Fig.9 から平行に端子位置をずらした磁気センサが従来の端子位置の磁気センサと比べ出力電圧が大きく変化していることが確認できる.

6.3.出力電圧評価

Fig.8 に示したパターンのうち①, ②, ④, ⑦, ⑧の磁束密度に対する出力電圧の測定結果を Fig10 に示す. Fig.10 から端子位置を変更した磁気センサは磁束密度に対して線形に出力電圧が変化していることがわかる. よって, 端子位置を変更した磁気センサは出力電圧向上に利用できると言える.

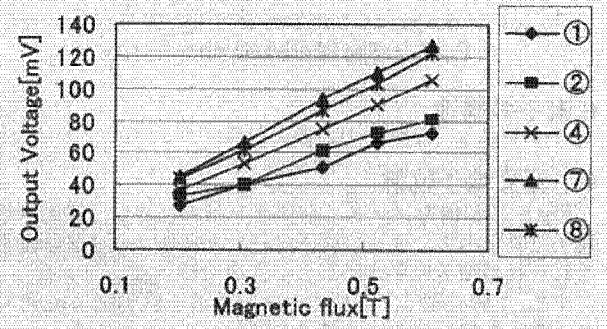

Fig.10 Magnetic flux – Output voltage

従来の端子位置である④に対する各端子の出力電圧比及び標準偏差を Table.1, Fig.11 に示す. Table.1, Fig.11 からドレイン寄りの端子位置である⑦, ⑧において約 20%の出力電圧向上が見られ, 標準偏差は④と比較してもなんら遜色ない.

Table.1 Output voltage ratio at each terminal position

	平均電圧[mV]	増幅率[%]	2ρ[mV]
①	54.15	55.71	7.20
②	70.47	73.02	10.64
③	85.25	88.84	11.09
④	96.82	100.00	10.02
⑤	104.51	109.74	10.75
⑥	110.88	115.05	12.51
⑦	117.32	119.68	10.74
⑧	117.26	119.13	10.16

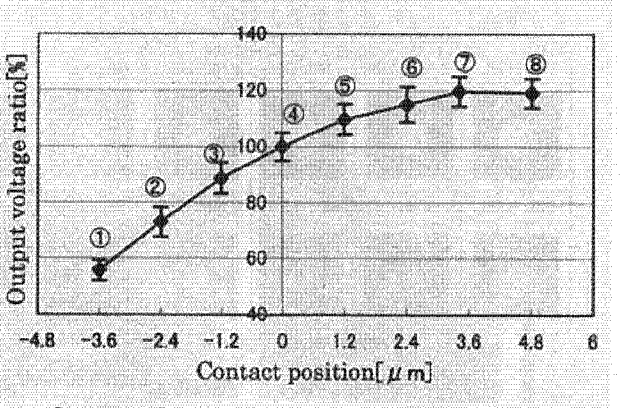

Fig.11 Output voltage ratio at each terminal position

7.まとめ

本研究では, MOS 構造を利用した集積化磁気センサについて検証を行った.

ダイナミックドライブ方式を導入し, 1 つの磁気センサの駆動力を増加させた結果, 位置測定の誤差を 1 画素(±25μm)以内でモーターの制御に必要とされる 1000frame/sec の読み出し速度を達成した.

端子位置を変更した磁気センサで測定を行った結果, 端子位置をドレイン近傍へ変更することで最大約 20%の出力電圧向上が可能であることがわかった.

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Collagen-phospholipid polymer hybrid gel designed for artificial blood vessel

Kwangwoo Nam[1], Tsuyoshi Kimura[1], Toshiya Fujisato[2], Takeshi Nakatani[2], Soichiro Kitamura[2], Akio Kishida[1,2].
[1]Division of Biofunctional Molecules, Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University
2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, JAPAN
[2]National Cardiovascular Center
5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, JAPAN
TEL: 03-5280-8029 FAX: 03-5280-8005 E-mail: bloodnam.fm@tmd.ac.jp.

Introduction: Collagen is often applied for diverse biomaterial, especially for cell related products, for its many advantages. However, use of collagen should be considered very carefully for its high thrombogenicity and low mechanical strength [1]. In order to use collagen as a artificial blood vessel, prevention of graft failure resulting from thrombus formation, and reinforcement of physical and mechanical properties is required. 2-methacryloyloxyethyl phosphorylcholine (MPC)-collagen network gel was developed. MPC polymer is a well-known material for its blood compatiblity and being applied in biomaterial field in various form [2]. In this study, we prepared a collagen hybrid gel using MPC polymer and collagen by immobilizing MPC polymer on the collagen gel surface and characterized the physical properties of this gel.

