# Supplementary Information

# Aptamer Selection against Alpha-defensin Human Neutrophil Peptide 1 on an Integrated Microfluidic System for Diagnosis of Periprosthetic Joint Infections

Rishabh Gandotra[1], Hung-Bin Wu[2], Priya Gopinathan[2], Yi-Cheng Tsai[2], Feng-Chih Kuo[3], Mel S. Lee[4**] and Gwo-Bin Lee[1,2*]

[1]*Institute of NanoEngineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan*

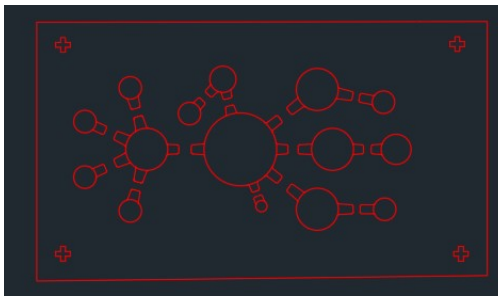
[2]*Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu Taiwan*

[3]*Department of Orthopaedic Surgery, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan*

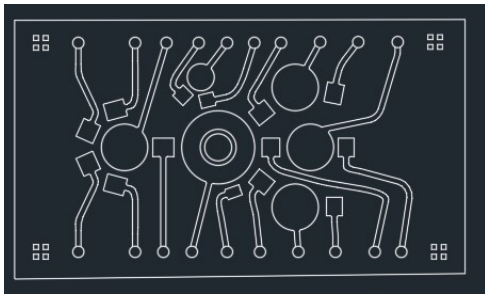
[4]*Department of Orthopaedic Surgery, Paochien Hospital, Pintung, Taiwan*

**Microfabrication of the microfluidic chip**

Microfabrication of the chip included 1) design of the poly-methyl methacrylate (PMMA) mold, 2) computer numerical control (CNC) engraving, 3) polydimethylsiloxane (PDMS; Sylgard



184 A/B, Dow Corning, USA) mold replication, 4) casting of PDMS layers, and 5) glass bonding. To design the PMMA mold, computer aided drawing (CAD) was used (AutoCAD 2017, USA). The 2D CAD drawings (Fig. S1) were converted into 3D solid-part files (Fig. S2) by Solidworks 2013 x64 (Dassault Systèmes SolidWorks Corporation, USA). These files were then simulated using VISI simulation software for mold preparation (VISI-Hexagon, USA) and uploaded to the CNC machine for mold microfabrication as in our prior works.



(a)

(b)

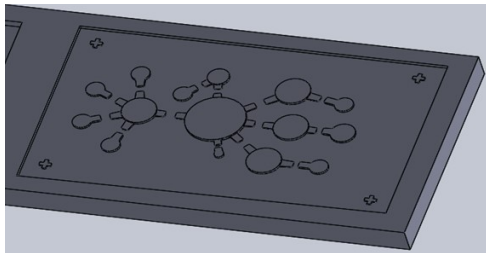


**Fig. S1.** Two-dimensional drawings of the (a) air control layer and (b) liquid channel layer.

**Fig. S2.** Three-dimensional drawings of the (a) air control and (b) liquid channel layers.

**Immobilization of magnetic beads with proteins**

For the immobilization of magnetic beads with target proteins, 100-µL aliquot of the



(a) (b)

Dynabeads (Dynabeads™ M-450 Epoxy, Invitrogen, USA; 2 x $10^9$ beads $mL^{-1}$, diameter = 4.5 µm) were collected and transferred into a micro-centrifuge tube under a bio-safety-level (BSL) cabinet or a ventilation hood to avoid any contamination. It was then washed twice with 100 µL of 0.01 M phosphate buffer saline (PBS; Merck, Germany) and suspended in 0.01 M sodium borate buffer (Merck, Germany; pH 9) to make the final volume of 1 mL. The beads were coated with human neutrophil peptide-1 (HNP 1) (D2043, HNP 1 human ≥80%, Sigma-Aldrich, Germany; 500 mg/L, 20 µL) protein as the target protein, as provided in the standard protocol. The micro-centrifuge tubes were then placed and mixed on a mini-tube rotator (RM-2, ELMI, Russia) for 30 mins at 45 RPM at room temperature (RT). The beads were then blocked with ethanolamine (E9508, ethanolamine, Sigma-Aldrich, USA, 0.5% w/v) that were gently mixed and incubated on the rotator with the same settings for 18-24 hr. The microcentrifuge tubes containing beads were

