国际生物信息与中医药论丛

SELECTED THESIS OF INTERNATIONAL SYMPOSIUM ON BIOINFORMATICS AND CHINESE MEDICINE

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國際生物信息與中醫藥論叢

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前 言

“法于阴阳”（取法于阴阳等中医学说）

“和于术数”（和调发展于科技与数理）

这是本次国际生物信息与中医药大会的主题。该文艺复兴式的命题，渊源于两千年前中医医典《内经》的开卷首篇——《素问·上古天真论》。本次大会努力贯通古今，融合中西。在坚持中医理论合理内核的大前提之下，将中医科学研究引向生物信息学、系统生物学、人类基因组学、分子遗传学、分子药理学、数理科学，在相互交叉与互相渗透中达成中医科学研究的突破性发展。尽管任务艰巨，困难重重，但本次学术会毕竟朝着这个方向迈出了坚实的一步。本次学术研讨会的主题是“法于阴阳，和于术数”，较之六年前上一届会议的主题“促进中医遗传学的分娩”，则更上一层楼。

本次国际生物信息与中医药学术研讨会，历经三年筹备，终于在成都如期召开了。本次大会得到了国家自然科学[国科金生外（03）30310303069]、四川省科技厅、成都中医药大学、新加坡医药卫生出版社（国际标准书号：ISBN981-04-8620-0）等单位的大力支持和帮助。会议先后收到论文 152 篇，从中择优 69 篇刊登于《国际生物信息与中医药论丛》，并在大会召开前出版。除中国（大陆、香港、台湾）外，还有来自于美国、英国、新加坡、挪威、韩国、泰国、马来西亚等国家的论文。

《国际生物信息与中医药论丛》开辟了十个栏目，几乎每一个栏目都有站在中医科学前沿的医学家、科学家发表新论：中国科学院沈自尹院士力倡将系统生物学与中医科学研究相结合；成都中医药大学王米渠教授将肾阳虚基因组的系列研究展示于众；清华大学长江学者程京教授在生物芯片高平台上前瞻医学科学的发展；全国优秀博士论文获得者李梢博士努力开辟生物信息学在现代中医研究中的新途径；北京 302 医院肖小河教授从数理科学的热力学角度深究中药四气药性；全国知名中医学家王琦教授透露了痰湿体质分子生物学研究的最新信息；清华大学王钊教授运用生物信息学方法分析与生殖相关基因的同源进化；中国中医研究院广安门医院吴志奎教授报告了补肾益髓法治疗地中海贫血的分子生物学研究进展；中国中医研究院西苑医院王阶教授广泛地辨析了不同疾病血瘀证的诊断方法；南京中医药大学蔡宝昌教授深入探讨了马钱子碱纳米脂质体的制备研究。此外，在冠心病血瘀证、骨关节炎虚寒证、再生障碍性贫血肾虚证、慢性胃炎阴阳两虚等等病证的诊治，在微观辨证、病理体质、七情应激等基础研究方面；在丹参、附片、麝香、银杏、马钱子等药物及其有效成分研究；在家系、双生子、聚类分析、易理术数方法应用等方面，都有一些新的信息和研究报导。

或许其中的一些研究离实际应用还有一定的距离；但是，在面对中医学这样复杂的科学问题，仍然需要这些“阳春白雪”似的前沿研究。从中医科学长远的观念来看，它不是太多，而是太少。创新性的基础研究，对中医科学将会产生深远的影响。虽然科学的探索之路总是艰辛而曲折，但以此论丛为开拓，促进中医药立足于生物信息平台，进而向现代科学穿插，其发展前景正如李白在《行路难》前瞻：“长风破浪会有时，直挂云帆济沧海”。

《国际生物信息与中医药论丛》编委会

二零零四年十一月一日

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*为方便大会交流，目录中文章作者署名次序有所调整

系统生物学和中医证的研究

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[摘要] 本文对自15世纪下半叶以来科学思想和科学方法论的回顾，阐明还原论是研究存在的科学，而涌现论则是生成的观点，科学的态度是把还原论和整体论结合起来。指出系统生物学理论和分子生物学相结合以及数学建模的方法是生命科学研究的新趋势，而"证"的研究正好和这个处于科学前沿、综合程度很强的系统生物学理论相结合，将几十年来用还原论方法在分子水平的研究成果用生物数学方法进行系统整合，将再次把"证"的研究推向科学前沿。

