An Educational Kit for Introducing Microfluidics-based Cell Adhesion Assay in Undergraduate Laboratory (Work in Progress)

Dr. Yan Wu, University of Wisconsin, Platteville

Yan Wu graduated from Tsinghua University, Bejing, China, in 1996 with a bachelor's degree in precision instruments and a minor in electronics and computer yechnology. She received her M.S. degree in mechanical engineering from the University of Alabama in 1998. She received her Ph.D. in electrical engineering from the University of Illinois, Urbana-Champaign, in 2005. Her Ph.D. thesis work was in the area of micro-electro-mechanical systems (MEMS) with a focus on effect of space charges on micro- to nano-scale electrostatic actuation. Upon receiving her Ph.D., she worked as a Postdoctoral Research Associate in the Department of Mechanical Science and Engineering in the University of Illinois, Urbana-Champaign, where worked in multiple projects using scanning probe microscopy to study material properties. In 2009, Yan Wu joined the faculty of the Department of Engineering Physics at the University of Wisconsin, Platteville. From fall 2015 to summer 2016, Yan Wu completed one year of sabbatical as a visiting scholar in the Department of Biomedical Engineering at University of Wisconsin – Madison.

Dr. Theodorus Evan de Groot, University of Wisconsin, Madison
Jorge Camacho
Patrick McMinn, University of Wisconsin, Madison

Graduate Research Assistant

Work in Progress: An Educational Kit for Introducing Microfluidics-Based Cell Adhesion Assay in Undergraduate Laboratory

Yan Wu[1], Ted de Groot[2], Jay Warrick[2], Patrick McMinn[2], John Guckenberger[2], Jorge Camacho[3], and Dave Beebe[2]

[1]Department of Engineering Physics, University of Wisconsin - Platteville
[2]Department of Biomedical Engineering, University of Wisconsin – Madison
[3]Department of Mechanical Engineering, University of Wisconsin – Platteville

Abstract

The study of the adhesion behavior of cells is an active area of academic research and as such, is an increasingly important component of biomedical engineering education. However, in delivering engineering courses, it is often challenging to provide laboratory experience of cell-based adhesion assays to undergraduate students, as the lab work involved is expensive, delicate, and usually requires substantial experimental skill. This article reports the development of a novel lab experience for undergraduate students for which a self-contained microfluidic assay kit was designed to deliver controlled shear stress of fluid flow for detaching adherent cells inside microchannels. Cell adhesion strength is measured by the fraction of cells that remain adhered after the application of a defined shear stress for a fixed duration. Each prepackaged kit consists of one micro-chip containing microchannels and several cartridges containing all of the reagents including cells for each step of the assay. The kit incorporates a simple surface tension-based fluid handling mechanism that enables exchange of fluids between the micro-chip and the cartridges without any handheld pipettes or external equipment. Instructional material was also developed for the kit. In addition to a detailed procedure for the experiment and suggested observation based discussion questions, the kit includes an introduction to microfluidic technology, basic fluidic dynamics concepts, and cell adhesion biology. The instructional material is suitable for junior and senior level undergraduate students in biomedical engineering or any closely related discipline. The relative simplicity and affordability of the kit made it accessible to undergraduate students in a laboratory course, who judged the lab as a strongly positive learning experience**.**

Cell-based assays play an important role in modern biology and biomedicine with applications in clinical diagnostics, drug development, and cell-biology research. The experience of carrying out cell-based assay in an undergraduate laboratory will help students build up the ability to make measurements on and interpret data from living systems, a critical outcome required for undergraduate programs in bioengineering or biomedical engineering in order to receive accreditation from the Accreditation Board for Engineering and Technology (ABET)[1]. However, in delivering engineering courses, it is often challenging to provide laboratory experience of cell-based assays to undergraduate students, as the lab work involved is expensive, delicate, and usually requires substantial experimental skills. We report the development of a microfluidic based assay kit to facilitate undergraduate laboratory experience of live cell measurements. It also serves as a tool to introduce microfluidic technology, a driving force in the current trend of miniaturization of analytical instrumentation. The educational kit

allows students to observe and analyze the change of adhesion behavior of live cells on the channel surface under biochemical digestion. Adhesive interactions between cells and their physical environments are central in developmental biology, tissue maintenance, tissue engineering, cancer progression, and many biotechnological processes. Cell adhesion assay with a microfluidic device allows students to demonstrate their ability to address problems associated with the interaction between living and non-living materials and systems, another critical outcome required by ABET for undergraduate programs in bioengineering or biomedical engineering.[1]

