APPLICATION OF A NOVEL HIGH RESOLUTION WIDEFIELD SURFACE PLASMON MICROSCOPE IN CELL ENGINEERING, WOUND HEALING AND DEVELOPMENT OF NEW BINDING ASSAYS

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APPLICATION OF A NOVEL HIGH RESOLUTION WIDEFIELD SURFACE PLASMON MICROSCOPE IN CELL ENGINEERING, WOUND HEALING AND DEVELOPMENT OF NEW BINDING ASSAYS

The Widefield Surface Plasmon Resonance Microscope was used for high lateral resolution imaging of: binding events between micropatterned extracellular matrix proteins and antibodies, and the cell/surface interface.

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BEng (Hons) Medical Engineering

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Submitted for the degree of Doctor of Philosophy

School of Engineering, Design and Technology

University of Bradford

2007
STATEMENT OF ORIGINALITY

To the best of my Knowledge, the material or the contents presented in this thesis are original except where otherwise noted within the text. None of this research has been submitted in whole or in part for any degree at this or any other university.

Muhammad Mahadi Abdul Jamil
ACKNOWLEDGEMENTS

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Words fail to express their contribution during this work since the day I began this research. Their continuous guidance and support with a high quality of supervision for which through their kindness and care so far, nine associated publications have been achieved from this research project and further publications are highly expected. The earliest publication was five months after commencing the research.

The attitude of both supervisors in demanding for regular meeting plus the setting of strict deadlines enabled the successful accomplishment of this research project. This certainly demonstrates their excellent etiquette of supervising postgraduate research students especially one like me coming from abroad who required a high motivational support to perform the research tasks allocated with a high degree of discipline. Indeed they have done their best and it is my believe that this collaboration will continue in the same way and in other directions, due to the valuable outcomes of this research program.

Nevertheless, these are only amongst the few great qualities of both supervisors which I am indebted to mention and I hopefully wish to adapt/follow these qualities especially when in the near future taking on the responsibilities of supervising postgraduate research students. Infinite amount of thanks also goes to both of them for their patience in correcting and proof reading this thesis.
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May God Bless You All.
I would like to dedicate this thesis to my beloved mother Aishah and father Abdul Jamil for their sacrifice in bringing me up and educating me since the day I was born, without their help it would not have been possible for me to reach this stage especially in pursuing a Doctorate Degree.

The dedication also goes to my wife Azlina Hussin who has always been there for me and our children (Huzaifah & Hanzalah) with her continuous encouragement, support and especially enormous sacrifice to enable the accomplishment of this study which will never be forgotten.
LIST OF ASSOCIATED PUBLICATIONS


Other journal papers in preparations (to be submitted later in 2007):


Application of a Novel High Resolution Widefield Surface Plasmon Microscope in Cell Engineering, Wound Healing and Development of New Binding Assays

Muhammad Mahadi Abdul Jamil

Keywords: Bio-molecular interaction, Antigens/Antibody interactions, Laminin, Fibronectin, Micro-contact printing, Surface Plasmons Microscopy, Transformation Growth Factor β three (TGFβ3), Cell guidance, Tetramethylrhodamine Isothiocyanate (TRITC), High resolution imaging, Cell on a substrate, Live cell imaging.

ABSTRACT: Surface Plasmon (SP) microscope systems are mostly built around the prism based Kretschmann configuration. In these systems the generation of Surface Plasmons (SPs) is achieved by p-polarized light striking a metallised prism surface at a specific angle and then monitoring the intensity of the reflected light. Thus in these systems, an image of the material can be obtained in terms of an intensity map, in which the intensity of the image is dependent on the way the light couples into the SPs. The drawback of these systems is that lateral resolution relies on the ability of plasmons to propagate along the metallised layer. The lateral resolution is thus limited to a few microns. Therefore, a new microscope system was developed, i.e. the Widefield Surface Plasmon Resonance (WSPR) microscope, that is not only capable of analysing molecular interactions at high vertical resolutions, but also enables SP imaging at much higher lateral resolution than prism based systems. The functionality of the novel (WSPR) microscope has been investigated by imaging a sequence of binding events between micropatterned extracellular matrix proteins and their specific antibodies both in air and real-time. Using the WSPR system a change in contrast was observed with each protein binding events. Images produced via the WSPR system were analyzed and compared qualitatively and quantitatively. The preliminary results acquired for these binding studies between antibody/antigens demonstrate that the WSPR system capable of resolving features down to 260nm although the theoretically proven lateral resolution of the WSPR system is ~500nm. Cell surface interactions under two different culture conditions, i.e. HaCaTs cultured on SPR substrate with Transforming Growth Factor β3 (TGFβ3) (50ng/ml) and without TGFβ3 were also investigated. It was found that HaCaTs cultured in the presence of TGFβ3 showed enhanced division and motility along with decreased cell attachment as compared with cells maintained in TGFβ3 free media. It is believed that cellular signalling by TGFβ3 is very important for enhancing tissue development in wound repair. It is confirmed that the WSPR microscope described here can be used to study sequential monomolecular layer of antibody/antigen interactions binding events and examination of cell surface interfacial interactions at lateral scales of less than one micron without the need for traditional immunofluorescent labelling. These results have significant implications in the development of new breed fast binding assays system and in enabling high resolution detailed examination of the cell surface couplings and cell signalling processes involved in cell attachment and migration.
LIST OF ABBREVIATIONS