[关键词] 系统生物学 还原论 涌现论 证 数学建模

Systems Biology and Studies on the ZHENG of TCM

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[Abstract] The idea of science and methodology of science since the later half 15th century was reviewed in this article. The great difference between reduction theory, which is used to study the components and nature of things, and emergence theory, which is mainly involved in studying the production of new things was precisely discussed. The better method in research work is to combinate these two theorys. The author pointed out that the combination of systems biology and molecular biology and application of mathematical modeling is a new trend in biology science. The connotation of ZHENG, a professional term of traditional Chinese medicine, is consistent with systems biology. The integration of findings at molecular level during the last several decades by reduction theory will push the research of ZHENG into the scientific frontier again.

[Key words] systems biology, reduction theory, emergence theory, ZHENG, mathematical modeling

古代中国和古希腊都把自然界当作一个统一体，用自发的系统概念考察自然现象，从整体角度说明部分与整体的关系，例如阴阳五行学说和天人合一，中医对于即使是一个局部的病变都要结合全身情况来考虑。古代辩证唯物的哲学思想包含了系统思想的萌芽。但由于对这一整体各个细节缺乏认识能力，因而对整体性和统一性的认识是不完全、不深刻的。

15世纪下半叶，近代科学兴起，自然科学从近代科学汲取了分析研究的实验方法把自然界的细节从总的自然联系中抽出来分门别类地研究形成形而上学自然观（把自然界看作彼此不相依赖的各个事物或各个现象的偶然堆积），还有牛顿力学问世以来，还原论的研究方法主宰了现代科学的众多领域，生物学和医学就是从整体到器官；组织、细胞、不断细分到分子生物学，甚至着力于生物单分子行为研究，是近10年国际上的热门课题。从唯物的历史观来看，这是时代的需要，无论形而上学的思维方式或是还原论的研究方法，在深入的、细节的考察方面它比古代笼统的整体观是一个进步。

19世纪上半叶，自然科学取得了一系列伟大成就，为马克思、恩格斯提出的辩证唯物思想"物质世界是由无数相互联系、相互依赖、相互制约、相互作用的事物和过程所形成的统一整体"提供了丰富的素材和坚实的基础。辩证唯物主义体现的物质世界普遍联系及其整体性思想，也就是系统思想。

20世纪由于生产力的巨大发展出现了许多大型、复杂的工程技术，都要求从整体上加以解决，系统科学便

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应运而生了。40年代出现的系统论、运筹论、控制论、信息论是早期的系统科学理论，科学家明确地直接把系统作为研究对象，一般公认为理论生物学家贝塔朗菲Bertalanffy，他认为必须考虑各个子系统和整体系统之间的关系才能了解各部分的行为和整体。

系统有三个特点。第一个特点是多元性和差异性，系统就是多样性的统一、差异性的统一。第二个特点是相关性，系统中不存在孤立的元素或组分，所有元素或组分都按照该系统特有的方式彼此关联在一起，相互依赖、相互制约，差异而不相关的事物构不成系统。所以系统是整合起来兼具多样性和统一性。第三个特点是整体涌现性（whole emergence），即若干组分按照某种方式整合成为一个系统就会产生这些组分单纯相加所没有的新东西，一旦把系统分解还原为各个组分，这新东西便不复存在。系统科学把这种整体才具有、而孤立的组分及其总和不具有的特性称为整体涌现性。例如单个分子没有温度，大量分子聚集为热力学系统就具有用温度表示的整体属性。一台安装好的机器具有它的零件总和所没有的功能。系统科学是探索整体涌现性发生的条件、机制和规律。

还原论的奠基者笛卡尔强调，为了认识整体必须认识部分，只有把部分弄清楚才可能真正把握整体。在这个意义上，还原论方法也是一种把握整体的方法即所谓分析--重构方法，但居主导地位的是分析、分解和还原，首先把系统分解为部分，用部分说明整体。在这种方法论指导下，400年来创造了一整套可操作的方法，取得巨大成功。对于比较简单的系统这样处理一般还是有效的，但现在面临的是大量复杂系统问题，把部分的认识累加起来的方法，已不适宜去发现整体涌现性。

