

Department of Biomedical Engineering  
Biomedical Research Tower, Room 504  
460 West 12<sup>th</sup> Avenue  
The Ohio State University  
Columbus, OH 43210  
leight.1@osu.edu  
614-685-9147

November 1, 2018

Dr. Avital Braiman  
Director of Editorial  
*Journal of Visualized Experiments*  
1 Alewife Center, Suite 200  
Cambridge, MA 02140

Dear Dr. Braiman:

I am pleased to submit an original research protocol entitled “Measuring Global Cellular Matrix Metalloproteinase and Metabolic Activity in 3D Hydrogels”, by Abdulaziz Fakhouri and Jennifer Leight, for consideration for publication in the *Journal of Visualized Experiments*. This manuscript builds on our prior work in which we developed a functionalized hydrogel which enabled 3D culture and facile measurement of matrix metalloproteinase (MMP) activity with a fluorogenic peptide sensor.

In this manuscript, we describe a protocol for measuring cellular matrix metalloproteinase (MMP) and metabolic activity in 3D poly(ethylene glycol) (PEG) hydrogels in a 96-well format. This manuscript provides an in-depth protocol as a companion to our currently reviewed article in *ASSAY and Drug Development Technologies*. This technique involves a fluorogenic MMP-degradable peptide covalently incorporated in a 3D degradable hydrogel, in which cellular MMP and metabolic activity can be measured utilizing a conventional microplate reader and without cellular retrieval nor further sample processing. The modular design of this assay makes it adaptable to the detection of other proteases through the introduction of different fluorogenic peptide sequences. Here, we demonstrate the use of this assay with human melanoma cells encapsulated across a range of cell seeding densities within the MMP sensor functionalized hydrogel assay to determine the appropriate encapsulation density for the working range of the assay. The assay demonstrated here combines 3D cellular culturing, MMP and metabolic activity detection, and is suitable for wide variety of applications.

We believe that this manuscript is appropriate for publication by the *Journal of Visualized Experiments* because it outlines an original technique which researchers in various disciplines can use to encapsulate cells in a 3D *in vitro* microenvironment and measure protease activity with minimal sample processing. Such an assay provides a practical, efficient and easily accessible 3D culturing platform for a wide variety of applications.

We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,



Jennifer L. Leight, Ph.D.  
Assistant Professor of Biomedical Engineering  
The Ohio State University