4. D. Kuroda, T. Hanawa, T. Hibaru, S. Kuroda and M. Kobayashi, "Torsion and Tensile Properties of Thin Wires of Nickel-Free Austenitic Stainless Steel with Nitrogen Absorption Treatment", Materials Transactions, **45** (2004) 112-118.
5. Chia-Hsien Chang, Hiroki Ogawaa, Akio Oki, Madoka Takaib, Masao Nagaia, Hideaki Hisamotoc and Yasuhiro Horiike, "Healthcare Chip Based on Integrated Electrochemical Sensors Used for Clinical Diagnostics of Bun",Japanese Journal of Applied Physics, (in press).
6. R. Ogawa, H. Ogawa, A. Oki, S. Hashioka,Y. Horiike "Fabrication of nano-pillar chips by a plasma etching technique for fast DNA separation" Thin Solid Films **515** (2006) 5167-5171.
7. N. Kaji, R. Ogawa, A. Oki, Y..Horiike, M. Tokeshi, Y. Baba "Study of water properties in nanospace" Anal. Bioanal. Chem. **386** (2006) 759~764.
8. 黒田大介、檜原高明、黒田秀治、藤原昌樹、堀池靖浩、"無痛針用 Ni フリーステンレス鋼細管の機械的特性"、高等専門学校の教育と研究、投稿中.
9. 黒田大介、"Ni フリーステンレス鋼"、金属 (2007) 30-36.
10. S. Fukumoto, T. Mastuo, D. Kuroda and H. Tsubakino, "Micro-Resistance Spot Welding of Nickel Free Austenitic Stainless Steel", Materials Science Forum, 593-543 (2007) 4081-4086.

2. 学会発表

1. 甲田裕子、張　嘉顕、沖　明男、小川洋輝、堀池靖浩、"ドライケミストリ法のマイクロ流路への適用によるバイオセンシングの基礎的検討"、日本バイオマテリアル学会シンポジウム (2004) 174.
2. H. Koda, C.H. Chang, A. Oki, H. Ogawa and Y. Horiike, "Biosensing with Dry Chemistry in Microchannel", Proc. 4th Asian Int. Symp. Biomaterials 220-221 (2004).
3. H. Ogawa, A. Oki, M. Nagai, J. Kikuchi and Y. Horiike, "Painless Venous Blood Collection System for Healthcare Chip", Proc. 4th Asian Int. Symp. Biomaterials (2004) 219-220.
4. C.C-Hsien, M. Nagai, H. Ogawa, S. Shinbashi, H. Hisamoto and Y. Horiike, "Development of Ammonia sensor", Proc. 4th Asian Int. Symp. Biomaterials (2004) 229.
5. M. Takai, S. Shinbashi, H. Ogawa, Y. Horiike and K. Ishihara, " Biocompatible Microglucose Sensor with Newly Designed Phospholipid Polymer for Healthcare Chip", Proc. 4th Asian Int. Symp. Biomaterials (2004) 228.
6. Y. Horiike, A. Oki, H. Ogawa, M. Nagai, M. Takai, C.C-Hsien, H. Hisamoto and K. Koda, "Development of Clinical Chips Checking Life Style-Related Diseases", Abstracts of Biosensors & Biomaterials Workshop 2005, (2005) O-28.
7. 堀池靖浩、"無痛針採血による在宅健康診断チップ"、第 19 回「大学と科学」公開シンポジウム「人体にやさしい医療材料」予稿集、(2004)、23-26.
8. 甲田裕子、張　嘉顕、沖　明男、小川洋輝、堀池靖浩、"ドライケミストリ法のマイクロ流路への適用によるバイオセンシングの基礎的検討"、日本バイオマテリアル学会シンポジウム (2004) 174.
9. 黒田大介、"生体用 Ni フリーステンレス鋼の開発"、日本バイオマテリアル学会シンポジウム 2004 予稿集、104.
10. 黒田大介、横山敦郎、山本玲子、塙　隆夫、廣本祥子、窒素吸収処理した Ni フリーステンレ

ス鋼の生体親和性、日本金属学会講演概要(第134回・東京)、(2004) 127.

11. C.-H. Chang, S. Hashioka, Hiroki Ogawaa, Akio Oki, Madoka Takaib, Masao Nagaia, Hideaki Hisamotoc and Yasuhiro Horiike, "Healthcare Chip on Integrated Droplet Electrowetting Sensors used for Clinical Diagnostics", 第53回応用物理学関係連合講演会, 3, 1387, (2006).

12. 堀池靖浩、甲田裕子、小川洋輝, "ドライ比色法による微量血液分析在宅診断チップ", 平成17年度　厚生労働科学研究費研究成果等普及啓発事業　萌芽的先端医療技術推進研究　ナノメディシン研究成果発表会(2006).

13. C.-H. Chang, H. Ogawa, M. Nagai, A. Oki, M. Takai, H. Hisamoto, and Y. Horiike, "Health Care Chip based on Integrated Electrochemical Sensors used for Clinical Diagnostics, Bun and Creatine", Proceedings of μTAS 2005 Conference, 2, 1312-1314, (2005).

14. Y. Horiike, A. Oki, M. Nagai, M. Takai, C-H. Chang, H. Hisamoto and H. Ogawa, "Development of Clinical Chips for Medical Diagnostics", Extended Abstract of the 2005 Intern. Conf. on Sol. St. Dev. And Mat., 2-3, (2005).

15. 堀池靖浩, "ナノテクノロジーによる医療の変革", つくば発ナノバイオ融合テクノロジーシンポジウム (2005).

16. 堀池靖浩, "無痛針による在宅健康診断チップ", 第43回茅コンファレンス(2005).

17. 黒田大介、"新しい金属系生体材料の開発と高機能化"、学術討論会-若手生体材料研究者が目指す金属とセラミックス、高分子とのインテグレート生体多機能材料 (2006) 92-114.

18. 黒田大介、織田直樹、塙　隆夫、"粉末射出成形による低アレルギーNiフリーステンレス鋼の製造"、第27回日本バイオマテリアル学会予稿集 (2005) 120.

19. 三部真智、黒田大介、檜原高明、黒田秀治、藤原昌樹、塙　隆夫、金澤健二、"Niフリーステンレス鋼のN吸収挙動に及ぼすCr添加量の影響"、日本金属学会講演概要集（第137回）(2005) 431.

20. 松尾太樹、福本信次、椿野春繁、黒田大介、"Niフリーステンレス鋼の抵抗マイクロ接合"、日本金属学会講演概要集（第137回）(2005) 433.

21. 黒田大介、織田直樹、塙　隆夫、" 歯科用Niフリーステンレス鋼のミクロ組織と力学的特性"、第49回日本学術会議材料研究連合講演会講演論文集 (2005) 82-83.

22. 黒田大介、織田直樹、塙　隆夫、"窒素吸収したNiフリーステンレス鋼MIM材の力学的特性"、日本金属学会講演概要集（第136回）(2005) 200.

23. 奥田順子、黒田大介、山本玲子、塙　隆夫、"生体用金属材料に対する細胞応答の遺伝子レベルにおける解析"、日本金属学会講演概要集（第136回）(2005) 240.

24. D. Kuroda, T. Hanawa, T. Hibaru, S. Kuroda, M. Kobayashi and T. Kobayashi, "New Manufacturing of Nickel-Free Austenitic Stainless Steel with Nitrogen Absorption Treatment", Proceedings of The 1st Japan-China Mini-Symposium on Biomaterials (2005) 98-101.