世界是演化的，一切系统都不是永恒的，还原论是研究存在的科学，而涌现论则是生成的观点，科学的态度是把还原论和整体论结合起来。按钱学森的说法："系统论是还原论和整体论的辨证统一"。

80年代非线性科学和复杂性研究的兴起对系统科学起了推动作用。非线性是数学概念，它研究系统内外结构与功能及其演化过程都是相互作用的显示，并给予数学上的量化。复杂性研究认为事物的复杂性是在适应环境的过程中从简单性发展而来的。故而要开展跨学科、跨领域的研究，3位诺贝尔奖获得者盖尔曼Gellmann、阿罗Arrow和安得森Anderson把经济、生态、免疫系统、胚胎、神经系统及计算机网络等称为复杂适应系统，认为存在某些一般性的规律控制着这些复杂适应系统的行为，这种认识向着宏观、交叉和复杂的整体的趋势发展已成为现代科学的主流。

还原论是把系统分解为大量的基本单元，认为这些单元的行为及其相互作用遵从普通而简单的自然法则，因为它仅仅适用于"简单系统"而不适于自然界中大量存在的复杂系统。人体是一个非线性复杂系统，所以必须以系统论和还原论相结合为特色的复杂性科学方法来研究，当今对于人体涉及生命科学的研究仍需要以还原论的思维例如单分子行为研究，继续向微观深入研究，把细节研究得更细致、更确切；但另一方面直接走向宏观层次，走向复杂和综合，系统科学必将有重大发展，改变科学世界的图景引起科学思维的革命。

莱洛伊·胡德Leory Hood（提出系统生物学）说：要把孤立的在基因水平、蛋白质水平的各种信息相互作用、各种代谢途径、调控途径、基因网络之间，所有的功能模块和系统都耦联、整合起来，用以说明生物整体，这是系统生物学的一个最基本想法。把这些数据集中起来不是简单的堆积，而是从中抽提出一些可供选择的数学模型，这些模型不仅可以模拟生物系统的行为，而且可以用来预测该系统受到干扰后未来的行为。概括起来，系统生物学第一步是整合数据，第二步是建立数学模型，第三步是预测系统行为。系统生物学研究方法的特点是通过层次与层次之间、网络与网络之间、系统与系统之间的联系和整合建立起来的复杂系统，并不是简单系统的叠加。这个复杂系统会出现一些涌现性行为、涌现性规律，出现一些单独系统所不能反映的新行为。系统生物学研究也会通过不同网络之间的贯穿特性，使得基因或蛋白质过渡到生物学功能（表型）。处于国际科学前沿的哈佛大学成立了系统生物学学院、加州理工学院成立了系统生物学研究院，国内如上海交通大学亦成立了系统生物学研究所，北京和广州亦成立相应机构，说明系统生物学在国际上已处于启动阶段。

中医学根源于华夏文明，是中国固有的传统医学，由于中国历史悠久，文化深厚，所培养形成的医学，既深且博，独成体系。春秋战国时期诸子百家富含哲理的争鸣，周秦阴阳五行学说盛行，都渗透到中医学的理论体系中，没有文化发展的影响是不能上升和逐步完善为系统的理论。

中医学对世界医学发展作出最独特的贡献，是建立了这个完整理论体系的认知方法，其中有两个最突出的核心，即整体观念和辩证论治。

整体观念认为人体是以五脏为中心，通过经络和精、气、血、津液把全身组织器官联系在一起，成为统一的整体来维持生命活动。整体观还体现在将人与自然界及周围环境也视为一个整体，所谓："人与天地相应也"、"天人合一"，关注人与自然的和谐。

辩证论治是又一特点，"证"是综合了产生病变的各方面（包括当时的气候和环境情况）因素和条件，结合个体的体质，然后作出判断。辩证论治和整体观念是密切不可分，即使是一个局部的病变，都要结合全身情况来考虑，始终从个体变化的角度来分析，中医就是擅长于个体化的治疗。

