Comparing these values, we can find the differences in diffusion coefficient between the bulk space and the nanospace.

3. EXPERIMENTAL

To investigate a diffusion coefficient and a hydrodynamic flow in nanopillar chips, we applied single-particle tracking (SPT) technique as shown in Fig. 1. Fluorescence polystyrene nanospheres that have 50 nm in diameter and have carboxylate groups on their surfaces were used as objective particles for measuring trajectory. Brownian motion of nanospheres was captured and their center of mass was analyzed and traced over 20 sec (Fig. 2).

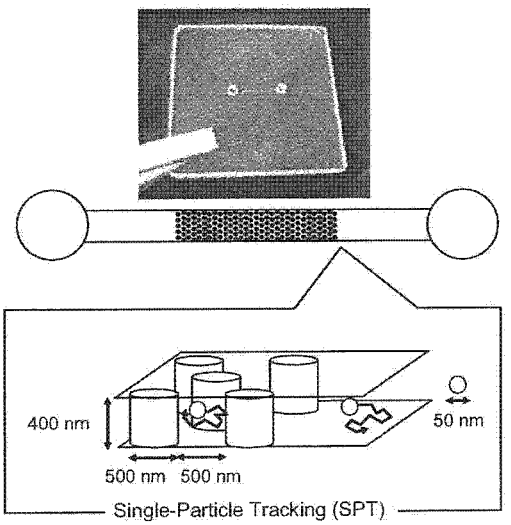

Figure 1. Schematic representation of the nanopillar chip used for a single-particle tracking (SPT) measurement.

4. RESULTS AND DISCUSSION

The diffusion coefficient at 132 ms time intervals were measured for 17 different particles over 20 sec, and the diffusion coefficients was D_{ch} = 3.15 $\mu m^2/s$ in the 400 nm-depth nanochannels and D_{pl} = 1.84 $\mu m^2/s$ in the nanopillars which have 500 nm diameter, 500 nm spacing and 400 nm height. These values could not be simply compared because geometrical differences, especially the occupied area of 500-nm wide nanopillar itself, were not factors into calculation. On the other hand, the diffusion coefficient estimated from the Einstein-Stokes equation was D_{th} = 9.81 $\mu m^2/s$ at

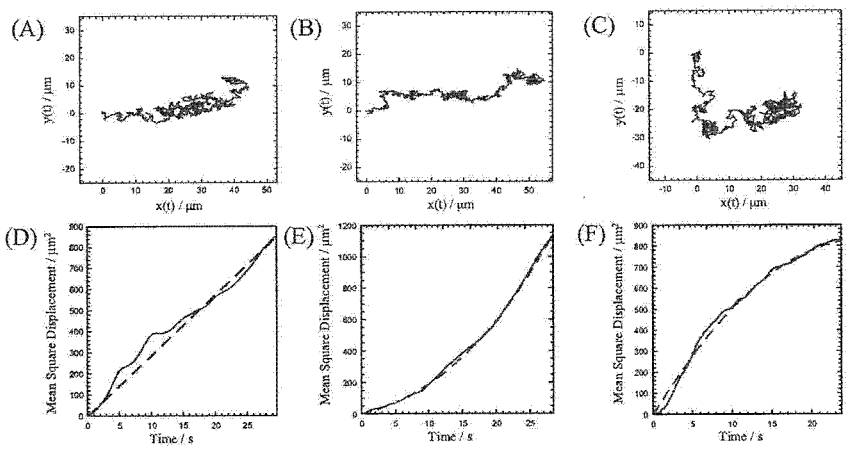

Figure 2. Typical trajectories of nanospheres (A, B, and C) and the corresponding plots (D, E, and F). Based on the result of least-square fitting, plots were classified into three motion modes: (D) a simple diffusion, (E) a directed diffusion, (F) others including a confined motion.

25 °C. Even if we could not simply compare the D_{ch} with D_{pl}, both of them are obviously smaller than the D_{th}. The smaller diffusion coefficient suggested higher viscosity in the nanometer-height channels and the nanopillars.

From the long-time tracking of the trajectory, we classified the motion of nanospheres into three modes: simple diffusion, directed diffusion which implies a presence of hydrodynamic flow and others. Consequently more directed diffusion was observed in the nanochannel than that in the nanopillars (Table 1). This result indicates that the nanopillars prevent a hydrodynamic flow to some extent when adjacent nanochannel has a hydrodynamic flow.

	Nanochannel	Nanopillars
Simple Diffusion	7 (41%)	9 (53%)
Flow + Diffusion	7 (41%)	4 (24%)
Confined Motion	3 (18%)	4 (24%)

Table 1. The number of classified motion modes of nanospheres and the percentage of total in parentheses. Most of the modes of nanospheres motion were classified into two modes, simple diffusion and directed diffusion, but their ratio was different between in the channel and the pillars.

5. CONCLUSIONS

The diffusion constants of nanospheres in nanometer-confined space suggested that the water in nanospace has higher viscosity as compared with bulk water. The classification of the modes indicated the presence of a hydrodynamic flow even in the nanospace. But it also indicated that the degree of a hydrodynamic flow in the nanopillars is less than that in the channel. These physicochemical properties in nanospace might contribute to realize high-throughput DNA separation system using nanopillars.

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ALLOCATION DEPENDENCE OF NANO-PILLARS FOR DNA ELECTROPHORESIS SEPARATION

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ABSTRACT

Different allocations of nano-pillars were investigated for DNA electrophoresis separation. The square allocation shows that DNA was extended and moved straight without disturbance of pillars, but DNA entangled around pillars in the case of the tilt allocation. The tilt allocation allowed us to separate DNA of 10 kbp and 38 kbp by their sizes, whereas square allocation demonstrated one broad peak alone. The separation in the tilt nano-pillars pattern is considered to result from the sieving effect for different sizes of DNA. These results suggest that any disturbance is necessary for electrophoretic separation of small size DNA.

Keywords: DNA Electrophoresis, DNA Separation, Nano-Pillar

1. INTRODUCTION

Fast DNA sequencing is necessary for the tailor made therapy and the genomics drug discovery in the near future. To realize the goal, the speed of DNA separation should be improved over 10^9 times than the present. Recently a channel filled by nano-pillars of 500 nm diameter and 500 nm spacing instead of an usual gel enabled us to achieve electrophoresis separation in 25 sec for λDNA (48.5 kbp) and T4 DNA (165.6 kbp), and furthermore the tandem arrangement of nano-pillars regions allowed to separate smaller DNA of 10 kbp [1].

This paper reports fabrication of nano-pillars in a channel that has different nano-pillar allocations and an important indication to reveal the mechanism of DNA separation by applying different allocations of nano-pillars for DNA electrophoresis. This work is expected to provide adequate nano-pillar structure so that different sizes of DNA are separated quickly and distinctly.

2. EXPERIMENTAL

The fabrication method of nano-pillars was reported already by our group [1]. Briefly, 500 nm-diameter holes pattern was delineated by a EB lithography on a posi-type resist spin-coated on a quartz plate. Ni was electroplated at holes and then after resist removal, pillars were fabricated by a dry etching using Ni mask. The diameter and the height of the nano-pillar were 500 nm and 4 μm, respectively. In this study, fabrication issues of irregular thickness of electroplated Ni pillars, narrowing of gaps among pillars due to resputtering of Ni pillars and unreliable bonding of 130 μ m thick quartz covering plate on the pillar fabricated quartz plate were overcome.

For acquiring the electrophoresis behaviour of single molecule DNA in nano-pillars, T4 DNA with 166 kbp was stained with intercalating fluorescence dye of YOYO-1 and the behavior within nano-pillars was observed by a fluorescence microscope, where the applied

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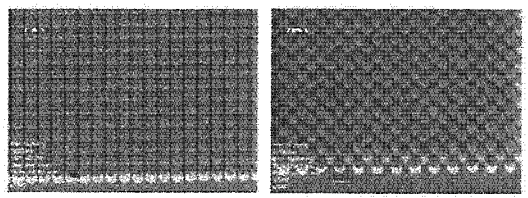

Figure 1. Allocation patterns of nano-pillars for (A) square pattern and (B) tilt pattern.