25. Y. Horiike, H. Koda, S-H. Chang, R. Ogawa, S. Hashioka, M. Nagai and H. Ogawa, "Calorimetric Measurement Clinical Chip For Home Medical Diagnosis", Proc. of μTAS 2006 Conference, Eds. T. Kitamori, et.al., Nov. 5-9, 2006, Tokyo, pp. 1558-1560 (2006)

26. 黒田大介、檜原高明、黒田秀治、藤原昌樹、堀池靖浩、"窒素により高剛性化した無痛針の機械的特性"、日本高専学会第12回年会講演会講演論文集(2006)55-56.

27. 小川洋輝、長井政雄、堀池靖浩 "微量血液分

析ヘルスケアチップの開発”第45回日本生体医工学会大会 2006年5月

3. 解説・総説

1. 堀池靖浩、一木隆範、沖　明男、”プラズマプロセスによるバイオチップの開発と展望”、応用物理、73(4)、470-475、(2004).
2. 小川洋輝、新橋里美、沖　明男、高井まどか、長井政雄、堀池靖浩、〃ヘルスケアチップの開発〃、化学装置、9、81-85、(2004).
3. 堀池靖浩、沖　明男、小川　洋輝、高井まどか、百瀬　俊、横川昭徳、高村　禅、”高齢化社会の
4. 沖　明男、堀池靖浩、”医療用バイオチップの現状と将来展望”、 電気学会誌、 124(4)、219-222、(2004).
5. 沖　明男、小川洋輝、堀池靖浩、”ヘルスケアチップ創製プロセッシングの基盤技術”、ナノバイオエンジニアリングマテリアル、フロンティア出版、133-140、(2004).
6. 堀池靖浩、沖　明男（分担執筆）、”ナノバイオ事典”、テクノシステム、(2005) (in press).
7. 堀池靖浩、小川洋輝, "株式会社アドビック - 在宅で健康診断できるヘルスケアチップの実用化開発 -", NIMS NOW, 3, 6, (2006).
8. 堀池靖浩、C.-H. Chang、沖明男、小川洋輝、長井政雄, "在宅で健康診断できるヘルスケアチップの開発", Bionics, 5, 30-34, (2005).
9. 小川洋輝、長井政雄、堀池靖浩, "μ-TAS応用によるバイオセンサ式ヘルスケアチップ", 電子材料, 11, 67-71, (2005).
10. 堀池靖浩, "無痛針採血による在宅医療健康診断チップ", 第19回「大学と科学」人体にやさしい医療材料, 52-60, (2005).
11. 堀池靖浩、甲田裕子、小川洋輝、長井政雄、“無痛針による微量採血分析から在宅で健康診断できるヘルスケアチップの開発”、臨床検査、(2006) 50、pp.1557-1565.

H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得

1. 特願2004-232638　発明の名称：血液分析装置及び血液分析方法　発明者氏名：堀池靖浩、沖明男
2. 特願2004-232639　発明の名称：血液分析装置及び血液分析方法　発明者氏名：堀池靖浩、沖明男
3. 特願 2004-238910　発明の名称：血液分析装置　発明者氏名：堀池靖浩、甲田裕子、沖　明男
4. 特願 2005-234992　“検体分析チップおよび検体チップの使用方法”
5. 特願番号未取得(平成18年2月27日提出)　“試薬調製方法”
6. 「血液分析装置」、特許第3847053号、公告日：平成18年9月1日

2. 実用新案登録

なし

3. その他

なし

II. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
堀池靖浩、沖　明男（分担）	比色チップ		ナノバイオ事典	テクノシステム	東京	2005	
沖　明男、小川洋輝、堀池靖浩（分担）	ヘルスケアチップ創製プロセッシングの基盤技術	石原一彦	ナノバイオエンジニアリングマテリアル	フロンティア出版	東京	2004	133-140
小川洋輝、長井政雄、堀池靖浩	血球・血漿分離チップ	馬場嘉信	ナノテク・バイオMEMS 時代のバイオ分離・計測技	シーエムシー出版	東京	2006	219-229
堀池靖浩 宮原裕二	バイオチップとバイオセンサー	高分子学会	高分子先端材料 One Point バイオチップとバイオセンサ	共立出版	東京都	2006年	1～65 139～173

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
A. Oki, H. Ogawa, M. Nagai, S. Shinbashi, M. Takai A. Yokogawa and Y. Horiike	Development of healthcare chips checking life-style-related diseases	Materials Science and Engineering C	24(6-8)	837-843	2004
M. Takai, S. Shinbashi, H. Ogawa, Y. Horiike and K. Ishihara	Biocompatible Microglucose Sensor with Newly Designed Phospholipid Polymer for Healthcare Chip	Proceedings of 4th Asian International Symposium on Biomaterials		228	2004
C.C-Hsien, M. Nagai, H. Ogawa, S. Shinbashi, H. Hisamoto and Y. Horiike	Development of Ammonia sensor	Proceedings of 4th Asian International Symposium on Biomaterials		229	2004

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Y. Horiike, A. Oki, H. Ogawa, M. Nagai, M.Takai, C.C-Hsien,	Development of Clinical Chips Checking Life Style-Related Diseases	Abstracts of Biosensors & Biomaterials Workshop 2005		O-28	2005
堀池靖浩、一木隆範、沖　明男	プラズマプロセスによるバイオチップの開発と展望	応用物理	73(4)	470-475	2004
沖　明男、堀池靖浩	医療用バイオチップの現状と将来展望	電気学会誌	124(4)	219-222	2004
堀池靖浩	無痛針採血による在宅健康診断チップ	自動車技術	58(7)	116-117	2004
堀池靖浩、沖　明男、小川　洋輝、高井まどか、百瀬　俊、横川昭	高齢化社会の到来とヘルスケアチップの創製－ドライエッチング技術の展開－	表面技術	55(6)	385-390	2004
小川洋輝、新橋里美、沖　明男、高井まどか、長井政雄、堀池靖浩	ヘルスケアチップの開発	化学装置	9	81-85	2004
堀池靖浩	無痛針採血による在宅健康診断チップ	第 19 回「大学と科学」公開シンポジウム「人体にやさしい医療材料」予稿集		23-26	2004
A. Yamamoto, Y. Kohyama, D. Kuroda, and T. Hanawa	Cytocompatibility evaluation of Ni-free stainless steel manufactured by nitrogen adsorption treatment	Materials Science & Engineering C -Biomimetic and Supramolecular Systems	24	737-743	2004
D. Kuroda, T. Hanawa, T. Hibaru, S. Kuroda and M. Kobayashi	Torsion and Tensile Properties of Thin Wires of Nickel-Free Austenitic Stainless Steel with Nitrogen Absorption Treatment	Materials Transactions	45	112-118	2004
黒田大介	生体用Niフリーステンレス鋼の開発	日本バイオマテリアル学会シンポジウム 2004 予稿集		104	2004
黒田大介、横山敦郎、山本玲子、塙　隆夫、廣本祥子	窒素吸収処理したNiフリーステンレス鋼の生体親和性	日本金属学会講演概要(第134回・東京)		127	2004