现在及以往对中医理论及实践的研究都是按照西医的还原论思维方法，从整体到器官、组织、细胞，不断细分到分子生物学，无法顾及到整体。还有一个西医的思维方法是采用现代科学计量统计的方法，从一部分样本中统计出数据，抽取共性，认为此共性代表总体的共性，就用于群体的治疗和研究，从而忽略了病人的个体差异。恰恰是**这两种西医思维对中医的研究方法成为发扬中医精华的瓶颈。**

中医药学本来就是先进的系统生命科学认知体系，"证"的研究正好可以和这个处于科学前沿、综合程度很强的系统生物学理论相结合，同时也是生命科学和临床医学相结合。这将是后基因组时代最重要的研究方向之一，从而把"证"的研究再次推向科学发展的前沿。

"证"是辩证论治的起点和核心。证是指在疾病的发生、发展过程中，一组具有内在联系的、能够反映疾病过程在某一阶段的病理病机，是机体对致病因素作出反应的一种功能状态。由于辩证是由外揣内，在具体运用上受到医患双方主观因素的影响，难以客观化和量化，所以必须通过"证"的内涵研究。我们在"肾"本质的研究中，借鉴了Basedovsky1977年提出的"神经内分泌免疫网络"学说，其核心是神经、内分泌、免疫三个系统已不是过去认为的彼此不相干，各司其功能，各行其职，而是构成了一个完整的网络系统，这是现代医学从局部观点到整体观念的一大发展和进步。但当时整个现代科学和现代医学还是被还原论的思维方法所笼罩着，我们对与"肾虚证"相对应的下丘脑－垂体－靶腺轴的研究仍循着还原论的思路不断往下细分，一直研究到分子生物学水平—基因的调控，找到了大量与"肾虚证"相关的活性物质及其相互作用的证据。但这只是"证明性的研究"，未能将"肾虚证"的外象与内涵从整体上统一起来。现在系统科学和系统生物学的崛起，才给"证"的整体观念和个体化的研究带来了机遇，当然，**没有以往大量而扎实的奠基性工作为基础，系统生物学失去整合的对象（生物体内大大小小有机联系的活性物质），亦将成为空架子，**要实现这外象与内涵的统一，对证加以量化，由此定出生物数学诊断标准，就必须要以系统生物学的理论和方法来引领，并且必须**系统生物学和分子生物学相结合。**

最近一期《科学》杂志在线刊登题为"科学的下一波热潮"的特辑，汇集了该刊2003年2月以来发表的关于生物数学的文章，从概念、发展方向和人才培养等方面详尽介绍了21世纪科学热点之一——生物数学。由于生命现象复杂，生物数学模型能定量的描述生命物质运动的过程，解决复杂的生物学问题。马克思当年说过："一门科学只有当它充分利用了数学之后，才能成为一门精确的科学"，中医学和生命科学当不能例外，只有更多的借助数学的威力，才有可能进入更高的境界。

每一个证候都有其外象（外候）与内涵，外候是用四诊——望、闻、问、切所获得的信息进行整理而得，但很难量化，即使用流行病学方法加以演释，亦是靠专家得经验打分，最多亦只是半定量。因此用生物数学方法将外候与内涵有机地整合起来，就能贯彻系统生物学和分子生物学相结合地思路。

《内经》有一段对"肾气"的经典描述，即女子以七、男子以八为基数，随着年龄的成倍增长，从生长、发育、壮盛以至衰老这样一条曲线是代表肾气由盛至衰的过程。"肾虚证"是根据中医理论在临床宏观层面辩证的结果，它是机体随年龄的增长体内各种功能衰退在宏观层面的表现，因此"肾虚辩证标准"带有明显的年龄依赖特征。

当然肾虚与衰老之间不能划等号，人体在衰老时除了有腰脊酸痛、胫酸膝软、耳鸣耳聋、发脱齿摇、尿有余沥、性功能减退等这些肾虚的外候，还可能因后天环境因素所造成的脾虚，或肾气虚衰导致的抉瘀证候。但肾为先天之本，与生长发育衰老的生命规律相关，故衰老应该是肾虚证的内涵。

