**Pioneering Successful Sybodies Immobilization On Sams Via An Innovative QCM Biosensing Approach**

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**Background and aims.** Synthetic single-domain antibodies, known as sybodies, created in vitro, possess the advantageous characteristics of nanobodies and are utilized in medical diagnostics. The urgent development of early diagnostic tools with high sensitivity and specificity is essential in cancer detection. This project aims to develop a quartz crystal microbalance (QCM) biosensor system for detecting cancer biomarkers in clinical samples by affixing sybodies to self-assembled monolayers (SAM).

**Methods.** The efficiency of three types of self-assembled monolayers (SAMs), namely thiourea, thioctic acid, and 3-mercaptopropionic acid, in immobilizing sybodies onto a gold-coated QCM sensor surface was examined. The sybodies VE01 (anti-VEGR2 - vascular endothelial growth factor receptor) and WHIC (anti-MBP - Maltose binding protein) were expressed and purified from E. coli W3110 bacteria carrying the plasmids pSB-sybodyVE01 and pSB-sybodyMBP (Addgene). The QCM system used QCM Au 5 MHz components combined with a multi-channel SP-150 potentiostat (Biologic, France) and the AFM 5500 atomic force microscope system (Agilent Technologies, USA). SAM formation and the binding of the sybody are evaluated using wetting angle measurement techniques, electrochemistry, and surface imaging of the sensor with an atomic force microscope (AFM).

**Results.** The thiourea SAM monolayer demonstrated superior attachment efficiency compared to thiotic acid and 3-mercaptopropionic acid, as indicated by a higher contact angle (𝜃thiourea > 𝜃thiotic acid > 𝜃3-mercapto), greater impedance (Zthiourea > Zthiotic acid > Z3-mercapto), and lower surface roughness. Similarly, among the tested sybodies, PVE01 showed better attachment efficiency on the SAM monolayer than WHIC, supported by its smaller contact angle (𝜃PVE01 < 𝜃WHIC), higher impedance (ZPVE01 > ZWHIC), and larger surface roughness.

**Conclusion/Discussion.** The findings demonstrate the successful functionalization of these SAMs with sybodies, establishing a robust framework for advancing highly sensitive and selective diagnostic technologies in the future.

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