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
T. Yamada, T. Uezono, K. Okada, K. Masu, A. Oki and Y. Horiike	RF Attenuation Characteristics for In Vivo Wireless Healthcare Chip	Japanese Journal of Applied Physics	44(7A)	5275-5277	2005
T. Yamada, T. Uezono, K. Okada, K. Masu, A. Oki and Y. Horiike	In Vivo Batteryless Wireless Communication System for Bio-MEMS Sensors	Japanese Journal of Applied Physics	44(4B)	2879-2882	2005
Chia-Hsien Chang, Hiroki Ogawaa, Akio Oki, Madoka Takaib, Masao Nagaia, Hideaki Hisamotoc and Yasuhiro Horiike	Healthcare Chip Based on Integrated Electrochemical Sensors Used for Clinical Diagnostics of Bun	Japanese Journal of Applied Physics			in press
S. Hashioka, R. Ogawa, A. Oki, Y. Miyahara and Y. Horiike	Simple and Quick Detection of Target DNA by Hybridization in Nano Gap Channel Array	Proceedings of μTAS 2005 Conference	1	730-732	2005
N. Kaji, A. Oki, R. Ogawa, Y. Horiike, Y. Baba	Water Viscosity and Hydrodynamic Flow in Nanopillar Chips	Proceedings of μTAS 2005 Conference	1	736-738	2005
R. Ogawa, A. Oki, S. Hashioka, N. Kaji, Y. Baba and Y. Horiike	Allocation Dependence of Nano-pillars for DNA Electrophoresis Separation	Proceedings of μTAS 2005 Conference	2	1012-1014	2005
C.-H. Chang, H. Ogawa, M. Nagai, A. Oki, M. Takai, H. Hisamoto, and Y. Horiike	Health Care Chip based on Integrated Electrochemical Sensors used for Clinical Diagnostics, Bun and Creatine	Proceedings of μTAS 2005 Conference	2	1312-1314	2005
Y. Horiike, S. Hashioka, R. Ogawa, A. Oki	Separation, Trapping and Single Molecule Detection of DNA Employing Nanostructures	International Symposium on Surface Science and Nanotechnology		359	2005
Y. Horiike, A. Oki, M. Nagai, M. Takai, C-H. Chang, H. Hisamoto and H. Ogawa	Development of Clinical Chips for Medical Diagnostics	Extended Abstract of the 2005 Intern. Conf. on Sol. St. Dev. And Mat.		2-3	2005

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
堀池靖浩、C.-H. Chang、沖明男、小川洋輝、長井政雄	在宅で健康診断できるヘルスケアチップの開発	Bionics	5	30-34	2005
小川洋輝、長井政雄、堀池靖浩	μ-TAS 応用によるバイオセンサ式ヘルスケアチップ	電子材料	11	67-71	2005
堀池靖浩	無痛針採血による在宅医療健康診断チップ	第 19 回「大学と科学」人体にやさしい医療材料		52-60	2005
R.Ogawa, H.Ogawa, A.Oki, S.Hashioka, Y.Horiike	Fabrication of nano-pillar chips by a plasma etching technique for fast DNA separation	Science.Direct	515	5167-5171	2006
Noritake.Kaji Ryo Ogawa Akio Oki Yasuhiro.Horiike Manabu Tokeshi Yoshinobu Baba	Study of water properties in nanospace	Anal Bioanal Chem	386	759~764	2006

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Development of healthcare chips checking life-style-related diseases

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Abstract

We investigated biochips that provide a home medical diagnosis as an application of miniaturized analysis systems. As the objective, an electronic blood collection system employing a painless needle was developed. Both the healthcare chip and hepatic function examining chip were studied based on the introduction of plasmas separated from a trace amount of blood. The former electrochemically measures pH, Na^+, K^+, glucose and BUN, and the latter measures the γ-GTP, GOT and GPT using a colorimetric analysis. The fluids of the blood in the micro-channel were controlled by centrifugal force. These chips are useful for checking life-style-related diseases.

Keywords: Healthcare chip; Hepatic function examining chip; Electrochemical; Colorimetric analysis; Mixer; μ-TAS

1. Introduction

It has become a serious issue in Japan that the recent increase in the aging society with fewer children especially increases the medical cost for person of advanced years. Prevention is important for them in order to live a healthy life. To realize such a society, we have to urgently establish biosensing technologies which allow simultaneous diagnosing of multiple items while at home. As the objective of the research field is called micro-Total Analytical System (μ-TAS) or Lab-on-a-chip [1–3], a large expectation is being placed for the development of various clinical chips for the quick diagnosis by a doctor at the patient's bedside, and in addition, a person's self examination at home.

Especially, we have to prevent kidney trouble and liver disease which are known as representative life-style-related diseases, which cause often serious illnesses. For this goal, in this report, our recent development situations are described for a healthcare chip [4–6] and a hepatic function examining chip [7,8].

2. Healthcare chip

2.1. Painless needle

The healthcare chip enables us to check our health/disease condition by analyzing multi components in a trace amount of blood. First, the needle collecting the blood should be painless. A stainless (SUS) tube 150 μm in diameter and with a 80 μm bore was used because the thin tube reduces cutting the nerves in the skin. The tube surface was hardened by plasma nitrification. The edge of the tube was polished at 10° and the chip with three planes was fabricated as shown in Fig. 1. Finally, the tip was electrochemically polished in a phosphoric acid solution. In addition, the inner wall of the tube was also polished. A SEM photograph after

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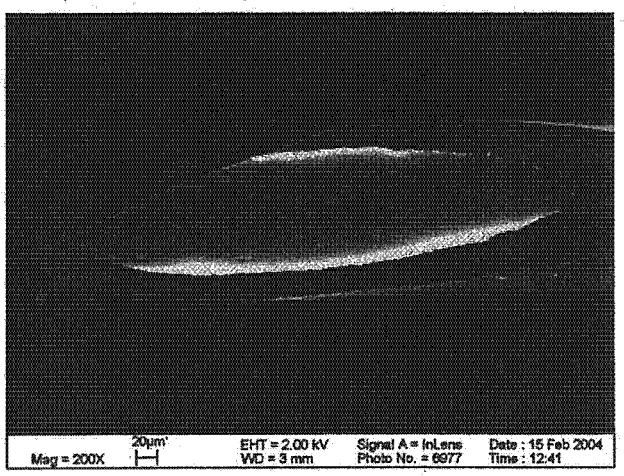

Fig. 1. Photograph of the tip of the painless needle.

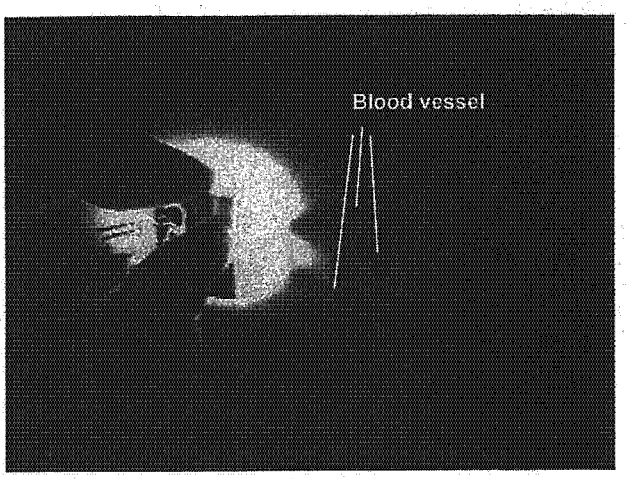

Fig. 3. IR image of veins irradiated by IR light emitting diode array.

polishing shown in Fig. 2(b) shows the smooth surface like a mirror as compared with a jagged feature shown in Fig. 2(a).