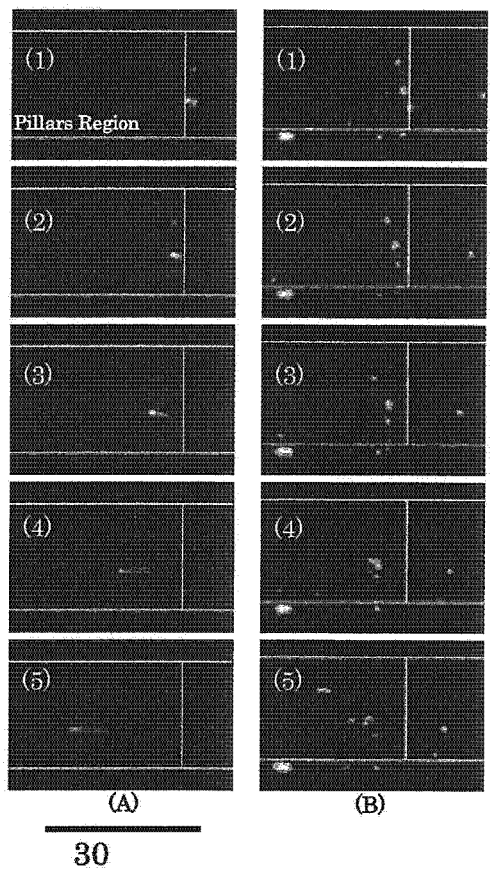

Figure 2. DNA (T4 DNA: 166 kbp) electrophoretic behavior for square (A) and tilt (B) allocations when DNA enters from no pillars region to pillars region.

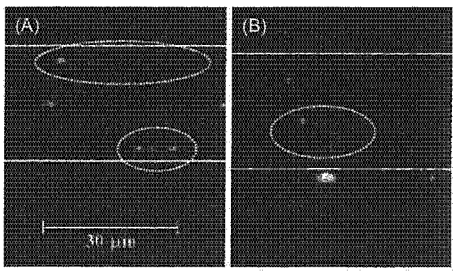

Figure 3. DNA (T4 DNA: 166 kbp) behavior for square (A) and tilt (B) allocations in nano-pillars.

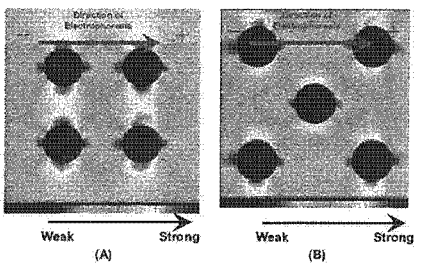

Figure 4. Electric field simulation for square (A) and tilt (B) allocations in nano-pillars.

electric field was 25 V/cm. Electrophoresis separation of 38 kbp and 10 kbp DNA obtained by digestion by ApaI was investigated by cross-injection into the nano-pillar regions, where the applied electric field for electrophoresis was 100 V/cm. The fluorescence intensity was observed at a point where was 500 μm distant from the entrance to the pillar region.

3. RESULTS AND DISCUSSION

The pillars were allocated at a square pattern and a tilt pattern, shown in Figure 1. A center-to-center distance between pillars was 600 nm.

Figure 2 shows a series of photographs that demonstrate conformation changes of DNA which enters from no pillars region to pillars region. Figure 3 shows detail movement of DNA in pillars region. Behaviors in square and tilt patters were compared. The square pattern shows that as soon as DNA entered to the pillar region, it was extended and moved straight without the disturbance of pillars. In the case of the tilt pattern, however, DNA

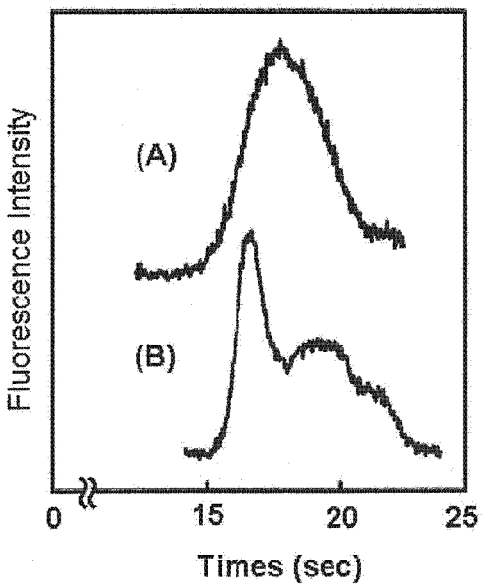

Figure 5. Electrograms in square (A) and tilt (B) nano-pillars for (10 kbp and 38 kbp obtained by DNA digested by ApaI. Intensity of fluorescence stained DNA was observed at a point where was 500 μ m distant from the entrance to nano-pillar region.

entangled pillars and could not move straight. Figure 4 shows electric field distributions for both allocations. The square pattern demonstrates strong electric field between pillars. This electric field is considered to enhance the DNA electrophoretic velocity. Thus DNA in the square pattern could pass through nano-pillars in faster than that in the tilt pattern. Indeed, when the velocity was estimated for chosen 30 DNA molecules, DNA velocities in square and tilt patterns was 26.95 μm/sec and 15.21 μm/sec, respectively.

The resul was shown in Figure 5. Opposed to the different velocities of single molecules of T4 DNA for two pillar allocations, the electrophoretic velocity of digested DNA did not depend on allocations of pillars. However, the square pattern showed only one broad peak, while the tilt allocation revealed the separated double peaks probably due to 10 and 30 kbp DNA within 20 sec as mentioned in the reference [1]. The separation found in the nano-pillars of tilt allocation is considered to result from the sieving effect for different size of DNA.

4. CONCLUSIONS

These results suggest that any disturbance is necessary for electrophoretic separation of small size DNA. According to the result, nano-pillars of tilt allocation fit to the size-based separation of DNA, and nano-pillars of square allocation contribute mainly to straight extension of DNA in nano-pillars, whereas they are presumed to have poor separation ability. The combination of square and tilt allocations is expected to lead to the control of separation and conformation of DNA in nano-pillars. Further work will make clear adequate allocation, size, gap and combinations of nano-pillars to separate efficiently DNA at wide range of sizes.

ACKNOWLEDGEMENTS

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HEALTH CARE CHIP BASED ON INTEGRATED ELECTROCHEMICAL SENSORS USED FOR CLINICAL DIAGNOSTICS, BUN AND CREATINE

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ABSTRACT

In this study, focus has been placed on potentiometric measurement of ammonia ions for BUN (blood urea nitrogen) and Creatine sensors by using new 19-membered crown ionophore, TD19C6 (3wt%), PVC (30wt%) membranes along with anionic additives (10mol %) K-TCPB and TFPB, and plasticizers (67wt%) BBPA and TOTM. The screen printed electrodes Ag/AgCl (250mm in diameter), newly developed non-liquid junction reference electrode, painless needle and disposable polycarbonate chip (PC), designed of using a trace of amount of whole blood extracted from painless needle is demonstrated.

Keywords: healthcare chip, electrochemical sensor, blood urea nitrogen (BUN), Creatine

1. INTRODUCTION

In the sensor development, concept of micro total analytical system (μ-TAS) and lab-on-a-chip system has been emerged as a means to provide vital technology. This paper reports a BUN (blood urea nitrogen) measurements based on detection of ammonia ions with an ionophore membrane and new development of non liquid junction type KCl-saturated reference electrode.

2. THEORY

In the previous study [1, 2, 3, 4] it showed that the BUN measurement was based on the detection of proton loss [H^+] during the reaction, NH_2CONH_2 (urea) $+2H_2+H^+ \rightarrow NH_4+HCO_3^-$, in the presence of urease. In this paper we report urease follows NH_2CONH_2 (urea) $+ 2H_2O \rightarrow 2NH_4^+ + CO_3^{-2}$ in the presence of urease and water. The released ammonia ions from hydrolysis are then detected by the ion-selective ionophore membrane measuring the potential difference between different concentrations of ammonia solutions. Consequently, the detected potential from various concentrations may have to fit the calculated results through Nernst Equation.

3. EXPERIMENTAL

In order to extract human blood from capillary vessels, painless needle made by using stainless (SUS) tube 150 μm in diameter with 80 μm bore (Figure 1), screen printed electrodes (Figure 2) along with disposable PC made microfluidic system were developed (Figure 1).