3D  three dimensional
Au  Gold
Ag  Aluminium
AFM Atomic force microscopy
ATP adenosine triphosphate
ATR attenuated total reflectance
Anti antibody
BFP back focal plane
BSA bovine serum albumin
BSE bovine spongiform encephalopathies
CCD charge-coupled device
DIC differential interference contrast microscope
DNA deoxyribonucleic acids
Dthiol 1,6-Hexanethiol
ER endoplasmic reticulum
EM electron microscope
ECM extra-cellular matrix
ESEM environmental scanning electron microscope
ELISA enzyme linked immunoabsorbant assay
FCS foetal calf serum
FGF fibroblastic growth factor
FIB fibronectin
GAGs glycosaminoglycans
HOBs human osteoblast
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>HaCaT</td>
<td>Human Keratinocytes</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s balanced salt solution</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>LAM</td>
<td>laminin</td>
</tr>
<tr>
<td>LCD</td>
<td>liquid crystal display</td>
</tr>
<tr>
<td>MCP</td>
<td>micro contact printing</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MIMIC</td>
<td>micromolding in capillaries</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acids</td>
</tr>
<tr>
<td>NA</td>
<td>numerical aperture</td>
</tr>
<tr>
<td>OWLS</td>
<td>optical waveguide lightmode spectroscopy</td>
</tr>
<tr>
<td>PCL</td>
<td>polycaprolactone</td>
</tr>
<tr>
<td>PEG</td>
<td>poly (ethylene glycol)</td>
</tr>
<tr>
<td>PEO</td>
<td>polyethylene oxide</td>
</tr>
<tr>
<td>PHA</td>
<td>polyhydroxykanoate</td>
</tr>
<tr>
<td>PHB</td>
<td>polyhydroxybutyrate</td>
</tr>
<tr>
<td>PLA</td>
<td>poly(lactic acid)</td>
</tr>
<tr>
<td>PLG</td>
<td>poly(glycolic acid)</td>
</tr>
<tr>
<td>PMNs</td>
<td>polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PPF</td>
<td>poly(propylene fumarate)</td>
</tr>
<tr>
<td>PPO</td>
<td>polypropylene oxide</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet derived growth factor</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>PLGA</td>
<td>poly(D,L-lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly (L-lactic acid)</td>
</tr>
<tr>
<td>PDLLA</td>
<td>Poly (D,L-lactic acid)</td>
</tr>
</tbody>
</table>
PHSRN  Pro-His-Ser-Arg-Asn
PLLACL  Poly (L-lactic acid-co-e-caprolactone)
PDLLACL  Poly (D, L-lactic acid-co-e-caprolactone)
QCM-D  Quartz crystal microbalance with dissipation
RER  rough endoplasmic reticulum
RGD  Arg-Gly-Asp
RNA  ribonucleic acids
RPMI  Rosewell Park Memorial Institute
RRETAWA (Arg-Arg-Glu-Thr-Ala-Trp-Ala)
SP  surface plasmon
SPs  surface plasmons
SAM  self assembled monolayer
SER  smooth endoplasmic reticulum
SEM  scanning electron microscope
SFM  scanning force microscope
SPR  surface plasmon resonance
STM  scanning tunnelling microscope
SCOM  scanning confocal optical microscope
TEM  transmission electron microscope
TGF-β  transformation growth factor beta
Thiol  2-Aminoethanethiol
TRITC  tetramethylrhodamine isothiocyanate
UV  ultra violet
WSPR  Widefield Surface Plasmon Resonance
IWSPR  Interferometer Widefield Surface Plasmon Resonance
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Figure 2-1: Detailed illustration of a cell with its organelles. Adapted from (www.biology.eku.edu/, 2005).  

Figure 2-2: Detail diagram of Nucleus. Adapted from (http://biology.uwinipeg.ca/, 2005).  

Figure 2-3: Mitochondrian and inner structures within mitochondria; attempting to show a 3D structure of mitochondrian. Adapted from (www.bio.miami.edu, 2005c).  

Figure 2-4: Shows the endoplasmic reticulum as seen under light microscope. Adapted from (www.bio.miami.edu, 2005a).  

Figure 2-5: Shows the Golgi apparatus along with transport vesicles from ER. as seen under light microscope. Adapted from (www.bio.miami.edu, 2005b).  

Figure 2-6: Shows details of Nucleus and Lysosome under light microscope. Adapted from (http://fajerpc.magnet.fsu.edu/, 2005).  

Figure 2-7: Drawing of cell membrane composition. Adapted from (www.biologylessons.sdsu.edu/, 2005).