

## **Direct Writing Biological Patterns & Constructs onto Fabrics**

**Project no.:** F06-CL02

**Competency:** Fabrication

**Project Team:**

Leader: Yong Huang/Clemson University/Advanced manufacturing process development, modeling, optimization, monitoring and control

Members: Douglas Chrisey/Rensselaer Polytechnic Institute/Direct writing of electronic circuits and biological systems

Xuejun Wen/Clemson University and Medical University of South Carolina/Bioengineering

Bhuvnesh C. Goswami/Clemson University/Textile engineering

### **1. GOAL STATEMENT**

The goal of this project is to explore knowledge of direct writing biological patterns and constructs such as biomaterials, proteins, and mammalian cells onto textile substrates using a novel matrix assisted pulsed laser evaporation direct-write (MAPLE DW) technique. Through this work, we expect to gain understanding of direct writing biological patterns and constructs onto fabrics to eventually create biological or medical microdevices embedded in fabrics. Such intelligent textiles are expected to find wide acceptance in healthcare and military applications.

### **2. ABSTRACT**

Intelligent textiles, also known as smart fabrics, have increasingly attracted attention due to their potential to provide platforms for additional functionality that will impact next-generation military and commercial market applications. Recent advances in genetically engineered biological constructs, tissue engineering, and implantable bio-sensors show great potential, when integrated with textile products, for healthcare and military applications. Through such advances, bio-textiles have a dual purpose role as both bio-actuator and bio-sensor. To efficiently integrate such bio-actuators and bio-sensors into a broad line of textile products based on different functional demands, novel fabrication processes must be pioneered. Among the different direct-write technologies, MAPLE DW has proven to be a rapid prototyping tool that forms mesoscopic patterns and structures of living cells as well as active proteins over various substrates. We have selected MAPLE DW to direct write biological patterns & constructs onto fabrics. MAPLE DW apparatus is being built at Clemson University to assist the proposed research on effectively direct writing biological patterns and constructs onto textile substrate. ABAQUS, finite element analysis software, has been used to model cell stress and strain information during cell direct writing, which is due to the impact between pulsed laser ejected cell droplet and receiving hydrogel coating buffer. The work thus far is laying down a foundation for future experimental and theoretical modeling effort in making fabric-based bio-actuators and bio-sensors.

### **3. INTRODUCTION**

Intelligent textiles, also known as smart fabrics, have increasingly attracted attention due to their potential to provide platforms for additional functionality that will impact next-generation military and commercial market applications. The significance and impact of

intelligent textiles on the US textile industry have been consistently recognized by NTC in fabricating bio-related intelligent textile products in the domain of both fabrication and materials competency. Recent advances in genetically engineered biological constructs, tissue engineering, and implantable bio-sensors show great potential, when integrated with textile products, for healthcare and military applications. Through such advances, bio-textiles have a dual purpose role as both bio-actuator and bio-sensor. They can be further connected with embedded computation and other non-bio sensing modules as wearable motherboards.

To efficiently integrate such bio-actuators and bio-sensors into a broad line of textile products based on different functional demands, novel fabrication processes must be pioneered, which also provide a complementary capacity to current NTC supported research. We selected direct-write technologies to tackle this challenge due to their powerful potential in writing electric circuits and biomaterials for tissue engineering and array-based biosensors based on computer-aided design [1]. Direct-write technologies include any techniques or processes capable of depositing, dispensing, or processing different types of materials over various surfaces. These technologies include plasma spray, laser particle guidance, matrix-assisted pulsed-laser evaporation (MAPLE), laser chemical vapor deposition (CVD), micropen, ink jet, e-beam, focused ion beam, and several novel liquid or droplet microdispensing approaches [1]. Each technique has its own merits and shortcomings. During a typical direct-write approach, patterns or layered structures are built directly without the use of masks, allowing rapid prototyping.