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Figure 1 painless needle (left), chip after insert painless needle (right)

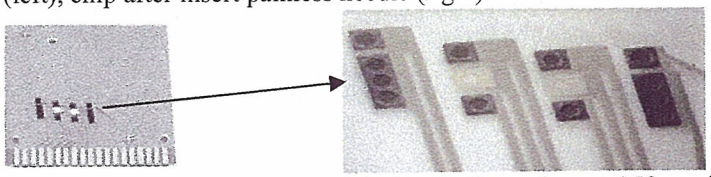

Figure 2 Screen printed electrode (carbon electrodes and Ag/AgCl electrodes are 250mm in diameter)

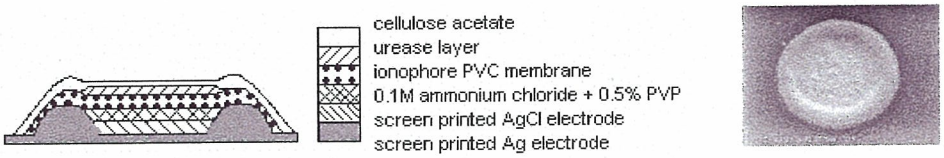

Figure 3: membrane formation of urea sensing membrane and electrode (left); surface formation of membrane on electrode by SEM (120×83.3μm WD 20.6mm) (right)

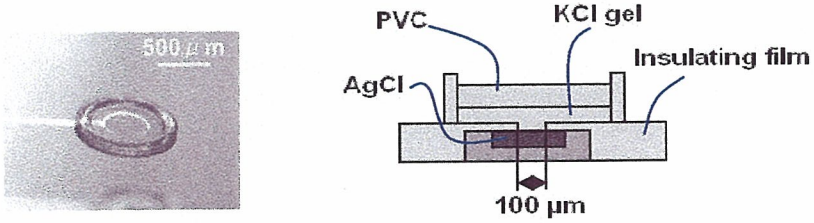

Figure 4 non-liquid junction reference electrode

4. RESULTS AND DISCUSSION

As one application of μ-TAS, we are developing a variety of clinical chips. One of them is a healthcare chip which enables us to check pH, Na+, K+, glucose and BUN from a trace amount of blood collected by a painless needle as shown in Fig. 1.

Four membrane recipes have been used and the results show reasonably stable measurements to various urea concentrations ranging from 1mM, 0.01M and 0.1M urea solutions (Fig. 5, (a)). In Fig. 5, calculated potential change per decade is about 35mV of Formula 1 while in it shows potential change of lower than 35 mV per decade of Formula 2, indicating that Formula 1 and 2 with proper inner layer anionic additives might provide better detection. In the BUN sensors study, Formula 3 and Formula 4 are not suitable due to their unstability to immobilize necessary components inside the membrane. In this study, Creatine measurement has also been carried out by using the same membrane configuration

with a mixture of Urease and Creatine amidinohydrolase. However, the results were tentative at a detection level of 0.01mM in concentration.

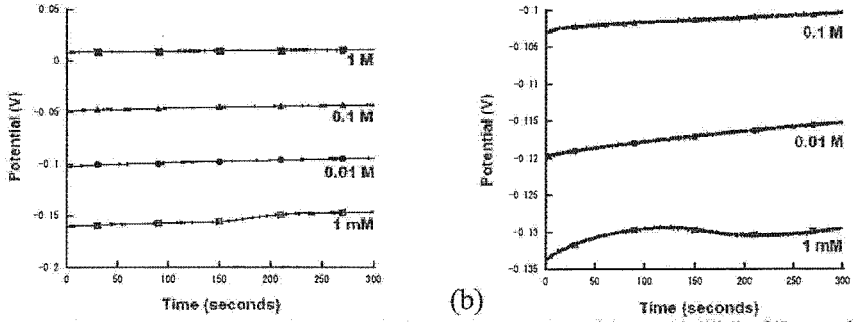

Figure 5: EMF measurement of ammonia ion of Formula 1 (a), and BUN of Formula 1 (b)

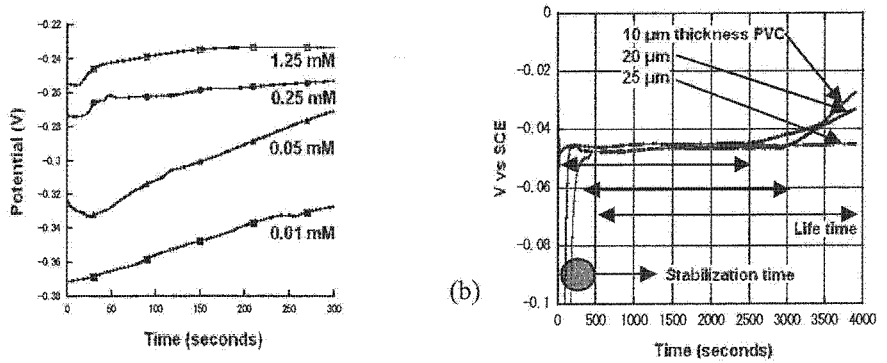

Figure 6: EMF measurement of Creatine (a) and measurement of non-liquid junction reference electrode (b) quick response time (<100 seconds) and long life time (>1000 seconds)

A reference electrode with rapid response less than 100 sec. long lifetime more than 1000 sec and no contamination of the solution are required for biochips which measure low level signals from trace amount of solutions such as a blood and thicknesses of 10 to 20 µm for the PVC film thickness was adoped for our on-chip measurement.

5. CONCLUSIONS

We successfully developed non-liquid junction reference electrode and achieved reasonable results on BUN and Creatine sensors based on ammonia ions detection.

ACKNOWLEDGEMENTS

This study was performed as a part of the Advanced Nano-Bio Device Project (P03011) supported by NEDO (New Energy and Industrial Technology Development Organization).

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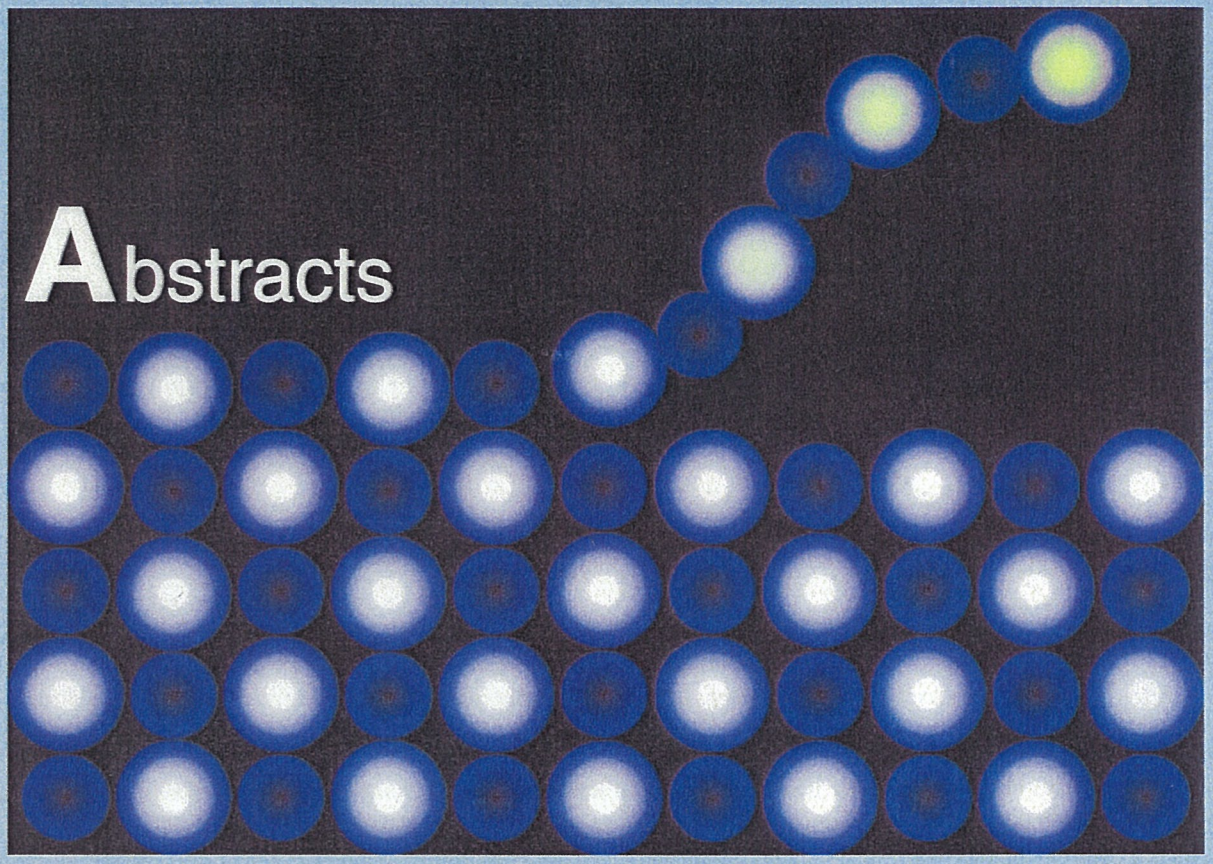

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Separation, Trapping and Single Molecule Detection of DNA Employing Nanostructures

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Faster DNA sequencing with 8-9 orders of magnitude over the present is required to realize the tailor-made medical treatment after 20-30 years. For the goal, we study fast separation of DNA extracted from cell lyses and subsequent detection of single molecule for sequencing of its DNA with nanostructures fabricated by EB lithography and RIE.

