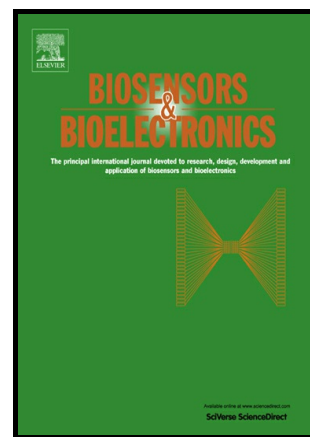


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The sensitive detection of ODAM by using sandwich-type biosensors with a cognate pair of aptamers for the early diagnosis of periodontal disease

Bang Hyun Lee¹, Sang Hoon Kim¹, Youngkyung Ko², Joo Cheol Park³, Suk Ji⁴, Man Bock Gu^{1*}

¹Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea

²Department of Periodontics, Seoul St Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

³Department of Oral Histology, School of Dentistry, Seoul National University, Seoul, Republic of Korea; Department of Dentistry & Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Republic of Korea

⁴Department of Periodontology, Institute of Oral Health Science, Ajou University School of Medicine, Suwon, Republic of Korea

*mbgu@korea.ac.kr

Abstract

This research aims to develop biosensors which could diagnose periodontal diseases in early phases and predict the illness stage of patients, in order to give them adequate treatment timely. Human odontogenic ameloblast-associated protein (ODAM), which is indicative for periodontal diseases, based on high correlation between the level of ODA in gingival crevicular fluid (GCF) and the degree of periodontitis. Many aptamers, including a cognate pair of aptamers which can bind to the different sites of ODA, were successfully screened in a very stringent condition employing saliva as a counter target through the graphene oxide-based systemic evolution of ligands by exponential enrichment (GO-SELEX). For the characterization of the aptamer candidates, GO-based fluorescence resonance energy transfer (GO-FRET) and surface plasmon resonance (SPR) assays were conducted. The sandwich-type binding of a cognate pair of aptamers to ODA was additionally confirmed by employing circular dichroism (CD) and magnetic beads-based fluorescence imaging methods. The resulting cognate pair of aptamers, OD64 and OD35, were found to have their dissociation constant (K_d), 47.71 nM and 51.36 nM, respectively. The minimum detectable concentrations of a sandwich-type SPR biosensor were found to be 0.24 nM and 1.63 nM, respectively, for both buffered and saliva samples. Finally, using this cognate pair of aptamers, a sandwich-type lateral flow strip biosensor was successfully realized. This research shows the potential for implementation of a cognate pair of aptamers on point-of-care biosensors which enables simple, rapid, and non-invasive saliva-based diagnosis of periodontal-related diseases that can overcome current diagnostic methods and improve health care system.

Keywords: Human odontogenic ameloblast-associated protein (ODAM); A cognate pair of aptamers; Graphene oxide-based systemic evolution of ligands by exponential enrichment (GO-SELEX); Lateral flow assay

1. Introduction

Periodontitis is a chronic inflammation induced by a multifactorial factors including pathogenic bacteria. It is a major cause of tooth loss in adults by destroying the underlying supporting teeth bone irreversibly (Pihlstrom BL et al., 2005). Although the global prevalence of a severe periodontitis is 11.2% (N.J. Kassebaum et al., 2014), mild to moderate periodontitis affects a majority of adults (Bruce A. Dye, 2012; Poul E. Petersen and Hiroshi Ogawa, 2012). Since it has been demonstrated that the degree of periodontitis is associated with a risk of various cancers (Meyer MS et al., 2008; Moraes RC et al., 2016; Tezal M. et al., 2007, 2009), there has been increasing interest in monitoring the periodontal diseases. Moreover, it is known to be highly related to many other local or systemic medical conditions, such as cardiovascular disease, metabolic disorders, rheumatoid arthritis, respiratory problems, chronic kidney disease, and impairment of cognitive function (E. Nordendahl et al., 2018; Gaetano Isola et al., 2017; John M. Liljestrand et al., 2018).

There have been different types of advanced technologies developed in the field of diagnosing periodontal diseases. Starting with Lab-on-a-Chip methods which can measure the salivary biomarkers, such as interleukin-1 β , C-reactive protein, and matrix metalloproteinase-8 (Christodoulides et al., 2007), integrated microfluidic platform for Oral Diagnostics (IMPOD) was also developed for the measurements of MMP-8, TNF- α , IL-6, and CRP in saliva (Herr et al., 2007). Moreover, Point of care (POC) device with a miniaturized sensor system which contains microbead array (Craig S. Miller et al., 2010) and a lateral flow chromatography for immunotesting active MMP-8 (Nilminie Rathnayake et al., 2017) reported as a current diagnostic methods. However, a current diagnostic way has lots of problems, such as inconveniency for patients and doctors regarding time and physical difficulties during diagnosing and treatment. (Gareth S. Griffiths, 2003; Hans-Peter Horz and Georg Conrads, 2014; IRA B. Lamster and Joseph K. Ahlo, 2007; Jun-ichi Kido et al., 2018; Pierluigi Coli et al., 2017). Therefore, early diagnosis of periodontal diseases was urgently needed in POC technology, especially in a non-invasive way with high specificity and sensitivity.