#### **4. MAPLE DW APPARATUS**

Developed at the Naval Research Laboratory, MAPLE DW has proven to be a rapid prototyping tool that forms mesoscopic patterns and structures of living cells as well as active proteins over various substrates [2-4]. Different from various established techniques in patterning cells, MAPLE DW forms cell patterns without the use of masks, stamps, etching, or other lithographic tools [3]. The depositing resolution can be small as 10  $\mu\text{m}$  [3] and the depositing volume per drop can be smaller than 10 pL [4]. Since biomaterials are embedded in a matrix to shield it from damage by the incident laser, the observations on the viability of living cells after direct writing over Si (111), glass and nutrient hydrogel plates are very promising [3]. These process attributes satisfy the main concerns such as resolution and cell protection in cell printing [5]. Compared with other laser assisted writing approaches [6,7], the MAPLE DW technique excels in providing more robustness in embedding the pattern/construct into fabrics, a laser machining function to modify fabric surfaces for better adhesion, and a higher direct write speed of greater than 1 m/sec.

Figure 1 shows a schematic of the MAPLE DW apparatus being assembled at Clemson University. The biomaterial or biological unit to be transferred is mixed at room temperature in a UV-absorbent matrix and coated onto a quartz disk that is UV transparent. A focused UV excimer laser pulse (wavelength of 193 nm, frequency of 10 Hz, FWHM width of 30 ns, and fluence of 0.01-0.5 mJ/cm<sup>2</sup>) is directed through the backside of the quartz support so that the laser energy first interacts with the matrix at the quartz interface. The laser pulse is focused at the matrix-support interface by a UV microscope objective that also serves as an optical guide to determine the area of the matrix to transfer. Layers of matrices near the support interface evaporate due to localized heating from electronic and vibrational excitation. This sublimation further releases the remaining material from the interface by uniformly ejecting it away from the quartz support to the conduit surface, which is 0.25 to 10 mm under and on the X-Y-Z translation stage. Different spatial arrangements of biological patterns and constructs are to be created by

controlling the translation stage. The MAPLE DW is being set up at Clemson University and is expected to be ready for bacteria writing trials at the end of this year.

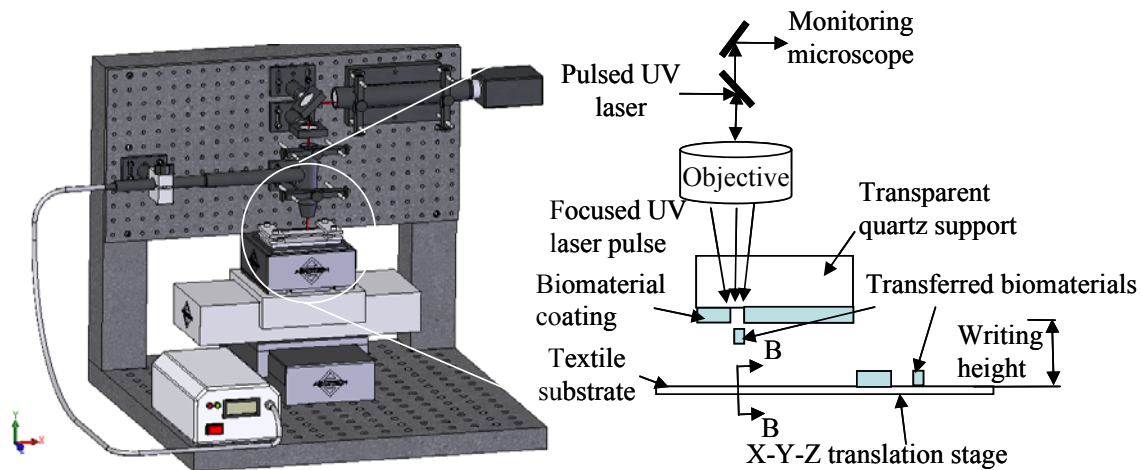


Fig. 1. Proposed MAPLE DW apparatus for biological pattern/construct direct writing