中医的各证候是机体不同的功能态，该功能态由一系列基因的表达所赋予，并被这一系列基因表达所

标识，也即证候的研究可以落实在基因表达谱上。我们利用符合生理性肾虚证的自然衰老大鼠模型（26月龄），并用中药淫羊藿总黄酮（EF）、补肾复方、活血复方分别进行干预、摘取大鼠下丘脑、垂体、肾上腺、脾淋巴细胞，采用美国Affymetrix Co.的大鼠基因芯片，研究衰老大鼠下丘脑－垂体－肾上腺－胸腺（HPAT）轴的基因表达谱以及药物干预的基因表达差异谱。研究结果提示：在"肾虚证"状态时，HPAT轴上出现了众多分子网络调控规律，表现为：A、EF上调多种神经递质受体的表达，通过神经－内分泌－免疫网络的下行通路激活神经内分泌和免疫系统。B、EF还通过生长激素轴、性腺轴、淋巴细胞凋亡三个方面的网络机制发挥分子网络效应。C、EF在淋巴细胞凋亡和增殖的网络机制中重塑凋亡相关基因及增殖相关基因的良性平衡。由此我们提出"证"的新概念："**证是一种有机综合的功能态，由一个调控中心及其所属众多分子网络所构成，作为对外界反应与自我调节的基础**"。补肾中药可以对此进行调整。正如美国加州理工学院新近创造了一种"基因调控网络"模型，以此可以解释海胆的胚胎发育不是单个基因，而是一种基因网络式的调控。这一原理同样适用于人类在发育或衰老时无数种不同类型细胞的调控方式和规律，也间接佐证了我们对"肾虚证"（衰老）提出的分子网络调控概念。

四十多年"肾虚证"的研究已逐渐向微观层面发展，揭示了"肾虚证"的内涵，并明确了许多可定量的标志性指标。当这些标志性指标尚未被整合时，它难以客观地反映"肾虚证"的整体情况。根据现代生物数学的观点，在生命科学中存在大量的非线性现象，**用类似人脑辨析思维的"神经网络"以及非线性动力学为主的理论和方法，建立数学模型有助于寻找生命科学内非线性现象的规律**。我们打算在对不同年龄段的健康人和不同月龄的大鼠进行宏观和微观检测分析后，应用"神经网络"、非线性动力学为主的理论和方法建立"肾虚证"数学模型，来寻找"肾虚"过程的规律。企望用系统生物学思维和方法去探索用还原论思维和方法所不能发现的新东西。

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生物芯片及其在生命科学中的应用*

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[摘要] 基于生物芯片的系统在过去的数十年中取得了极大的进步。人们发明了多种制造工艺以及各种的材料来满足苛刻的要求来制造这样的生物芯片系统。传统的微加工工艺以及一些新近发展的技术，例如塑料成型和微阵列法，常被用于制造具有各种分析功能的基于硅、玻璃和塑料的芯片，它们在临床诊断和基础研究中有着重要应用。人们用这些芯片来把所有生物分析过程中都涉及到的三个经典的步骤——样品制备，生物化学反应以及结果检测和分析整合到一起，最终的目的是构建完全集成的、更小的、更高效的实验台，甚至是手持式分析仪——这些都可归为"芯片上的实验室"系统。同时，基于芯片的分析系统已向人们展示了它多功能的一面，例如分析小分子化合物、核酸、氨基酸、蛋白质、细胞和组织。这一章将对生物芯片的不同功能和基于芯片的集成系统进行论述。

[关键词] 生物芯片 应用 芯片上的实验室

Biochips and Applications

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[Abstract] Biochip-based systems have enjoyed impressive advancement in the past decade. A variety of fabrication processes have been developed to accommodate the complicated requirements and materials for making such a device. Traditional microfabrication processes and other newly developed techniques such as plastic molding and microarraying are being explored for fabricating silicon, glass or plastic chips with diverse analytical functions for use in basic research and clinical diagnostics. These chips have been utilized to facilitate the total integration of three classic steps involved in all biological analyses, i.e., sample preparation, biochemical reaction, and result detection and analysis, and finally construct fully integrated smaller, more efficient bench-top or even handheld analyzers – laboratory-on-a-chip system. Meanwhile, biochip-based analytical systems have demonstrated diversified use such as the analyses of small chemical compounds, nucleic acids, amino acids, proteins, cells and tissues. In this chapter, aspects related to biochips with different functionality and chip-based integrated systems will be reviewed.