Eventhough a lot of different kinds of salivary biomarker candidates have been found and studied for this multifactorial oral inflammation diseases (Baeza M. et al., 2016; Buduneli N. and Kinane DF., 2011; Mario Taba Jr. et al., 2005), little is known about the specific and reliable biomarker which is related only to the periodontal diseases. However, most recently, the protein ODAM in GCF is known to be a representative biomarker only for the periodontal diseases (H.K. Lee et al., 2015, 2018). ODAM is a tooth-associated epithelia protein which plays a significant role in odontogenesis at the last step of mineralization by combining with enamel matrix. Since it allows that the tooth surface can attach to its junctional epithelium, when the inflammation happens, it can come out from the periodontal pocket that can be exposed to the surface (Daniel P. Kestler et al., 2008). To date, the most common method for the ODAM measurement is using commercialized ELISA kit, which is based on the quantitative sandwich enzyme immunoassay method generating colorimetric signals. It has been reported the high sensitivity for detection of ODAM protein in different samples, such as serum, plasma, and tissue homogenate. However, still the cross-reactivity with detection antibody cannot be completely removed from this detection platform. Moreover, this assay requires people to have professional skills and knowledge to utilize all the biochemical components included in the kit which makes them hard to apply as a POC diagnosis. Therefore, in this study, for diagnosing the periodontal diseases, we present novel biosensors based on a cognate pair of aptamers binding to this biomarker protein, ODAM in a sandwich form. Contrary to the conventional

2. Material and methods

2.1. Materials and reagents for GO-SELEX

To find the ODAM-specific aptamer, the GO-SELEX process was performed (B.H. Lee et al., 2017; J. W. Park et al., 2012) which starts with 200 pmoles of a random library with a randomized region 20 (N_{20}) nucleotides edged by two constant regions assigning primer annealing and PCR amplification (primer sequences: FP 5'-CCATTCGTACGCAACAGG-3' and RP 5'-GCATTCAGAGCCATCCAC-3'). After denaturing the random library pools to get the best conformational structure of themselves, we added 5 mg/ml, 200 μ l of GO and incubated on the rotator for 30 minutes at room temperature. After this, the mixture was centrifuged to remove all the supernatant which includes unbound random library to GO. And we added the main target, ODAM recombinant protein, 200 pmoles in 10 mM PBS to the precipitated GO solution, to see that a target-induced detachment of ssDNAs could happen upon the target addition for 30 minutes at room temperature. Then, this mixture was centrifuged again to get the supernatant where the ODAM-bound ssDNAs resides. All these processes are called, positive selection, since we only used the main target for the screening. Followed by two times of positive selection, a negative selection which uses counter target (human saliva, 200 pmoles), was done twice to increase the specificity of aptamer candidates stringently. At this time, we first incubated ssDNAs with the counter targets for 30 min, and then with GO and the main target during the same period to get the specific ssDNAs only to the main target. At every selection round, the last supernatant has gone through ethanol precipitation, and the amount of ssDNAs for each round was measured by a spectrophotometer (ND-2000, Nanodrop, U.S.A.). When the final selection round finished, which shows the saturation state regarding the recovery ratio, cloning and sequencing steps has come after to obtain the sequences of the selected ssDNA, aptamer candidates. All oligonucleotides were sequenced and synthesized from the company Genotech and Cosmogenetech, Korea, and all chemicals including counter target molecules (α -amylase, human serum albumin) used in the SELEX process were purchased from Sigma Aldrich (U.S.A.). The main target, ODAM recombinant protein was purchased from Cusabio, China, and all the clinical samples, human saliva from periodontally health subjects (minimal bleeding on probing with normal gingival sulculs depths) were obtained from Seoul St. Mary's Hospital and Ajou University Hospital, Korea, with the approval of IRB (1040548-KU-IRB-17-154-A-3).

2.2. Assays for the characterization of aptamer candidates

2.2.1. GO-FRET assay

By using one of the characteristics of GO, quenching fluorescence signal, the affinity to ODAM of 18 sequences from sequencing was characterized. For this test, 4 nM of each candidate labeled with FAM molecule at the 5'-end (GenoTech Co., Korea) was incubated for 30 min at room temperature with 1.6 μ g/ml of GO solution to make them nonspecifically attach to GO by π - π stacking, which causes the emission energy of FAM that is maximized at 520 nm to be quenched. However, if the sequence has an affinity to ODAM, it will be detached out from the GO surface since it will be conformationally changed upon adding ODAM into the mixture and makes the fluorescence of FAM recovered. The emission spectrum of each resulting solution was measured by microplate reader (SpectraMax i3x, Molecular Devices, U.S.A.) with the excitation wavelength of 480 nm. Each mixture of aptamer with or without GO was also analyzed as a negative and positive control, respectively. This assay was also done with different concentration of ODAM (0, 0.25, 1, 2, 4, 6, 8, 12, 16 nM) to see the dose-dependency to the ODAM by each sequence. The autofluorescences from all the components themselves including ODAM were checked and the amount of each component was optimized as well in advance.

2.2.2. SPR assay and a sandwich-type SPR biosensor

The selected aptamers by FRET assay were further identified by SPR (BioNavis, Finland) by immobilizing them on to the gold chip (BioNavis, Finland) surface via avidin-biotin linkage. First, a gold chip was freshly prepared by washing with ethanol and distilled water. This clean chip was then submerged in 50 mM DTPA solution at room temperature overnight. With this step, the COOH functional groups can be activated which can make reaction with the 200 mM of N-ethyl-N'-(dimethylaminopropyl) carbodiimide (EDC) and 50 mM of N-hydroxysuccinimide (NHS) in 10 mM PBS for 1 h at room temperature. And 100 µg/ml of streptavidin was incubated for 90 min. After removing excess streptavidin by washing with distilled water, 50 mM of ethanolamine was used to block the unreacted functional groups for 30 min. Then 1 µM of 5'-T₁₀-biotinylated aptamer was incubated for 1 h. For the last step, the chip was blocked again with 50 µg/ml of BSA for 30 min and washed thoroughly with distilled water. All SPR analyses in this research were done by using these aptamers modified gold chips. To get the stable data, 10 min of baseline measurement was done by flowing the same buffer that we used in SELEX process. Injection of ODAM protein was followed to see the SPR signal change for the additional 10 min. Washing the unbound targets were followed by flowing buffer through the channel. By analyzing the change of the reflective index degree upon the binding of the target, we could see the affinity level of each aptamer candidate to ODAM. This assay was also conducted by using a different concentration of ODAM (0, 1, 2, 4, 8, 16, 32, 64 nM) in both buffer and spiked-in-saliva condition to confirm the aptamer candidates' dose-dependency. Furthermore, a cognate pair of aptamers were found by the same analytical procedure by flowing secondary aptamer candidate through the channel.