Our previous paper reported that tilt patterned nanopillars achieved 25 sec of fast electrophoresis separation of T4 (166 kbp) and λ (48 kbp) DNA [1]. To study the origin, allocations of tilt and square patterns with 500 nm in diameter and pitch were fabricated as shown in Fig. 1. Electrophoresis of 38 kbp and 10 kbp DNA digested by ApaI was investigated for both patterns. To contrast with only a bunched peak due to strong electric field among pillars in the square allocation, clear separation of them was observed by the tilt allocation. The result implies that the sieving effect caused by the snaked tilt pattern is responsible for the separation.

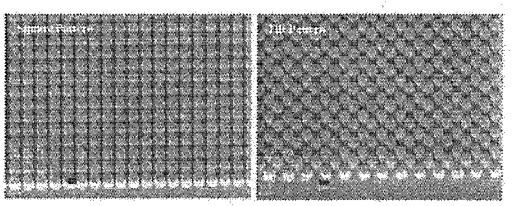

FIG. 1 Nanopillars with square (left) and tilt (right) patterns.

To trap the separated DNA, the triangle shaped channels with 50 nm gaps were fabricated on a quartz plate. Fig. 2 shows hybridization of a target DNA with T4 DNA trapped at nanogaps channel array. The technique is useful for a simple and quick detection of infection diseases coming from virus.

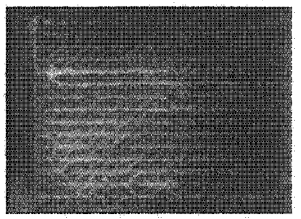

FIG. 2 Hybridized target DNA with trapped DNA at nanogaps.

To sequence this trapped DNA, we study detection of one DNA molecule using a nanopore. D. Branton et al. demonstrated electrical sequencing based on blockade of DNA translocating through the pore with 2 nm in diameter [2]. We study the nanopore detection of a mono-nucleotide cut by a reaction of λ exonucleotide. A hole (see Fig.3) opened in a Si wafer by anisotropic etching was shrunk by the digital CVD of Si nitride [3]. Besides, the pore size was reduced to 2nm by Ar^+ beam re-sputtering as monitoring ion currents with a MCP detector set at the backside of the pore.

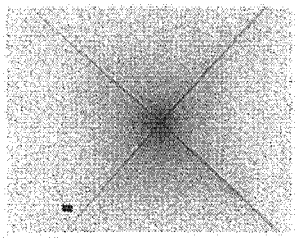

FIG. 3 Nanopore at bottom of Si anisotropic feature.

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PL-1 (Plenary)

Development of Clinical Chips for Home Medical Diagnostics

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1. Introduction

The aging society proceeds rapidly in many countries, thus pressing each national total budget by a medical cost spent for persons of advanced years. Early prevention is very important for them to live a healthy life. Clinical diagnostic is one of the promising applications of μ-TAS (micro Total Analytical System) or Lab on a Chip [1], especially in a point of care setting. We are developing a healthcare chips which enable us to check our health condition by analyzing a trace amount of blood collected by a painless needle [2]. This paper reports the simple blood collection, an electrochemical measurement chip for bio-chemical items and a calorimetric measurement chip for life style-related diseases.

2. Electronic blood collection by painless needle

The needle collecting the blood should be painless. The edge of a 150μm diameter SUS tube was polished at 10 degrees for three planes. The inner wall of the tube was also polished ultra-smoothly, leading to natural collection of the blood by our blood pressure. To collect the blood by ourselves, the location of the blood vessel was visualized using a NIR (850 nm) light emitting diode array. In addition, measurement of the potential between the skin and the blood vessel allowed us to detect depth of the blood vessel. The combination led to the electronic collection. Figure 1 demonstrates actual collection using the electronic collection system, where the man does not look at his own arm, but observed a display.

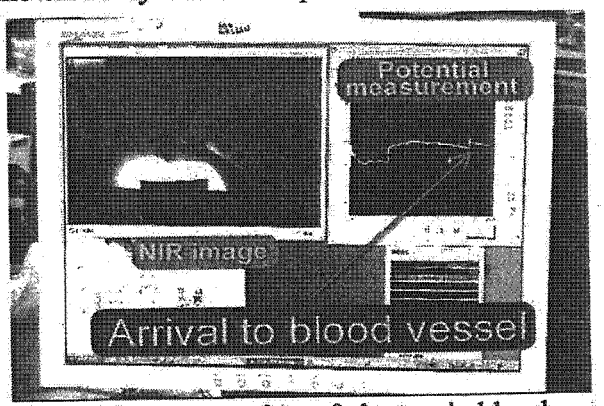

Fig. 1 Demonstration of electronic blood collection. Left; NIR image, Right; Potential variation. A person looks at the display during blood collection.

3. Electrochemistry measurement chip

We planed to measure the pH, Na^+, K^+, glucose, BUN and creatinine concentrations by an electrochemical method. The chip was fabricated by bonding two plates of a channel pattern and a screen-printed electrode patterns. Figure 2 shows this measurement chip, (a), and a magnified photograph of integrated chemical

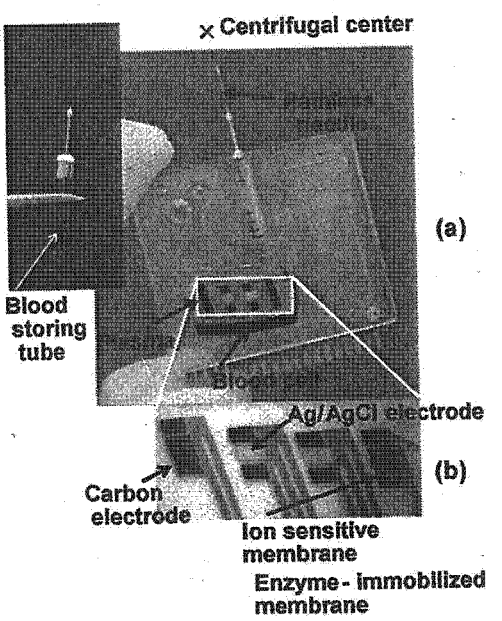

Fig. 2 Photograph of healthcare chip. A blood collected tube is inserted into the chip and centrifugall separated (a). Magnified integrated bio-censors (b)

sensors. (b). Both correction solution before the measurement and blood for the separation were conveyed by centrifugal operation, because individual blood should not be handled. The blood collection assembly was inserted into the chip after the correction and then separated to plasmas at the upper regions of the sensors and blood cells at the channel bottom.

First a non-liquid junction type KCl saturated reference electrode was developed for the electrochemical method to generate a standard potential for pontentiometric sensing. Next, Bis[(12-crown-4)methyl]-2dodecyl-2-methylmalonate and bis[(benzo -15-crown-5) -4-methyl]pimelate were used for Na^+ and K^+ ionophores. Selectivity of $Na^+/K^+=10^2$ and $K^+/Na^+=10^3$ are obtained due to the adequate anion exclusion agents. To improve the poor adhesion of glucose oxidase (GOD) at the glucose sensing, homogeneity of ferrocene was improved using VFc-co-HEMA polymeric mediator coated on a carbon electrode and chemical cross linking of glutaraldehyde on the mediater surface immobilized GOD. BUN was measured by ammonia generated from the unease reaction based on the ammonia measurement using 19-membered crown ionophore, TD19C6, PVC membranes along with anionic additives K-TCPB and TFPB, and plasticizers BBPA and TOTM. The creatinine measurement using creatine amidinohydrolase instead of unease requires more improvement at lower concentration. Now, automatic measurement of Na^+, K^+, glucose and BUN are available.