Sample Processing

Sample preparation is the first stage of a lab-on-a-chip system. It generally implies the ability to process crude biological samples such as blood, urine, water, etc. to isolate target molecules or bioparticles of interest such as nucleic acids, proteins or cells. Currently, most of the analytical methods used in biomedical research and clinical applications analyze samples at volumes of >2 μL. Handling and processing of micro-samples (e.g., μL and sub-μL volume) is difficult. Analysis of sub-μL volumes of sample has known problems such as loss of sample

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on the walls of pipette tips, loss by evaporation, loss of the targeted analyte because of adsorption onto the tubingwalls or containment vessels during manipulation and processing period, difficulty in obtaining a representative sample from an nonhomogeneous specimen. Additionally, the low concentration of the analyte may restrict the scale of miniaturization. In many cases the analytes are usually present at extremely low concentration, e.g. 100 molecules/mL. Hence, in a 1μL sample there is less than one molecule of the analyte, and thus this degree of miniaturization is impracticable. Sample miniaturization is suitable for molecular analysis of genomic targets. Generally speaking, there are approximately 4,400-11,000 white cells in a 1 μL of adult human blood. In theory the DNA molecules from a single white cell are sufficient to allow theamplification of the region of interest millions of times through the use of molecular technologies such as PCR. For a blood specimen, suppose the white blood cell count is 10,000/μL, the average volume for a sample to contain one white blood cell is 100 pL. In the event of detecting rare cell types or microorganisms (e.g., detection of cancerous cells, fetal cells in maternal circulation, assessment of minimal residual disease), insisting on the use of reduced volume of samples is no longer practical. Under these circumstances, sample sizes compatible with detection will have to be determined by the expected cell frequency or microbial load and sample volumes ranging from 100 μL to 5 mL may be desired. Moreover, specific selection (e.g., dielectrophoresis technology) or a preconcentration step has to be adapted to ensure the presence of the desired cells or microorganisms.

Microfiltration

To analyze nucleic acid by a lab-on-a-chip system the nucleic acids released from white blood cells usually have to be amplified by various amplification technologies such as PCR or SDA. However, these amplification processes might be inhibited by hemoglobin released from red blood cells. Hence a fundamental consideration in designing the microfilter chips for sample preparation is to facilitate the largest possible isolation of white blood cell populations or nucleic acids with very low red cell or hemoglobin contamination. All microfilter chips so far have been fabricated directly from silicon using both conventional wet etching and reactive ion etching. Different structural designs were explored, including simple arrays of posts [1], tortuous channels, comb-shape filter and weir-type filters [2]. The general structure of a microfiltration chip is an etched chamber that contains the filter element across the entire width of the chamber. The study of microfilter-facilitated cell separation soon revealed that the deformability of cells plays a critical role in the separation efficiency. The filter dimensions were initially designed according to the reported sizes of blood cells obtained from morphological measurements of the stained cells. Yet, filtration of white and red blood cells was found to be influenced by the cell concentration, applied pressure, viscosity of the medium, and the size of the filter port. And it was discovered that red blood cells with relatively stable discoid architecture readily align themselves to facilitate passage through a 3 μm gap while highly deformable white blood cells with spherical diameters in excess of 15 μm will pass through filter gaps of only 7 μm. Thus, optimization of filter geometry was performed and weir-type filters with a filter gap of approximately 3 μm were proved to be effective in isolating large sized-white blood cells with relatively high yield [2]. For the isolation of cells with very small sizes (e.g., bacterial or viral particles) or specific types or subtypes (e.g., CD4+), microfilter chips may be incompetent despite its effectiveness in removing red blood cells from blood. The following two isolation approaches may be found useful.