2.2.3. CD spectrum analysis

CD spectrum investigation was conducted to study the structural change of aptamers upon ODAM binding. Quartz cuvette (Hellma Analytics, Germany) was used in this assay, and 1 µM of each components; primary aptamer (OD64), ODAM, counter target (α -amylase), and secondary aptamer was measured in a different combination as shown in Fig. 4a. Each of the peak difference could be interpreted as it comes from the structural changes of ssDNAs when they make reaction with specific molecules.

2.2.4. Imaging of a cognate pair of aptamers in sandwich formation by using confocal laser scanning microscopy (CLSM)

Confocal microscopy was used to see the image of aptamer duo work with a sandwich way by using confocal laser scanning microscope (LSM 700, Carl-Zeiss, Germany). Cy5-5'-labeled secondary aptamer was used in this test as to see the fluorescence signal. By making reaction with three kinds of solutions, with and without target protein in different condition, we could see the fluorescence signal which works with sandwich assay by a cognate pair of aptamers.

2.2.5. Lateral flow strip biosensor (LFSB) preparation

The LFSB was designed and done as shown in Scheme 1. Sample and adsorption pad (CFSP203000), NC membrane (HF180MC100) were purchased from Milipore, U.S.A. Control and test line were drawn by using dispenser (ZX 1010 B0056, Biodot, U.S.A.) As a blocking solution, sample pad blocking solution (3% BSA, 0.05% Tween-20 in 0.01 M PBS), NC membrane blocking solution (1% BSA, 10 mM Tris, 100 mM NaCl) were used. For the NC membrane washing solution (0.1% Tween-20, 10 mM Tris, 100 mM NaCl) was used for removing excess particles which might be present after the blocking step. In the assay for buffer and spiked-in-saliva condition, 10 mM PBS and normal human saliva were used, respectively. All the LFSB studies were conducted in a dip-stick method by using 96 well plates followed by putting an assembled strip into each well which contains different test samples. In case of the buffer condition, 25 µl of different concentration of ODAM mixed with the same amount of OD35-AuNP were used as a test sample. For the saliva condition, 1/10 diluted human saliva sample together with OD35-AuNP spiked with 25 µl of different concentration of ODAM were used as a test sample. Immobilizing of DNA on the gold nanoparticle

3. Results and discussion

3.1. Screening of ODAM-specific aptamers

By using the immobilization-free GO-SELEX as mentioned in Materials and Methods section, the screening process of ODAM-binding aptamers was conducted. As a result, from the random ssDNA library, aptamers which can specifically bind to ODAM were proficiently sorted out. The overall selection process consisted of seven rounds, including five positive selection with the main target protein, ODAM, and two negative selection with the counter target, human saliva in this study. Human saliva was added to our sample as a counter target, which should be very stringent, because of its future usage in application works. And this negative selection using human saliva helps to increase the main target selectivity of ssDNAs at each selection.

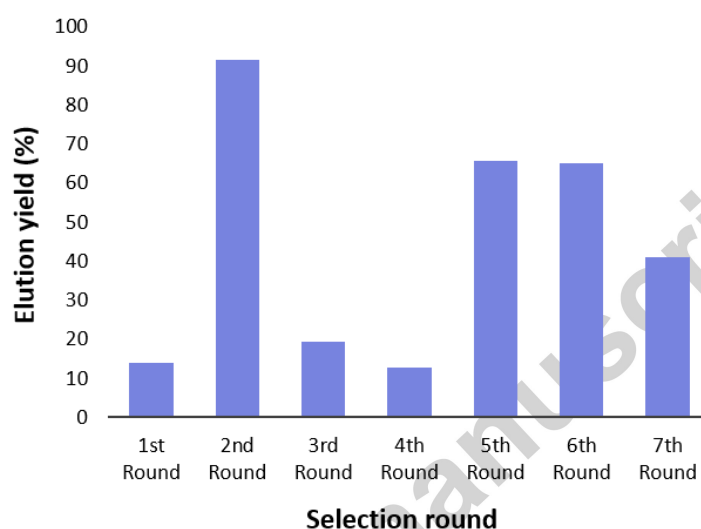


Fig. 1. SELEX progress of ODAM-specific ssDNAs by GO-SELEX

As shown in Fig. 1, the recovery ratio of bound ssDNAs only to ODAM, which was measured at every selection round by the nanodrop, increased up to 91.62% at 2nd round. However, since we used human saliva sample in the negative selection, the recovery ratio decreased, when this harsh condition of the process was applied at 3rd and 4th round. Eventually, the ssDNA pool of 5th round showed the highest recovery ratio (65.80%) and the cloning and sequencing steps were carried out to acquire the aptamer candidates. In summary, we got the 18 different sequences of ODAM-binding aptamer candidates (Table S1).