## 5. NUMERICAL STUDY OF CELL DROPLET AND HYDROGEL COATING IMPACT DYNAMICS

### 5.1. Problem Statement

Cell viability after direct writing need consider both mechanical and biochemical damages. Mechanical damage to cells, especially fragile mammalian cells, still poses a significant challenge to achieve a perfect cell viability post cell transfer. For example, it was found that the transferred cell viability depends on the cell droplet ejection speed and substrate culture coating thickness in MAPLE DW, in which cell droplet was ejected from a quartz carrier to a receiving substrate due to the pulsed laser generated evaporation pressure [8]. High-speed imaging discovered that the velocities of MAPLE DW-ejected material can range from 50 to 1000 m/s [9]. The transferred cell droplets decelerate after ejection, and sometimes die if the impact between cell and receiving culture coating/receiving substrate leads to cell membrane rupture. For such circumstances, receiving coating, if necessary, is typically selected based on a trial-and-error approach to avoid cell damage. For safe and reproducible cell direct writing, the impact induced cell damage/membrane rupture must be understood in addition to biological property research. The following study aims to understand cell stress and strain conditions during direct writing using finite element method (FEM).

### 5.2. Computational Procedure

The proposed problem is formulated for a generic cell direct writing process during the MAPLE DW process. Once a cell droplet, typically enclosed by a hydrogel, is ejected from printer orifice or matrix quartz support with an initial velocity, it travels through the air first. Eventually, the cell droplet reaches a receiving substrate, typically a glass slide coated with hydrogel that allows for cell adhesion and growth, and reduction of cell impact during deposition as shown in Fig. 2. This study assumes the cell is enclosed by hydrogel to form a droplet, and the receiving coating is also hydrogel-based.

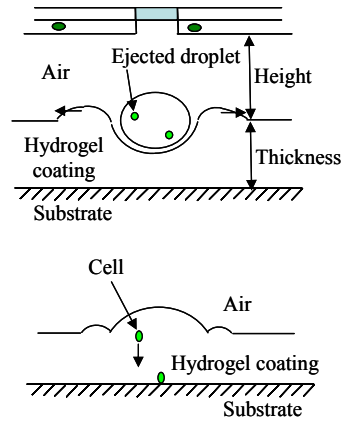


Figure 2. Schematic of laser direct writing

During the whole impact process, cells undergo significant deceleration and survive a much higher maximum force than they are capable of under steady state conditions. The governing equations for this impact process can be described using the conservation equations of mass, momentum and energy, respectively as follows:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho V) = 0 \quad (1)$$

$$\rho \frac{\partial V}{\partial t} + \rho (V \cdot \nabla) V = \nabla \cdot \sigma + \rho f \quad (2)$$

$$\rho \frac{\partial E}{\partial t} + \rho (V \cdot \nabla) E = \sigma : (\nabla V) + \rho \frac{\partial Q}{\partial t} \quad (3)$$

where  $t$  is used to denote the time,  $\rho$  is the density,  $V$  the velocity,  $\sigma$  the Euler stress tensor,  $f$  the body force per unit mass,  $E$  the specific internal energy,  $Q$  the energy supplied from environment, while  $\nabla$  is the gradient operator,  $\nabla \cdot$  the divergence operator,  $:$  the tensor double dot product operator. The above equations hold true for cells and both hydrogels of the droplet and receiving coating. Besides boundary and initial conditions, proper material models, which include equation of state, constitutive model and failure criteria, are also indispensable in solving Equations (1-3). An equation of state is used to define the corresponding functional relationship between pressure, density and internal energy. A constitutive model defines the stress dependence of related strain, strain rate and temperature. In addition, a material model generally includes failure criterion, i.e. an equation describing stress and/or strain condition, when obtained, causes the material to fracture and lose its ability to support stresses.