Magnetic cell sorting

A microfluidic structure has been made in silicon to enable the magnetic cell sorting [3]. An enrichment rate of more than 300-fold has been achieved. However, it was impossible to control the interaction time of particles with magnet due to the parabolic flow profile in microchannel. In addition, build-up of magnetic particles increased the magnetic field gradient inside the channel and consequently entrapment of particles was observed.

Electronic cell separation

Spiral gold electrodes were fabricated on the glass substrate. The electrode array consists of four parallel spiral electrode elements energized with phase-quadrature signals of frequencies between 100 Hz and 100 MHz. Depending on the frequency and phase sequence of applied voltages, the three dimensional forces generated by spiral electrodes could result in cell radial motion, levitation, and trapping. The chip bearing spiral electrodes has

been used for the enrichment of breast cancer cells and isolation of breast cancer cells from blood [4]. Complicated design of platinum/titanium and indium tin oxide electrodes have been fabricated also on glass substrate for cell manipulation [5]. Negative dielectrophoresis has been employed in this case for concentrating and switching the particles at flow speed up to 10 mm/sec. In addition, planar microelectrodes were used to trap viral particles when a phase-shifted high frequency AC signal is applied [6]. Moreover, individually addressable microelectrode array fabricated on silicon substrate has recently been used for the isolation of cultured cervical carcinoma cells from human blood [7]. This demonstrated the possibility of further integrating cell isolation devices with other microdevices through the use of established silicon processing technologies.

Biochemical Reaction

Biochemical reaction may include various types of chemical or enzymatic reactions such as chemical labeling, DNA amplification using PCR or SDA (strand displacement amplification) or DNA restriction enzyme digestion.

Amplification of Nucleic Acids

The amplification of nucleic acids has been performed in microchips fabricated from different substrates such as glass [8], silicon-glass [7] and plastics [11]. Both thermal and isothermal amplification techniques were demonstrated [9]. The reaction volumes varied from a 1 μL [12] to > 25 μl [14]. The glass-silicon microchips were bonded by using either silicone rubber [10] or anodic bonding [13]. The size of the amplification products ranges from approximately 50 bp to 1600 bp. Thermal cycling was achieved either by an on-chip polysilicon thin film heater or externally by means of a Peltier heater-cooler or an infrared irradiation [14]. Nucleic acids have been amplified in these microchips using conventional hot-start PCR, LCR, DOP-PCR [14], multiplex PCR and SDA [9]. RNA has been amplified using the single step RT-PCR protocol [15]. Rapid PCR was achieved recently on a microchip-based PCR device using flexible printed circuit technology. An new digital temperature control system was developed by introducing a heater/sensor switching procedure. Temperature stability within ±0.3 °C and a transitional rate of 8 °C/s during heating/cooling was achieved [16].

Surface chemistry plays a significant role in microchip amplification reactions [13]. Various passivation procedures have been tested and several identified that are PCR and LCR friendly. Covering a silicon surface with a thermally induced silicon dioxide layer (thickness of 2000 Å) is the most effective passivation procedure discovered so far for nucleic acid amplification reactions [14]. Isothermal nucleic acid amplification techniques (e.g., nucleic acid sequence-based amplification and strand-displacement amplification) are candidate techniques for a microchip format. These techniques do not require the use of the heater-cooler system and therefore greatly simplify the construction and operation of a microchip for nucleic acid analysis and should prove energy saving.

Other chemical reactions

Apart from the DNA/RNA amplification performed in various microchips, other chemical reactions have also been investigated using microchips. For example, both quartz and glass microchips have been fabricated for performing capillary electrophoresis and postcolumn reaction [17]. On-chip postcolumn reaction of o-phthaldialdehyde and amino acids generated theoretical plate numbers up to 83000 and approximately 90 ms peak widths. Approximately 10% degradation efficiency was due to the reactor geometry. Apart from that, it was found through the study that pH differences in the mixing solutions play a role in the efficiency of the postcolumn reactions. In another report, enzymatic reactions were performed within a microfabricated channel network [18]. Precise concentrations of substrate, enzyme, and inhibitor were mixed in nanoliter volumes using electrokinetic flow. Reagent dilution and mixing were controlled by regulating the applied potential at the terminus of each channel, using voltages derived from an equivalent circuit model of the microchip. The β-galactosidase-catalyzed hydrolysis of resorufin β-D-galactopyranoside was used as a model system for enzyme kinetic and inhibition determinations. The microchip approach assay allowed the studies to be completed with significant time-savings and reduction of reagent consumption by more than 4 orders of magnitude while delivering results consistent with conventional approaches.