3.2. Screening of aptamer candidates using GO-FRET and SPR assays for their specificity analysis

GO-FRET assay was conducted to test the affinities of the selected aptamer candidates to ODAM protein using GO-based quenching method (Chun-Hua Lu et al., 2009). By calculating the fluorescence ratio emitted at 520 nm, $(F-F_0)/F_0$, (F is recovered state, and F_0 is quenched state), aptamer candidates which show the affinity to target could be screened out among the candidates. Moreover, with the dose-dependency test, we could figure out the tendency of each aptamer candidate binding to the target. Among 18 candidates, 8 sequences, which are OD17, OD29, OD32, OD35, OD39, OD64, OD78, and OD82, showed the significantly high recovery ratio, indicating that these are the aptamer candidates with high affinity and specificity (Fig. S1). Those 8 sequences with the images of their secondary structures were saved up for further characterization studies (Fig. S2).

To characterize the selected aptamer candidates more precisely, SPR assay was conducted (N. H. Ahmad Raston et al., 2015). In this method, specific concentrations of ODAM and the counter target molecules flowed through SPR channel to the surface of the gold chip, where each aptamer immobilized on. The mass changes upon the binding of ODAM to aptamers could be detected by SPR machine through the change of the reflective index. And the amount of change that the degree of the reflective index showed the level of binding affinity of each aptamer to ODAM or counter target molecules proportionally. Fig. 2 shows the SPR response units for 8 aptamer candidates in response to target, ODAM, and its counter targets, α -amylase and human serum albumin.

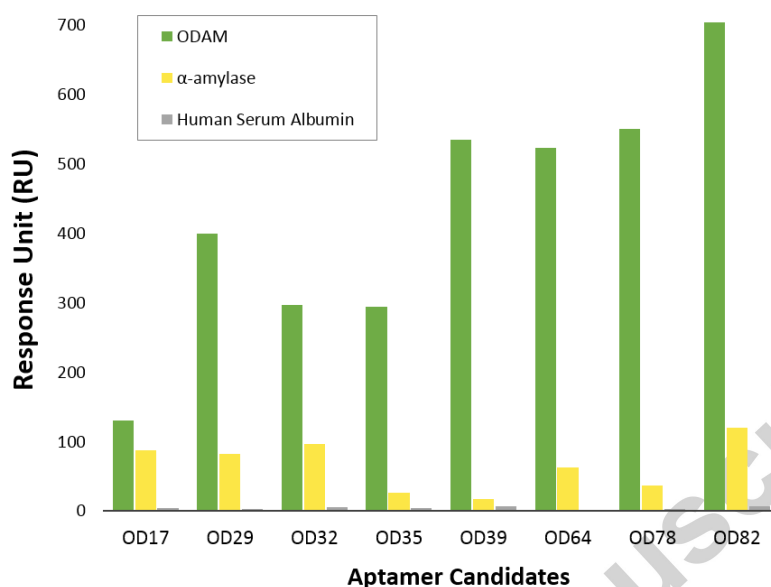


Fig. 2. The SPR-based characterization of 8 selected aptamer candidates showed their specificity and sensitivity to ODAM. Based on the response units in response to ODAM, α -amylase, and human serum albumin, 5 aptamers were finally selected as OD29, OD39, OD64, OD78, and OD82.

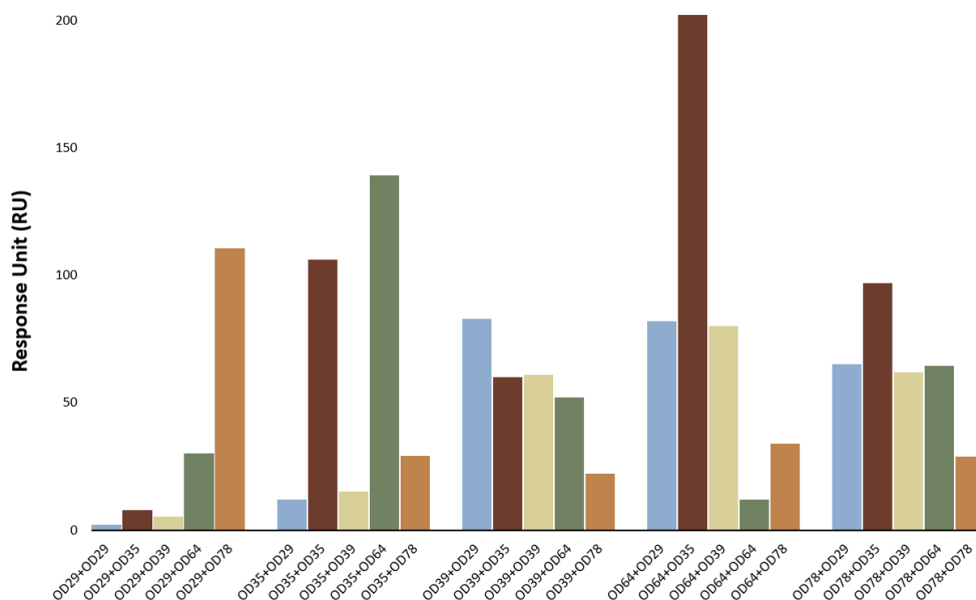


Fig. 3. Total 25 combinations of 5 selected aptamers as a primary capture and secondary aptamers were analyzed by using SPR assay, in order to find a cognate pair of aptamers working in a sandwich format. Over the certain values of the response units in response to ODAM in a sandwich format on SPR analysis indicates the possible combinations to be a cognate pair of aptamers.