then collected and placed on a magnetic rack (DynaMag™-2 Magnet, ThermoFisher Scientific, USA) and the supernatant were discarded. The beads were then washed twice with 100 µL of 0.01 M PBS containing 0.01% Tween-20 (Sigma-Aldrich, USA). For the final step, the coated beads were suspended in 100 µL of 0.01 M PBS with 0.3% sodium azide and stored at 4°C till further use.

Beads coated with 8 µL of immunoglobulin G (IgG) (I4506, IgG from human serum, Sigma-Aldrich, USA, 1 mg/mL), 8 µL of human serum albumin (HSA) (A3782, albumin from human serum, Fluka Analytical, Switzerland, 1 mg/mL) and synovial fluid (SF) used the similar conditions to immobilize proteins on Dynabeads M-450 epoxy beads as mentioned above. The extracted clinical sample from patients (i.e. SF) was not diluted and kept in its original concentration while coating the beads. The concentration for the above two proteins (IgG & HSA) were kept at 1 mg/mL. The binding buffer used was 0.1 M sodium borate buffer (997 µL) + 3 µL of protein-coated Dynabeads in a micro-centrifuge tube. The reagents were then mixed gently and rotated for 30 mins at 45 RPM at RT. For the next step, the beads were blocked with 1 µL of 20 µM bovine serum albumin (BSA, A7030, Sigma-Aldrich, USA) for overnight at 40 RPM for 10-12 hr on the rotator. For the final step, the beads were washed and suspended with 100 µL of PBS and were stored at 4°C till further use.

**TA cloning, transformation and screening of colonies**

For the assessment of final SELEX products after the complete procedure that involved 5 rounds of positive selection, 2 rounds of negative selection and one round of competitive selection, TOPO™ TA cloning kit (Invitrogen, USA) was used such that the samples were purified using

vectors. The plasmids prepared after TOPO reaction mix were cloned and transformed using chemically competent *E. coli*, One Shot® Competent Cells (Life technologies). They were then plated on a LB culture plate and colonies were further extracted and cultured in culture medium. The transformed cultures of individually extracted colonies were screened using a PCR process and confirmed for the presence of a band at 72 base pairs (bp) for the defined affinity (Fig. S3).

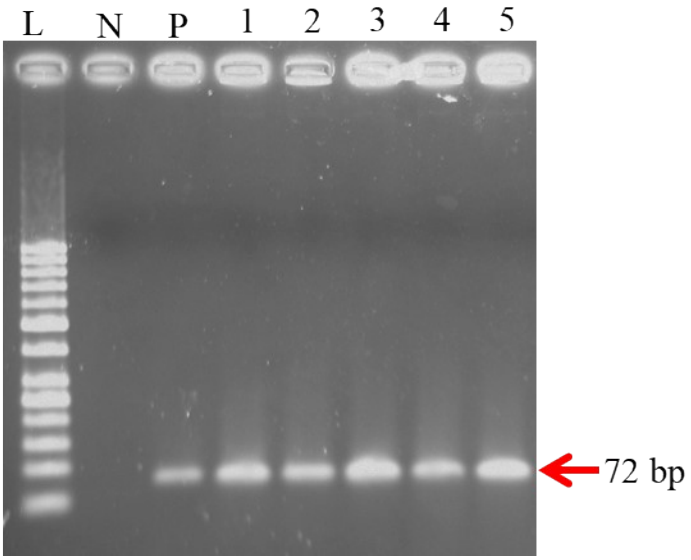



**Fig. S3.** Screening of TA-cloned colonies after PCR on a 2% agarose gel. A red arrow denotes the desired band size.

**Aptamer candidates and sequence**

Based on the screened TA colonies after PCR, 1 mL of cultured broth was sent for sequencing (Genomics Inc, Taiwan). The sequences were obtained as given below. We identified the sequence by using the sequence of forward and reverse primers used in the process of PCR in SELEX.