The needle did not cause any pain but skill was needed to pierce the needle into the blood vessel through the muscle, because we frequently lost sight of the vein and did not have information on its location from the skin surface. Therefore, the position of the blood vessel was visualized using an array of near infrared (NIR) light emitting diode with a wavelength of 850 nm as shown in Fig. 3. Furthermore, measurement of the potential between the skin and the blood vessel surfaces allowed detecting the depth of the blood vessel, where one terminal was an electrode which was pasted on the skin surface and elapsed time for the piercing of the needle into the arm (Fig. 4). When the needle did not hit the blood vessel, the potential did not change, while it drastically dropped once it touched the blood vessel surface. Fig. 5 shows photographs demonstrating the collection of the blood with the needle and the needle assembly after collecting the blood. This man, who was collecting his blood, did not look at his own arm, but observed a display in which the position of the blood vessel was imaged by the NIR irradiation and the potential change was monitored during piercing of the needle. The blood flows out due to his own blood pressure without a pump after the needle pierces the blood vessel, because the inner surface of the needle is extremely smooth. Indeed, this may be called electronic blood collection [8].

2.2. Healthcare chip operation

Fig. 6(a) and (b) shows photographs of the recently developed healthcare chip, where (a) shows the chip before inserting the needle assembly, and (b) shows introduction of the blood to the biosensor region. The chip is fabricated by bonding two plates. One is the channel pattern which is printed on a 27×24×1.5 mm polycarbonate plate using an injection mold method. The other is the electrode pattern screen-printed one on a 27×27×1.5 mm polyester plate. In this state, when the chip is rotated around the 1st rotation axis of ×1, the centrifugal force introduces a calibration solution into the channels equipped with biosensors. After calibration, the rotation around the 2nd rotation axis of ×2 conveys

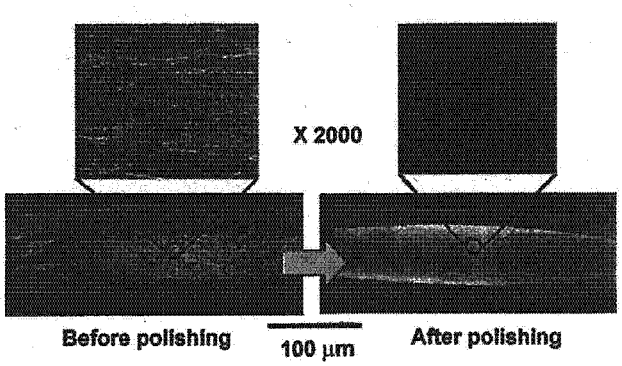

Fig. 2. SEM photograph before and after polishing the SUS needle.

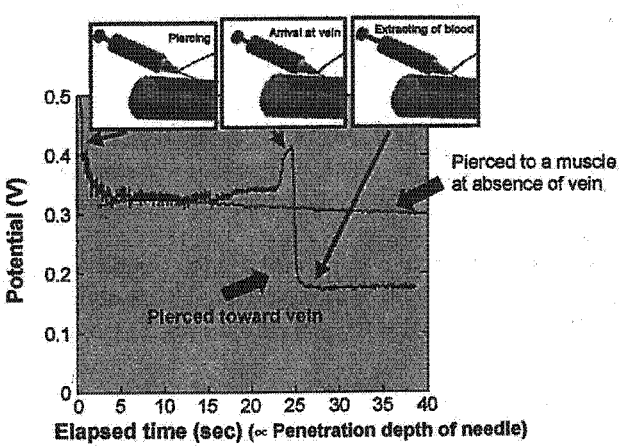

Fig. 4. Potential between the skin surface and the needle vs. elapsed time of piercing the needle.

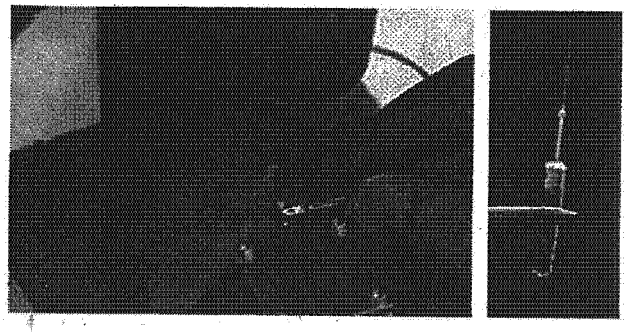

Fig. 5. Photograph of the blood collection with the needle and the needle assembly after collecting the blood.

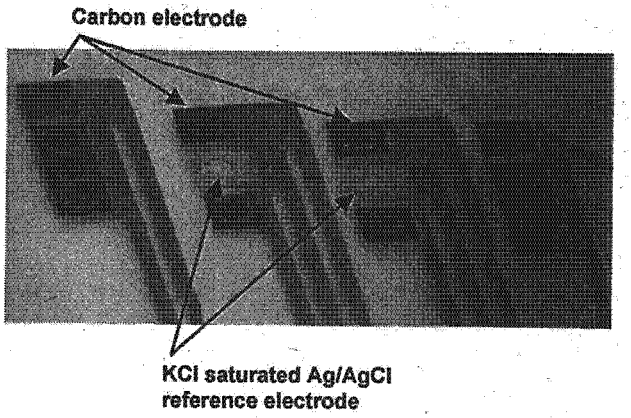

Fig. 7. Photograph of electrodes printed on a polyester plate.

the solution to a waste channel. Subsequently, the needle assembly filled by the blood is inserted into the top of the chip. The rotation around the 1st rotation axis of ×1 introduces the blood to the biosensor channels and simultaneously separates the blood into plasmas in the upper regions of the channels and blood cells to the bottoms of the channels as shown in Fig. 6(b). The biosensor surfaces are covered by plasmas, thus measuring the following components.

2.3. Biosensors and measurements

We plan to measure the pH and concentrations of Na^+, K^+, glucose, BUN (blood urea nitrogen), creatinine and lactate. These items are measured by an electrochemical method. Fig. 7 shows a photograph of part of the electrodes printed on the polyester plate. Carbon electrodes and KCl saturated Ag/AgCl reference electrodes were formed on carbon–silver wires. The Ag/AgCl layer was also a screen-printed. A Teflon membrane was coated on the AgCl surface to protect the AgCl from dissolving in the electrolyte. These membranes were coated using an automatic dispenser. The ion or enzyme sensitive membranes were coated on the carbon electrodes. The membrane structures for measurement of the pH, Na^+, K^+, glucose and BUN are listed in Table 1. Bis[(12-crown-4)methyl]-2dodecyl-2-methylmalonate and bis[(benzo-15-crown-5)-4-methyl]pimelate were used for Na^+ and K^+ ionophores. The selectivites of $Na^+/K^+=10^2$ and $K^+/Na^+=10^3$ are obtained due to the adequate anion exclusion agents shown in Table 1.

Fig. 8 shows the measurement results of the Na^+ and K^+ ions regarding their concentrations. The concentrations of the Na^+ and K^+ ions in human plasma are slightly lower than those in PBS (phosphate buffer solution), which are 144 and 4.2 mmol dm^{-3}, respectively, while the reliability of the sensors is considered to be satisfactory for actual use. The BUN and glucose concentrations were measured using calibration curves whose data were obtained by the on-chip measurement as shown in Figs. 9 and 10 [9].