### 5.3. Simulation Results and Discussion

#### FEM Model Setup

ABAQUS 6.4 Explicit solver, coupled with an auto-adaptive remeshing approach, is used to model the proposed impact process. The FEM explicit technique was developed to solve the inefficiency of the implicit solution for complex, dynamic problems. The most attractive feature of the explicit method is that it gets rid of the global tangent stiffness matrix which is indispensable for implicit methods. As a result, it reduces computational cost. The explicit procedure integrates through time by using many small time increments. The central-difference operator is conditionally stable.

To simplify the problem, only a cell is considered inside a droplet, and this cell is in the middle of the droplet. The cell droplet is assumed to impact the hydrogel coating in a normal

direction, and the impact process can be modeled as axisymmetric. The distance from the symmetric axis, where impact happens, to the lateral perimeter of coating is assumed very large compared with the hydrogel coating thickness, so the infinite element of the coating edge is selected to minimize the boundary effect. The initial condition is the droplet velocity after traveling in air with the forward velocity of jetting away from the biological layer. The positive velocity direction is specified vertically downward here. Heat conduction was not considered in this study for simplicity, so the internal energy change only results from the mechanical work.

The schematic of the elements is shown in Fig. 3. For the problem's axisymmetry nature, axisymmetric plane elements are used. Totally 6, 18, and 680 elements are used for the cell, the droplet hydrogel and the hydrogel coating, respectively. Element 1 and Node 1 in Fig. 3 denote the cell portions of interest in the following analysis. Around the potential contact domain (around the selected symmetric axis) in the substrate, dense meshes are used, while meshes become relatively coarse away the selected symmetric axis. This is done for the two reasons: (1) The accuracy at the contact domain can be achieved since denser meshes could better capture the contact feature of interest, and (2) The computation cost can be greatly reduced. Severely distorted element deformation is the main hurdle in this numerical implementation. In order to mitigate this possible severe distortion, the ratio of height to width of the elements in the contact domain is set as large as 3 such that the elements here could undergo large deformation before the simulation stops. Further, the Arbitrary Lagrangian–Eulerian (ALE) formulation based the auto-adaptive re-meshing technique is adopted for its potential to maintaining high quality mesh of large material deformation. The ALE parameters are set as Frequency = 5 and Mesh Sweeps = 3.

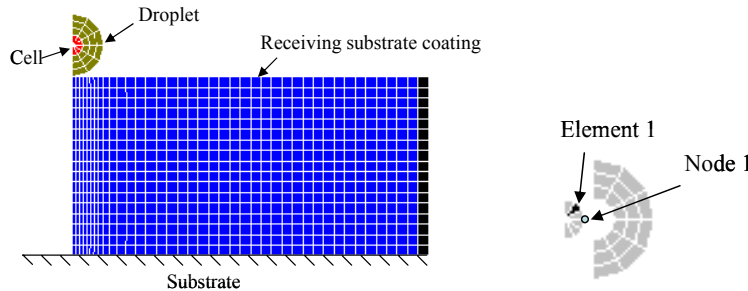


Figure 3. Proposed finite element mesh

Suitable mesh size selection is of importance in capturing the impact process using the explicit procedure for the sake of computational efficiency and accuracy. The mesh size is selected at the order of  $1\ \mu\text{m}$  based on the structure geometry of interest here.

The hyperelasticity model is selected for the hydrogels and the elastic model for the cells. The Mooney-Rivlin model is used to capture the hydrogel hyperelasticity response, and its parameters are interpreted based on the published uniaxial test data [10]. In addition, the indispensable material parameters for the equations of state are also assumed as  $c_0 = 21\ \text{m/s}$ ,  $s = 0.25$ ,  $\Gamma_0 = 2.15$  for hydrogel and  $c_0 = 110\ \text{m/s}$ ,  $s = 0.25$ ,  $\Gamma_0 = 2.15$  for cell [10, 11]. The shear modulus for cell is  $0.6047\ \text{MPa}$  [11, 12]. Density of cell and hydrogel are both taken as  $1000\ \text{kg/m}^3$ . The hydrogel droplet diameter is assumed  $18\ \mu\text{m}$ , and the cell diameter  $6\ \mu\text{m}$ . The failure of hydrogel is controlled by tensile stress, which is given by the hydrostatic stress in ABAQUS. This hydrostatic value ( $25\ \text{KPa}$ ) is obtained through the hydrogel axial tension test data [10].