The result indicates that 5 aptamers, named OD29, OD39, OD64, OD78, and OD82, showed the best results in terms of both specificity and sensitivity. In fact, the specificity of 8 aptamer candidates was determined first based on the response unit to the main target, ODAM, and the cut-off was set to 300. Next, their response unit to the α -amylase, which is the counter target, were compared as well to choose the specific aptamers which can show the significant response difference between main and counter target. Therefore, these 5 aptamers which passed the criteria in terms of both sensitivity and specificity are used for further study.

3.3. Successful Finding of a cognate pair of aptamers and its confirmation using CD and imaging analysis

In order to see if any pairs among 5 aptamers act as a cognate pair of aptamers, which can bind to the target, ODAM, together at different binding sites in a sandwich formation, SPR-based sandwich-type assays were conducted for 5 aptamers selected in the previous steps. After completing 25 combination analysis (5 aptamers x 5 aptamers) using SPR-based sandwich type analysis, it was found that the combination of the aptamer OD64 as a primary capture aptamer and the aptamer OD35 as a secondary aptamer showed the highest signal difference in their response units on SPR analysis, indicating that this combination act as a cognate pair of aptamers strongly (Fig. 3). Interestingly, the reverse combination, in which the aptamer OD35 as a primary capturing moiety and OD64 as a secondary signaling moiety, also showed the similar but less sensitive signal change, compared to the original combination. This suggests that this reverse set of cognate pair of aptamers is also working, regardless of their order. In this study, we used the aptamer OD64 as a primary and OD35 as a secondary receptor, since this combination showed the best result, while this cognate pair of aptamers have the possibility that they can be applied flexibly to different kinds of assay platforms consequently.

For the further experiments, the variation of the structural change upon the addition of ODAM to its cognate pair of aptamers was studied by analyzing CD spectrums. As can be seen in Fig. 4a, when the ODAM interacts with single aptamer, OD35 (OD35 + ODAM) or OD64 (OD64 + ODAM), a high peak of aptamer itself (OD64, OD35) at around 220 nm becomes significantly decreased. In sandwich-type binding cases (OD64 + ODAM + OD35, OD35 + ODAM + OD64), they also showed peak differences compared to those with single aptamer only (OD64, OD35). More importantly, when the non-target molecule, α -amylase, comes in, this combination of a cognate pair of aptamers didn't show any peak shifting (OD64 + α -amylase + OD35), which means they are specific to its main target, ODAM, only. Through these spectra, we could assume that the conformational change of the aptamers which is represented as an altered spectrum might occur, due to the interaction between the target and its aptamers in a specific way of a cognate pair of aptamers working.