Since the present BUN measurement follows the reaction of NH_2CONH_2 (urea)$+2H_2+H^+ \rightarrow NH_4+HCO_3^-$ in the presence of urease, urea is measured by the decrease in the proton (H^+). However, many protons exist in blood. Therefore, we are now developing a detection method of ammonia ions in urea. Creatinine is not measured yet, because ammonia ions generated from creatinine and intrinsic urea present in the blood have to be separated

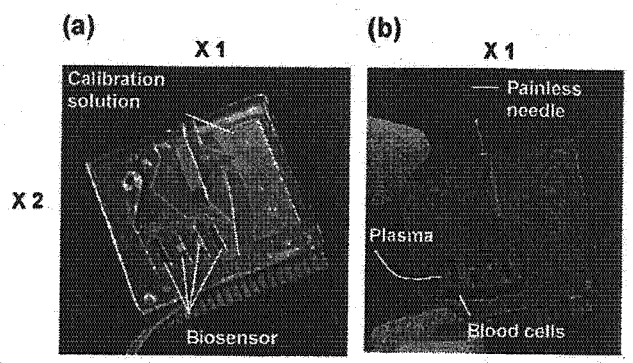

Fig. 6. (a) Photographs of the healthcare chip before inserting the needle assembly. (b) Photographs of the healthcare chip after centrifugal introduction of the blood from the needle assembly to the biosensor region.

Table 1
Composition of the ion selective and enzyme membranes

pH	Non-conductive poly-pyrrole (PPy)
Na^+	Na^+ ionophore (bis[(12-crown-4)methyl]-2 dodecyl-2-methyl malonate), plasticizer (2-nitrophenyldodecyl ether (NPOE)), anion-exclusion agent (K-TCPB) and poly vinyl chloride (PVC)
K^+	K^+ ionophore (bis[(Benzo-15-crown-5)-4-methyl] pimelate), plasticizer (NPOE), anion-exclusion agent (K-TCPB) and PVC
BUN	Immobilized urease by poly-ion-complex method/ non-conductive PPy
Glucose	Immobilized glucose-oxidase by poly-ion-complex method/ferrocene

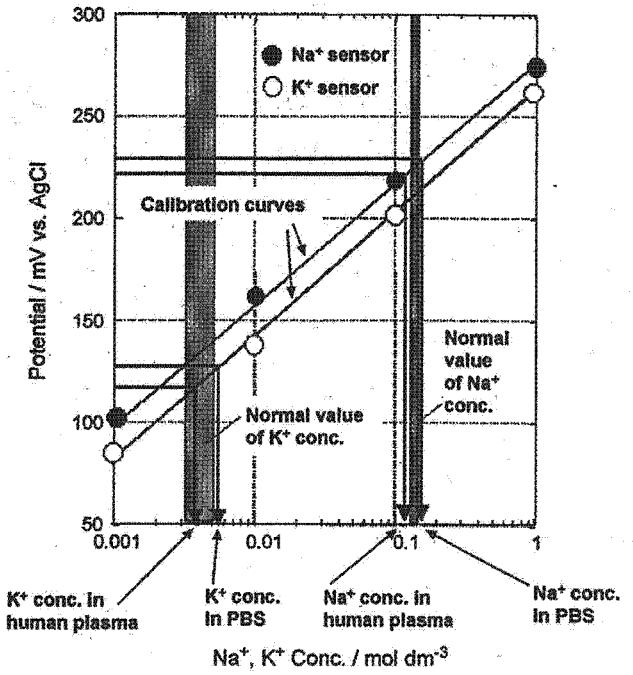

Fig. 8. On-chip measurement results of Na^+ and K^+ ions.

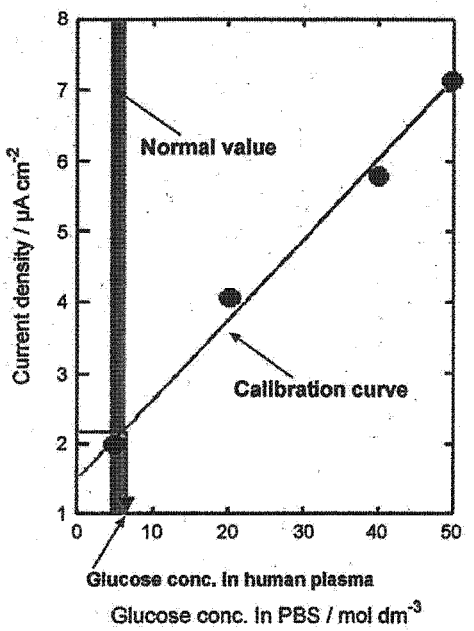

Fig. 10. On-chip measurement results of glucose.

[10]. The ammonia ion detection will be used for the measurement of creatinine.

3. Hepatic function chip

3.1. Mixer

The hepatic function chip measures the three enzymes of γ-GTP, GOT and GPT, which flow into the blood from liver

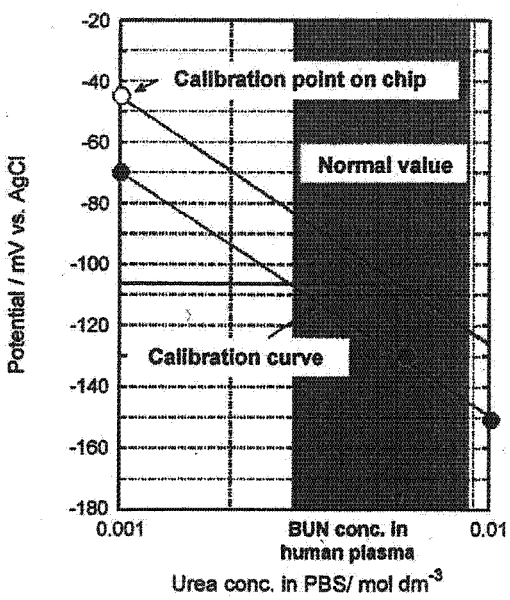

Fig. 9. On-chip measurement results of BUN.

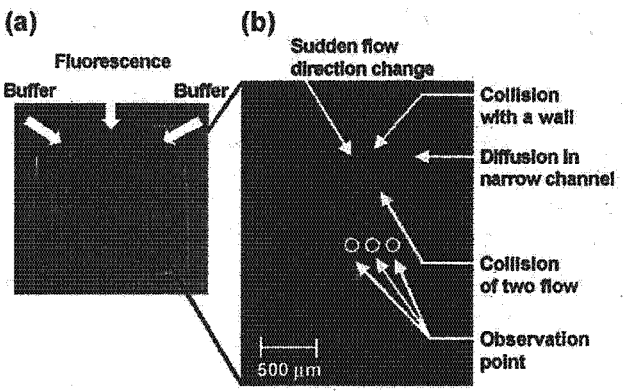

Fig. 11. (a) Experimental chip with multi-stage mixing chambers. (b) Mixing chamber connected to an observation chamber.

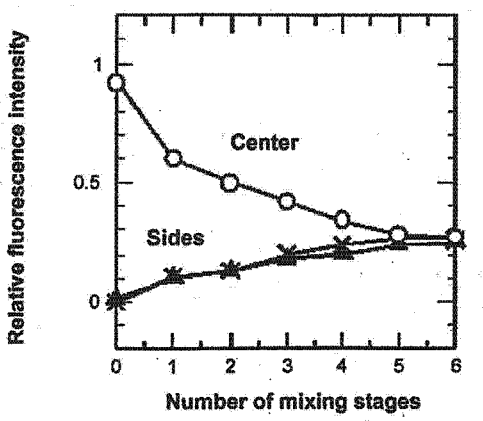

Fig. 12. Relative florescence intensity at three positions in the observation chamber shown in Fig. 11(b) vs. the number of mixing stages for the flow rate of 10 μL/min.

