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Development Of A Bioreactor For In Situ Optical Imaging

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Introduction

The National Institute of Standards and Technology's (NIST) goal is to develop measurement techniques (metrologies), which will aid in the advancement of emerging technologies. The goal of this project is to develop an *in situ* optical imaging technique that is capable of imaging interactions at the cell/scaffold interface. The ability to image live cells and also image their interactions with the surrounding environment will provide information about the spatial organization of the cells within the scaffold with respect to scaffold design and culture conditions. To do this, a nondestructive/noninvasive *in vitro* imaging technique that is able to penetrate the highly scattering materials of tissue engineering scaffolds is needed. In our laboratory, we have successfully used collinear optical coherent microscopy/confocal fluorescence microscopy (OCM/CFM) to image the 3-D interconnected porous structure of polymeric scaffolds (1,2). In order to perform live cell imaging, a system or bioreactor that can sustain cell viability and allow for imaging was constructed.

Methods and Materials

The bioreactor is made from medical grade stainless steel and is easily sterilized and assembled. A glass coverslip acts as a window to allow for imaging. A coverslip corrected, high numerical aperture (NA = 0.8) objective with a 2 mm working distance is being used for imaging (Fig. 1). The temperature of the bioreactor and the media is being maintained by circulating water through a copper heating element. The combined height of the bioreactor and the heating element must be small enough to allow for imaging in the OCM/CFM as well as in a conventional confocal microscope.

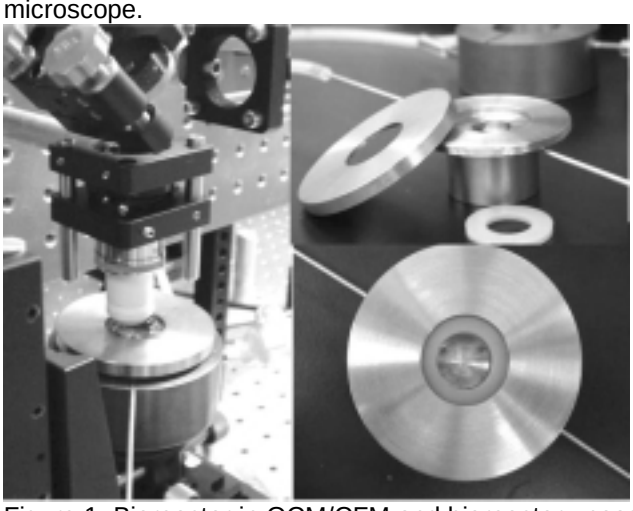

Figure 1. Bioreactor in OCM/CFM and bioreactor unassembled. The bioreactor has three components for assembly - top, coverslip/washer, and base.

Results and Discussion

There are several critical parameters that need to be considered in bioreactor design, all of which are related to maintaining cell viability. The temperature stability of the bioreactor was monitored

via a thermocouple. The circulation bath was able to maintain the temperature of the bioreactor and the media at (37±1)°C for over 5 d.
Another key element in the design of the bioreactor was medium exchange. It has been shown in numerous studies that dynamic cell culture enhances cellular activity by supplying fresh nutrients, removing waste, and applying shear stress to the cells (3). The flow rate through the bioreactor was measured with a flow meter. The flow rate varied between 4 mL/min and 25 mL/min for an empty cell growth chamber. For a cell growth chamber containing a scaffold the flow rate would be significantly lower. Therefore the shear forces on the cells within the scaffold would be lower with the aim to match physiological forces. Figure 2 illustrates how the cell growth chamber fills with media. The media is flowing in from the upper left corner of each image. Within 10 s the chamber is completely filled with media.

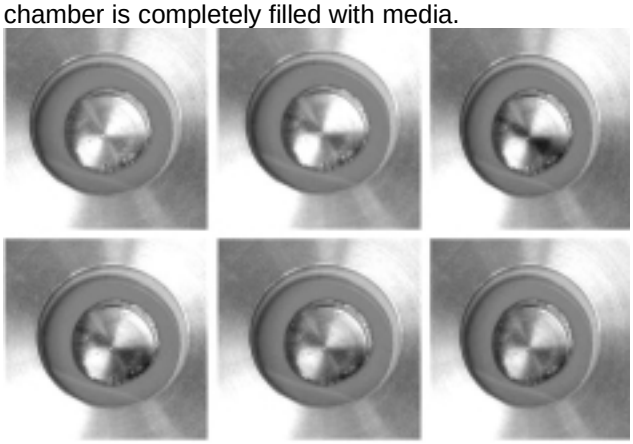

Figure 2. Example of flow of media and filling of the cell growth chamber.

Conclusions

Collinear OCM/CFM is capable of imaging the 3-D interconnected porous network of scaffolds and fixed cells within the scaffold. With the development of the bioreactor it will be possible to image the cell/scaffold interaction real time. The next step in the development of the *in situ* imaging technique and the bioreactor is to relate the flow rate to shear force and further verify cell viability over several days. This will help to further elucidate the connection between structure and function in scaffolds for tissue engineering.

References

1. J.P. Dunkers, M.T. Cicerone and N.R. Washburn, *Optics Express*; 11(23) p. 3074, 2003.
2. J.P. Dunkers and M.T. Cicerone, *Biomaterials Forum*, 25(3) p. 8, 2003.
3. G.N. Bancroft, V.I. Sikavitsast, J. van den Dolder, T.L. Sheffield, C.G. Ambrose, J.A. Jansen, A.G. Mikos, *Proc. Nat. Acad. Sci. USA*, 99(20) p. 12600, 2002.

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$^{+}$Non-statistical data