### Effect of Coating Thickness and Drop Velocity on Cell Stress and Strain

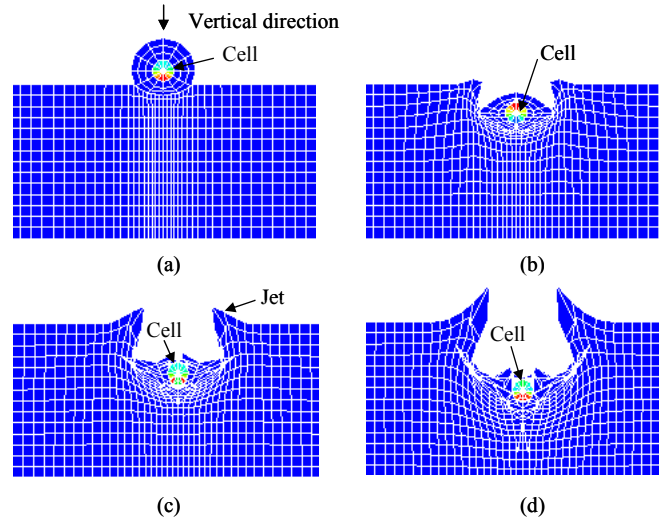


Figure 4. Illustration of the impact process at 0.10490, 0.40497, 0.60688, and 0.85547  $\mu\text{s}$  sequentially

Some representative simulation results of this cell droplet and hydrogel coating impact process are shown in Fig. 4. For this simulation, cell droplet has an initial impact velocity of 50 m/s and hydrogel has a thickness of 51  $\mu\text{m}$ . It can be seen that as the contact time increases, the hydrogel drop enclosing the cell gradually merges into the substrates. Meanwhile, a jet is formed at the interface of the droplet and hydrogel coating contact area. Fracture of the material at the jet front as seen in Figs. 4(b-d) is controlled by a hydrostatic tensile stress condition. Due to the stress wave propagation, the different portion of the cell experiences the stress oscillation. Due to the small stiffness of the hydrogel when comparing with that of cell (KPa vs. MPa), low stress level appears in the substrate as observed from the FEM simulation results.

Different thickness substrates (15, 30, and 51  $\mu\text{m}$ ) are selected to study the effect of hydrogel coating thickness on cell von Mises stress profiles. Element is chosen to output the von Mises stress variation, and the von Mises stress is calculated based on the integration points in the element. As seen in Fig. 5, stress wave is observed. For Fig. 5(a), which representing the impact process with a 51  $\mu\text{m}$  thick coating, the first stress wave peak occurs around 0.33  $\mu\text{s}$  while the second peak around 0.58  $\mu\text{s}$ . The first peak value is obviously higher than the others following, which demonstrates that viability of the cells is mainly determined during the early impact stage. During the incipient stage of the impact, the relative velocity of contact areas is significantly larger compared with those following, thus the pressure impulse acting on the cell is higher. The velocity evolution can also be seen in Fig. 7, which is to be discussed later.

