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Computational Modeling of Extracellular Matrix Protein Stamping on Hard Substrates for Biomedical Micro-Devices.

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Abstract

We model the direct transfer of ECM protein to hard substrates that are relevant to biomedical micro devices. To better understand the processes involved in the contact and transfer of protein, mechanics model and finite element simulations were used to simulate the protein transfer process over hard substrates with different stamped–strike layer thickness ratios. We also considered the direct transfer of the ECM protein to bare substrates. Interfacial fracture analyses are used to determine the minimum stamping pressure required for direct transfer. A conservative model is also presented for the stamping of ECM protein on hard substrate. This provides the opportunity for better designs of new stamps and optimization of existing ECM Stamps

Keywords: ECM Protein, stamp, Substrates.

1. Introduction

The native environment of living cells is a 3D scaffold comprising an insoluble aggregate of several highly organized , multifunctional large proteins and glycosaminologlycans(GAGs), collectively known as extracellular matrix (ECM). The ECM provides mechanical support for cells. Most mammalian cells must adhere to a surface to survive. It also profoundly influences the fundamental cellular functions such as migration, proliferation, differentiation and apoptosis of the cells with which it is in contact.

By interacting directly with the ECM, Cells gather information about the chemical and physical nature of the environment, integrate and interpret it, before generating the appropriate physiological response. This behavior expands the application of micro-patterning the ability to direct cells behavior through precisely designed environments, by patterning ECM proteins on substrates [1].

ECM protein are relatively soft materials that provides opportunities for alternative fabrication methods that preserve their low cost potential. The techniques for applying proteins to substrate surfaces include: soft lithography, complimentary photo lithographic techniques, spot arraying, direct writing using tip of an atomic force microscope, and colloidal arrays [2-9]. Micro contact printing [10-12] from the family of the soft lithography methods is mostly used.

Prior studies [13, 14] have shown that agragose gels can be micro-patterned for the stamping of protein and affinity capture of protein from solution and their dissociation by contact printing [15]. Micro-patterning by local transfer of a metal film has also been carried out using cold welding processes [16, 17]. Van der waals forces have also been used to facilitate direct transfer of an organic thin film from a stamp to a substrate [18].

In this work, we model the direct transfer of ECM protein to hard substrates that are relevant to biomedical micro devices that are relevant to implantable devices. The effects of such ordered textures, in particular microgroves and ridges on different cell types have been analyzed by many authors[19, 20] Typically the cells become elongated and aligned in the direction of grooves this phenomenon is known as contact guidance [1] The degree of alignment has also been shown to depend on the groove depth and width.

To better understand the processes involved in the contact and transfer of protein, we model and simulate the direct transfer of ECM protein to hard substrates with different stamped–strike layer thickness ratios. We also

consider direct transfer of the ECM protein to bare substrate surfaces. Interfacial fracture mechanics is used to determine the minimum stamping pressure required for direct transfer.

2. **Theory**

The stamp consisted of a polydimethylsiloxane (PDMS) layer supported on a silica wafer. It was coated with the ECM protein for direct transfer to a photoresist grated glass. A thin layer of ECM protein Strike layer of the same composition as that of the stamped layer was deposited on the grated substrate before the stamping. Figure 1 shows a three dimensional view of the strike layer deposited on the photoresist-grated substrate.

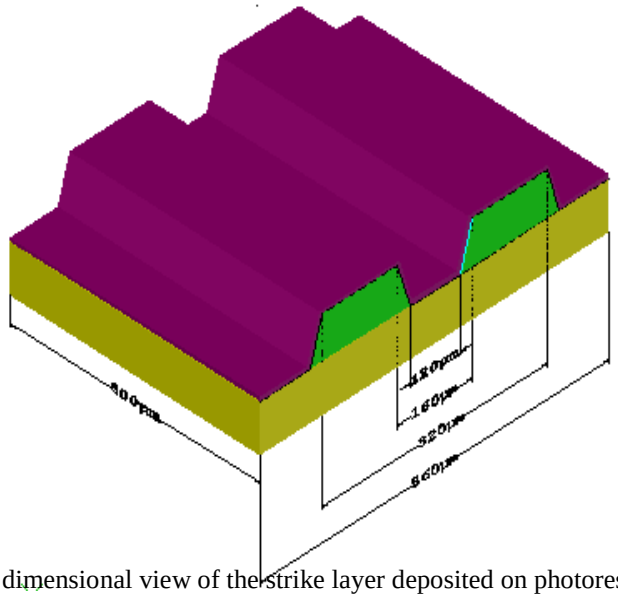

Figure 1. The three dimensional view of the strike layer deposited on photoresist-grated substrate.