F-ACAGCACCACAGACCA

R-TGTTTGTCTTCCTGCC

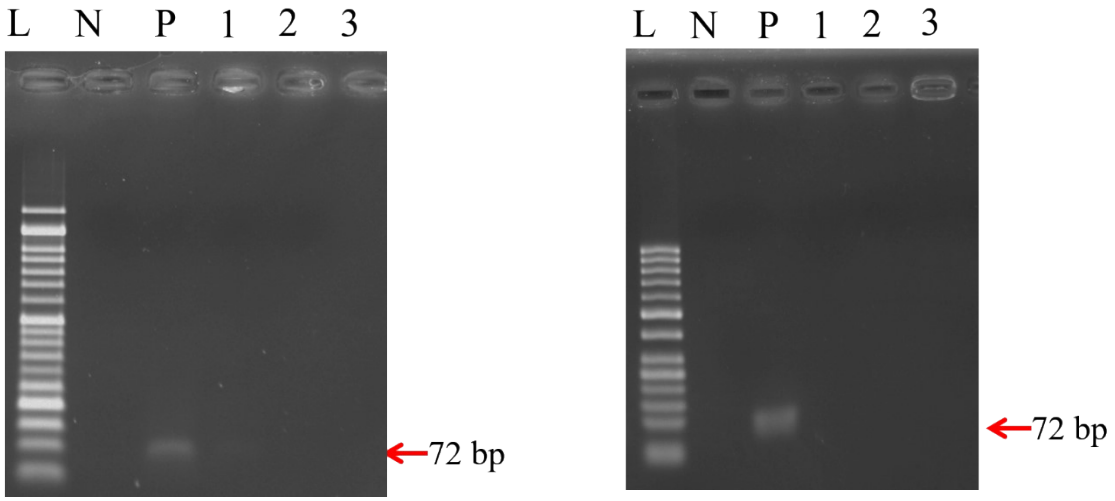
F-GGCAGGAAGACAAACA

R-TGGTCTGTGGTGCTGT

Once the strand was isolated, the sequence of 72 bp was obtained for further analysis.

AGGCCTAGCTGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTG
GCAGGAAGACAAACACCGCACAATGCACGAGCTAGTCATACCGGTTACTGATCGGT
GGTCTGTGGTGCTGTAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATC
CGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATA
GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAAT
TCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAG
TGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACC
TGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGT
ATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGC
GGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGG
GATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA
AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACA
AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAG
GCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCG
GATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTG
TAGGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAAC
CCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC
CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTACAGGATAGCAGAGC
GAGTATGTAGCGTGCTACAGAGTTCTTG

From the process of SELEX, we have identified 33 aptamer candidates, out of which the best sequence (number 9, or S9) has re-occurred 3 times and its reverse compliment also occurred 3 times. Based on several key factors such as G-C pairs, lower Gibbs free energy, hydrogen bonding etc., the S9 sequence was selected to be the most suitable candidate for further experimentation.




**Affinity test of aptamer towards HNP 1 and non-specific proteins (IgG & HSA)**

**Fig. S4.** Affinity test of the aptamer towards non-specific proteins under different PCR conditions, where L=50-bp DNA ladder, N=negative control (water), P=positive control (ssDNA), and 1, 2, and 3 represent 20, 15, and 10 PCR cycles, respectively.

**Testing of binding efficiency of aptamer (S9) while compared with scrambled ssDNA library**

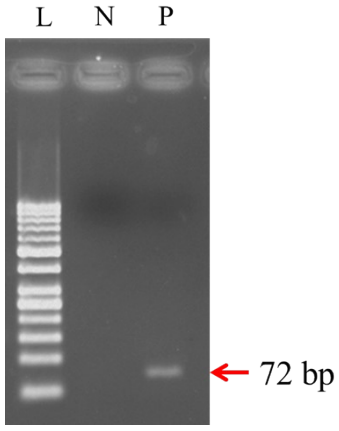



**Fig. S5.** Affinity test of the aptamer towards HNP 1 protein for 1 round of SELEX with PCR

performed for 20 cycles, where L=50-bp DNA ladder, N=scrambled aptamer (random sequence of S9 aptamer), P=positive control (S9 aptamer).