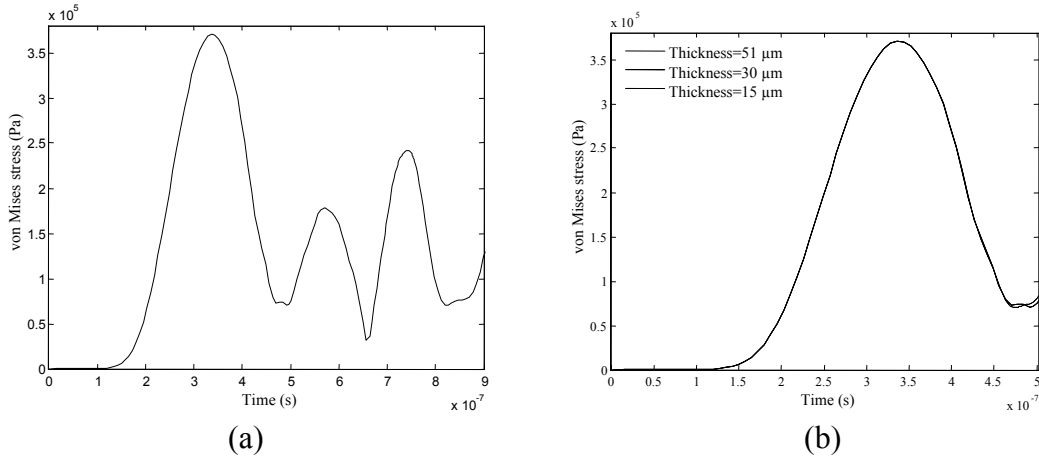


Figure 5. Cell von Mises stress comparison (Node 1 of Figure 3)

It is also found from Fig. 5(b) that the stress wave peak value varies little using different coating thickness. It indicates that the thickness has little impact on the magnitudes of external forces executing on the cell body if the coating thickness value is larger than a certain number, which is to be determined in a future study. As observed, when the coating thickness is larger than 40  $\mu\text{m}$  in writing pluripotent embryonal carcinoma cells, a 100% cell viability can be achieved [8]. More studies on the effect of coating thickness on stress wave profile will be addressed in a future study. However, it should be recognized that the cell damage may be due to the impact between the cell droplet and the hydrogel coating as well as the impact between the cell droplet and the substrate after the droplet travels through the whole coating. Future damage studies on cell damage in direct writing need consider both of two impacts.

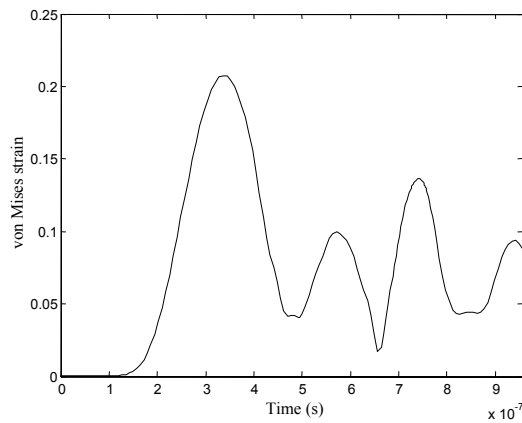


Figure 6. Cell von Mises strain

Fig. 6 also depicts the Element 1 von Mises strain, which matches that of the cell von Mises stress since the linearly elastic model is selected as the cell constitutive equations.

Cell Velocity and Acceleration

Cell center vertical downward acceleration after impact (thickness = 51  $\mu\text{m}$  and  $V_0 = 50$   $\text{m/s}$ ) is shown in Fig. 7. It indicates the cell decelerates as high as  $10^8$   $\text{m/s}^2$ , which is comparable with other predictions (at the order of  $10^7$   $\text{m/s}^2$ ) [8]. Maximum acceleration value depends on material properties of the hydrogel and cell pair and droplet initial velocity.

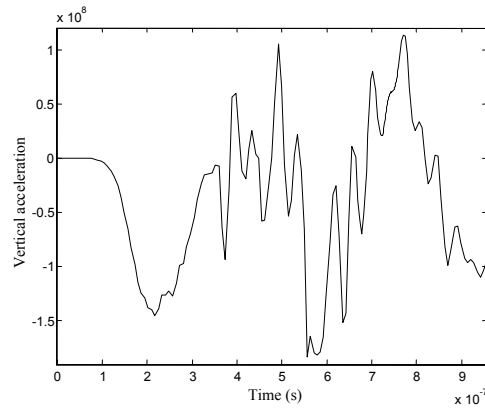


Figure 7. Cell center acceleration

Fig. 8 shows the Node 1 vertical velocity using different coating thicknesses. It shows that the velocity magnitude varies little, a similar tendency observation as that of Fig. 5(b). Generally, the thicker the coating, the lower the cell velocity after penetrating through the coating, if can. Then, generally thicker coating still alleviates the cell impact intensity when cell reaches the bottom of the coating. It is also seen that the cell transient velocity is not monotonically decreasing, but exhibits velocity oscillations during deceleration. The reason for oscillation is to be further identified.