4. Calorimetric measurement chip

A hepatic function examination chip which detected γ-GTP, GOT and GPT enzymes using a calorimetric method was reported [2]. However this chip had problems such as an additional process needed for metering of plasma after the separation and high 50:1 mixing ratio of substrate buffers to plasma for GPT and GOT measurements. To solve these problems, a new calorimetric measurement chip has been developed. The chip consists of 3 layers. All process is carried out by the centrifugal force. In a 1st layer, blood separation/ plasma metering and metering of a 1st reagent are done simultaneously. Here, a long zigzag-like channel allows to separate blood cells and plasma during the transportation efficiently, thereby collecting

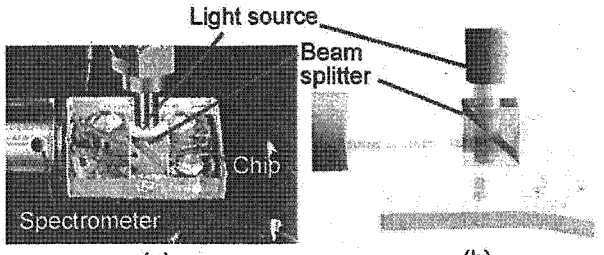

Fig. 3 Reflective optical absorption measure -ment system at the 3rd layer consisting of the mixing and the optical cell.

blood cells and apportioning plasma of the top clear layer to three chambers with a definite volume of 0.4 μL. A 2nd layer conveys both plasma and 1st reagent to two reservoirs with almost same volume. A 3rd layer introduces a 2nd reagent to reservoirs, and then plasma and two kinds of reagents are injected to a measurement optical cell through a mixing channel. The mixing is performed through a long transport channel equipped with many protuberances in the inner wall which cause the chaotic advection. Figure 3 shows an experimental measurement system of the 3rd layer where a white light is incident to the optical cell. An absorbed light reflected on an Al coated cell bottom was detected by a spectrometer though a beam splitter. Not only γ-GTP, GOT and GPT, but cholesterol and neutral fat were measured successfully.

5. Conclusion

To establish the home medical diagnosis, a variety of clinical chips together with the electronic blood collection system have been studied. These chips will lead to preventions of life style-related diseases such as kidney trouble, diabetes and liver disease. When the home medical diagnosis system is realized, the diagnostic data communication with clinical centers through a medical network is expected to change not only medical treatment, but the society system significantly.

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[2]A. Oki, H. Ogawa, S. Shinnbashi, M. Takai, A.Yokogawa and Y. Horiike, Mat. Sci. & Eng. C24 (2004) 837-843.

ポストゲノム技術が，健康管理に役立つときがくる

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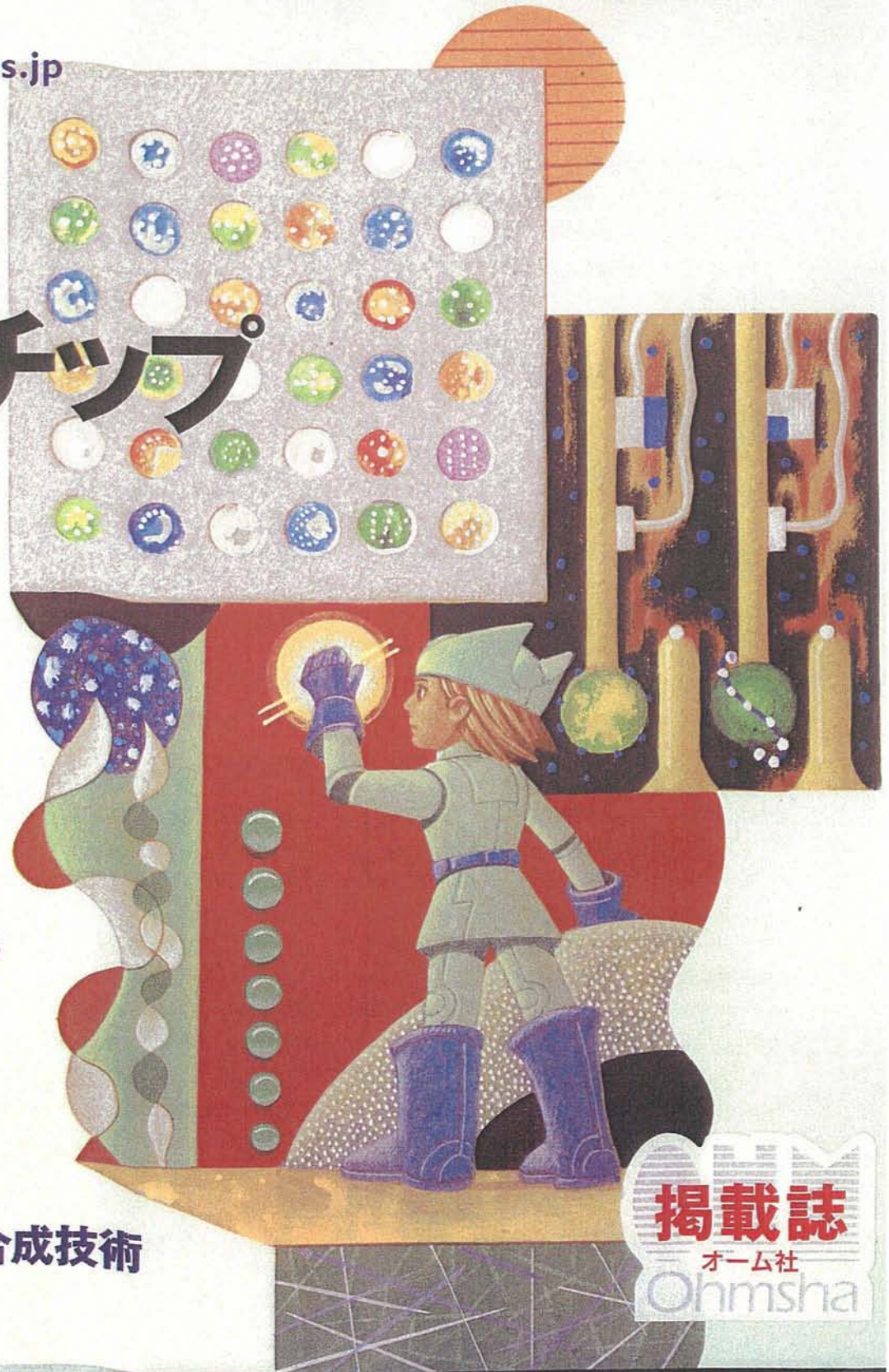

Special Issue

診断バイオチップの未来

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- タンパク質解析チップ
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Special Issue 03 診断用バイオチップの未来

在宅で健康診断できるヘルスケアチップの開発

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高齢化社会の到来がまぢかにせまっている。医療費の軽減や，早期発見・診断の必要性が増し，在宅で簡便・確実に検診できるバイオチップ技術の発展が期待されている。それを可能とする，ヘルスケアチップ技術はここまできている。

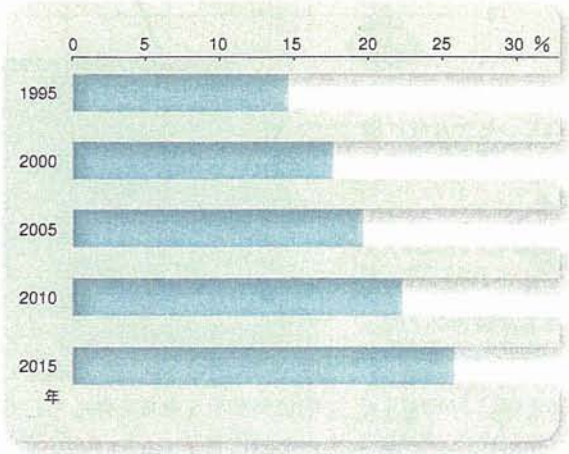


図
65歳以上の人口の総人口に対する割合

わが国では近年，高齢者層の医療費が増大し続けている。高齢者が元気で毎日を送るためには，予防が大切である。そのために，微量の採血から在宅で簡便・確実に同時多項目を診断できる，μTAS (micro Total Analytical System)やラボオンチップ[1]の応用として種々の診断用バイオチップの開発に大きな期待が寄せられている。

図1に本開発の目的とその展開をまとめた。種々の診断チップが整うと，計測された多項目のマーカー値を医療施設に通信回線で送り，医療ブロードバンドネットワークと高精細ディスプレイを介して，医師による問診が在宅で可能になる。さらに，長期間の使用によって検出マーカーを増やし，医療施設に多数の人の健康・疾病マーカーの推移が蓄積されたデータベースが構築され，マーカーと疾病との相関関係が解明できるようになる。さらには，医師不在の寒村や離島に住む人々への遠隔診断が実現される。

われわれは，本目的を実現すべく，8年ほど前から無痛針で微量の血液を採取して，その血漿から電気化学センサによってマーカーを測定するヘルスケアチップ[2]や，比色法による肝機能診断チップ[3][4]などを開発してきた。紙面の都合で本稿では，前者について最近の開発状況を述べる。この両チップを用いると，糖尿病，腎臓病，肝臓病のような生活習慣病の診断が可能になる。現在この種の電気化学的センサによって多項目を測定するチップとしてi-STATが広く病院で使われているが，100μLの血液を必要とするうえ，採血は医師がおこなわねばならず，在宅では到底使用できない。われわれのチップでは，6μLの全血，すなわち3μLの血漿から多項目の測定が可能である。

