Real-time cancer cell detection and cell adhesion on implant materials surface

Implants are covered with proteins and cells when introduced into the body. To understand interactions on these interfaces, in vitro tools are utilized. Real-time label-free platforms allowing dynamic and static flow-conditions are exploited to understand cell adhesion and in this way improve compatibility of implants. The same features are advantageous for biosensor development based on cell detection (e.g., important for cancer research) as well.

Attachment of human mesenchymal stem cells (AD-MSC) and lysozyme protein on a few tens of micrometers thick hydroxyapatite (HA), coated by plasma spraying, was measured by Multi-Parametric Surface Plasmon Resonance (MP-SPR). Hydroxyapatite is a component present in teeth and bones, and in its synthetic form it is widely used in orthopedic prosthesis to enhance implant osseointegration (connection to a living bone). The results of MP-SPR measurements showed that cells favor binding on HA coating as opposed to gold.

In a separate experiment, a biosensor was developed to detect tumor cells. Binding of breast cancer cells (MCF-7) and non-cancerous cells (MCF-10A) to a surface bound targeting peptide (18-4) and a reference peptide were measured. The biosensor surface was able to distinguish cancer cells from normal cells.

Introduction

Biomolecular interactions are routinely determined in the fields of drug discovery and biosensor development. Surface Plasmon Resonance (SPR) is a well-established method to measure real-time label-free molecular interactions. Powerful Multi-Parametric Surface Plasmon Resonance (MP-SPR) instrument can perform measurements in a wide angular range (40-78 degrees) and at more than one wavelength, which extends applicability of SPR also to tissue engineering and biosensing using whole cells and nanoparticles.

The wide angular range makes MP-SPR capable of measuring films that are significantly thicker than the penetration depth of the SPR evanescent field. This applies to mirometer thick polymer and ceramic films or living cells, for example (figure 1).

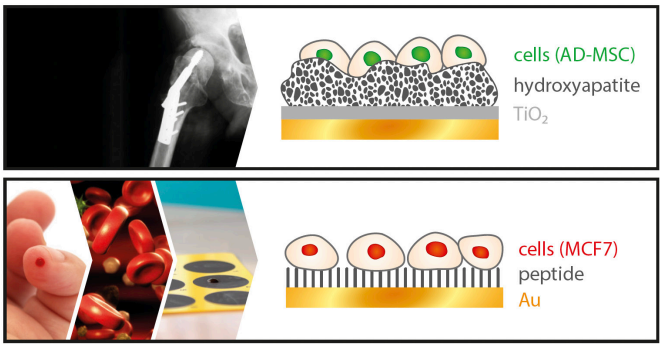

Figure 1. (Top) Binding of stem cells (AD-MSC) on ceramic hydroxyapatite surface was determined for orthopedic prosthesis development. (Bottom) Binding of breast cancer cells (MCF-7) to target peptide was measured to develop biosensor for cancer detection from blood sample.

Materials and methods

Study 1: Hydroxyapatite (HA) coatings were plasma sprayed onto a titanium dioxide (TiO_2) coated gold sensor slide (Vilardell *et al*. 2016). Binding of human mesenchymal stem cells, derived from adipose tissue (AD-MSC), on the HA coating was measured using MP-SPR Navi™ 210A VASA instrument. A baseline was established using pure cell culture media before the cell sample (0.1 million cells/ mL in cell culture medium) was injected and interaction was measured in static mode. Signal response was monitored until the cell attachment reached a plateau value. For comparison, lysozyme adsorption on the HA coating was measured.

Study 2: A breast cancer specific peptide 18-4 (WxEAAYQrFL) and reference peptide (XEPAYQRFTC) were covalently attached on gold sensor slides using thiol chemistry (Etayash *et al*. 2015). The sensor slide was immersed in peptide/PBS solution (1mg/mL) for 12 h at room temperature. Binding of cancer cell line MCF-7 (100 cells/mL) and the corresponding normal cell line MCF-10A to peptide surfaces were measured in flow. Cells were injected using a flow rate of 10 µL/min at 25°C in MP-SPR Navi™ 200 OTSO instrument.

Results and discussion

Hydroxyapatite (HA) formed tens of micrometer thick and highly porous layer on the substrate (Figure 2). Based on scanning electron microscopy (SEM), thickness was found to be 24 ± 6 µm. On the gold surface, cells adhesion reached a plateau signal response value in 10 minutes. In comparison, binding on HA surface was clearly slower and plateau was reached only after 90 minutes (Figure 3). AFaster cellular adhesion was observed on the HA surface within the 10 minutes after injection, relative to the bare gold substrate. The effective surface area of porous HA coating is larger than the effective surface area of gold surface.

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This affects binding rates and the adhered amount of cells. HA is favourable for cell attachment and thus implant osseointegration.

Lysozyme protein bound strongly to the HA coating (Figure 4). However, part of the proteins dissociated from the surface during rinsing, indicating fairly weak interaction between the proteins and the HA coating.