Methods: Collagen was made into film and was cross-linked by immersed into the 0.05M 2-morpholinoethane sulfonic acid (MES) buffer (pH 9) *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) for 4 hours at 4°C. And then this gel was immersed into MES buffer containing EDC/NHS-preactivated poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-methacrylic acid] (PMA) and immobilized PMA on the collagen gel to make a collagen hybrid gel. To increase the mole ratio of PMA immobilized on the collagen, EDC/NHS activated PMA was re-added to the collagen hybrid gel and immobilized under same condition as written above to make a double-immobilized collagen hybrid gel (DIC hybrid gel). X-ray photoelectron spectroscopy (XPS) and Scanning electron microscope (SEM) was used to characterize the surface of the hybrid gel. Swelling ratio and free amine group analysis was used to characterize the cross-linking efficiency. Collagenase was used to measure the stability of collagen hybrid gel against degradation. And cell adhesion test was executed to characterize the cell compatibility of the collagen hybrid gel.

Results / Discussion: PMA was successfully immobilized on the surface of the collagen gel. XPS result showed that the phospholipids group was mainly deposited on the surface of the collagen gel. And when the surface morphology and the razor-cut surface was observed by SEM, the surface became much smooth compared to that of uncross-linked surface. The razor-cut surface showed that the porous layer, indicating collagen layer, was placed between non-porous layers of PMA. This implies that the collagen hybrid gel would be phase separated, with PMA layer totally covering the collagen gel.

The free amine groups exist in the microfibrils was about 60%. The percentage of unreacted amine group decreased maximum 20% when the gel is cross-linked once again by same procedure. The cross-link of the collagen gel using EDC and NHS is known stop reacting after 1 hour [3], but the re-activation of carboxylic group by EDC and NHS made the collagen to cross-link with PMA and formed much denser network.

The shrinkage temperature increased for the collagen hybrid gel compared to uncross-linked collagen gel. This implies that immobilization of PMA occurred on the surface of the collagen gel, but made the gel tougher and protect the gel from the thermal degradation by forming much denser network. Formation of the denser network increased the elastic modulus also.

The collagen gel would be completely degraded by collagenase within 3 hours. In the case of collagen hybrid gel, approximately 40% would be degraded after 3days. And for DIC gels, it would be left undegraded for more than a week. The leak of PMA was not detected, for DIC gel, indicating that the high cross-link network would be stable.

The contact angle of the collagen gel decreases as the PMA is immobilized on the collagen. The surface of the gel is turning hydrophilic, indicating that the surface is becoming more blood compatible and able to control the cell adhesion. The cell adhesion test using L929 showed that the number of cell adhered on the surface decreases as the density of PMA increased. This is not due to the toxicity, for phospholipids polymer is already proven to be non-toxic [4]. The morphology of the cells was round, indicating the interaction between the cell and surface was suppressed. This result was also observed by other groups [5].

Conclusions: The collagen that was immobilized on the collagen gel was firmly cross-linked with collagen microfibrils. The adoption of phospholipids polymer increased the mechanical property, while maintaining its soft tissue viscoelastic behavior. Increase in the hydrophilicity made the cell difficult to adhere, which is the affect of phospholipid head group.

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P1

Influence of nano-vibration stimuli on cell differentiation for tissue engineering

TK Kimura[1], YI Ito[1], TF Fujisato[2], TM Masuzawa[3], AK Kishide[1]
[1]Tokyo Medical and Dental University, TOKYO, Japan
[2]National Cardiovascular Center Res. Ins, OSAKA, Japan
[3]Ibaraki University, HITACHI, Japan

The handling of cells is one of important factors for tissue engineering. Recently, physical stress and stimuli, such as 2-D stretch, hydrostatic pressure and shear stress, have been extensively studied for controlling cell function. In this study, we report the influence of nano-vibration stimuli as physical stress on cell differentiation. Here, we adopted nano-vibration stimulation system as a novel physical stimulation method. The piezo-electric actuator is employed to apply micrometer- to nanometer amplitude. To investigate the influence of nano-vibration on cell differentiation, PC12 cells were used as model cell and stimulated using nano-vibrator. The cells were seeded on multi culture plate and then nerve growth factor (NGF) was added at final concentration of 50 ng/ml. The cell culture plate was set on nano-vibrator and shaken at various frequencies for 1 hour from day to day. The morphology of live cells was observed by light microscope for 4 days. The cells having at least one neurite with a length equal to the cell body diameter were defined as differentiated cells. The degree of cell differentiation was expressed as a percentage of the total cells. In the case of no addition of NGF, the cells were hardly differentiated with and without nano-vibration stimuli. On the other hand, with NGF, the cell differentiation was observed and promoted with nano-vibration in the initial stages. The length and number of neurite per a cell were investigated using NIH image. The no different in number and length of neurite was observed with or without nano-vibration stimuli. These findings may lead to novel cell culture systems controlling cell differentiation of some of stem and progenitor cells.

