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論文内容の要旨

Ion channels play key roles in functions and dysfunctions of all cells. Single channel recording methods using planar (black) lipid membranes (BLM) and patch clamp techniques are widely used at present, but they are not suited for automation and miniaturization. Therefore, in order to develop electrophysiology library arrays, several groups have recently reported on wafer-based devices that can replace the more traditional glass pipettes and Teflon partitions that are employed for investigating ion channels activities in cells and artificial membrane. The supported planar lipid bilayer (SPLB) is a lipid bilayer supported on solid surfaces. Concerning the ion-channel biosensors, single ion channel recording has been succeeded in suspended membranes made by the painting method on micro-machined supports. In the case of the suspended membrane, it is not easy to make a single bilayer with a small pore diameter (several „m), which is necessary for high speed recording and low noises. In the SPLBs made by vesicle fusion however, single channel recording have not yet been reported. The SPLB on the silicon based microelectrode are extremely attractive since small pore can be easily made, thus it has a potential of high stability, high sensitivity and high density of integration. It is considered that a major challenge in the production of tightly sealed bilayers to reduce leakage currents to the levels found in the suspended membrane, and most likely, this will require the reduction of the substrate surface roughness and the elimination of edge effects. The efforts to get high resistivity in the tethered supported membrane on Au surface have been done by several groups. Recently, the tethered lipid bilayers with a high electrical resistance of ~130 M„ and the subsequent detection of only a few synthetic ligand-gated ion channels incorporated in the tethered lipid bilayer, have been reported. [S. Terrettaz *et al.* Langmuir 19 (2003) 5567] In spite of these efforts, it is clear that a much higher resistivity (G„ seal) is required to realize a supported membrane biosensor which can be applied to the single ion channel recording.

In the present thesis, based on these backgrounds, he has developed several elementary processes to realize an ideal SPLB with G„ resistance on Si-based microelectrodes. He has developed the techniques to fabricate a hole (well) with a diameter of about 1 μm for microelectrodes on a $SiO_2/CoSi_2/Si$ substrate, while maintaining the SiO_2 surface roughness at less than 1 nm using a femtosecond laser microfabrication technique and synchrotron radiation etching. The SPLB membrane was formed on the surface of a microelectrode area by the fusion of giant unilamellar vesicles. He has characterized the stability, electrical resistance, capacitance, and current noise of the bilayers.

After the deposition of Co on the Si(100) surface, the SiO_2 thin film consists of spin on glass (SOG) (400 nm thickness) and sputtered SiO_2 (200 nm thickness) was formed on the Co/Si. Then by annealing at 540°C for 10min, the Co/Si layer was changed to $CoSi_2$ keeping the SiO_2 surface roughness less than 1 nm. A 300 nm of Co layer was deposited on the SiO_2 surface by

sputtering as an etching contact mask and circular patterns were made on the Co mask using the femto-second laser ablation. The SR etching of the SiO_2 layer to make the wells on the electrode was carried out at the beam line 4A2 of the SR facility (UVSOR) at the Institute for Molecular Science, using a mixture of SF_6 (0.05 Torr) and O_2 (0.002 Torr) as an etching gas. The SR etching results in a vertical wall and completely stops at the surface of the $CoSi_2$/Si(100). SR was used because of its unique features such as high spatial resolution, extremely high material selectivity between $CoSi_2$ and SiO_2, anisotropic etching, low damage, and clean etching atmosphere. Finally the Co contact mask was removed without damaging the substrate by immersion into 0.1 M HNO_3 aq. AFM images of the SiO_2 surface after the removal of the Co mask showed that the surface was very flat (Ra=0.8 nm), which is essential for the formation of the defect-free SPLB on the surface.

Ag (50 nm) was deposited by electroplating on the surface of $CoSi_2$ which was exposed at the bottom of the etched well. Then the surface of the Ag was changed into AgCl also by electroplating. The giant unilamellar vesicles were prepared by adding a buffer solution (10 mM KCl, pH = 6.6) to vacuum-dried films of dipalmitoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-3-phosphor-L-Serin (POPS) (9:1, w/w) and agitating at room temperature. Mixing of negatively charged lipid POPS to neutral lipid DPPC was essentially effective to form unilamellar giant vesicles without aggregation. Formation of SPLB covering the well-type electrode by the rapture of the giant vesicle was confirmed by fluorescence microscope. When substrates are immersed in an aqueous solution of lipid vesicles, the vesicles adhere to the surface, rupture, and spread to form a bilayer on hydrophilic surfaces of SiO_2. It has been suggested that a thin water layer approximately 1-2 nm is trapped between the support and the headgroups of the lower leaflet of the bilayer. [Bayerl, T. M.; Bloom, M. Biophysical Journal, 58 (1990) 357]

Fluorescence microscopy images showed that the diameter of the SPLB formed on the SiO_2/Si(100) surface by the rapture of the giant vesicles was typically about 150 - 300 „m, large enough to cover the electrode area (10 - 30 „m diameter). AFM images of the bilayer showed that the thickness of the SPLB membrane was 4.5 nm, corresponding to the height of a single bilayer. The electric characteristics were measured by a patch clamp amplifier through the AgCl/Ag electrode. The resistances before and after the lipid bilayer formation were 10±3 M„ and 1.2 G„ , respectively. This confirmed the G„ seal formation of SPLB on the microelectrodes. The capacitance of the bilayer measured by using a patch clamp amplifier was 10.7 pF. These values were observed with extremely good reproducibility during our experiments for more than 5 hours.