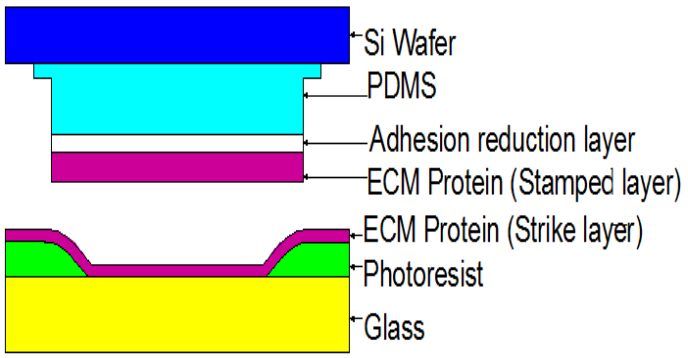

Figure 2. The contact transfer of ECM protein from a stamp to hard or soft substrate.

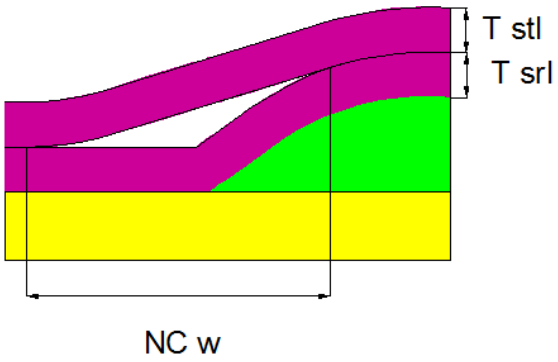

Figure 3. Schematic of non-contact width NCw formation after ECM stamping.

Different stamped layer thickness, $T_{stl,}$ to Strike layer thickness, $T_{srl,}$ ratios were modeled to obtain final ECM layer thickness of 700 nm. The ratios considered were: 7/0, 6/1, 5/2 and 4/3. Note that 700 nm is the natural ECM thickness required for cell fundamental functions. Also the hard substrate has a breadth of 560 µm and length of 600 µm. The model also assumed a typical grate depth of 12 µm and exposed substrate surface width and length of 120 µm and 600 µm respectively. These were modeled on glass substrates.

The stamp pattern was modeled as rectangular shaped stamp with a length of 600 µm and a width of 160 µm. The alignment tolerance was set to 20 µm. Figure 2 shows a diagram of the stamp set up for the stamping of ECM protein . Figure 3 shows the non contact width NCw created as a result of defect from the stamping process.

The Griffith theory of crack propagation was used to obtain the critical crack length from the simulation. Crack propagation occurs when the released elastic strain energy is at least equal to the energy required to generate new crack surface. The stress σ required to create the new crack surface is given by:

$$\sigma = \left(\frac{2E\gamma}{\pi a}\right)^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Under plane strain conditions, equation 1 becomes:

$$\sigma = \left(\frac{2E\gamma}{(1-v^2)\pi a}\right)^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where E is the Young's Modulus of the material, γ is the surface energy density of the material, a is the crack half-length and υ is the poisson's ratio of the material.

Irwin and Orowan suggested that Griffith's equation could be modified by including the plastic work, $\gamma_{p,}$ into the total elastic surface energy required to extend the crack. This results in the modified Griffith equation given below

$$\sigma = \left(\frac{2E(\gamma_s + \gamma_p)}{\pi(1-v^2)a}\right)^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Since $\gamma_s + \gamma_p$ is equal to the energy release rate, G, equation 3 can be expressed as:

$$\sigma \approx \left(\frac{EG}{(1-v^2)a}\right)^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

The critical crack length was fed back to the model to determine the minimum pressure required for stamping for different stamped layer-strike layer thickness ratios.

3. Modelling and Simulation

Finite element simulations were performed using the Abaqus software version 6.12-2. Abaqus is a trademark of Dassault Systemes, Providence, RI, USA. The materials data required for this simulations were obtained from relevant literature and summarized in Table 1.

Since collagen is the main structural component and the most abundant glycoprotein in ECM Protein. Collagen material properties were used to simulate the structural behavior of ECM Protein. The interaction between the stamped layer surface and strike layer pre-deposited glass surface were modeled as hard contact.

Table 1. The material data used for stamping model**.**

Material	**Poison's ratio**	**Young Modulus (GPa)**
ECM Protein	0.30	5.0
Au	0.42	106.0
PDMS	0.49	0.003
photoresist	0.30	8.0
Glass	0.30	68.0

The materials were assumed to be isotropic and elastic and adhesive interaction between the ECM protein surfaces ECM-substrate surfaces were ignored prior to contact. Stamping Pressure ranging from 5MPa to 40 MPa were simulated for the ECM stamping.

4. Results and Discussion

Figure 4 shows the plot of stamping pressure against the resulting non contact width NCw. We need to know the quantitative relationship between NCw and Stamping pressure for a given ECM stamped layer-strike layer thickness ratio. The NCw decreases with increase in stamping pressure. This is because of the increased deformation of ECM stamped layer as result of increased stress in the layer when the stamping pressure is increased. There is decrease in the NCW with reduced Tstl/Tsrl. The reason for this is due to reduction in the stiffness of the ECM stamped layer. If the pressure remains constant ,the reduction of the Tstl will produce higher deformation.