P29

Development of bioscaffold preserving collagenic structure in biological tissue

DT Terada[1], K Sawada[2], H Ogata[1], K Yoshida[3], S Funamoto[4], T Fujisato[1], A Kishida[4], N Nagaya[1], T Nakatani[1], S Kitamura[1]
[1]National cardiovascula center, SUITA-CITY OSAKA, Japan
[2]Osaka Seikei College, OSAKA, Japan
[3]Found for Biomed Res & Innovation, KOBE, Japan
[4]Tokyo Medical & Dental Univ, TOKYO, Japan

The development of regenerative vascular grafts is strongly desired especially for the pediatric patients. There are many research works related to artificial grafts made of biodegradable synthetic materials. However, it is still difficult to control the biodegradability due to their hydrolysis, adapt the mechanical properties required in the artery, and reproduce complex shape such as aortic arch. In this study, a regenerative collagenic vascular graft was developed from porcine aorta by removing cells and structural proteins other than collagen from the tissue.
Porcine aorta was isolated from the Clawn miniature pig (Japan Farm, Co. Ltd.). The tissue was placed in a vacuum oven at 120°C to cross-link collagen fibers. Elastin fibers were then taken away form the tissue by enzymatic digestion using elastase of 0.56 u/ml in tris buffer solution including CaCl2 of 10 mM and NaN3 of 0.02% at 37°C with gentle stir. The obtained tissue was subjected to histological and biomechanical studies.
The mechanism of cross-linking by the dehydrate heat treatment in vacuum atmosphere may be attributed to condensation reaction between a carboxyl or hydroxyl group and an amino group of the protein. However, the elastic fibers were digested enzymatically even after the treatment and it was confirmed histologically that the obtained tissue has no elastic fiber and cellular components inside. The collagen fibers remaining in the tissue were also degraded completely by collagenase. The tensile strength certainly decreased after the enzymatic treatment, however an appropriate cross-linking could reduce the decline in tensile strength. The tensile strengths of the obtained vascular graft, a porcine native aorta, and pulmonary artery were 1.09, 2.45, and 0.87 MPa, respectively. The graft may applicable not only to the pulmonary artery but to the other arteries. Also, the graft may have better ability to promote cell infiltration and tissue remodeling compared with the acellular tissue without elastin digestion since the tissue may have more porous structure. The original structure was well preserved all through the process.
The collagenous grafts were prepared by the cross-linking followed by elastin digestion of the porcine vascular tissue. This may be adapted to the vascular tissue regeneration.

P30

Myogenic Pre-Differentiation of Bone-Marrow Human Mesenchymal Stem Cells By in Vitro Electrostimulation

A Genovese[1], R Giambroni[2], O Schussler[2], A Patel[1], T Ravikumar[1], E Lauret[2], A Carpentier[2], JC Chachques[2]
[1]University of Pittsburgh Medical Center, PITTSBURGH, United States of America
[2]Pompidou Hospital, University of Paris., PARIS, France

Objective
Cell therapy using bone marrow stem cells (BMSCs) is emerging as a potential new therapy for myocardial regeneration. These cells could be capable of proliferation and differentiation into cardyomyocytes; however the differentiation of BMSCs into fibroblasts after implantation into an infarct area may increase the risk of ventricular tachyarrhythmia. The cytological differences between bone-marrow stem cells and muscle cells could be a limitation for graft success and a reason for the initial reduction of implanted cells. We investigated the effects of cell preconditioning with bipolar 'ex vivo' electrostimulation of human bone-marrow mesenchymal stem cells (HMSC).

Methods
HMSC cells were collected from patients undergoing CABG procedures or purchased (Poietics, hMSC, Cambrex BSR, Inc.). In the first case, four ml of sternal bone marrow were collected after sternotomy and HMSC selected and purified. Cell cultures were divided in two groups, one without stimulation and one electro stimulated during 3 weeks using two electrodes submerged into the cell culture medium and connected to a pulse generator delivering 9Volts, 0.54 ms pulses at a rate of 120 ppm, similar to fetal cardiac frequency. After each week, cell differentiation was evaluated by changes in cell morphology and with desmin, connexin43 and troponin I-C antibodies.

Results
The electrostimulation of HMSC resulted in myogenic morphologic and immunologic modifications with positive anti-desmin, anti-troponin stain, and changes in the expression and distribution of connexin 43. Cell multiplication was increased in the long-term electrostimulated cultures.

Conclusions
Electrostimulation was investigated for driving the conditioning process of HMSC towards myogenic cells. We observed an increase in cell multiplication, improved cell organization, and primary myogenic pre-differentiation. This method might be used to precondition stem cells or a matrix (grafted with cells) before implant in the ischemic heart with potential benefits in graft survival and therapeutic effects.