Although the resistance value fulfills the condition required for the measurement of single channel measurements, even then it is much smaller than those (> 30 G„) realized in the planer or suspended membranes. This may be due to the edge leak current. Therefore, by depressing the edge leak current, much higher resistance of lipid bilayer is expected to be obtained.

He has considered to use the hydrophobic self-assembled monolayers (SAM) as a "guard ring" to reduce the edge leak current of SPLB. He has developed a patterning method of octadecyltrichlorosilane (OTS) SAM by photo-lithography and UV ashing. OTS-SAM was formed on the sputtered SiO_2 surface by immersing the sample into a 1.0 mM solution of OTS in toluene for 10 s at 22°C. Then negative resist (7„m height) pattern was made with lithography technique. After 30 min of UV ashing, resist pattern was removed with remover. The OTS-SAM on the open area, which was not covered with the resist, were completely removed by UV ashing, while no change was observed in the OTS-SAM on the area covered with the resist. The height of the OTS-SAM was ~2.5 nm and the roughness of the SiO_2 surface without OTS-SAM was Ra=0.8 nm. SPLB was formed on this patterned OTS-SAM by rapture of giant unilamellar vesicles. AFM and fluorescence microscopy images have shown that SPLB forms bilayer on hydrophilic SiO_2 surfaces and a monolayer on OTS-SAM hydrophobic surfaces. This technique has been considered to use for the formation of tightly sealed bilayers to reduce leakage current of the SPLB.

論文の審査結果の要旨

Mashiur Rahman君の学位論文は、固体基板表面に平面脂質二重膜を乗せこれに膜タンパクの一種であるイオンチャンネルを再構成した、バイオセンサー、いわゆるサポーテッドメンブレンバイオセンサーの製作を目指して、その最も重要な要素技術である、無欠陥脂質二重膜（ギガオームシール）の製作と、さらに脂質二重膜と基板との隙間を流れるエッジリーク電流をおさえるための自己組織単分子膜によるガードリング構造の形成を試み、いずれも世界に先駆けて成功に至った成果をまとめた報告である。全6章から構成されている。

膜タンパク質は全創薬ターゲットの50%以上を占めており、ポストゲノム創薬のうえで極めて重要なタンパク質とされているが、スクリーニングなどに必要な適切なバイオセンサーが無く、サポーテッドメブレンは有望なバイオセンサーとして、あるいは細胞膜表面反応をインビトロで研究するための好都合な反応場を提供するものとして、世界的に興味がもたれ活発な研究が展開されているところであるが、単一イオン電流（single ion channel current）を計測する事が出来るレベルの無欠陥脂質二重膜の形成にはこれまで成功した報告例が無かった。勿論、ガードリングの形成は試みられた例は無い。今回Rahman君は、従来外国では表面化学修飾技術の確立した金基板を用いることが主流であったのを、表面をナノレベルで加工が容易なシリコン基板を用いることを提案し、これにその1nm以下の表面凹凸を保持したまま表面の電極穴形成技術を確立するとともに、その上にベシクルフュージョンにより脂質二重膜を形成する技術を開発し、見事1.2ギガオームの無欠陥膜の形成に成功するに至った。

第一章では、本研究の背景、基礎知識、研究の目標などについて記述している。第二章では、各種膜形成技術、パターン形成技術、放射光エッチング技術、電極形成技術、脂質二重膜形成技術など実験上の問題を議論している。第三章では、放射光による基板加工の過程でRahman君が発見した現象である、放射光誘起シュリンキング（放射光照射によりスピンオングラスSOGとよばれるSiO2 膜が縮む）の現象とその応用としての3次元加工の例を記述している。第四章はギガオームシール形成について、基板の加工技術、ベシクルフュージョン技術を詳細に記述している。表面の平坦度を凹凸1nm を維持して電極ホールの穴開けに成功しており、そのために放射光エッチングのマスクであるCoフィルムをフェムト秒レーザー加工により穴開けをし、この穴パターンを利用して放射光エッチングによりSiO2の穴開けを行うという独創的な手法を開発した点が成功に至った大きな理由となっている。従来の世界最高値が200MΩであったものを1.2GΩを達成した。これが単一二重膜であることも蛍光顕微鏡観察や電気特性の測定により確認している。またこの章ではエッジリーク電流を抑制するために、分子の長さが脂質分子のカーボン鎖の長さとほぼ等しいオクタデシルトリクロロシロンの自己組織単分子膜を形成しこれをホトリソグラフィによりパターン形成を行いこの上で脂質二重膜のフュージョンを行い、パターン化したSAM膜をアンカーとする形で二重膜が形成することに成功している。

以上いずれも独創性の高いアイデアと粘り強い実験により世界に先駆けて重要な技術の達成に成功した物で、非常に高いレベルの研究成果である。公開発表もきちんとしており、審査委員会は出願論文が博士（学術）の授与に値すると全員一致で判断した。