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Biosensors for fertility and pregnancy in cattle

A thesis presented
in partial fulfilment of the requirements
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Yu-ting Hsu

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Abstract

This project is focused on progesterone sensing, using both surface plasmon resonance (SPR) and lateral flow immunoassay (LFIA) methods with a new progesterone (P4) sensing material to develop cost effective assays for progesterone sensing in bovine serum and milk samples.

P4-PEG-OVA was synthesised, characterised and used for P4 detection. The P4-PEG-OVA sensor surface showed an improvement in surface response compared with two shorter ligand 4TP-P4-OVA and 4TPH-P4-OVA in SPR studies.

An analysis method has been developed and modified for bovine serum and milk analyses. The results indicated the P4-PEG-OVA ligand allowed sensitive P4 detection in SPR sensing and allowed bovine P4 cycle profiling. The SPR analysed data was compatible with the ECLIA and ELISA independent analyses and the P4 cycle of each of the three bovine milk samples showed a very similar trend and the extraction level was also consistent.

The P4-PEG-OVA ligand was used to develop a LFIA sensor strip, and the inhibition assay for bovine serum and milk analyses established. The results indicated that, after appropriate sample pre-treatment, the bovine estrous cycle profile could be detected. The LFIA method can be a potentially quick, easy and cost effective semi-quantitative P4 analysis for serum and milk samples.

A new material, polyhydroxyalkanoate (PHA) granules has been investigated for the possibility of developing a new surface biosensor. From the surface studies, the results indicated that the 3GNZZPhaC beads have the potential to become an alternative binding material for SPR sensing due to its unique gold binding property. A flow cell was designed, constructed, and tested on 3GNZZPhaC beads prior the preliminary SPR investigations.

The ZZPhaC beads also showed the gold binding property and ZZPhaC beads were used for SPR studies. The results suggested a possible application for them as a new SPR binding material for antibody detection.
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Table 4.1

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### Abbreviations

<table>
<thead>
<tr>
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<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4TP-P4</td>
<td>3-(pregn-4-ene-3,20-dione-4-yl)thiopropanoic acid</td>
</tr>
<tr>
<td>4TPH-P4</td>
<td>6-[3-[(pregn-4-ene-3,20-dione-4-yl)thiopropano-yl] amino]hexanoic acid</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>Anti-P4</td>
<td>progesterone antibody</td>
</tr>
<tr>
<td>AuNPs</td>
<td>gold nanoparticles</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>C</td>
<td>control line</td>
</tr>
<tr>
<td>CBG</td>
<td>corticosteroid binding globulin</td>
</tr>
<tr>
<td>CM5</td>
<td>carboxymethylate dextran</td>
</tr>
<tr>
<td>CL</td>
<td>corpus luteum</td>
</tr>
<tr>
<td>DCC</td>
<td>1,3-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dpm</td>
<td>disintegration per minute</td>
</tr>
<tr>
<td>EC20</td>
<td>the lowest concentration that can be distinguished from the background noise</td>
</tr>
<tr>
<td>EC50</td>
<td>the half maximal effect concentration</td>
</tr>
<tr>
<td>EC80</td>
<td>the highest concentration that can be distinguished from the background noise</td>
</tr>
<tr>
<td>ECLIA</td>
<td>electrochemiluminescence immunoassay</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3(-3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EIAs</td>
<td>enzyme immunoassays</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>FC1</td>
<td>flow cell one</td>
</tr>
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</table>
FC2       flow cell two
FSH       follicular stimulating hormone
GC-MS     gas chromatography–mass spectrometry
GEPIs     genetically engineered polypeptides for inorganics
HBS-EP+   SPR buffer contained 0.1 M HEPES, 1.5 M NaCl, 30 mM EDTA and 0.5% v/v Surfactant P20
hCG       gonadotropin
HEPES     4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC      high performance liquid chromatography
IFC       integrated micro fluidic cartridge
IgG       immunoglobulin G
kDa       kilodalton
LC-MS     liquid chromatography–mass spectrometry
LFDs      lateral flow devices
LFIA      lateral flow immunoassay
LH        luteinising hormones
LOD       limit of detection
NHS       N-hydroxysuccinimide
NZVP      New Zealand Veterinary Pathology Limited
P4        progesterone
P4-PEG    N-(13-(carbonylamino)-4,7,10-trioxatridecanyl)- 3-(pregn-4-ene-3,20-dione-4-yl)thiopropanamide
PHA       polyhydroxyalkanoate
PHB       polyhydroxybutyrate
PHBA      poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
pI        isoelectric point
pK        disassociate constant
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
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<tr>
<td>RIAs</td>
<td>radioimmunoassays</td>
</tr>
<tr>
<td>RU</td>
<td>response unit</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SPR</td>
<td>surface plasmon resonance</td>
</tr>
<tr>
<td>SPRI</td>
<td>surface plasmon resonance imaging</td>
</tr>
<tr>
<td>T</td>
<td>test line</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TIR</td>
<td>total internal reflection</td>
</tr>
<tr>
<td>ZZ domain</td>
<td>antibody binding domain of protein A</td>
</tr>
<tr>
<td>$\theta_{\text{SPR}}$</td>
<td>surface plasmon resonance angle</td>
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<tr>
<td>$\lambda_{\text{max}}$</td>
<td>lambda(max)</td>
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</table>