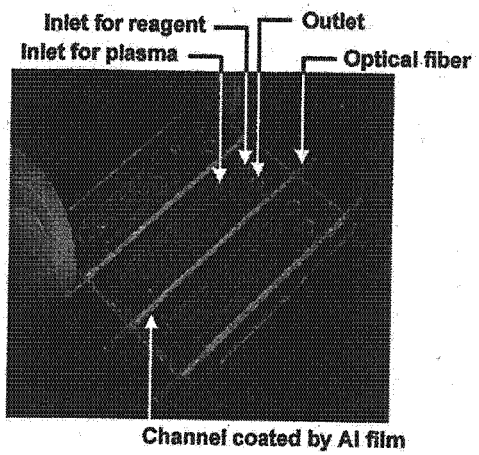

Fig. 13. Chip measuring three components for hepatic functions.

cells destroyed by a liver infection. The measurement method used a colorimetric analysis. This method requires good mixing of the plasma or serum with a substrate buffer solution. In general, however, the mixing is not easy in the microfluidics which are dominated by a laminar flow. So far, a lot of studies have been reported in the field of the microTAS [11]. We have also developed a novel mixer for the hepatic function examining chip.

Fig. 11(a) and (b) shows an experimental chip with multi-stage mixing chambers and a mixing chamber connecting to observation chamber, respectively. The inlet consists of three channels with the middle one for a florescence reagent (FITC) and both sides for PBS. The channel width ratio of the middle to both sides was 1 to 10. As shown in Fig. 11(b), the mixture collides with a wall and then its flow direction is suddenly changed at a corner. The diffusion of molecules is also promoted in the narrow channels. The process is sequentially repeated for six stages. Fig. 12 shows the relative florescence intensity at three points in the observation chamber (see Fig. 11(b)) vs. the number of mixing stages at the flow rate of 10 μL/min. Complete mixing occurred in the 6th chamber over a wide range of drawing velocities.

3.2. Chip fabrication and measurement

Fig. 13 shows a chip examining three components for hepatic functions. Since an incident light should propagate with minimal loss of the light for colorimetry, an Al film was coated on the inner walls of the channels as follows: (1) Channel patterns with a half depth were fabricated using a SU-8 resist and then molded on two PET plates. (2) The Al film was sputter-deposited on the surfaces with the channel pattern of both PET (polyethylene terephthalate) plates. (3) The Al film deposited on the upper PET surface was removed by chemical mechanical polishing (CMP) and both plates are thermally pasted together. Optical fibers were inserted into the inlet and outlet of the channel.

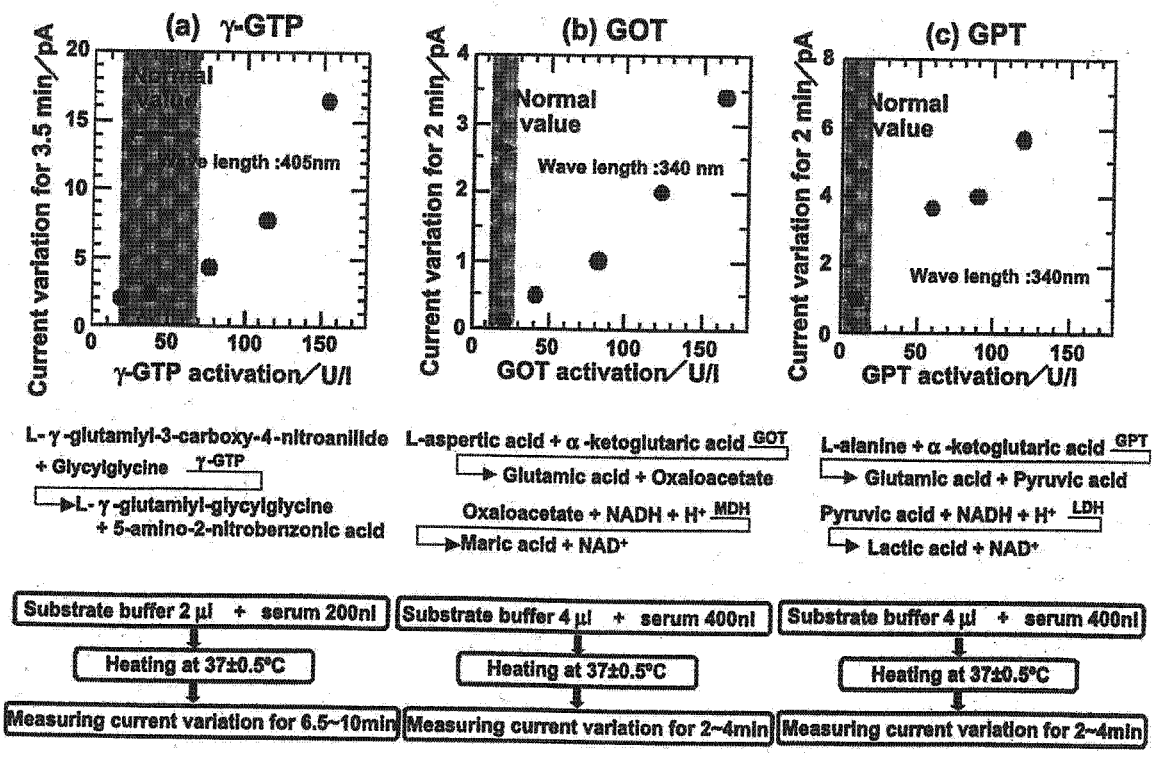

Fig. 14. (a, b and c) Calibration curves obtained for γ-GTP, GOT and GPT activities.

The temperature of the chip was maintained at 37 °C using a Peltier element. Lights of 340 and 405 nm, which were generated by the band-pass filtering of a D_2 lamp, were detected with a photodiode through the measurement channels. The plasmas to be mixed were obtained by the centrifugal separation of the blood. Fig. 14(a), (b) and (c) shows the obtained calibration curves for the γ-GTP, GOT and GPT activities. The photocurrent changes during 3.5, 2 and 2 min were measured for γ-GTP, GOT and GPT, respectively. The measurement methods and reagents are listed in each figure. Abnormal values as well as the normal ones were successfully measured [7].

3.3. Chip integrating plasma separation, metering, mixing and measurement

The colorimetric analysis method needs a wide range mixing ratios of 1:10–40. In the experiment for the chip shown in Fig. 13, the amounts of the serum and the substrate buffer were correctly measured in advance. Therefore, a series of processes for the plasma separation, the metering, the mixing and the measurement has to be integrated into one chip for practical use. Fig. 15 shows a photograph of an integrated chip developed for the one item measurement of γ-GTP. This processing is as follows: (1) The blood is introduced into one end of a U-shape channel. The rotation of the chip around the 1st rotation axis of x1 separates the blood into the plasma in the U-shape channel and the blood cells into a storage portion at the bottom of this channel. (2) The chip is rotated counterclockwise at 90° and the rotation around the 2nd rotation axis of ×2 introduces the plasma into the metering chamber. The residual plasma is simultaneously removed to the waste portion. (3) The chip is returned clockwise at 90° to the original position. The strong rotation of the chip around the 1st rotation axis of ×1 conveys the metered plasma to the substrate buffer solution storing chamber through the capillary valve. (4) The plasma and the substrate buffer stored in the accumulation chamber are drawn into the mixing channel by aspiration from an outlet of a measurement channel and subsequently into the measurement chip. (5) 405 nm light is transmitted to the measurement channel.