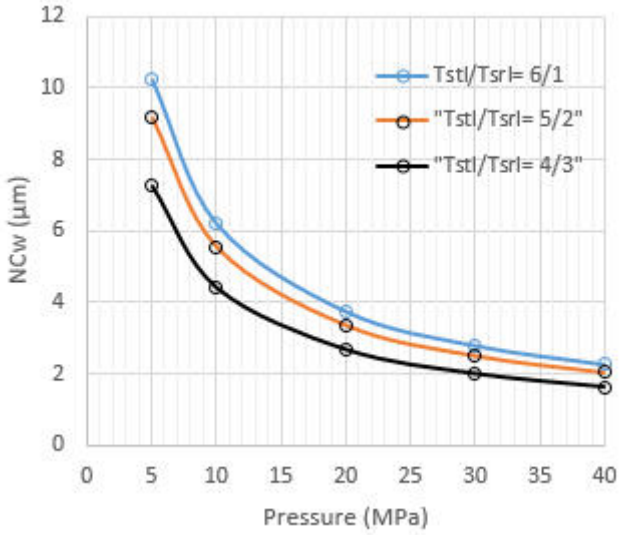

Figure 4. Plot of Stamping pressure against the resulting Non contact width NCw as result of the stamping defect.

(a) (b)

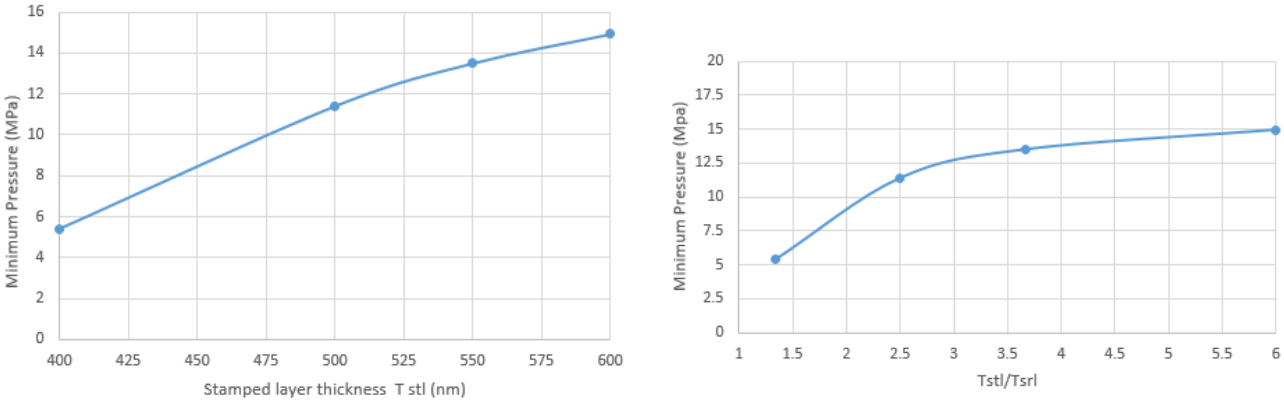

Figure 5. (a) The plot of the stamped ECM layer thickness Tstl against the minimum required pressure for stamping. (b) The plot of the ECM stamped layer-strike layer ratio Tstl/Tsrl against the required minimum pressure required for stamping.

Figure 5(a) shows the dependence of the stamped layer thickness on the minimum pressure required for stamping. At low pressure ranging from 10 kPa to 4 MPa, Ncw >0. At higher pressures, NCw →0. The minimum pressure required for stamping ranges from 5.4 MPa to 15 MPa for 700nm ECM protein transfer. The pressure required for stamping increases gradually with increased stamped layer thickness.

From the plot of minimum pressure versus Tstl/Tsrl as shown in Figure 5(b), the minimum pressure increases with increase in Tstl/Tsrl with pressure increase relatively small for thickness ratios greater than 4.5. This shows

that the stamping pressure required for ECM composite can be optimized by clever adjustment of Tstl. This provides the opportunity for design optimization of ECM Stamps.
A conservative model was obtained for ECM protein stamping.
NCw=f(Tstl,Tsrl,P)……5
Since the effect of Tsrl is negligible for hard contact modelling, Equation 5 could be expressed as:
$NCw = [511787ln(Tstl)P^{-0.723}]$ 6

5. Conclusion

We modeled 700 nm thick ECM protein transfer on hard substrate. The ECM was modeled as composite and the transfer of ECM protein on hard substrate was investigated using a mechanics model and finite element simulations. By considering the effect of stamping pressure on NCw for different ECM protein thickness Tstl and Tsrl, we have shown the stamping defect can be reduced by adjusting the stamping pressure and stamping pressure required for ECM composite can be optimized by clever adjustment of Tstl. This provides the opportunity for design optimization of ECM Stamps. A conservative model was presented for stamping of ECM protein on hard substrate.
The processes involved in the transfer of ECM protein stamping to soft substrate has not been studied. Soft contact modeling of ECM protein on soft substrates like Polyethyleneglycol PEG and PDMS needs to be explored and cellular functions on stamped ECM studied.

Acknowledgements

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