Result Detection

Result detection may be facilitated by microchannel–based separation approach, microarray-based affinity binding approach, etc.

Microchannel-based separation methods

One distinct advantage for microfabricated chips is that they can be utilized as platforms for multi-purpose liquid sample handling and analysis. As a result, a variety of separation methods have been developed for use with microchips. The methods implemented on chips include free-solution capillary electrophoresis, capillary gel electrophoresis, micellar electrokinetic chromatography, isotachophoresis, isoelectric focusing, open-channel electrochromatography and free-flow electrophoresis.

Nucleic acid analyses

The analyses of nucleic acids can be divided into two main categories. One is the fragment sizing in most cases related to the detection of DNA mutations. The other one is DNA sequence analysis. For the first category polymer solution gel capillary electrophoresis has been used as the main separation media. One of the earliest separation case, the rapid sizing of PCR amplified HLA-DQα alleles as well as the spiked DNA marker with size ranging from 72 to 1353 bp was obtained in approximately 2 minute in a glass capillary electrophoresis device [20]. Hydroxyethyl cellulose (HEC) was used to form the entangled free-solution sieving matrix in this study. Apart from glass chip plastic chips have been fabricated and used for fragment sizing [21] and detection of single DNA molecules [7]. Using injection-molded acrylic capillary electrophoresis chip and HEC as sieving matrix all fragments in the DNA marker with size ranging from 72 to 1353 bp was baseline resolved in 2.5 minutes. The standard deviation for run-to-run is less than 1% and for chip-to-chip is between 2-3% [21]. Also PDMS molded capillary electrophoresis chip has been used together with hydroxypropyl cellulose as sieving media in the separation of the marker same as that used in reference 6 and 36. In the study of single DNA molecule detection efficiency larger than 50% was obtained using the same device [7]. Fused silica capillary electrophoresis chip has been fabricated and used for fast DNA profiling [21]. In this case a replaceable denaturing polyacrylamide matrix was employed for baseline resolved separation of single-locus short tandem repeats amplicons. The complete separation of four amplicons containing loci of *CSF1PO*, *TPOX*, *THO1* and *vWA* was achieved in less than 2 minutes representing a 10- to 100-fold increase in speed compared to the conventional capillary or slab gel electrophoresis systems. In another report glass capillary array electrophoresis chip filled with HEC solution was used for high-speed DNA genotyping [22]. Twelve DNA samples with the largest fragment size of 622 bp were separated in parallel in less than 3 minutes. For the detection of all lanes a laser-excited confocal fluorescence scanner was developed for achieving a temporal resolution of 0.3 seconds. In a recent case, ribosome RNA samples were separated in an injection-molded plastic microchannel with a cross-section of 100x40μm and a effective length of 1 cm [23]. The sieving matrix employed is hydroxypropylmethylcelulose and the detection of RNA less than what can be obtained from a single cell is achieved using a fluorescent microscope equipped with a photometer. Recently, a PDMS chip-based temperature gradient capillary electrophoresis was developed for fast screening of single nucleotide polymorphisms. A temporal temperature gradient with a precision of 0.1 °C per step was applied on the chip during the separation. The homoduplexes and heteroduplexes were base-line resolved [24]. Ultra high speed DNA sequence analysis has been achieved on a glass capillary electrophoresis chip where a denaturing 9%T and 0% C polyacrylamide solution was used as the separation media [25]. When a four-color detection scheme was used the readout of approximately 200 bases was obtained in 10 minutes in an effective separation length of 3.5 cm. After optimization of both electrophoretic channel design and methods much higher readout in four-color DNA sequencing was obtained, i.e., 500 bases in less than 20 minutes [26]. For the purpose of fast DNA sequence analysis if a 96-channel array chip is used one can then easily see how significant the production rate could be compared to the conventional DNA sequencers.