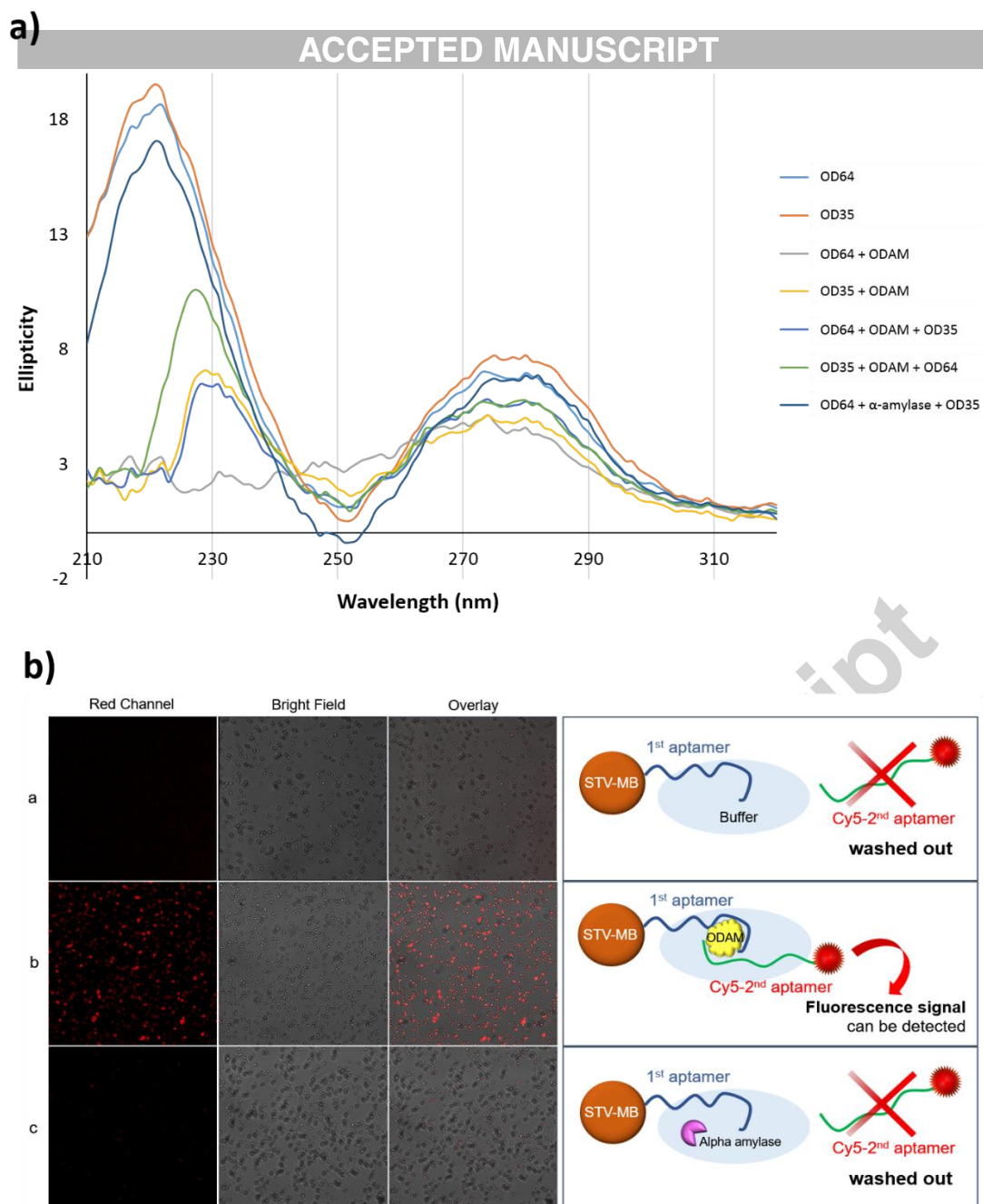


Fig. 4. (a) Circular dichroism (CD) analysis of OD64 and OD35 with or without ODAM and α -amylase, (b) CLSM analysis of a cognate pair of aptamers forming sandwich formation.

Added to this indirect evidence of CD spectrum analysis, we used magnetic beads immobilized with primary aptamers (OD64) and the fluorescence dye Cy5-labeled secondary aptamer OD35, in order to have direct evidence that this cognate pair of aptamers makes the sandwich-type binding to the target ODAM. For visualizing this combination of a cognate pair of aptamers with the target ODAM, confocal laser scanning microscopy (CLSM) was used to image the fluorescence signal from Cy5-OD35, when it makes the sandwich formation together with OD64 and ODAM. It is clear from Fig. 4b that only when the target protein ODAM is present in the sample, the fluorescence signal could be observed. The overall results shown in Fig. 4 reaffirmed with the same results in previous GO-FRET and SPR assays.

After reaffirmed a cognate pair of aptamers, both aptamers forming sandwich-type binding were further characterized in their specific affinities. A binding affinity to ODAM for each cognate pair of aptamers, OD64 and OD35, was analyzed by SPR

assay with different concentrations of ODAM protein samples. As shown in Fig. S3, both aptamers showed the dose-dependency to their target molecule, ODAM. From this dose-dependency curves, the K_d values of OD64 and OD35, estimated by adopting non-linear regression analysis, were 47.71 nM and 51.36 nM, for OD64 and OD35, respectively.

3.4. Development of a sandwich-type SPR biosensor using a cognate pair of aptamers: its specificity and the limit of detection

In the implementation of this cognate pair of aptamers to a sensing platform, a sandwich-type SPR biosensor was completely developed by immobilizing the primary aptamer OD 64 on an SPR sensor chip. In this SPR-based biosensor, immobilized primary aptamers, OD64, bind the target ODAM and the secondary aptamers, OD34, which eventually make the combination of a cognate pair of aptamers with the target ODAM in the middle in a sandwich format. The detection limit of this sandwich-type SPR biosensor, based on a non-linear regression analysis, was found to be 0.48 nM (for single aptamer OD64) and 0.24 nM (for a cognate pair of aptamers) in buffer (10 mM PBS) (Fig. 5a), respectively, while they were 2.49 nM (for single aptamer OD64) and 1.63 nM (for a cognate pair of aptamers) in human saliva (Fig. 5b), respectively.