Cancerous cells (MCF-7) bound significantly stronger to the target peptides than to the reference peptides, as desired (Figure 5). Both cell types bound equally to the reference peptide surface, indicating cells low unspecific binding on the substrate. Binding of non-cancerous cells to the target peptide was even lower than to the reference surface, thus indicating desired peptide selectivity for cancerous cells. The sensitivity of peptide surface against cancer cells was found to be as low as 5 ± 3 cells/mL.

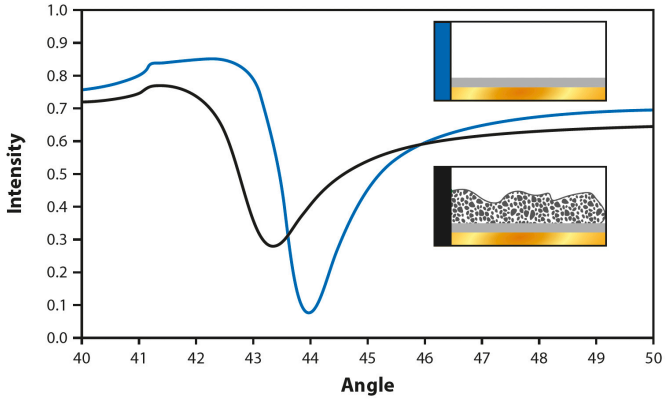

Figure 2. Complete SPR curve of TiO_2 sensor slide **with** and **without** HA coating measured in air. Thick layer of HA forms a waveguide phenomenon-based SPR curve.

Conclusions

MP-SPR measures adhesion of cells on coatings of several micrometers thick as well as on surface immobilized target molecules. This realtime technology is suitable for measurement of binding kinetics on various materials, ranging from metals (TiO_2) and ceramics (HA) to soft materials (PDMS). MP-SPR measures in controlled temperature (15 to 45 °C) and in both static and dynamic flow conditions, thus making instruments powerful for living cell sensing.

See how MP-SPR measures layer thickness and refractive index (Application Note #128) and how nanoparticles cell uptake is measured (Application Note #156).

References:

Vilardell *et al.*, Journal of Functional Biomaterials, 7 (3), 2016
Etayash *et al.*, Nature Scientific Reports, Vol. 5, 2015

Acknowledgement:

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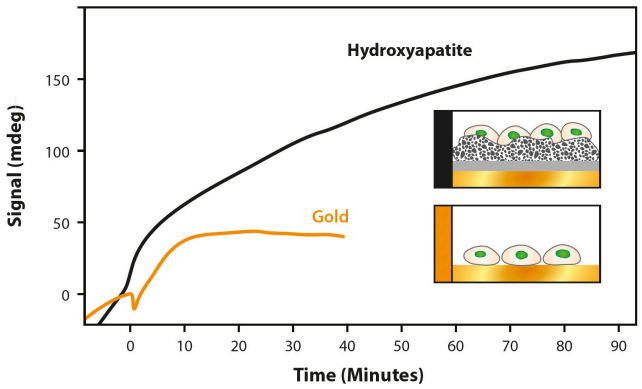

Figure 3. AD-MSC cells adhered to a higher extent on **hydroxyapatite (HA)** when compared to gold. Plateau level of binding was reached in 10 minutes on gold whereas it took over 90minutes on HA coating.

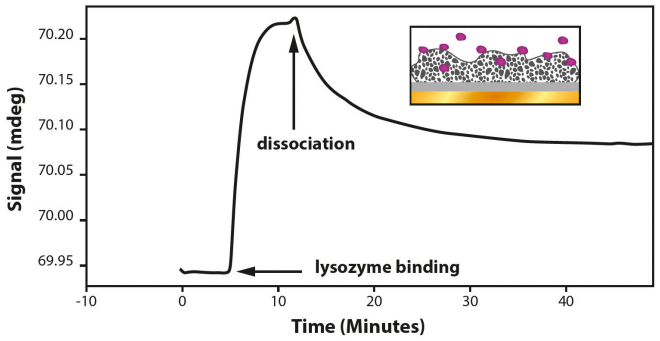

Figure 4. Binding kinetics of lysozyme on the hydroxyapatite coating.

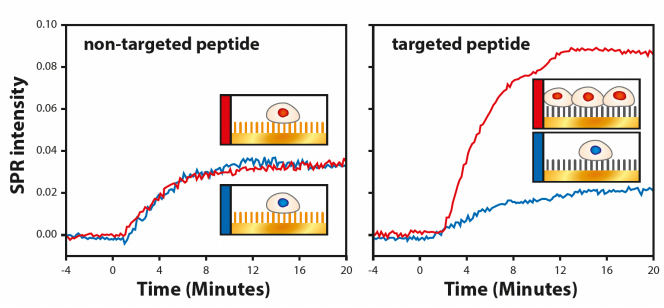

Figure 5. Binding of cancer cells (MCF-7) and non-cancerous cells (MCF-10A) to target peptide and reference peptide. Binding of cancer cells to the targeted surface occured to a higher extent (red curve) than to the non-targeted surface (blue curve).

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA or 220A NAALI

Sensor surface: Au, TiO_2, other metal or inorganic coating

Software: MP-SPR Navi™ Controller, DataViewer and TraceDrawer™ for MP-SPR Navi™

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