The kit is a new application of the previously published microfluidic-assay platform called ''Kit-On-A-Lid-Assay'' (KOALA)[2] in collaboration with the original inventors. As shown in Figure 1, each KOALA set consists of one KOALA micro-chip (containing 12 microchannels, called the lid) and several KOALA cartridges (called the kit). The cartridges, containing all of the reagents for each step of the assay, can be stored for extended periods of time at temperatures as low as -80° C. Each step of the assay is performed in two simple operations. First, the cartridge is prepared by quickly thawing (less than 1 min is required given the microliter volumes) and removing a protective seal. Second, the reagents from the cartridge are delivered by "clicking" it onto the micro-chip. This fluid exchange takes about 5-30 seconds, after which the cartridge can be disposed of and a new cartridge applied for each of the following steps. The use of surface tension-based methods to drive fluid exchanges in the KOALA platform permits a high level of precision without electronic actuation or external equipment. The source of cells can be from the pre-packaged cells frozen in cryopreservation fluid in a special cryopreservation KOALA cartridge, or a freshly prepared cell suspension cultured in conventional cell culture facility. Using a series of cartridges, a whole cell-based assay can be pre-loaded into the KOALA set. To apply the KOALA platform to cell adhesion assays, we designed a series of micro-channels with different channel depths to deliver different levels of shear flow stress in order to detach the adherent cells inside the micro-channel (Figure 2, right y-axis). Microscope images are used to analyze cell adhesion strength by calculating the percentage of cells that remain adhered after the application of a defined shear flow stress for a fixed duration. Practical applications of cell adhesion assays often involve assessing the effect of certain treatment (e.g., exposure to chemicals) on the ability of cells to adhere. In designing the kit, we chose to use trypsin as the biochemical treatment. Trypsin is an enzyme that weakens cell adhesion by cleaving peptides chains of the cell adhesion molecules. Students can study the change of adhesion strength of cells as a function of trypsin treatment time and temperature. This gives an opportunity for students to design experiments and build data interpretation skills from inquiry-based learning.

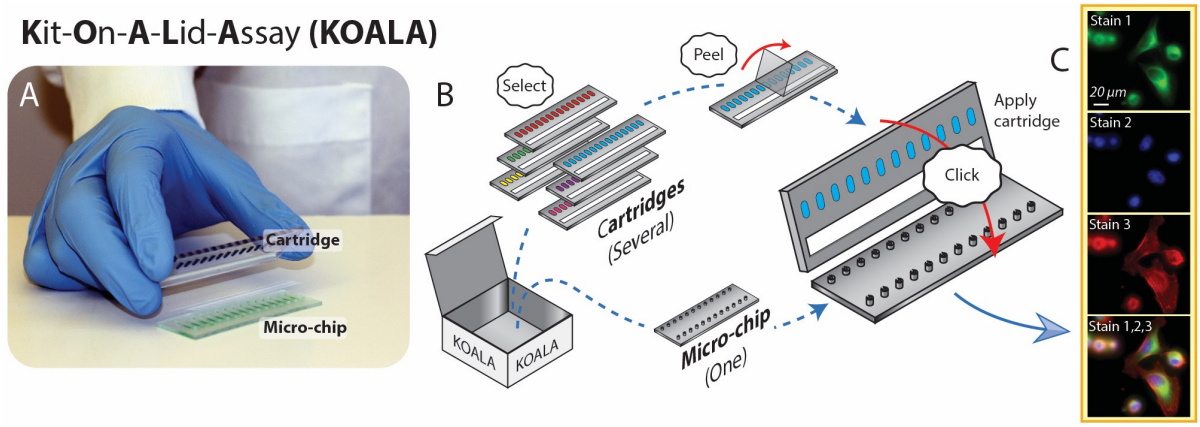

Figure 1. (A) Operation of the KOALA is as simple as pressing a cartridge onto a micro-chip. (B) Each kit consists of several cartridges prefilled with reagents, and one micro-chip containing the microchannels. Each step of the assay is completed by "clicking" one cartridge onto the micro-chip. The cells are cultured and imaged in the micro-chip. (C) An assay performed in KOALA demonstrating immunostaining capabilities (Stain 1 – Anti-tubulin (tubulin stain) / Stain 2 – DAPI (nuclear stain) / Stain 3 – Phalloidin (actin stain))

