(ECM)蛋白的特异性结合所调节。胶原作为含量最丰富的骨胞外基质蛋白,常被用于生物材料表面的预吸附,以促进细胞粘附。而培养基质中往往含有血清以及其它蛋白和表面活性物质,因而细胞外基质在材料表面的吸附受蛋白质竞争性吸附调节,包括基底预处理、培养基以及细胞分泌等许多不同来源的蛋白质竞争吸附。材料表面亲疏水性影响蛋白在表面的吸附,从而影响细胞在材料表面的粘附。研究蛋白在材料表面的吸附及亲疏水性对吸附的影响,有助于研究细胞-材料表面相互作用,并为细胞组织工程中构建有利于细胞粘附和生长的生物材料提供有用信息。本文以胶原和牛血清蛋白(BSA)为模型蛋白,研究了两者的吸附和竞争吸附对细胞粘附和生长的影响。

在单组分溶液中,胶原和牛血清蛋白在疏水处理的硅片上的吸附分别是亲水表面的3倍。但当胶原和BSA的竞争吸附(胶原和BSA的浓度分别为0.1 mg/ml和1 mg/ml)时,光学椭偏成像法和原子力显微镜研究表明胶原优先吸附于亲水表面,而BSA则优先吸附于疏水表面。随着疏水性增加,BSA与胶原竞争吸附的结果是BSA含量增加,而胶原含量降低,在较疏水表面(接触角约80oC时),BSA吸附量占所吸附蛋白的90%。

ROS 17/2.8成骨细胞在胶原和BSA竞争吸附表面的粘附表明:在未经蛋白预处理的亲、疏水表面粘附率均不利于细胞粘附,经BSA预处理的表面抑制细胞粘附,胶原在亲水和疏水表面吸附均能促进细胞粘附,而胶原和BSA竞争吸附或培养基中含血清时,亲水表面由于能优先吸附胞外基质蛋白而促进细胞粘附,而疏水表面由更易吸附BSA或其它非胞外基质蛋白而不利于细胞粘附。因此蛋白的吸附和竞争吸附是影响细胞粘附的直接原因。

在胶原预吸附表面,ROS17/2.8细胞呈现长的、相互连结的胞质突起;细胞的增殖期提前,增殖加快;在汇合期细胞的碱性磷酸酶活性是无胶原处理表面的约1.3倍,说明胶原的处理可以促进细胞的部分分化表型。其原因可能是胶原与整合素特异性相互作用诱导的信号传导与生长因子诱导的信号传导共同作用的结果。

Kinetics and Force Dependence of P-selectin/PSGL-1 Interactions Measured by AFM

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Leukocytes roll along the endothelium of postcapillary venules in response to inflammatory and thrombotic processes. The rolling under hydrodynamic shear forces is a first step in directing leukocytes out of the blood stream into sites of inflammation and is mediated by the selectins, a family of extended, modular, and calcium-dependent lectin receptors[1,2]. The interactions between P-, E- or L-selectins and their counter ligand P-selectin glycoprotein ligand-1 (PSGL-1) play important a crucial role in the processes. Kinetics of selectin/PSGL-1 was extensively studied by using such the state-of-the-art techniques as flow chamber, micropipte aspiration, and atomic force microscopy (AFM)[3-6]. A pioneering work found that the dissociation of P-selectin/PSGL-1 bond increased with external force, demonstrating the catch bond existence using an AFM assay[6]. Here the interactions of P-selectin with PSGL-1 under external forces were further investigated by measuring force dependence of bond dissociation and binding kinetics on the dislodging rate.

P-selectin and PSGL-1 were purified from human platelets and neutrophils, respectively. PSGL-1 was then reconstituted into a supported lipid bilayer, which was formed on a glass coverslip above a polymer cushion[7,8]. P-selectin was immobilized on a Si3N4 tip via the capture antibody S12, which was pre-absorbed on the cantilever tip by overnight incubation followed by incubation in 1% BSA to block nonspecific binding. Experiments were done by repeatedly moving the P-selectin into contact and away from the PSGL-1. Each adhesion test cycle consists of three phases: 1) Approach, 2) Contact, and 3) Retraction, and measurements were reproducible for more than 100 cycles. The probabilities of adhesion and rupture force were simultaneously obtained from such cycles.

Adhesion probability of P-selectin/PSGL-1 interactions was measured at different contact durations and the kinetic parameters were predicted using a small system probabilistic model[4,5,9]. Rupture force data for P-selectin/PSGL-1 dissociation followed a normal distribution. The peak value of rupture force continuously increased with increase of dislodging rate. Data reported here agreed well with those from previous works[10,11]. The work provided new insights into biomolecular dynamics of ligand-receptor interactions process.

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Telomerase Activity and Microgravity - Dependent Apoptosis in Human Leukemia HL - 60 Cells

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Mammalian cells subjected to conditions of spaceflight and the microgravity environment ofspace; manifest a number of alterations in structure and function. Among the most notable changes incells flown on the Space Shuttle are reduced growth activation and decline in growth rate in the totalpopulation[1-3]. Other changes include chromosomal aberrations[4], inhibited locomotion[5], alteredcytokine production[6,7], changes in PKC distribution[8], and increased apoptosis[3]. Human lymphocytesrespond poorly to mitogenic stimulation in microgravity[2] and cells of the lymphoblastoid T cell line,Jurkat, are growth arrested[3].

Telomerase is a ribonucleoprotein enzyme that maintains protective structures at the ends of eukaryoticchromosomes and implicated in cellular resistance to apoptosis.[9]. Telomerase activity can be up regulatedrapidly of in human leukemia HL - 60 cells treated with clinical doses of the DNA - damaging drug etoposide[10]. Few study show telomere and telomerase activity under microgravity[11]. We conducted the present study to determine the relationship between microgravity - dependent apoptosis and telomerase activity in humanleukemia HL - 60 cells.

To examine the increased apoptosis of the human leukemia cells in simulated microgravity, we submitted cultured HL - 60 cells to the rotary cell culture system (RCCS) of varying duration for 2hours,4hours, 6hours, 12hours, 24hours, 48hours. To assess telomerase activity, a PCR - based telomeric repeatamplification protocol assay (TRAP) was used. RT - PCR was performed to examine the mRNA levels of hEST2/hTERT (human telomerase reverse transcriptase), bcl - 2 (B cell leukemia/lymphoma 2 gene) in HL - 60 cells. Apoptosis was determined by microscope, agarose electrophoresis and flow cytometry analysis. We found: after 2hours at microgravity, numerous nuclei underwent the classical morphological alterations (chromatin condensation, nuclear fragmentation, apoptotic bodies) that lead to the programmed cell death, and flow cytometry analysis also found the apoptosis in HL - 60 cells. And at same time, telomerase activity was changed in a time - dependent manner during the microgravity - induced apoptosis of HL - 60 cells. These lines of evidences indicated that regulation of the telomerase activity in HL - 60 cells was closely related to microgravity - induced apoptosis.

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切应力作用下联合培养的血管平滑肌细胞对内皮细胞合成分泌 PDGF - B 的影响*

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为探讨切应力作用下联合培养的血管平滑肌细胞(VSMC)对内皮细胞(EC)PDGF - B 表达的影响。本文应用