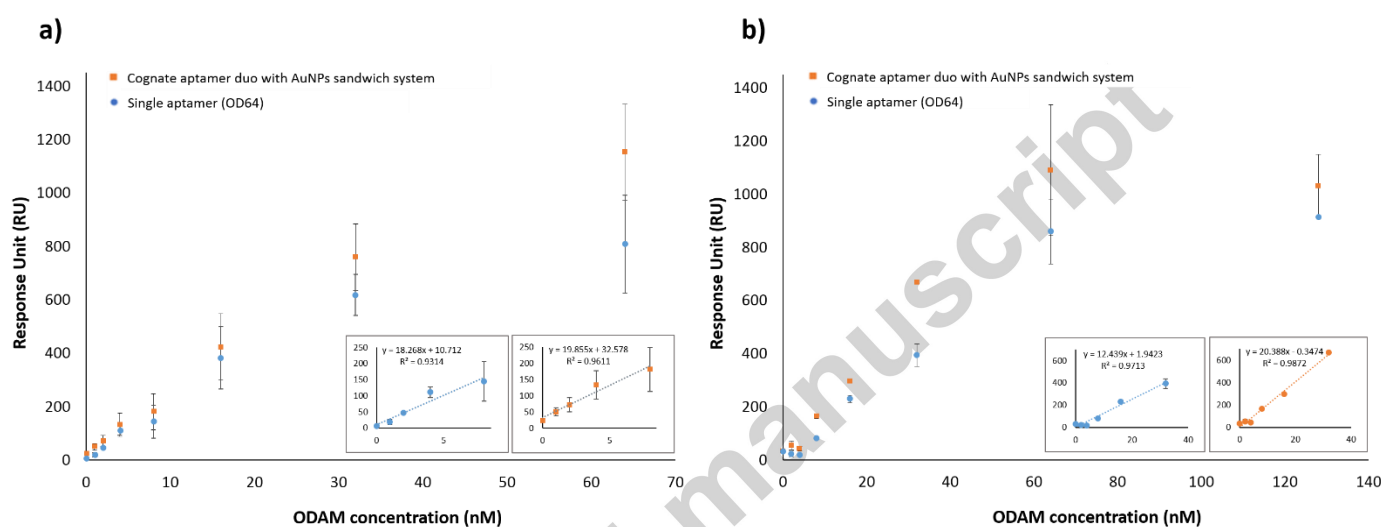
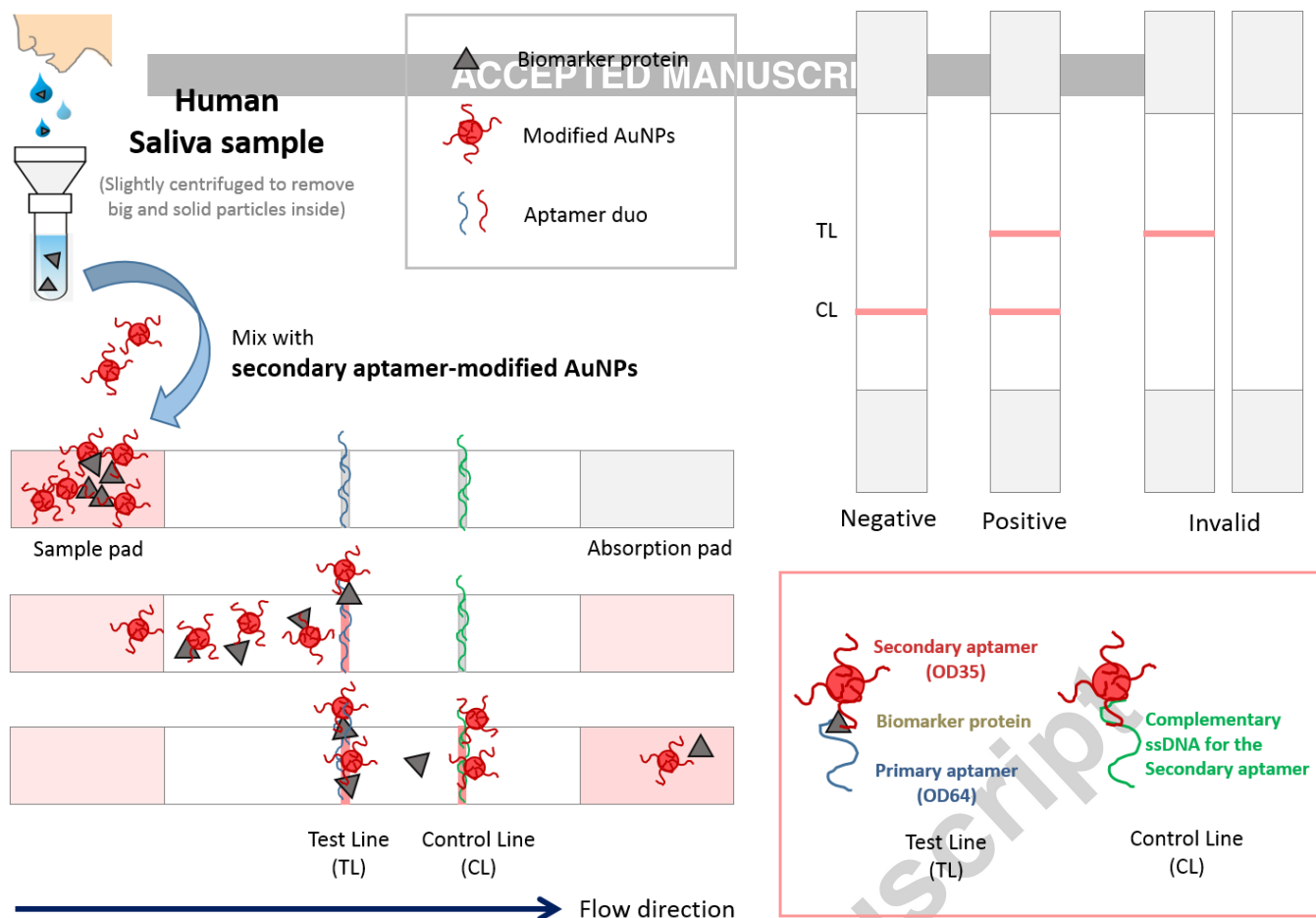


Fig. 5. Comparison of the response units of SPR biosensors in response to target ODAM (a) in the buffer and (b) in human saliva between the use of a single aptamer (OD64) and the use of a cognate pair of aptamers (OD64 and OD35).

3.5. Development of a sandwich-type lateral flow strip biosensor (LFSB) using a cognate pair of aptamers: its specificity and the limit of detection

Since all these different characterization assays agreed well with its first discovery, confirmation of a cognate pair of aptamers, we successfully developed a lateral flow strip biosensor (LFSB) which can be used to become an improved diagnostic tool for the periodontal diseases. More specific information about experimental concepts are given in Scheme 1.



Scheme 1. Schematic diagram of ODAM detection using a lateral flow strip biosensor.

These assembled paper strips shown in Scheme 1 were used for sandwich-type lateral flow strip biosensor using a cognate pair of aptamers. The feasibility of ODAM detection by using this sandwich-type LFS biosensor was studied by testing various concentrations of ODAM (2940, 1960, 980, 490, 245, 122.5, 61.25, 30.62, 15.31, 7.65, 3.82, 0 nM) in buffer (10 mM PBS) and human saliva. The Fig. 6 indicates that the detection limit of this cognate pair of aptamers-based LFS biosensor was found to be 8.32 nM and 14.59 nM of ODAM in buffer and saliva condition, respectively by naked-eye. Additionally, the specificity of this LFS biosensor was also confirmed by testing with other non-target molecules, such as α -amylase, human serum albumin, normal human saliva sample. (Fig. S4)