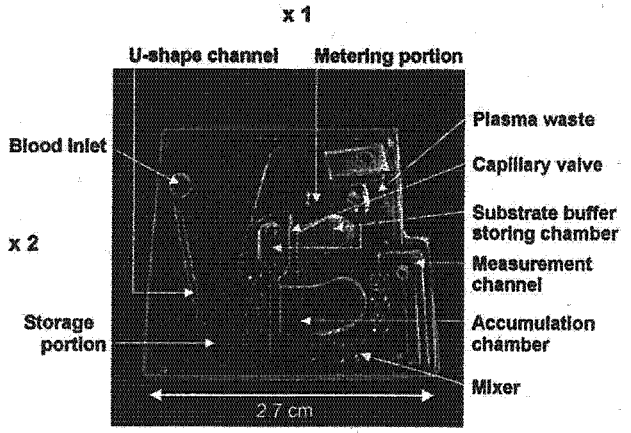

Fig. 15. Photograph of an integrated chip.

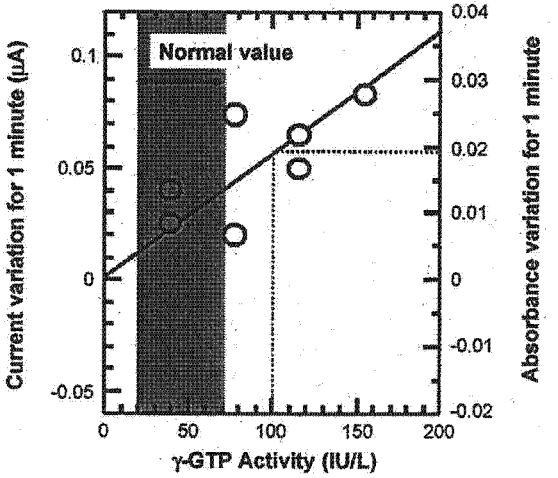

Fig. 16. Calibration curve of γ-GTP by the integrated chip.

Fig. 16 shows a calibration curve of γ-GTP. The measurement was carried out using the rate assay method, where variations in a detector current and absorbance during 1 min are plotted. Although scattering of the values are seen, the calibration curve is obtained for a wide range of γ-GTP activities. We are now developing a chip system to simultaneously measure three items.

4. Conclusion and future prospect

To establish the home medical diagnosis, the electronic blood collection system necessary for this goal as well as the healthcare chip checking Na^+, Ka^+, BUN, etc., and the hepatic function examining chip for γ-GTP, GOT and GPT have been studied. However, we have some issues of utilizing both chips for personal diagnosis at home. One is the higher hardness of the needle to collect the blood at a 100% reliability. Furthermore, BUN and creatinine sensors by measuring ammonia ions and the simultaneous measurement of three items including γ-GTP, GOT and GPT have to be developed. These measurements will lead to recognizing kidney trouble, diabetes and liver disease when the home medical diagnosis is realized by various biochips and the diagnostic data are communicated to clinical systems through a medical network, and not only medical treatment but also the society system are expected to be significantly changed.

Acknowledgement

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References

[1] D.R. Reyes, D. Lossifidis, P. Auroux, A. Manz, Anal. Chem. 74 (12) (2002) 2623.
[2] P. Auroux, D. Iossifidis, D.R. Reyes, A. Manz, Anal. Chem. 74 (12) (2002) 2637.
[3] R. Edwin Oosterbroek, Albert van den Berg (Eds.), Lab-on-a-Chip Miniturized System for (Bio)Chemical Analysis and Synthesis, Elsevier B.V., The Netherland, 2003.
[4] A. Oki, S. Adachi, Y. Takamura, K. Ishihara, H. Ogawa, Y. Ito, T. Ichiki, Y. Horiike, Electrophoresis 22 (2001) 341.
[5] A. Oki, M. Takai, H. Ogawa, Y. Takamura, T. Fukasawa, J. Kikuchi, Y. Ito, T. Ichiki, Y. Horiike, Jpn. Appl. Phys. 42 (2003) 3722.
[6] A. Oki, H. Ogawa, Y. Takamura, Y. Horiike, Jpn. Appl. Phys. 42 (2003) L342.
[7] A. Yokogawa, A. Oki, T. Shimasaki, H. Takasu, Y. Horiike, Proc. μTAS2003, Squaw Valley, USA, 2003, p. 895.
[8] H. Ogawa, M. Nagai, J. Kikuchi, Y. Horiike, Proc. μTAS2003, Squaw Valley, USA, 2003, p. 741.
[9] M. Takai, S. Shinbashi, H. Ogawa, A. Oki, M. Nagai, Y. Horiike, Proc. μTAS2003, Squaw Valley, USA, 2003, p. 403.
[10] H. Suzuki, Y. Matsugi, Sens. Actuators, B 98 (2004) 101.
[11] N.-T. Nguyen, S.T. Wereley, Fundamentals and Applications of Microfluidics, Artech House, Boston, 2002, p. 394.

4TH ASIAN INTERNATIONAL SYMPOSIUM ON BIOMATERIALS (AISB4)

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BIOCOMPATIBLE MICROGLUCOSE SENSOR WITH NEWLY DESIGNED PHOSPHOLIPID POLYMER FOR HEALTHCARE CHIP

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Introduction

Checking our daily blood-glucose value is a first step to prevent diseases, that is a typical lifestyle-related disease. A blood glucose analyzer for using of the daily health check should be portable, fast measurement, cheap, and high accuracy from a small amount of blood sample. Under the concept, several kind of healthcare chip having multi-item sensors has been developed [1-3]. In this paper, we report a design of membrane structures with phospholipid polymer on electrochemical microglucose sensor for blood analysis in a small volume less than 1μL.

Materials and Methods

Microglucose sensor was fabricated on screen-printed carbon electrode on polyester sheet. Electrode window (500μm x 700μm) was controlled by photolithography. Typical membrane structure of the glucose sensor was illustrated in Figure 1. Ferrocene entrapped with vinyl polymer was used as an electron mediator and glucose oxidase (1500unit/mL) was immobilized by polyioncomplex(PIC). Poly(solium 4-stylen-sulfonate)(PSS) and poly-L-lysine hydrobromide(PLL) were used as anionic and cationic polymers, respectively. Finally, phospholipid polymer: poly(MPC-*co*-n-butyl methacrylate)(PMB) (0.3wt% EtOH solution) was coated on the PIC layer.

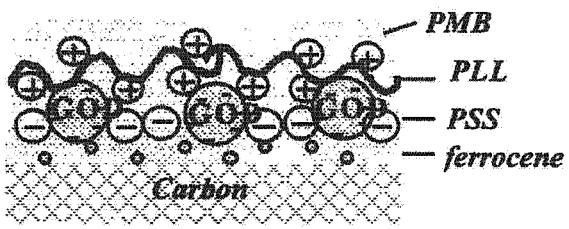

Figure 1: An image of membrane structure on microglucose sensor.

Results and Discussion

In our previous study, it was concluded that effect of PMB coating on microglucose sensor was a suppression of protain absorption in human plasma[4]. Figure 2 shows onother results of PMB coating. Current density measured at 0.35V vs. Ag/AgCl on glucose sensor with PMB coating shows linear response toward glucose concentration even after second measurement. While, current response of the sensor without PMB coating was unstable. Current density in second measurement, furethermore, was drastically decreased comparing with that in the first measurement. In present understanding, it was not clear the cause of obtaining high reliability on the glucose sensor with PMB coating.

