Biomimetic Detection of Progesterone by Novel Bifunctional Group Monomer Based Molecularly Imprinted Polymer Prepared in UV Light

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Supplementary Materials

Successful formation of IA based MIPs film was analyzed by Fig. S1 (A), giving the real time kinetic curve for the polymerization. First of all, the film was grafted on modified gold plated chip by utilizing RAFT polymerization in UV light which was indicated by coupling angle shift from 59° to 69° (see Fig. S2.). Then the film was rinsed with washing solution for 20 min which resulted in the removal of template molecules and the reflectivity value decreased with time as shown in Fig. S1 (B). Consequently, the angle shifted from 70.03° to 69.84°, see Fig. S2.

![Fig. S1. (A) Polymerization of IA based MIPs film and (B) Washing of template molecules](image)
Fig. S1. SPR senso-gram for IA based MIPs: (a) Before grafting of MIPs film, (b) After grafting of MIPs film, (c) before treating with washing solution and (d) after treating with washing solution.

Real time monitoring of IA based MIPs film was done by SPR to investigate the specificity of biosensor. Rebinding curve of Progesterone exhibited maximum variation in reflectivity while treating with IA based MIPs (Fig. S3 a). On the other hand, Testosterone, β-estradiol and Estrone presented negligible response (see Fig. S3 b, c &d). Hence this specific behavior indicated the presence of highly selective progesterone-imprinted recognition sites.

Fig. S3. (A) Response of SPR for absorption of: (a) Progesterone, (b) Testosterone, (c) β-estradiol and (d) Estrone (each at 10\(^{-8}\) mol/L) (B) Structures of progesterone analogues

To ensure the stability of prepared biosensor, the IA based MIPs films were stored at room temperature for 30 days. After 30 days the same chip was implied for detection of progesterone in sample solution. The real time monitoring in SPR is shown in Fig. S4. Expectedly, the biosensor chip displayed high degree of stability and retained its efficiency by 83%.
Fig. S4. The rebinding kinetics of Progesterone (10^8 mol/L) in PBS buffer solution after 0-30 days storage at room temperature

For the investigation of recoverability of IA based biosensor, adsorption of sample solution was made with rinsing of chips after adsorption by using washing solution to recover its blank state (Fig. S5).

Fig. S5. Recoverability of MIPs film for adsorption in 10^8 mol/L progesterone solution (n = 2)

After confirmation of selectivity and efficiency of IA based MIPs, the Biosensor was applied for the detection of progesterone in saliva. Fig. S5 shows the reflectivity changes of different (10%, 20% and 50%) V/V % solution of saliva in distilled water. The reflectivity changes at 20% and 50% V/V concentrations are much higher to be fitted in the SPR senso-gram owing to greater extent of suspension. The higher reflectivity binding affinity at 50% and 20% in IA based MIPs reveals the unfeasible utilization of these solutions. So, the 10% V/V solution of saliva in water was selected for further observations.
Fig. S6. (A) Real time kinetics of reflectivity signals in 50%, 20% and 10% saliva. (B) Real time monitoring of kinetics by reflectivity changes in SPR due to affinity binding of progesterone on MIPs film.