1K11-4　Migration promotes myoblast proliferation by reducing myotube formation

○Shiplu CHOWDHURY[1], Yuichi MUNEYUKI[2], Masahiro KINO-OKA[2], Masahito TAYA[3]
([1]Dept. Biotech., Grad. Sch. Eng., Osaka Univ., [2]Grad. Sch. Eng. Sci., Osaka Univ., [3]Dept. Biotech., Grad. Sch. Eng., Osaka Univ.; Grad. Sch. Eng. Sci., Osaka Univ.)

[Objective] Human skeletal muscle myoblast cells explored a new era in the field of cardiac tissue engineering. For transplantation, the expansion of myoblast cells is still challenging due to their lower growth potential accomplished with the formation of myotubes during culture. In this study we attempted to improve the migration activity of myoblast cells by surface modification and medium supplementation that reduce myotube formation resulting in the promotion of cellular proliferation.[Experimental and results] Myoblast cells were cultured in DMEM (containing 10% FBS), with or without EGF, on plain and laminin surface. The population of proliferative cells was detected using BrdU. Modification of surface with laminin, when cultured in DMEM without EGF, increased the migration and also decreased myotube formation which in turn enhanced growth rate 1.3 times as compared to that on plain surface. In addition, online observation revealed that migration was remarkably promoted on laminin surface with EGF supplementation in DMEM and growth rate also were 1.6 times higher than that on plain surface cultured in DMEM without EGF supplementation. These results demonstrate that synergy effect of laminin and EGF facilitates myoblast migration that improves the growth potential.

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○Shiplu CHOWDHURY[1], Yuichi MUNEYUKI[2], Masahiro KINO-OKA[2], Masahito TAYA[3]
([1]Dept. Biotech., Grad. Sch. Eng., Osaka Univ., [2]Grad. Sch. Eng. Sci., Osaka Univ., [3]Dept. Biotech., Grad. Sch. Eng., Osaka Univ.; Grad. Sch. Eng. Sci., Osaka Univ.)

Key words Migration, Myotube formation, Laminin, EGF

1K11-5　高密度三次元培養における培養担体の違いによる細胞機能の変化

○本郷 有克[1], 石田 誠一[2], 小澤 正吾[2], 大野 泰雄[2], 澤田 純一[3], 和田 昌憲[1], 石川 陽一[1]
([1]エイブル株式会社, [2]国立衛研・薬理部, [3]国立衛研・機能生化学部)

【目的】薬物代謝は重要な肝機能の1つである。ヒト組織由来初代肝細胞を用いた薬物代謝酵素誘導に関する研究が盛んに行われているが、再現性や経済的な理由からより簡易で再現性の高いシステムが求められている。我々はこれまでラジアルフロー型バイオリアクター（RFB）を用いた肝癌由来細胞株HepG2の高密度三次元培養においてDNAマイクロアレーによる遺伝子発現解析を行い、平面培養と比較し肝組織に特異的な遺伝子の発現が増強されていることを見出してきた。今回、RFB培養下での薬物代謝酵素誘導について検討を行った。【方法と結果】5mlRFBにてHepG2を培養し、増殖期と定常期まで培養を行い、rifampicin及びdexamethasoneを培地中にそれぞれ添加し添加後3日目（rifampicin）または2日目(dexamethasone)にmRNAを回収した。ヒドロキシアパタイトを培養担体として用いた場合CYP3A4の誘導倍率をRealtime-PCRにて算出した結果、増殖期においてはCYP誘導が確認されたが、定常期においては誘導が確認されなかった。CYP誘導倍率は平面培養と比較し高かったが、PVAスポンジを培養担体として用いた場合では高いCYP誘導は確認できなかった。以上より、培養担体により細胞機能発現が変化することが確認された。

Different modulation of cellular functions under the various scaffolds in three-dimensional high density cell culture system

○Tomokatsu HONGO[1], Seiichi ISHIDA[2], Shogo OZAWA[2], Yasuo OHNO[2], Jun-ichi SAWADA[2], Masanori WADA[1], Yoichi ISHIKAWA[1]
([1]ABLE Corp., [2]NIHS)

Key words Radial-flow bioreactor, CYP3A4, high density cell culture

1K12-1　招待講演
Insulin microcrystals for pulmonary delivery

○Chan-Wha KIM
(Sch. Life Sci/Biotechnol., Korea Univ., Korea)

Pulmonary delivery provides the most promising non-parenteral route for the administration of protein and peptide therapeutics. Insulin was used as a model protein to demonstrate the feasibility of using protein crystals for the pulmonary delivery. Insulin microcrystals with a mean diameter of 3 μm were prepared using a seed zone method. The yield of crystallization was very high (>95%), and the microcrystals were recovered with high efficiency (>98%) by centrifugation. Morphological examination using scanning electron microphotography showed the microcrystals to be of a homogeneous rhombohedral shape, with some rhombus forms, without aggregates. After the administration of 32 U/kg of the microcrystal suspension to STZ-induced diabetic SD rats by intratracheal instillation, the blood glucose levels were reduced and hypoglycemia was prolonged over 13 h, as compared to the insulin solution. The percent minimum reductions of the blood glucose concentration (%MRBG) produced by microcrystal suspension and insulin solution reached 36.5 and 37.2%, respectively, of the initial level, and the percent total reductions in blood glucose ($\%TRBG_{13h}$) were 34.4 and 25.0%, respectively. In the case of inhalation using a sieve-type ultrasonic rebulizer, the %MRBG produced by the microcrystal suspension and solution were 21.7 and 26.3%, respectively, of the initial level, and the $\%TRBG_{13h}$ were 66.7 and 58.4%, respectively. However, the hypoglycemic effects of the microcrystal suspension were prolonged over 7 h, which compares favorably with the insulin solution ($P<0.05$ by unpaired t-test). These results could be attributed to the sustained-release of insulin from the microcrystals, which were deposited widely throughout the entire lung.

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○Chan-Wha KIM
(Sch. Life Sci/Biotechnol., Korea Univ., Korea)

Key words insulin, inhalation, microcrystal, diabetes