無痛針の開発と電子採血

在宅診断を可能とするためには，痛みをともなわずに，素人でも採血できることが不可欠である。痛みは，針を皮膚に穿刺する際，神経網を切断する時に感じる。そこで，外径0.15mmのステンレス(SUS)製管を用いて無痛針を作成した（**図2**）。従来の針の外径に比して4分の1程度なので，腕に刺しても痛みは感じない。管内壁を平坦化したため，針を皮膚に刺すと自分の血圧だけで血液が針を通過するので，ポンプを必要とせずに採血が可能になった。

しかし静脈採血の場合，その所在が目視できないことが多い。そこで，近赤外光（Near Infrared: NIR, 850nm）を照射して血管を可視化することにした。NIR光は，水を透過し皮下を伝播できるが，血液中のヘモグロビンは透過できない。そのため，

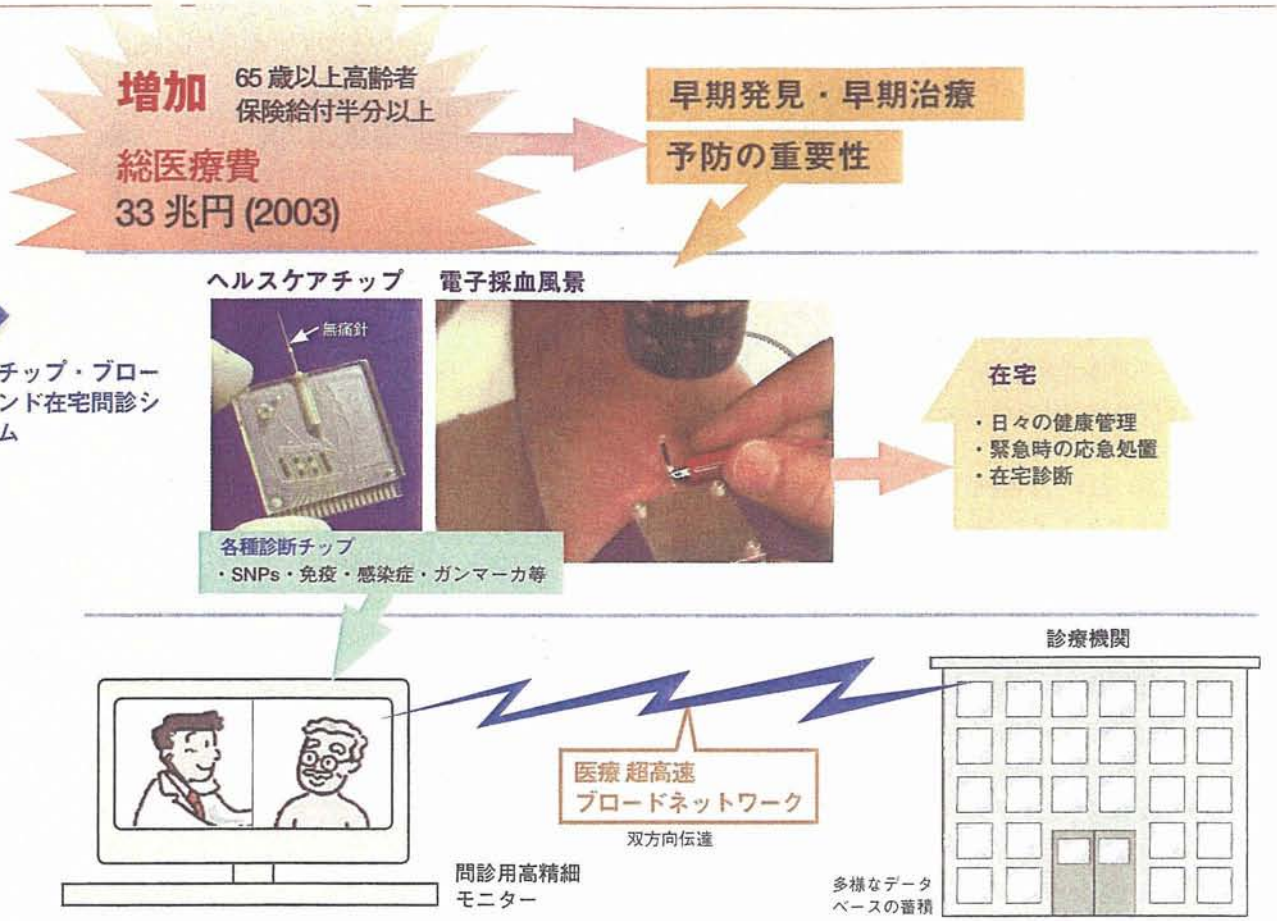


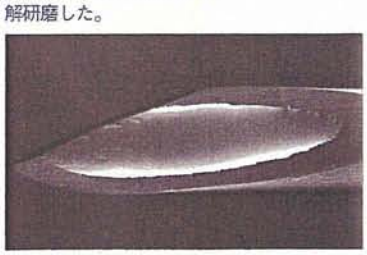
図1
診断チップ・ブロードバンド在宅問診システム

血管の存在がわかる。さらに，皮膚表面から静脈がある位置までの深さを知ることも重要である。その深さを知るために，針と皮膚表面との間に生じる電位を測定し，針が血管に到達したことを検出できるようにした[5]。

血管の位置と深さを知ることができたので，採血者は自分の腕と針を見ずに，ディスプレイ上のNIRイメージと電位変化を見るだけで採血が可能になった（図3）。電圧は，針が皮膚に穿刺すると少し上がり，筋肉内の移動中は一定に保たれる。静脈に達すると再び上がり，その時採血が始まったことを示している。将来的には画像処理を駆使して自動採血をめざす。

図2
無痛針

細管化時に生じた粗い管の内壁を特殊な方法で超平滑化し，先端の3面を10度に研磨し，最後に電解研磨した。



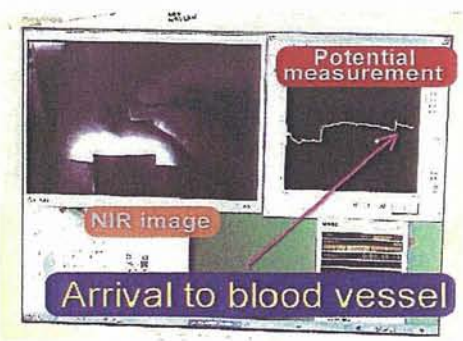


図3
NIRイメージと電位変化を見るだけで採血が可能

採血者が自分の腕でなく，液晶画面を見ながらNIR像で静脈を探し（左図），穿刺し，皮膚表面と針との間の電圧を右図で検知しながら，静脈位置を検出して採血する。

バイオセンサ

診断チップの心臓部はバイオセンサである。われわれは，図4に示すバイオセンサを開発し，センサ材を電極上に塗布するために，専用の自動ディスペンサーも開発した。

電気化学的計測では，基準電位を発生する参照電極が極めて重要となる。参照電極では，検体溶液，イオン種およびイオン強度にかかわらず常に一定の電位を出力することが求められる。同時に，微量の血液を含む液体をあつかう使い捨てチップの微小電極においては，①100秒以下の迅速応答，②1000秒以上の長寿命，③検体溶液を汚染しないなどの条件をも満足しなければならない。

微小電極には，液絡型と非液絡型があるが，本チップでは，イオンセンサのメンブレンとして用いられるPVC（塩化ビニール）を使って非液絡型を開発した。基板上に金属マスクでAu/Cr膜をスパッタ堆積し，その上にポリイミド膜をコートし，さらに100 μ mの開口部を開け，その開口部のAgを溶液処理でAgClにした。また，その上にSU-8レジストでリング状井戸を形成し，その中にPVA（ポリビニールアルコール）とKClを混ぜたものを塗布し，乾燥した。その上に，可塑剤含有のPVCを