There have been many approaches to improve conventional diagnostic methods of periodontal diseases by developing digitalized and supplemental diagnostic tests which can provide the information about ongoing inflammation process by detecting various well-known biomarker candidates. However, despite these technical improvements, the majority of researchers and clinicians are steadily relying on elemental assessments such as probing and radiography. Based on the studies which state a mechanism of ODAM expression in various pathologies and its potential abilities to be used as a periodontal disease biomarker (Nishio C et al., 2000; H. K. Lee et al., 2011, 2012, 2016, 2018; R. M. Wazen and P. Moffatt et al., 2015; Yu M, Mu Y, Qi Y et al., 2016), we chose ODAM as a biomarker for diagnosing periodontitis. Especially for the human ODAM, measuring its exact concentration in human saliva is still in the problem under investigation. By using ODAM ELISA kit, researchers and clinicians are able to detect ODAM, but this assay system needs to be more improved due to its reproducibility and questions in its accuracy. The most noticeable thing is that, since no SPR or LFS biosensors have been studied for the target ODAM as its biomarker, this cognate pair of aptamers-based SPR and LFS biosensor development will positively impact the early diagnosis of periodontal diseases in an efficient way.

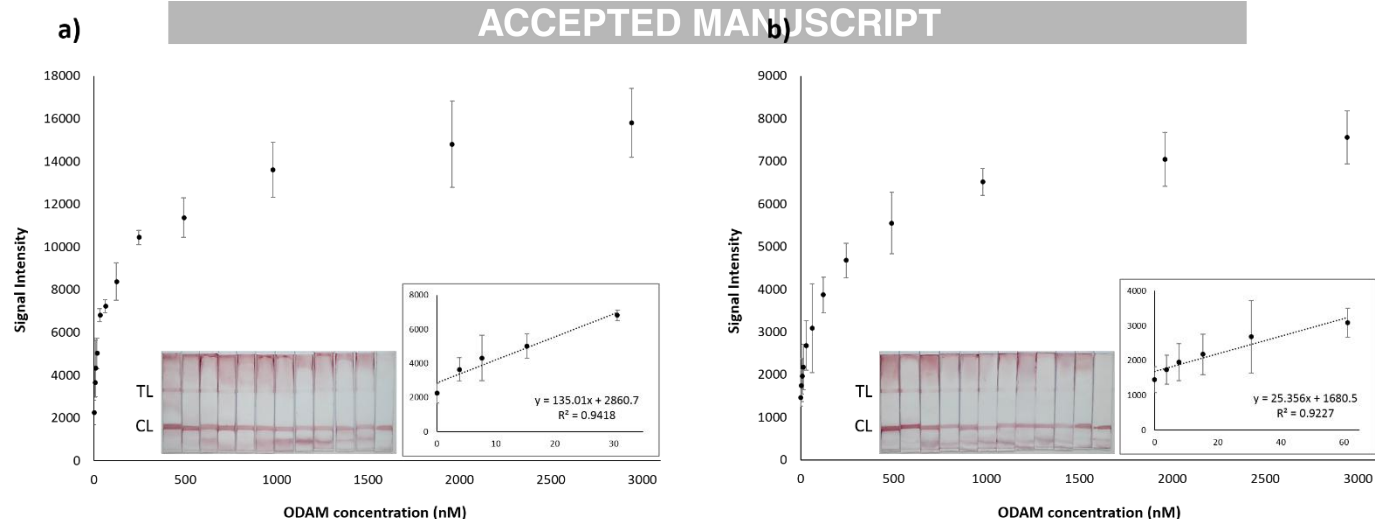


Fig. 6. Development of a sandwich-type lateral flow strip biosensor using the selected cognate pair of aptamers OD64 and OD35 for the detection of the target, ODAM. (a) Dose-dependent saturation of this LFS biosensor in PBS buffer spiked with ODAM and (b) in real saliva samples spiked with ODAM.

Conclusions

This study clearly represents that a cognate pair of ssDNA aptamers, which can specifically bind to the target ODAM protein at different sites by immobilization-free GO-SELEX, was favorably screened followed by confirming their characteristics with different assays. By using this cognate pair of aptamers, a sandwich-type SPR biosensor was developed with having a limit of detection at 0.24 nM and 1.63 nM in the buffer and saliva samples, respectively. In addition, this cognate pair of aptamers was successfully utilized to develop a sandwich-type lateral flow strip biosensor which shows its limit of detection at 8.32 nM and 14.59 nM in the buffer and saliva samples, respectively. More importantly, we could detect the target ODAM protein quantitatively, not only in the buffer but also in human saliva samples which ensures faster outcomes when comparing to current diagnostic methods. In conclusion, we have demonstrated the ability of the cognate pair of aptamers-based biosensors for the sensitive detection of ODAM, a potential biomarker for an early and non-invasive diagnosis of periodontitis. From continuing studies on the complete confirmation of ODAM as a biomarker of periodontitis along with the clinical tests with healthy and patient saliva samples, a sandwich-type binding pair of aptamer based biosensors with higher sensitivity will be finally developed for the early diagnosis of the periodontal diseases.

Acknowledgments

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Ethical approval

Researches about human-oriented samples are contained in this article. Every procedure was performed according to the ethical standard of all universities under the law of IRB (1040548-KU-IRB-17-154-A-3). Written informed consent was obtained

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Highlights

- Human odontogenic ameloblast-associated protein (ODAM)-binding a cognate pair of aptamers with high specificity and sensitivity was developed by GO-SELEX under the harsh condition of human saliva in a negative selection.
- By CD analysis and CLSM, a cognate pair of aptamers forming sandwich-type binding to ODA was confirmed and visualized.
- This cognate pair of aptamers was successfully utilized to develop a sandwich-type of SPR and lateral flow strip biosensor with high sensitivity.
- These sandwich-type aptasensors work well in real saliva samples.