In the above process, one round of the SELEX (details mentioned in the manuscript) was performed with S9 aptamer with the above-discussed SELEX by using HNP 1 coated epoxy beads where the ssDNA was replaced by the scrambled aptamer sequences, i.e. randomized sequence of ssDNA library. The final products were analyzed by agarose gel electrophoresis. A clear band observed for S9 aptamer at 72 bp indicates the strong affinity towards the HNP 1 protein while no band was observed for scrambled ssDNA indicating no significant affinity towards the target protein. The present assay suggests enough evidence of the affinity of S9 aptamer and scrambled ssDNA as negative control towards HNP 1.

**Table S1**. Operating conditions for aptamer-based ELISA-like assay for the dissociation constant (Kd) value assay and the calibration assay.

| **Assay conditions** | **Kd** | **Calibration assay** |
|---|---|---|
| Concentration and (number of beads) used for coating | 1 mg/100 µL (~$8.5 \times 10^8$) | same |
| Volume of aptamer | 5 µL of 100 µM stock | 7.5 µL of 100 µM stock |
| Number of beads per assay | 2.55 x $10^6$ in 3 µL | 5.95 x $10^6$ in 7 µL |
| Number of aptamers per bead (assuming all aptamers were coated on beads) | ~3.5 x $10^5$ aptamers/bead | ~5.3 x $10^5$ aptamers/bead |
| Dynamic range of HNP 1 | 3-300 nM (0.01-1 mg/L) | 0.1 mg/L – 100 mg/L |
| First incubation time (aptamer-coated beads+HNP 1) (mix 1) | 60 min | 30 min |

| | | |
|---|---|---|
| Primary antibody dilution factor | 1:500 | 1:1,000 |
| Second incubation time (mix 1+primary antibody) (mix 2) | 60 min | same |
| Secondary antibody (dilution factor) | 1:2,500 | 1:5,000 |
| Third incubation time (mix 2+secondary antibody) | 60 min | same |
| Reaction volume of assay | 200 µL | 50 µL |
| Washing buffer (twice after every mixing step) | 0.01 M PBS + 0.01% Tween 20 (200 µL) | 0.01 M PBS + 0.01% Tween 20 (50 µL) |

**Commercial kits and established assay for AD detection**

At present, only few works focused on HNP 1 for PJI detection using synovial fluid could be found in literature, but all of them are for HNP 1-3 alpha defensins. Some kits like Synovasure target the negative and positive detection of samples. The comparison for detection ranges at such stage with our present work becomes invalid due to different parameters used such as detection from serum obtained from blood, 1000 x dilution for the clinical samples in assay conditions.

Unit presented in ng/mL scale = samples were used as 1/1000 dilution

Unit presented in mg/L scale = samples original concentration

1. Assay using HPLC to determine HNP 1-3 in synovial fluid is (2-100 mg/L) [2].
2. Detection limit for HNP 1-3 in PJI detection from immunoassay is 1.5 mg/L [13].
3. Alpha defensins HNP 1-3 using lateral flow test is 1.56 mg/L

HNP 1 has been detected using the tests with serum/SF dilutions.

Threshold value (HNP 1) = 00.03 mg/L or 30 ng/mL [49]

**Table S2**. Detection limit of various commercialized products

(The information is obtained from company's website and product manual)

| **Company** | **Detection range** |
|---|---|
| Biomatik | 0.625 ng/mL- 40 ng/mL |
| LifeSpan BioSciences | 0.3 ng/mL-20 ng/mL |
| MyBioSource | 0.5 ng/mL-32 ng/mL |
| R&D Systems | 0.5 ng/mL-32 ng/mL |
| Abbexa Ltd | 0.312 ng/mL-20 ng/mL |
| CUSABIO Technology LLC | 0.625 ng/mL-40 ng/mL |
| BosterBio | 0.3 ng/mL-20 ng/mL |
| Hycult Biotech | 0.15 ng/mL-10 ng/mL |