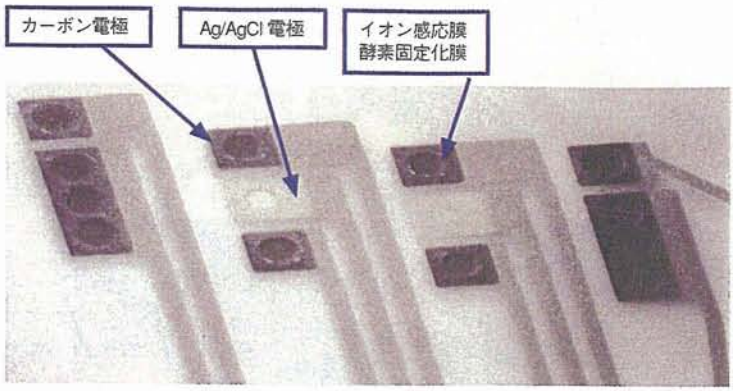


図4
診断チップの心臓部となるバイオセンサ

センサ電極にカーボン，参照電極に銀／塩化銀を使用し，配線やパッドは銀ペーストをポリエステル基板にスクリーン印刷法で形成している。

THF（テトラハイドロフラン）に溶かして覆った。

図5は，0.1M リン酸緩衝液中で3種類の PVC 膜厚に対する出力電圧の時間変化を示す。いずれの電極でも，まず水が下層の Ag/AgCl 電極に到達し，-4mV vs. 標準カロメル電極（SCE）という，飽和 KCl 溶液中で Ag/AgCl 電極が本来の出力電圧を出すまで時間を必要とする。安定してから一定時間後，KCl が PVC 膜を通してバルク液に流れ出すと，KCl が希釈されて電圧が上がることが分かる。この結果から，われわれのセンサでは PVC の最適膜厚 10～20 μm と決定した。

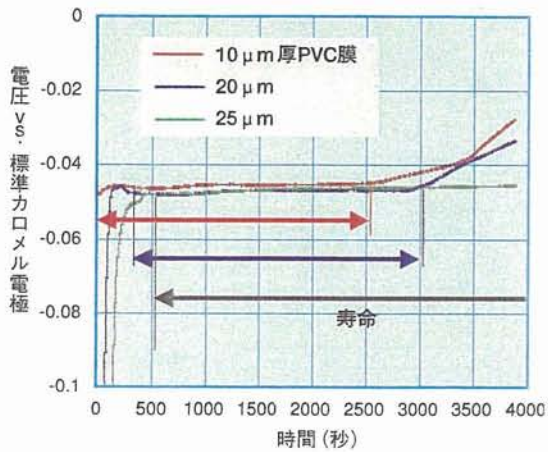


図5
PVC 膜厚に対する出力電圧の時間変化

0.1M リン酸緩衝液中で3種類の PVC 膜厚に対する出力電圧の時間変化を計測。この結果から，われわれのセンサでは PVC の最適膜厚 10～20 μm と決定した。

pH センサとして，電解重合によりカーボン電極上に絶縁性ポリピロールを形成した。Na^+ と K^+ イオン濃度の測定には，それぞれ Bis（12-crown-4）と Bis（benzo-15-crown-5）のイオン感応膜を使用した。図6は Na^+，K^+ のイオン濃度の時間依存性を示す。

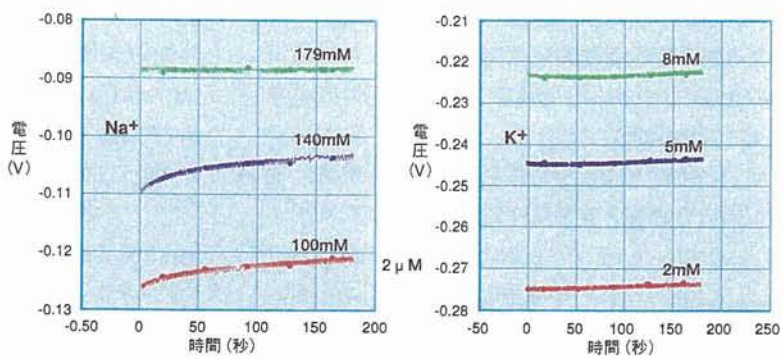


図6
Na^+, K^+ のイオン濃度の時間依存性

イオン感応膜は，アニオン排除剤と可塑剤を THF で溶解した PVC で固定化した。

グルコースセンサは，当初グルコースオキシダーゼ（GOD）を絶縁性ポリピロールとポリ-L-リシン膜のポリイオン複合体（PIC）により，同時にメディエータとしてフェロセンを含ませたものをカーボン電極上に固定化していたが，GOD 酵素と PIC 膜の固定化が弱いため，フェロセン層が不均一になるという問題があった。そこで，VFc-co-HEMA 重合メディエータを使用してカーボン電極との固定化を改善し，さらに GOD の固定化のためグルタルアルデヒド（GA）を用いて化学的架橋を形成した結果，図7のように，GA 濃度が3% の時に良好な特性を得ることができた。

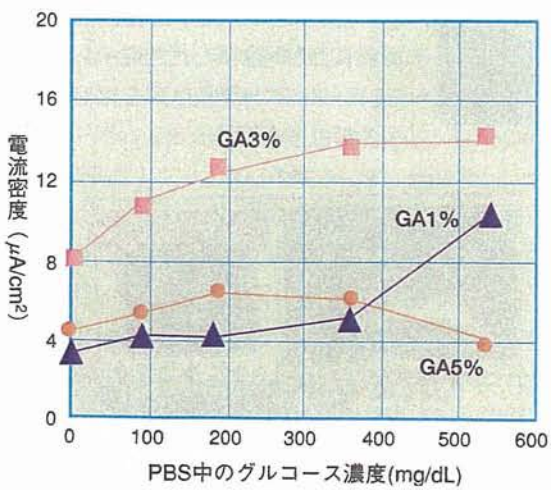


図7
グルタルアルデヒド濃度変化に対する電流とグルコース濃度の関係

VFc-co-HEMA 重合メディエータを使用してカーボン電極との固定化を改善し，さらにグルタルアルデヒドを用いて化学的架橋を形成した。

BUN センサも，以前は尿素がウレアーゼ酵素反応によって H^+ が減少することで検出していたが，血液中には H^+ がたくさんあるため，それが干渉して測定値が安定しなかった。そこでアンモニアの検出法にかえ，図8の左図のように，製作した。クレアチニンでも，クレアチニンアミディノヒドロラー

図8

尿素窒素 (BUN) センサ

TD19C6 のイオノフォアを可塑剤とアニオン排除剤，および 0.1M NH_4Cl + 0.5%PVP を PVC 膜に取り込み，AgCl 電極上に固定化し，さらに BUN 検出のためのウレアーゼと，固定化膜としてセルロースアセテートを塗布した。右図は，尿素検出の結果を示す。