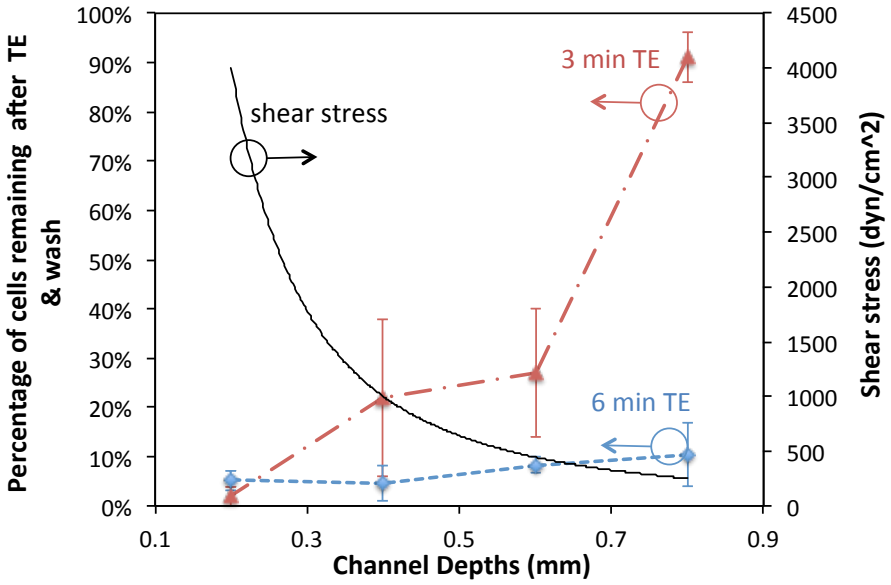

Figure 2 Experimental results (left y-axis) show that remaining cell percentage is depended on the channel depths of the fluid flow. Dashed line is data from 6min TE treatment and dash-dotted line is from 3min TE treatment. Decreasing channel depth increases the shear stress of fluid flow (solid line, right y-axis) for detaching cells.

As shown in Figure 2 (right y-axis), the shear stress due to fluid flow inside the micro-channels increases from 160 dyn/cm^2 to 2700/cm^2 as channel depth decreases from 0.8mm to 0.2mm according to the Poiseuille model.[3] We carried out experiments using mouse epithelial cell line NMμMG and 0.25% Trypsin-EDTA (TE) solution. Results in Figure 2 (left y-axis) show that the high flow shear stress in the channel with 0.2mm depth almost completely washed

off cells. The adhesion strength dependence on the time of trypsin treatment is obvious in channels with lower shear stress. The blue dashed line is the data from 6min trypsin treatment and the red dash-dotted line is from 3min trypsin treatment, all at room temperature. Cells show weaker adhesion after 6min trypsin treatment than after 3min trypsin treatment.

The instructional material developed for the kit serves as a resource for educators to adapt the kit to their own curriculum. There is a background information section that covers microfluidic technology, basic fluid dynamics concepts, and cell adhesion biology. It also contains a detailed procedure for the experiment and suggested discussion questions. Table I shows the topics covered in the instructional material. The content of the instructional material is highly multi-disciplinary. It is suitable for junior and senior level undergraduate students in biomedical engineering or any closely related discipline. Skills that are desirable for students to have before using the kit include microscope operation, cell counting, image processing skills with software tools such as ImageJ, and data analysis skills such as data plotting and basic statistical analysis using Excel. We applied the kit as a lab module to the students who enrolled in a senior level course on Microfluidics. Results of student self-assessment survey according to six ABET learning outcomes show that students judged the lab as a strongly positive learning experience. Direct assessment data of student learning is not available at this point of time. The authors plan to make the kit available to Biomedical Engineering Education community after further testing and development.

Table I Topics covered in the instructional material

Subject	Topics
Cell Biology	Extracellular Matrix (ECM) and its impact to cell function
	Cell adhesion mechanism
	Biomedical implications of cell adhesion behavior, example: *anoikis* and change in cell adhesion behavior of cancer cells
	Cell morphology
Biomaterial	Surface modification of material for enhancing cell adhesion
Biochemistry	Trypsin and the mechanism for trypsin to weaken cell adhesion
	Temperature dependence of biochemical reaction.
Physiology	Osmotic balance in cells, the function of PBS buffer
Fluidic dynamics	Laminar flow
	Shear stress calculation in micro channels.
Microfluidic technology	Lab-on-a-chip devices and the reasons for the current trend of miniaturization of analytical instrumentation
	KOALA device design, surface tension based micro-scale fluid handling

References

1. *Handbook of research on biomedical engineering education and advanced bioengineering learning: interdisciplinary concepts*. (Medical Information Science Reference, 2012).
2. Berthier, E. et al. Kit-On-A-Lid-Assays for accessible self-contained cell assays. *Lab Chip* **13,** 424–431 (2013).
3. Lu, H. et al. Microfluidic shear devices for quantitative analysis of cell adhesion. *Anal Chem* 76, 5257-5264 (2004).