, without cells. Online, real-time parameters monitoring enabled observation of progressive pH drop during cell dynamic culture and of sudden drop both in pH and pO2 due to induced bacterial contamination.

The different cell densities, estimated by DNA and MTT assays at the end of the experiment, could also be discriminated by different ΔpH values, detected by the system, thus giving an index of culture progression, assessable in real-time.

Conclusions. Our perfusion bioreactor, with automated, online monitoring of culture parameters, can represent a step forward towards a reliable device for the safe and automated manufacturing of biological tissues.

Keywords. perfusion bioreactor, online monitoring, automation

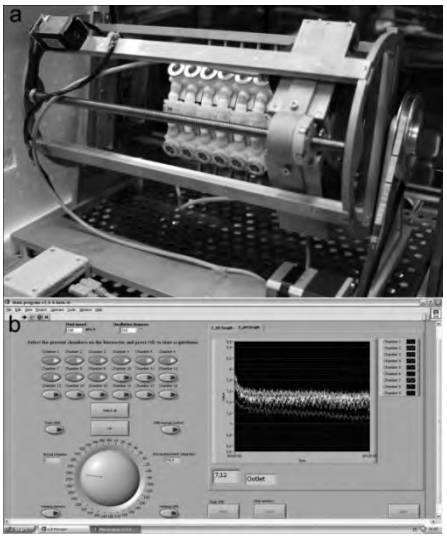

Figure 1: a) The sensorized bioreator system; b) Front panel of the cusutomized software.

(7.O4) MODELING OF FLOW-INDUCED SHEAR STRESS APPLIED ON 3D CELLULAR POROUS SCAFFOLDS

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Introduction. Novel tissue engineering bioreactor systems are designed to overcome the size limitations of engineered tissue, which are dictated by oxygen and nutrient diffusion rates. Our bioreactor system employs direct perfusion through porous biopolymer scaffolds, which is meant to simulate physiological interstitial flow conditions. In order to properly estimate the flow-induced shear stress to which the cells are exposed, a computational fluid dynamics (CFD) model was developed. This model takes into account the complex 3D structure of the porous biopolymer scaffold and the growth of the cell layer and calculates the shear stress distribution as a function of the controllable flow rate and culturing time.

Goals. Develop a CFD model to estimate flow-induced shear stress applied on cells seeded on a porous biopolymer scaffold in a direct perfusion bioreactor, as a function of inflow rate and growing tissue layer thickness. The current model was designed to predict high shear stress values within the physiological range naturally sensed by vascular cells (1–10 dyne/cm^2).

Results. Representational maps of velocity and 3D shear stress distribution were obtained for each of the models. Analysis of the calculated wall shear-stress distribution in the acellular scaffold model shows that while the shear stress values positively correlated with increasing inflow velocities, the distribution pattern remained largely unvaried. As is expected in low Reynolds flow (Re<0.02), the flow regime, while convoluted, was absolutely laminar, and mean shear-stress remained proportional to the inlet velocity.

Conclusions. Our model provides an estimation of the dynamic microenvironment to which cells are exposed in our direct perfusion bioreactor. As such, it represents a useful tool for perfusion bioreactor system design, and provides an added level of control over experimental setups.