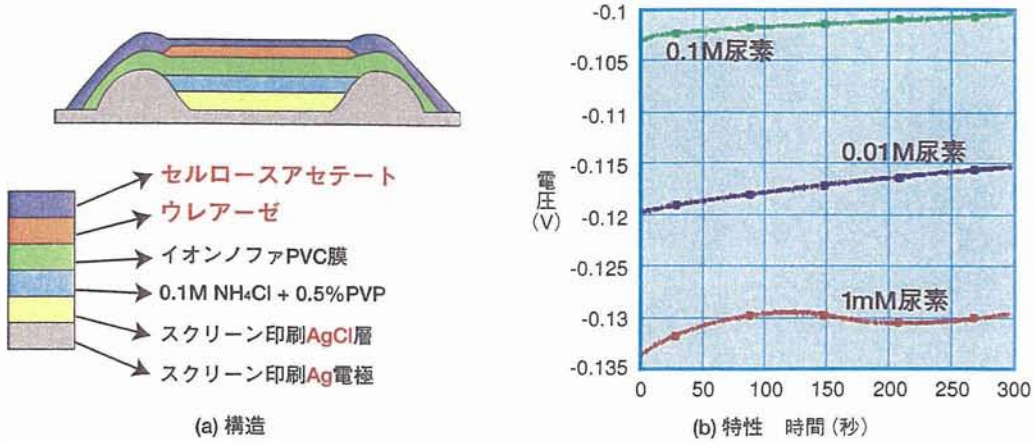


図9

ヘルスケアチップの構造

(a) に (b) の採血済み採血アセンブリを挿入して、遠心分離後 (c) に示すように血漿と血球が分離される。

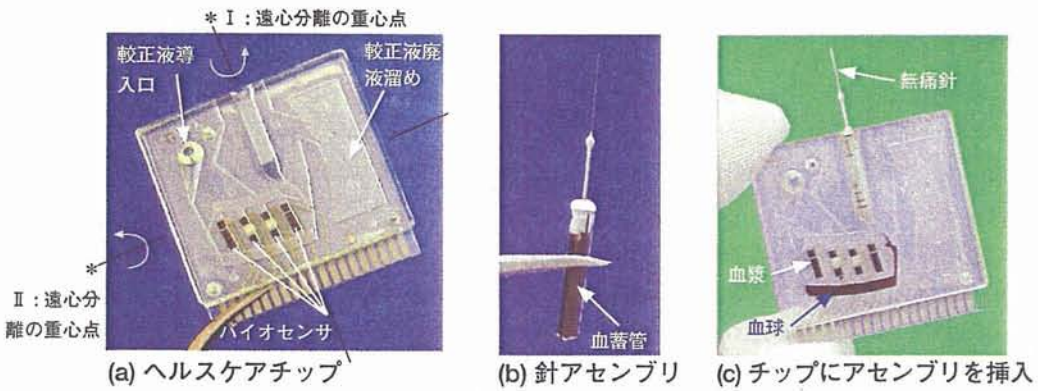
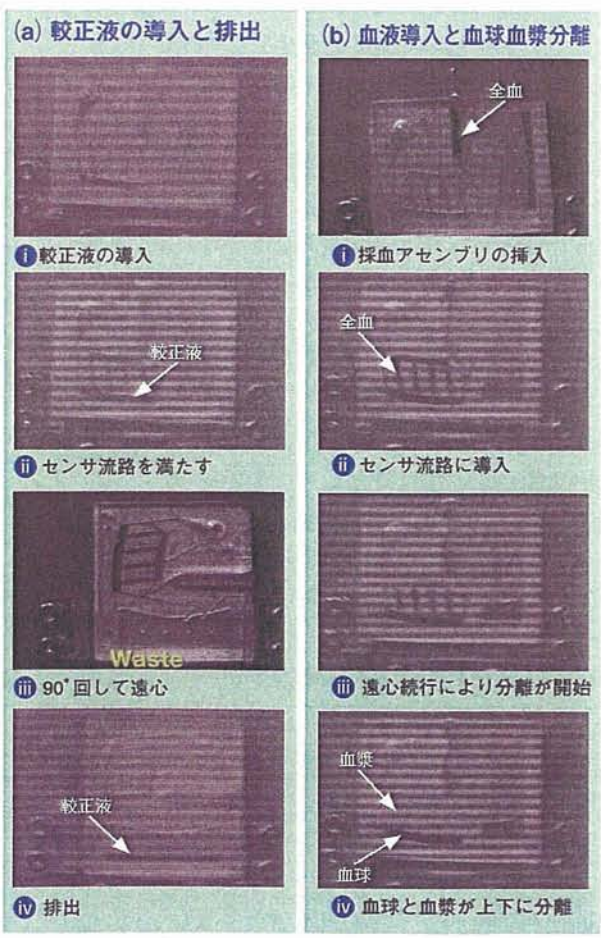


図10

チップ流路への溶液の流れ

(a) の較正液（試験液）の移送と排出では，(i) 較正液導入口から較正液を導入し，(ii) 図 9a の重心点（Ⅰ）を中心として遠心力をかけセンサ領域に移送し、較正後，(iii) チップを 90 度半時計回りに回転し，(iv) 重心点（Ⅱ）を中心として遠心力によって排液溜めに排出する。
(b) の血球分離では，(i) 針アセンブリを導入し，(ii) 図 8c の重心点（Ⅰ）を中心として遠心力をかけ全血がセンサ領域を満たした後，(iii) さらに回転をし続けると，(iv) 血球のみが下方に分離していき，最終的には血漿がセンサ領域のみに残る。



ゼ酵素反応を経由して尿素生成後のアンモニア検出を試みているが，まだ 0.1mM 以下の検出には不安定性が残っている。

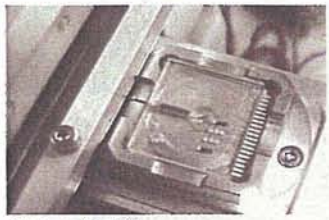
チップ構造

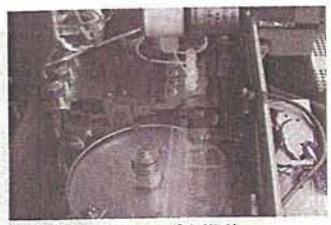
図 9a に示すヘルスケアチップは，ポリエステル基板上に電極をスクリーン印刷法で形成したものと，射出成型で形成したポリカーボネート製の流路用基板とを張り合わせたチップである。2000 年に始めて開発したチップでは，無痛針から採血して U 字型流路に全血を導入する際，流路の末端に設けた石英製の EOF（電気浸透流）ポンプを用いて，U 字型流路の後に設けた ISFET（イオン感応電界効果型トランジスタ）と参照電極によって Na^+，K^+ をはじめて測定した[2]。しかし，血漿は U 字流路の片側しか使えず，また本チップは使い捨てであるため高価な ISFET と石英の使用は不可である。更に非ニュートン性液体の血液は，多分岐した流路中で，輸送時に一本の流路に偏って流れるなどの振る舞いをし，結局，図 9a の形に落ち着いた。

当初，針を付けていたが滅菌時にバイオセンサを損ねることが危惧され，図 9b に示す針アセンブリで一旦採血後，それを図 9c に示すように挿入後，遠心分離した。針アセンブリでは，無痛針を

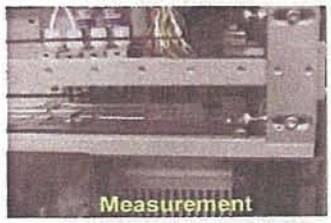
外径2.3mmのプラスティク管に接続した。この血蓄管に溜まる採血量は6μL（1mm³×6）であり，在宅診断はこの採血量程度で達成すべきと考えている。


①採血筒を装着


②チップの設置


③ローターにチップを搬送

④血球血漿分離



⑤チップを測定端子部に搬送，測定

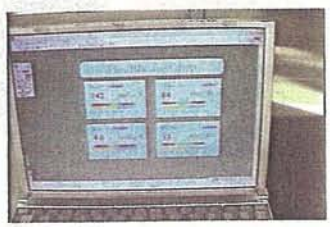

⑥結果表示

図11 試作計測器によるマーカ 測定プロセス

ヘルスケアチップ計測の動作

電気化学法では，バイオセンサの校正が必要である。チップ流路への溶液の流れを知り，流路の最適設計をするため，ストロボ観察をおこなう。**図10**は，その動画から抽出したものである。

次に，較正後のチップを，試作した計測器で測定した例を図11に示す。まず①採血アセンブリをチップに装着し，②チップを計測装置に設置し，③遠心分離用回転器にチップを搬送し，④血球・血漿分離をおこない，⑤チップを測定用端子部に搬送し，⑥測定結果が画面に表示される。ここでは，Na^+，K^+，BUN，グルコースの4項目が表示されている。

今後の展望

診断チップを家庭に持ち込むためには，個人差にかかわらず無痛で確実に採血できるシステムや，微量血液から正確に健康・疾病マーカーが測定でき，安価・小型・安全で操作が簡単な測定器が要求されると思う。

採血針では，現在のSUS304から，アレルギーを引き起こすNiを除いたSUS管の細管化を開発している。窒素アニールによって剛性が向上し，より信頼性の高い無痛針が期待される。

また，診断チップの検査項目では，現状にコレステロール，中性脂肪なども加え20項目程度は測定できるようにする。測定装置もCDプレイヤーのようなものにしたいが，専門メーカのご支援をいただきたい。

さらに，現在廃棄している血球成分から，免疫検査，DNA解析などをするための基礎研究もしている。種々のバイオチップによって，診断機関と直結した在宅診断が実現すると，医療，さらには社会システムを大きく変革すると期待される。その基盤が確立されると，幼少のころからデータが蓄積されることで，各人の成長に応じた食事や，社会環境などに起因する病気への予防体制が確立され，少子化問題の解決にも寄与する。発展途上諸国の疾病診断・予防にわが国が大きく貢献できる。

これを実現するためには，多くの電子部品・システムが開発されねばならず，バイオとエレクトロニクスが融合する新学問，新技術，新産業の創出が必要とされる。

Profile

長年，半導体の微細加工技術を研究・開発を行ってきたが，1994年ごろ大腸がんを患い，入院中，自分の技術を医療に活用できないかと思った。退院後，SiO_2に200nmの高アスペクト比の溝を掘ることができ，これを分析器に使用しようと思い立った。現在の流路は数百μmであるが，発端はナノバイオの発想だった。ヘルスケアチップを実用化し，在宅診断を是非実現したいと念願している。
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電子材料

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【特集】流体MEMSのすべて
―マイクロ流体デバイスの応用展開とその実用化―