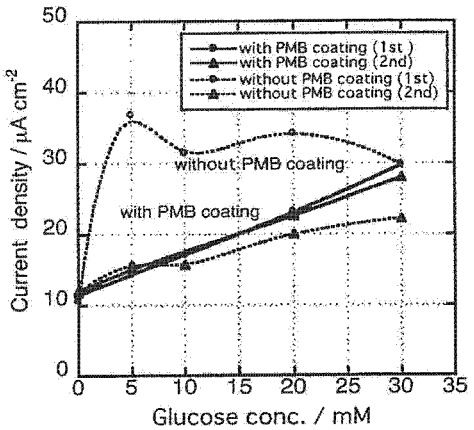

Figure 2: Current responses on microglucose sensors with and without PMB coating.

Conclusion

High reliability and stability on microglucose sensor for healthcare chip were obtained by the PMB coating on the sensor membrane consisted of PLL/GOD/PSS/Ferrocene.

References

[1] A. Oki et.al., (2003) *J. Jpn. Appl. Phys.* **43(6)**, 3722
[2] H.Ogawa et.al., (2003) *Proceedings of micro-TAS* **Vol.1**, p.741-743
[3] M. Takai et.al., (2003) *Proceedings of micro-TAS* **Vol.1**, p403-405
[4] M. Takai et.al., (2004) *Abstract of 7th World Biomaterials Cong.*, p138

DEVELOPMENT OF AMMONIA SENSOR

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Introduction

In the development of biosensor, concept of micro total analytical system (μ-TAS) or lab-on –a-chip system[1] has been emerged as a means to provide vital technological support to make home medical self check-up possible. In our group, researches based on health marker sensors, such as Na^+, glucose, K^+ have been carried out using integrated ISE[2, 3]. In this study, focus has been placed on ammonia sensor in terms of stable measurement of low concentration of ammonia using modified chemicals cocktail on PVC membrane[4, 5]. In addition, this study also aims to use a trace amount of whole blood, selectivity, shape and thickness of the membrane is also observed critical and important to the chip designed by the research group. The chip used in this study is based on previous study[6, 7], using disposable poly(ethylene terephthalate) PET plate.

Materials and Methods

Reagents and Solutions. PVC 30 wt%, plasticizers Bis(1-butylphenyl)adipate BBPA and Tris(2-ethylhexyl)trimellitate TOTM 67 wt%, anionic additives Potassium tetrakis(4-chlorophenyl)borate k-TCPB, Sodium tetrakis(4-fluorophenyl)borate Dihydrate TFPB 10 mol%, were bought from Fluka. Ammonia ionophore [22] TD19C6 was bought from DOJINDO. All aqueous solutions were prepared with deionized water.

Measurement of ammonia concentration. Potential measurements were made at room temperature (20-25°C) and the data recorded by Labview software were collected by under stopped-flow conditions with NH_4Cl concentrations (ranging from 1mM, 0.01M, 0.1M to 1M).

Results and Discussion

Measurement of ammonia concentration.

In this study, new type of chip has been developed in which only 4μl of blood is necessary to run the analysis (see Figure 1).

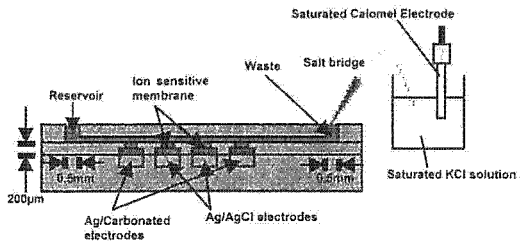

Figure 1: Cross section of a chip fabricated for the evaluation of selectivity.

Stable potential at low concentration of 1mM is achievable during the measurement with inner layer 0.01M ~ 0.2M NaCl and in the presence of 0.5% PVP.

Conclusion

Ammonia sensor has been successfully developed in the presence of inner layer (NaCl and PVP), especially stable measurement at low concentration. Markers like BUN and creatine/creatitine will be further invetigated on top of the ammonia sensor in the near future.

Reference

[1] A. Manz, N. Graber and H. M. Widmer: Sens. Actuat. **B 1** (1990) 244.

[2] Y. Horrike, S. Adachi, Y. Takamura, K. Kataoka, T. Ujiie and T. Ichiki: Int. Symp. Capillary Chromatogr., Gifu, Japan, 1999, OP409.

[3] A. Oki, S. Adachi, Y. Takamura, K. Kataoka, T. Ichiki and Y. Horrike: Proc. μTAS 2000, Enschede, The Netherlands, 2000, p. 403.

[4] K. Suzuki, D. Siswanta, T. Otsuka, T. Amano, T. Ikeda, H. Hisamoto, R. Yashihara and S. Ohba, Anal. Chem. **72** (2000) 2200-2205.

[5] S. Sasaki, T. Amano, G. Monma, T. Otsuka, N. Iwasawa, D. Citterio, H. Hisamoto and K. Suzuki, Anal. Chem. **74** (2002) 4845-4848.

[6] A. Oki, S. Adachi, Y. Takamura, K. Ishihara, H. Ogawa, Y. Ito, T. Ichiki and Y. Horrike: Electrophoresis **22** (2001) 341.

[7] A. Oki, M. Takai, H. Ogawa, Y. Takamura, T. Fukazawa, J. Kikuchi, Y. Ito, T. Ichiki and Y. Horiike, Jpn. J. Appl. Phys., **42** (2003), 3722-3727.

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(Biosensors 2005)

ABSTRACTS

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In Cooperation with
National Institute of Advanced Industrial Science and Technology (AIST)

Development of Clinical Chips Checking Life Style-Related Diseases

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We are studying clinical chips allowing the home medical diagnosis from a trace amount of a blood as an application of miniaturized analysis systems. For the goal, first of all, even armature has to collect the blood without any pains at home. Both sharp edge and smooth inner wall of a SUS tube with 150 μm in diameter led to a painless needle. Besides irradiation of NIR (near infra-red) to an arm and potential measurement between the arm surface and the needle enabled us to silhouette the blood vein and to detect its depth, thereby developing the electronic blood collection system. The blood is separated to cells and plasmas by centrifugal force on a chip. The chip called "healthcare chip" measures electrochemically pH, Na^+, K^+, glucose, BUN and creatinine. The fluidics of the blood in the micro-channel was controlled by a centrifugal force [1]. Recently, sensing of glucose has advanced considerably by improving the chemical cross linking of GOD (glucoseoxidase) using Glutaraldehyde over a polymeric mediator of VFc-*co*-HEMA on a carbon electrode. High sensitive measurement of BUN using ammonia ions was also achieved. A hepatic function examining chip for γ-GTP, GOT and GPT were studied. A new multi-staged H shaped-mixer of plasmas with substrate buffers achieved wide range measurements of activities of three enzymes successfully [2]. However, it was difficult to meter and introduce definite volumes of both plasmas and buffer solutions for the calorimetric type chip. Therefore, we are now developing another calorimetric type chip which uses the dry-chemistry. In this method, reagents which are dissolved in a gel and freeze-dried are react with plasmas in a channel and then measured. At present, glucose, BUN, γ-GTP, GOT and GPT are measured. These clinical chips lead to preventions of a life-related diseases such as diabetes, kidney trouble and a liver disease. When such home medical diagnosis is realized by various clinical chips, and diagnostic data are communicated to clinical systems through a medical network, not only the medical treatment, but the society system is expected to be changed greatly.

References

1. A. Oki, H. Ogawa, M. Nagai, S. Shinbashi , M. Takai, A. Yokogawa, and Y. Horiike, Mat. Sci. & Eng. C 24 (2004)837-843.
2. A. Yokogawa, A. Oki, T. Shimasaki, H. Takasu and Y. Horiike, Proc. μTAS2003, Squaw Valley,USA, (2003) 895-897.

プラズマプロセスによるバイオチップの開発と展望

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