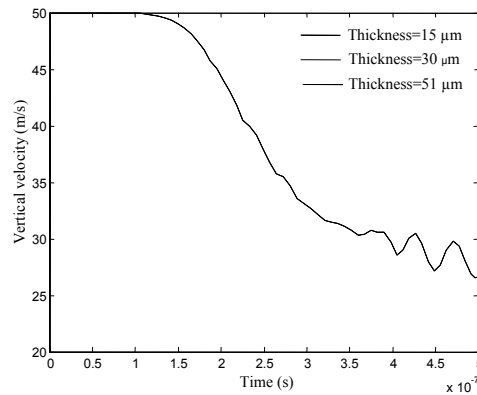


Figure 8. Node 1 vertical velocity

Effect of Substrate Preparation

In order to appreciate the significant effect of hydrogel coating, a contrast between impacts of cells on rigid body substrate is also analyzed. Fig. 9 gives the von Mises stress level difference between a 15 μm thick coating and a purely rigid substrate. The stresses are zero at the early beginning of impact since the element of interest is an upper portion of the cell. It takes 0.1 μs for the stress wave to reach Element 1. It can be seen that the magnitude of von Mises stress when using a rigid substrate is much higher than that of using the hydrogel coating. It is concluded that the substrate hydrogel coating not only provides a growth culture for cells, but also serves as a cushion layer to protect the cell viability in direct writing by significantly reducing the impact-induced von Mises stress.

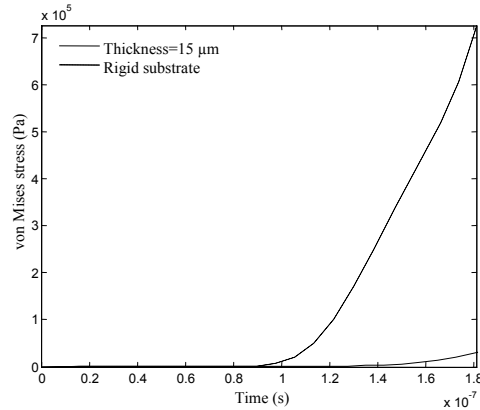


Figure 9. Stress comparison using hydrogel coating and rigid substrate

## 6. CONCLUSIONS AND FUTURE WORK

A study of cell penetration into a hydrogel coating layer has been studied using FEM. The study shows that auto-adaptive mesh based FEM can capture the impact process even when a low elastic modulus hydrogel is involved. Some conclusions can be drawn from this study:

- Hyperelasticity model works for hydrogel nonlinearity large deformation modeling,
- Once the hydrogel thickness is higher than a certain value, there are no pronounced differences among the stresses and velocities profiles at the beginning of the impact, and
- Even a thin hydrogel layer such as 15  $\mu\text{m}$  can significantly reduce the impact-induced stress magnitude.

The future work will focus on:

- MAPLE DW apparatus setup and operation at Clemson University,
- *E. coli* bacteria direct writing onto fabrics with satin weave structure, and
- Receiving hydrogel coating thickness optimization during cell direct writing using FEM.

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**PROJECT WEB SITE ADDRESS:**

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**ACKNOWLEDGEMENTS:**

The project was assisted by graduate students: K. Foy, Y. Lin, and W. Wang at Clemson University. The discussion with Dr. Ty Dawson of Milliken Research Corporation (Spartanburg, South Carolina) is also highly appreciated.